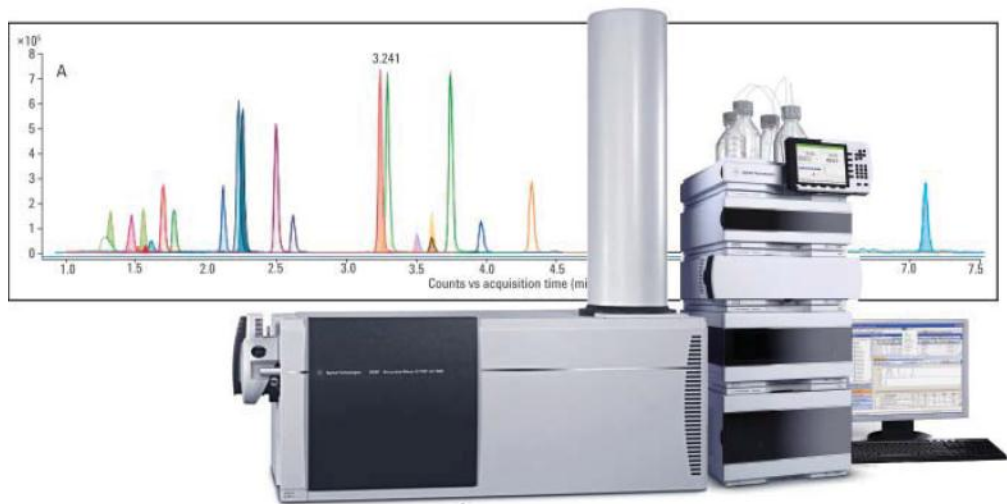


# An Effective Workflow for Impurity Analysis Incorporating High Quality HRAM LCMS & MSMS with Intelligent Automated Data Mining

**Dave Weil, Ph.D. and  
Jim Lau, Ph.D.**



**Agilent Technologies**

# Typical Method Conditions: 1260 UHPLC

**UHPLC:** Agilent 1260 Binary pump, well plate autosampler, thermostatted column compartment

**Column:** Agilent Zorbax Eclipse Plus C-18, 1.8  $\mu$ m, 2.1 mm x 50 mm

**Column temperature:** 40°C

**Injection volume:** 1 or 2  $\mu$ l Oxytetracycline standard

**Autosampler temp:** 4 °C

**Needle wash:** flushport (water:methanol 50:50), 3 seconds

**Mobile phase:**  
A = 0.05% Trifluoroacetic acid in H<sub>2</sub>O  
B = 0.045% Trifluoroacetic acid in ACN

**Flow rate:** 0.5ml/min

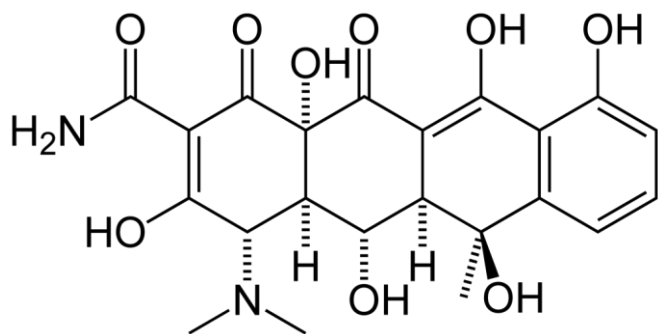
**Gradient:**

Time (min)	%B
0.00	10
7.50	50

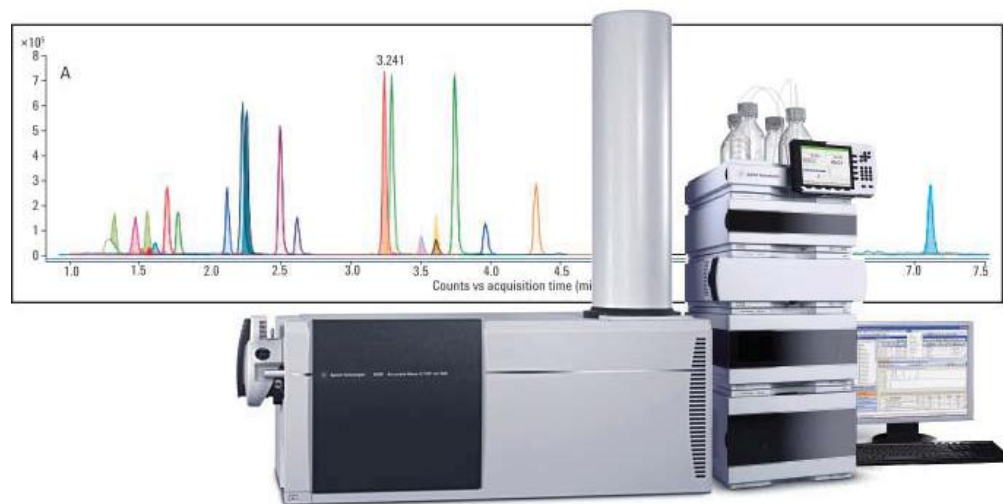


# Impurity Analysis of Oxytetracycline and H2O Stressed (30 min at 90 deg C)

## Oxytetracycline

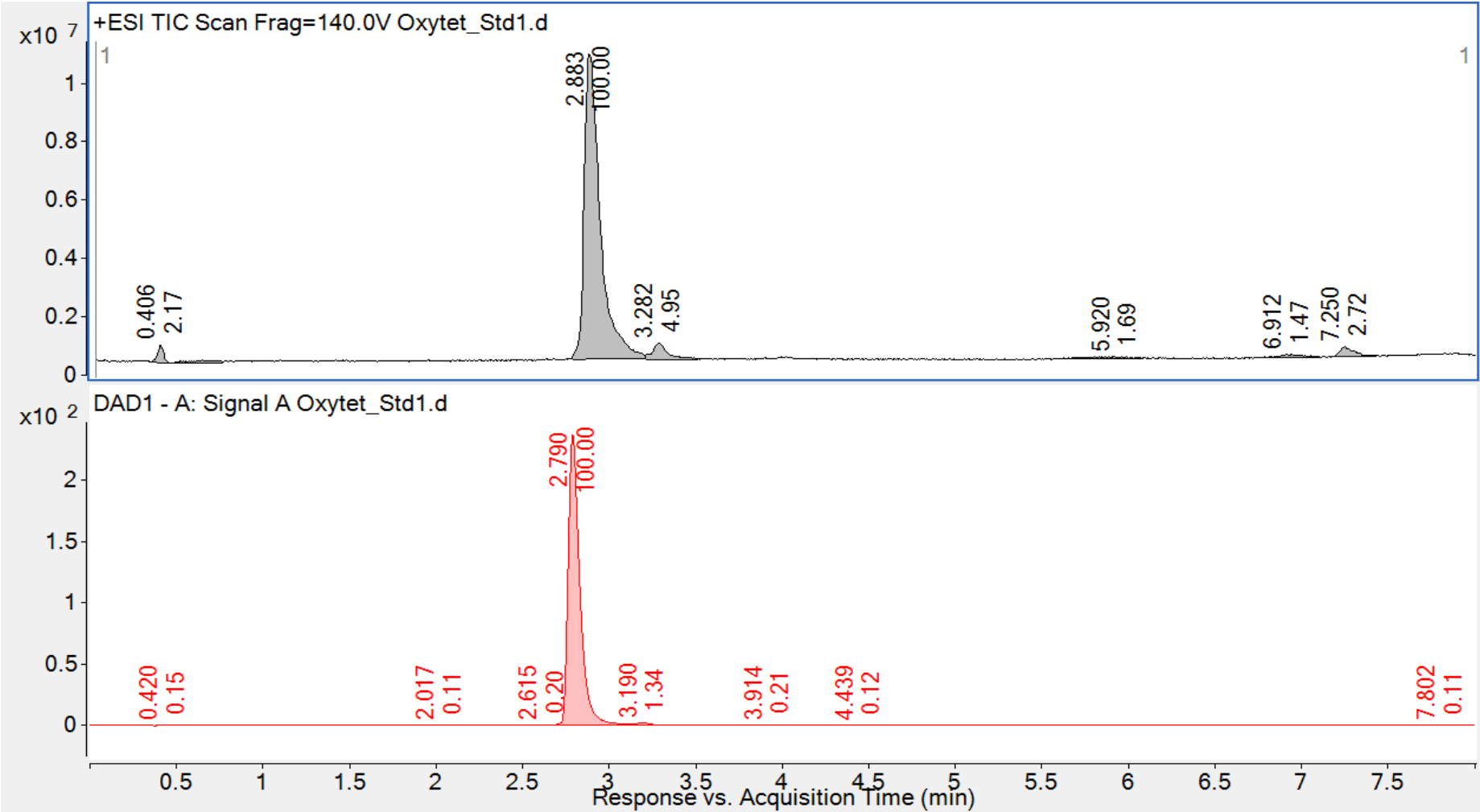


trade name: Terramycin  
C<sub>22</sub>H<sub>24</sub>N<sub>2</sub>O<sub>9</sub>  
460.148183

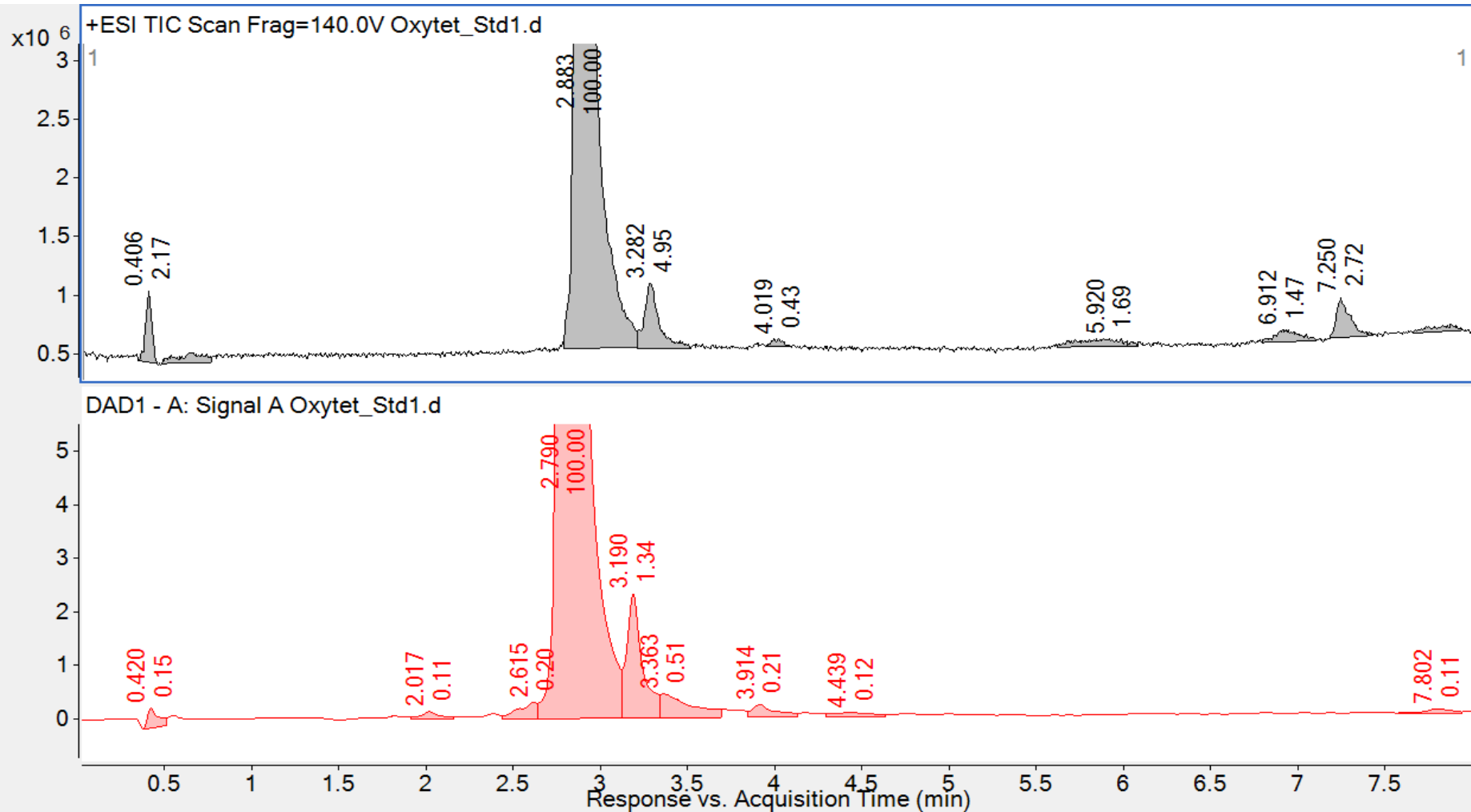


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# Pos ESI LC/MS TIC and UV Chromatograms of Standard Oxytetracycline:



# Expanded TIC and UV Chromatograms of Standard Oxytetracycline:

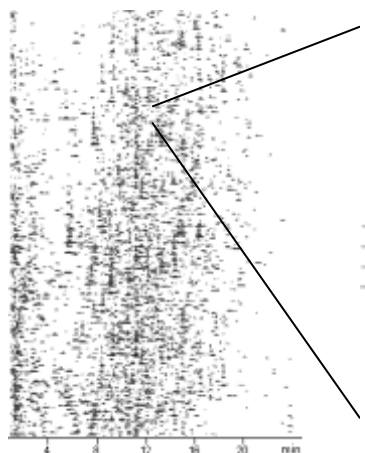
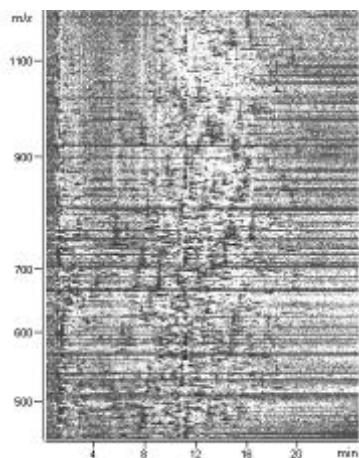


**1st Step – Use MassHunter Qual software to find all components in the sample using the automated MFE (Molecular Feature Extraction) chromatographic deconvolution algorithm.**



# Molecular Feature Extractor (MFE)

## Transforming Accurate MS Data to Chemical Information



species	RT	m/z	mass	abund.
M+3H	13.248	921.5025	2761.4856	356764
M+3H+1	13.247	921.8373	2761.4874	548320
M+3H+2	13.248	922.1722	2761.4897	476790
M+3H+3	13.249	922.5072	2761.4924	256680
M+3H+4	13.249	922.8416	2761.4934	117149
M+3H+5	13.251	923.1744	2761.4892	45705
M+3H+6	13.252	923.5058	2761.4810	15721
M+3H+7	13.246	923.8387	2761.4775	4492
M+3H+8	13.263	924.1649	2761.4535	677
M+2H+Na	13.248	928.8293	2761.4842	29521
M+2H+Na+1	13.249	929.1643	2761.4863	46140
M+2H+Na+2	13.247	929.4957	2761.4781	43276
M+2H+Na+3	13.247	929.8283	2761.4737	33217
M+2H+Na+4	13.258	930.1614	2761.4706	23203
M+2H+Na+5	13.285	930.5000	2761.4842	11741
M+2H+Na+6	13.277	930.8343	2761.4846	3970
M+2H+K	13.247	934.1483	2761.4673	8064
M+2H+K+1	13.241	934.4888	2761.4863	13793
M+2H+K+2	13.244	934.8196	2761.4759	11070
M+2H+K+3	13.250	935.1566	2761.4847	5695
M+2H+K+4	13.247	935.4933	2761.4926	3721
M+2H+K+5	13.280	935.8045	2761.4237	1182
M+H+2Na	13.238	936.1547	2761.4783	6951
M+H+2Na+1	13.241	936.4933	2761.4913	9925
M+H+2Na+2	13.244	936.8294	2761.4853	8407
M+H+2Na+3	13.251	937.1567	2761.4769	7074
M+H+2Na+4	13.256	937.4817	2761.4497	3386
M+H+2Na+5	13.242	937.8123	2761.4392	2586

Export	features: 4664 / groups: 1167				
	RT	mass	abund.	height	#ions
463	7.715	1271.5348	11146	1329	3
464	7.716	1573.7000	1223	188	2
465	7.716	740.3860	18753	1216	4
466	7.716	1970.7335	2788	281	3
467	13.242	2826.4049	11853	662	4
468	13.244	2814.4034	60168	1698	8
469	13.245	2816.4119	8707	680	3
470	13.245	2331.1504	3191	291	3
471	13.247	1407.6968	4377	385	2
472	13.249	2761.4877	4778072	79795	78
473	13.250	2433.2736	3736	399	3
474	13.250	2806.4575	16286	650	7
475	13.252	1123.6411	4246	416	2
476	13.253	2068.0019	14029	665	7
477	13.253	2783.4528	27728	1212	8
478	9.159	677.2975	8801	714	2
479	9.159	2173.8394	3492	427	3
480	9.161	1310.6477	1838172	70500	23
481	9.161		12218	1585	1
482	9.162	1334.6137	75374	2869	10
483	10.385	1540.6853	2023	344	2
484	10.387	1076.6421	27577	4103	4

Raw data



Background noise removed



Individual m/z peaks grouped into isotope clusters



Isotope clusters grouped into molecular features

Identification, Quantification, Differential Analysis are performed on *chemically qualified compound data*



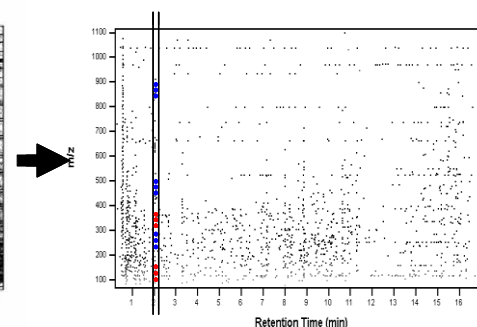
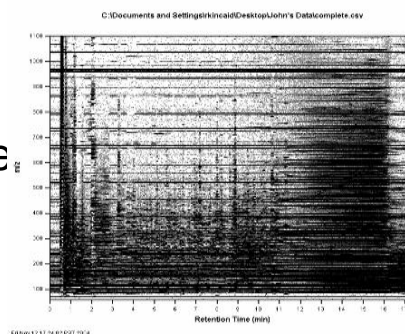
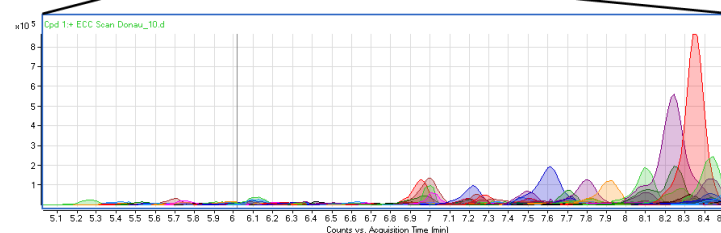
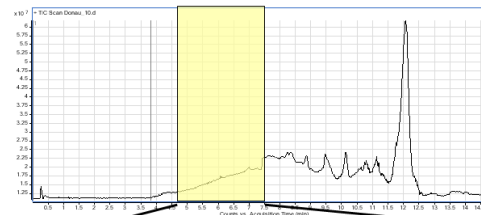
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# Molecular Feature Extraction (MFE)

## Compound Finding Algorithm

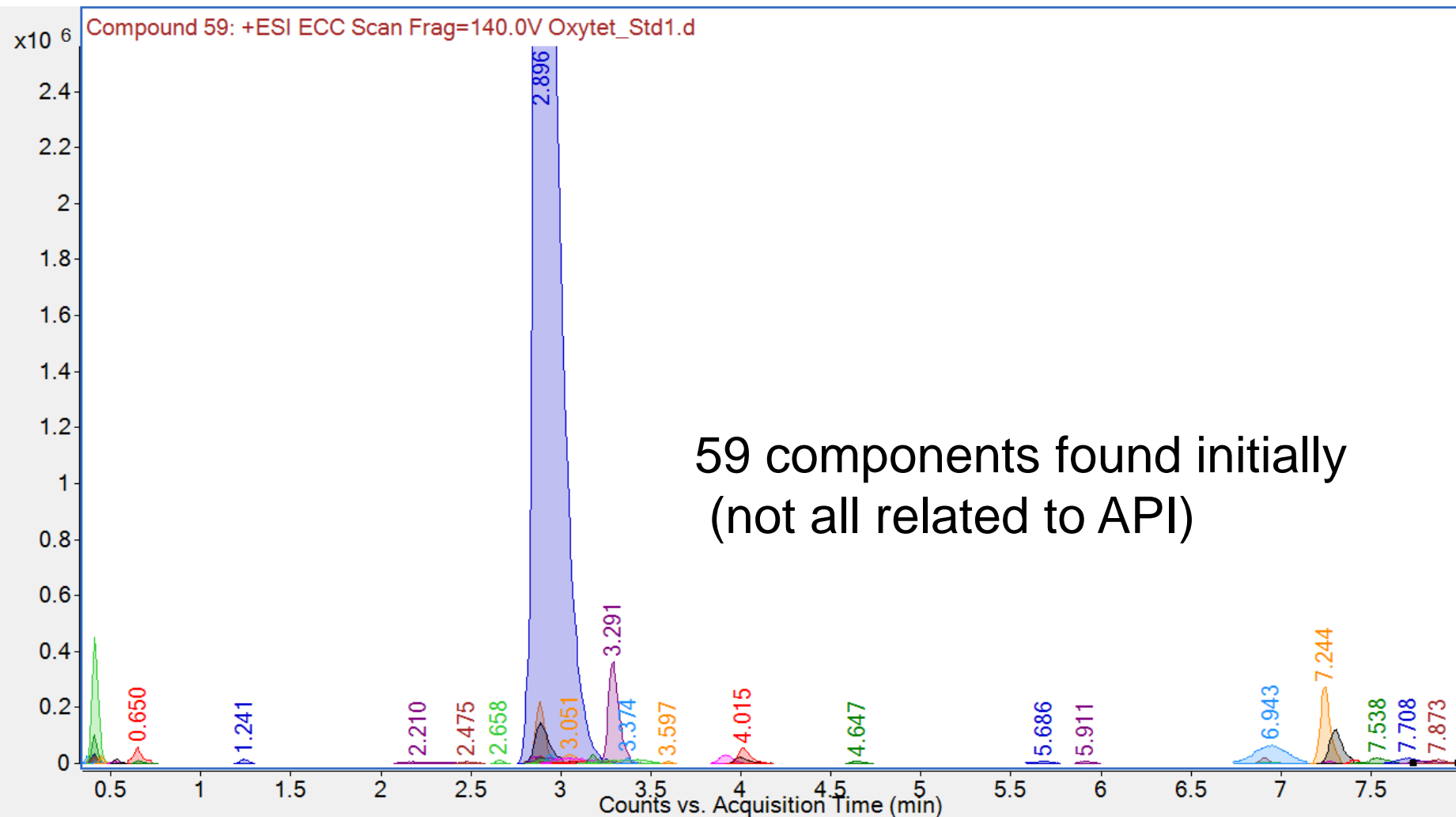
Data is processed by a proprietary feature-finding algorithm

- Finds chromatographic peaks
  - Finds all ions that are related
  - Includes any adducts, such as  $\text{Na}^+$  or  $\text{K}^+$
  - Includes isotopes  $A+1$ ,  $A+2$  for all adducts found...
  - Includes different charge states
  - Checks for dimers
  - Creates a compound spectra (ECC)
- Sums all ion signals into one value (Feature with associated Volume)
- Fully automated processing
- Creates data file for export to MassHunter





# Molecular Feature Extracted (MFE) Component Chromatograms for Oxytetracycline Standard:



# Several filters are available to find API relevant impurities (Mass Defect, Common MS/MS Fragment, and MSMS Neutral Loss):

**Method Editor: Find Compounds by Molecular Feature**

Find Compounds by Molecular Feature | Method Items

Extraction | Ion Species | Charge State | **Compound Filters** | Mass Filters | Mass Defect | Peak Filters (MS/MS) | Results

Mass defect filtering

☒ Filter results on mass defects

Expected mass defect

Constant

0.1600 Da + ( 0.0000 per 100.00 Da )

Calculate from formula

Mass defect tolerance

Constant (symmetric)

+/- 0.0300 Da



# Variation from Nominal Mass for Selected Elements

H = 1.0078

F = 18.9984

H+ = 1.0073

O = 15.9949

Br = 78.9183

Cl = 34.9689

C<sub>12</sub> H<sub>18</sub> N O Br = 271.0572

Si O<sub>3</sub> C<sub>13</sub> H<sub>28</sub> = 280.1808



# How Mass Defect Allows Discovery of Related Components

**Biotransformation Mass Defects**

Parent Compound  
 Parent Compound Molecular Formula:


Mass Defect  
 Mass defect range of the selected biotransformations:  -  Da  
 Difference of selected biotransformation mass defects from parent:  -  Da

Biotransformations

Use	Name	Phase	Formula	Mass	Req.	Result Formula	Result Mass	Defect	Δ Defect
<input checked="" type="checkbox"/>	Parent	I		0.00000		C25 H32 Cl N5 O2	469.22445	0.22445	0.00000
<input checked="" type="checkbox"/>	2x Hydroxylation	I	+O2	31.98983		C25 H32 Cl N5 O4	501.21428	0.21428	-0.01017
<input checked="" type="checkbox"/>	Hydroxylation	I	+O	15.99491		C25 H32 Cl N5 O3	485.21937	0.21937	-0.00509
<input checked="" type="checkbox"/>	Second/Third Amine to Hydroxylamine/N-Oxide	I	+O	15.99491	N	C25 H32 Cl N5 O3	485.21937	0.21937	-0.00509
<input checked="" type="checkbox"/>	Aromatic Ring to Arene Oxide	I	+O	15.99491		C25 H32 Cl N5 O3	485.21937	0.21937	-0.00509
<input checked="" type="checkbox"/>	Alkene to Epoxide	I	+O	15.99491		C25 H32 Cl N5 O3	485.21937	0.21937	-0.00509
<input checked="" type="checkbox"/>	Oxidative Dechlorination	I	+O+H-Cl	-17.96611	Cl	C25 H33 N5 O3	451.25834	0.25834	0.03389
<input type="checkbox"/>									

7 of 120 biotransformations selected

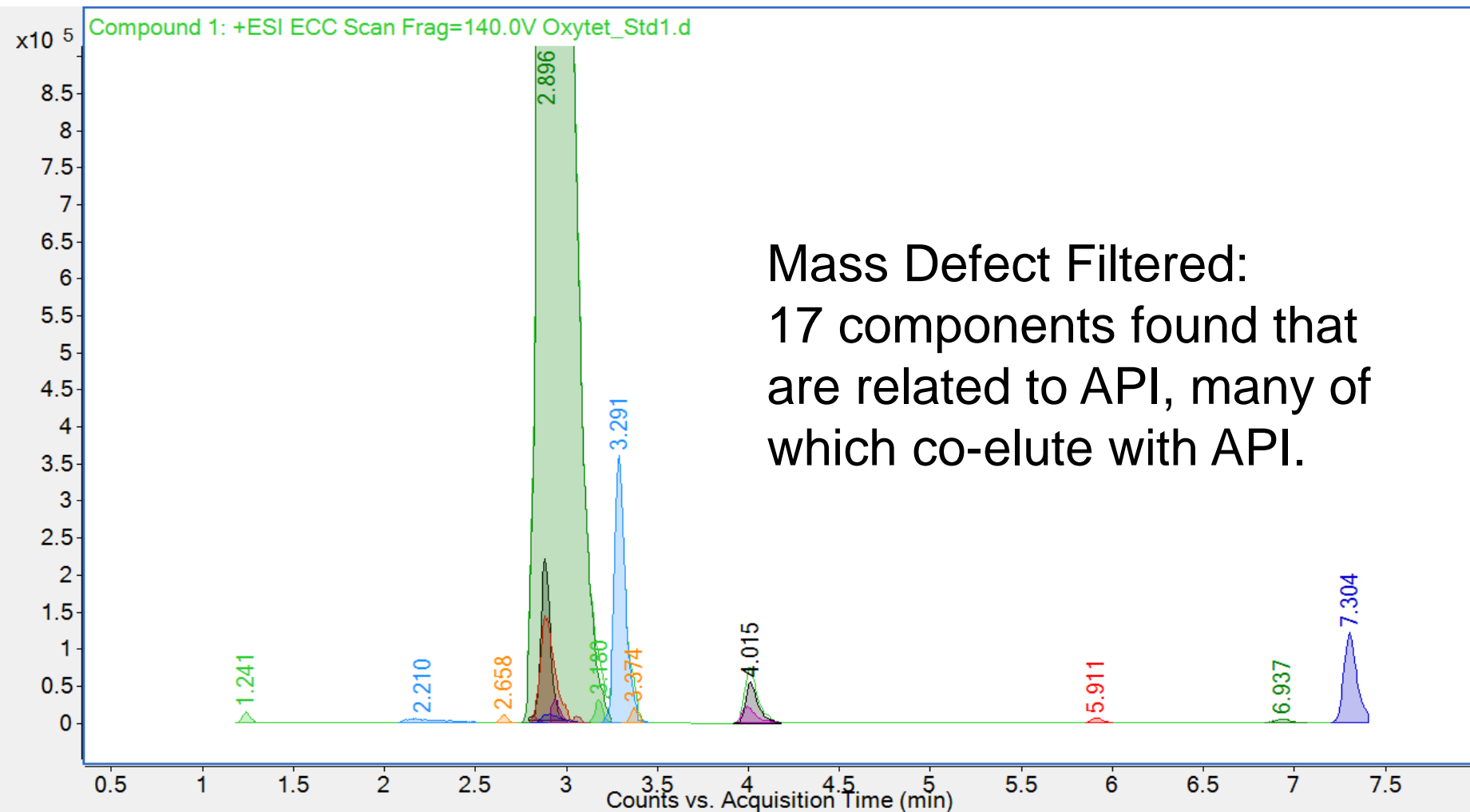
☒ Restrict to possible  
☒ Hide not selected

 **Agilent Technologies**

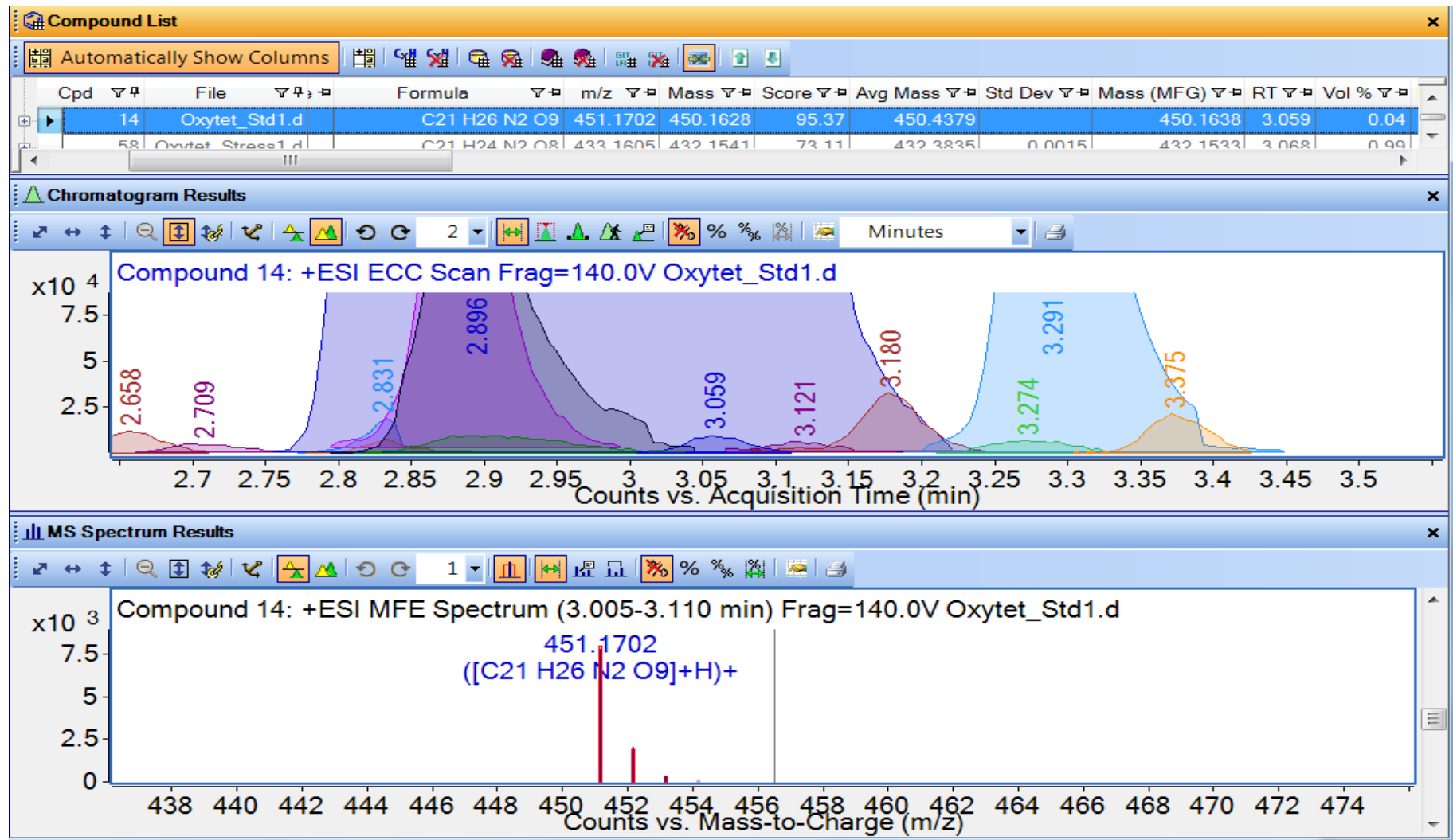
**2nd Step – Use Mass Defect Filter to eliminate compounds not related to structure/formula of API**



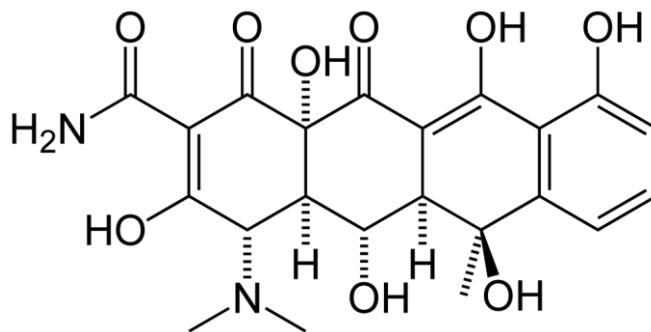
# Molecular Feature Extracted Component Chromatograms for Oxytetracycline Standard



# Note impurities hidden under API UV signal, all found by MassHunter MFE:



**3rd Step – Use Molecular Formula Generator (MFG)  
to propose elemental formula for all the  
compounds (“features”) related to API**





# Molecular Formula Generation (MFG) Scoring

## Use All Available Information – Consolidated Format

Scoring based on

**Method Editor: Generate Formulas**

Method Items

Allowed Species Limits Charge State Scoring

Contribution to overall score

Mass score: 100.00

Isotope abundance score: 60.00

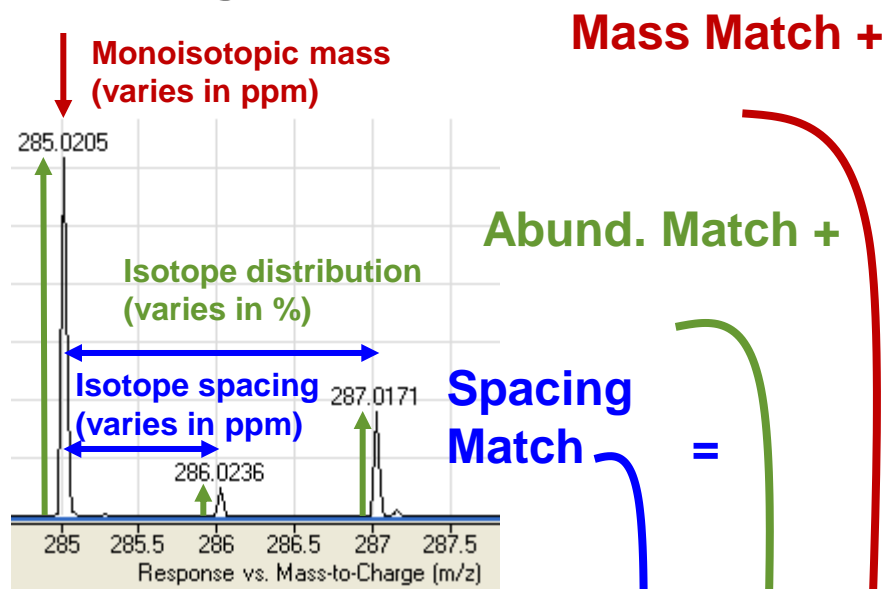
Isotope spacing score: 50.00

Expected data variation

MS mass: 2.0 mDa + 5.6 ppm

MS isotope abundance: 7.5 %

MS/MS mass: 5.0 mDa + 7.5 ppm



**Overall Score**

**MS Formula Results: Cpd 2: C<sub>10</sub>H<sub>9</sub>CIN<sub>4</sub>O<sub>2</sub>S**

	m/z	Ion	Formula	Abundance
	285.021	(M+H) <sup>+</sup>	C <sub>10</sub> H <sub>9</sub> CIN <sub>4</sub> O <sub>2</sub> S	24506.1

Best	Formula (M)	Calc m/z	Score	Cross Score	Mass	Calc Mass	Diff (ppm)	Abs Diff (p)	Spacing Matc	Abund Matc	Mass Match	m/z	DBE
<input checked="" type="checkbox"/>	C <sub>10</sub> H <sub>9</sub> CIN <sub>4</sub> O <sub>2</sub> S	285.0208	99.55		284.0137	284.0135	-0.71	0.71	99.19	99.26	99.69	285.021	8
<input type="checkbox"/>	C <sub>7</sub> H <sub>12</sub> N <sub>2</sub> O <sub>6</sub> S <sub>2</sub>	285.021	77.28		284.0137	284.0137	0.01	0.01	99.54	1.93	100	285.021	3
<input type="checkbox"/>	C <sub>7</sub> H <sub>13</sub> CIN <sub>4</sub> O <sub>2</sub> S <sub>2</sub>	285.0241	75.57		284.0137	284.0168	11.12	11.12	99.87	83.87	46.22	285.021	3

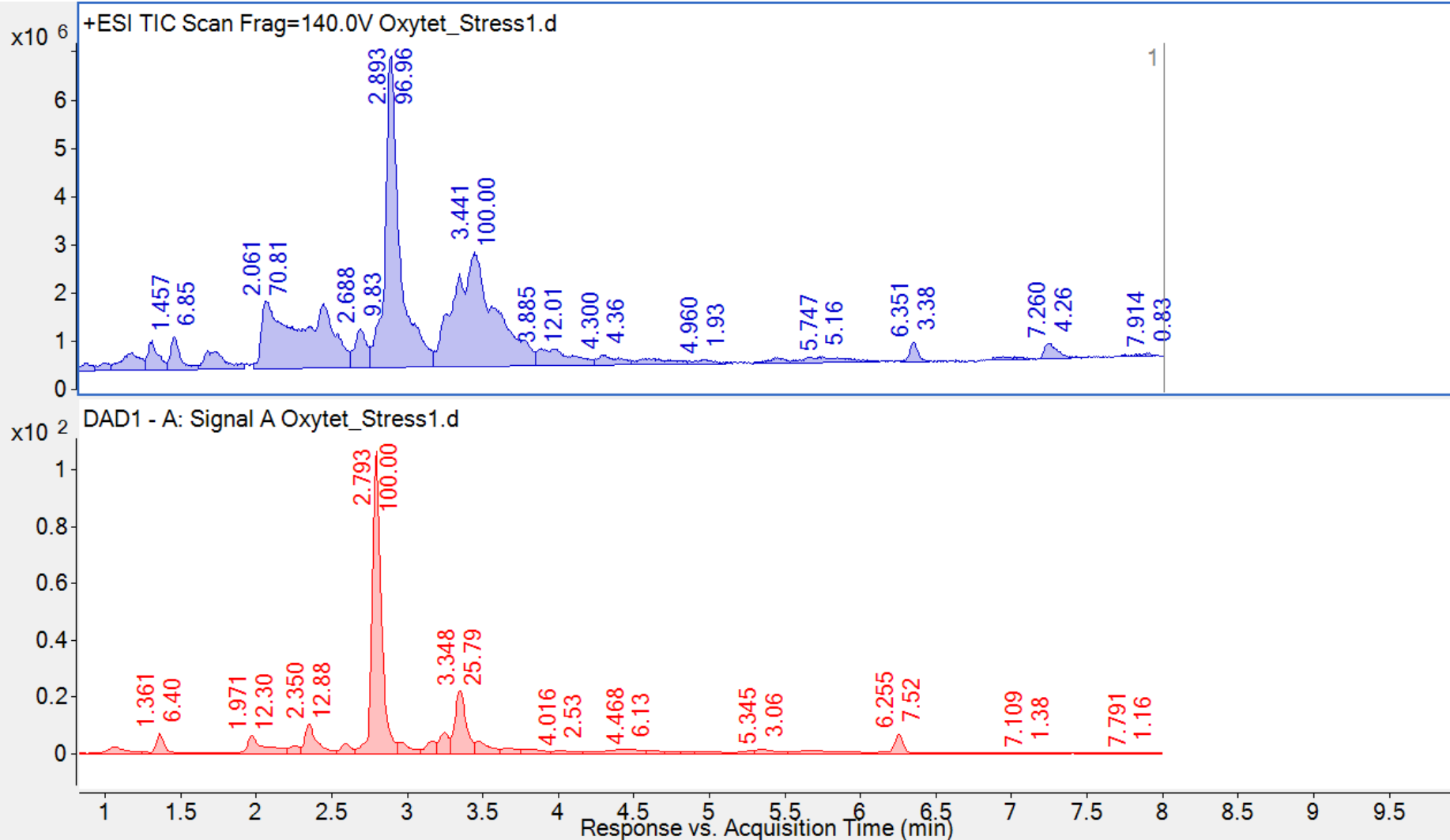


# Mass Defect Filtering yields high proportion of API related components.

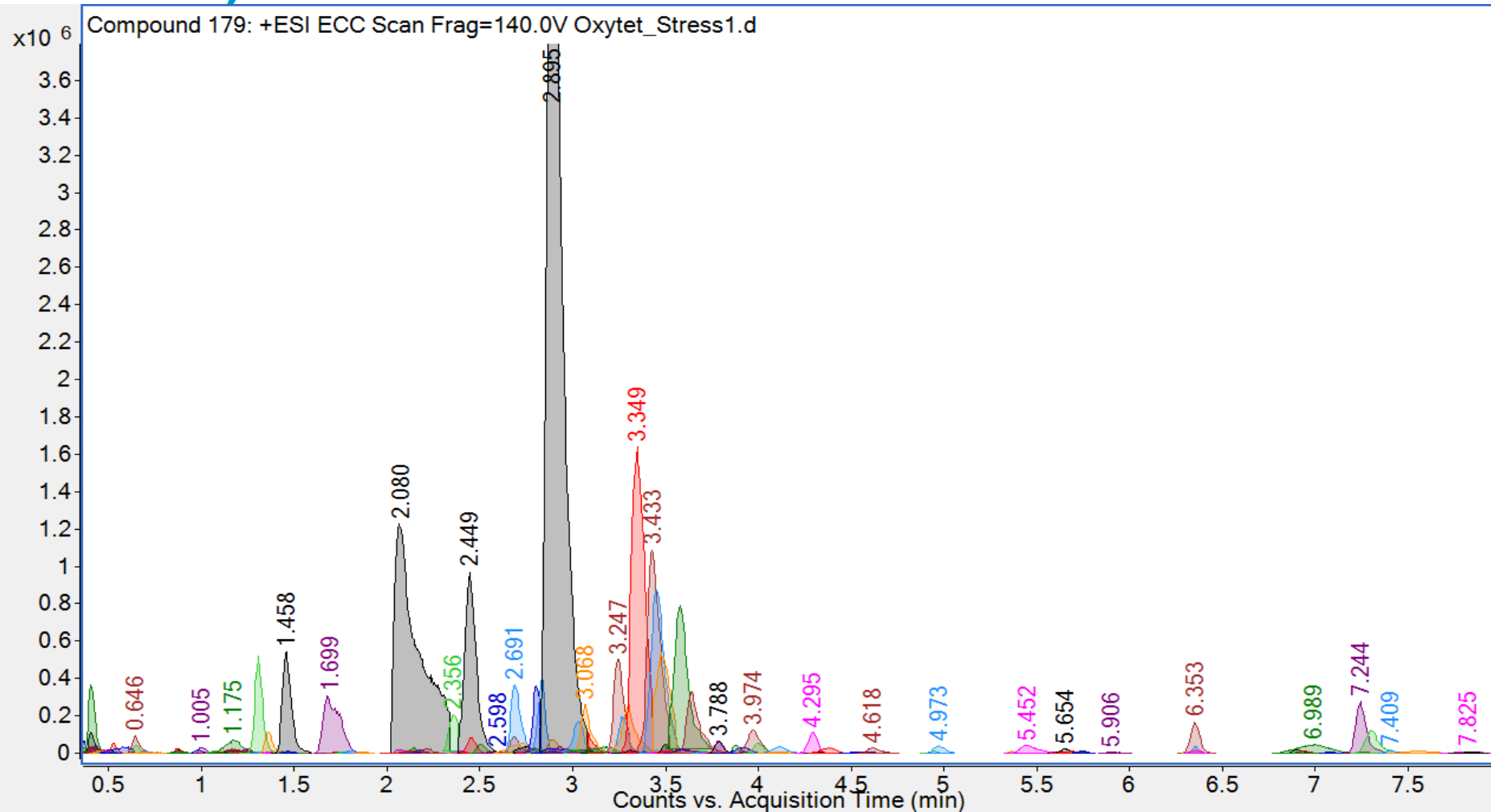
Compound List														
Automatically Show Columns														
	Label	Formula	RT	Score	Mass	Std Dev	Vol %	Vol	Width	Height	Base Peak	Mass (MFG)	Diff (MFG, ppm)	
+	1.d Compound 4	C21 H24 N2 O8	2.834	76.62	432.1523		0.03	20768	0.075	5959	433.1596	432.1533		
+	1.d Compound 5	C23 H25 N O9	2.885	47.38	459.1533	0.0003	1.23	838963	0.066	220359	460.1605	459.1529		
+	1.d Compound 6		2.89		442.1371		1.21	825158	0.102	85860	443.1443			
+	1.d Compound 7	C22 H24 N2 O9	2.896	99.07	460.1486	0.0003	93.06	63438896	0.128	6767952	461.1558	460.1482		
+	1.d Compound 8	C22 H24 N2 O10	2.906	77.96	476.1428		0.09	61483	0.095	7499	477.15	476.1431		
+	1.d Compound 9	C21 H26 N2 O9	3.059	95.37	450.1628		0.04	29271	0.049	7759	451.1702	450.1638		
+	1.d Compound 10	C21 H26 N2 O8	3.18	99.53	434.1686		0.14	97326	0.049	25494	435.1759	434.1689		
+	1.d Compound 11	C22 H24 N2 O8	3.291	99.1	444.1532	0.0001	2.29	1559485	0.075	279864	445.1607	444.1533		
+	1.d Compound 12	C21 H26 N2 O8	3.375	95.65	434.168		0.09	62432	0.048	16640	435.1749	434.1689		
+	1.d Compound 13	C22 H24 N2 O9	3.998	97.07	460.1472		0.18	125180	0.111	18037	461.1544	460.1482		
+	1.d Compound 14	C22 H24 N2 O8	4.015	98.46	444.1527		0.39	262542	0.087	43869	445.16	444.1533		
+	1.d Compound 15	C16 H25 N O2	5.911	81.44	263.1878		0.05	33687	0.079	5804	264.1953	263.1885		
+	1.d Compound 16	C9 H19 N O	6.936	86.06	157.1464		0.06	38913	0.118	5275	158.1538	157.1467		
+	1.d Compound 17	C13 H24 N2 O	7.304	87.26	224.1888	0.0004	0.9	615018	0.085	104506	225.1961	224.1889		



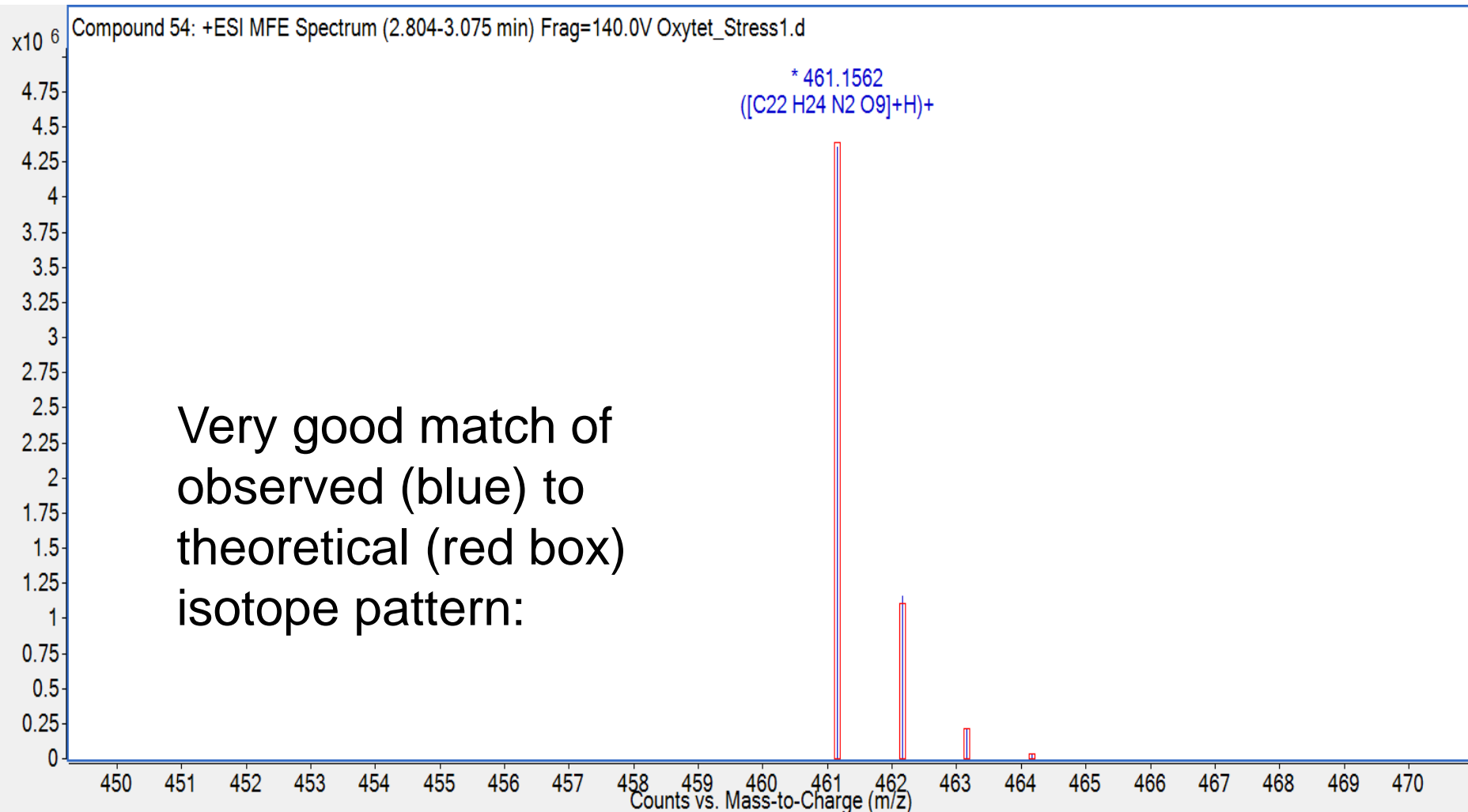
# TIC and UV Chromatograms of H2O Stressed Oxytetracycline



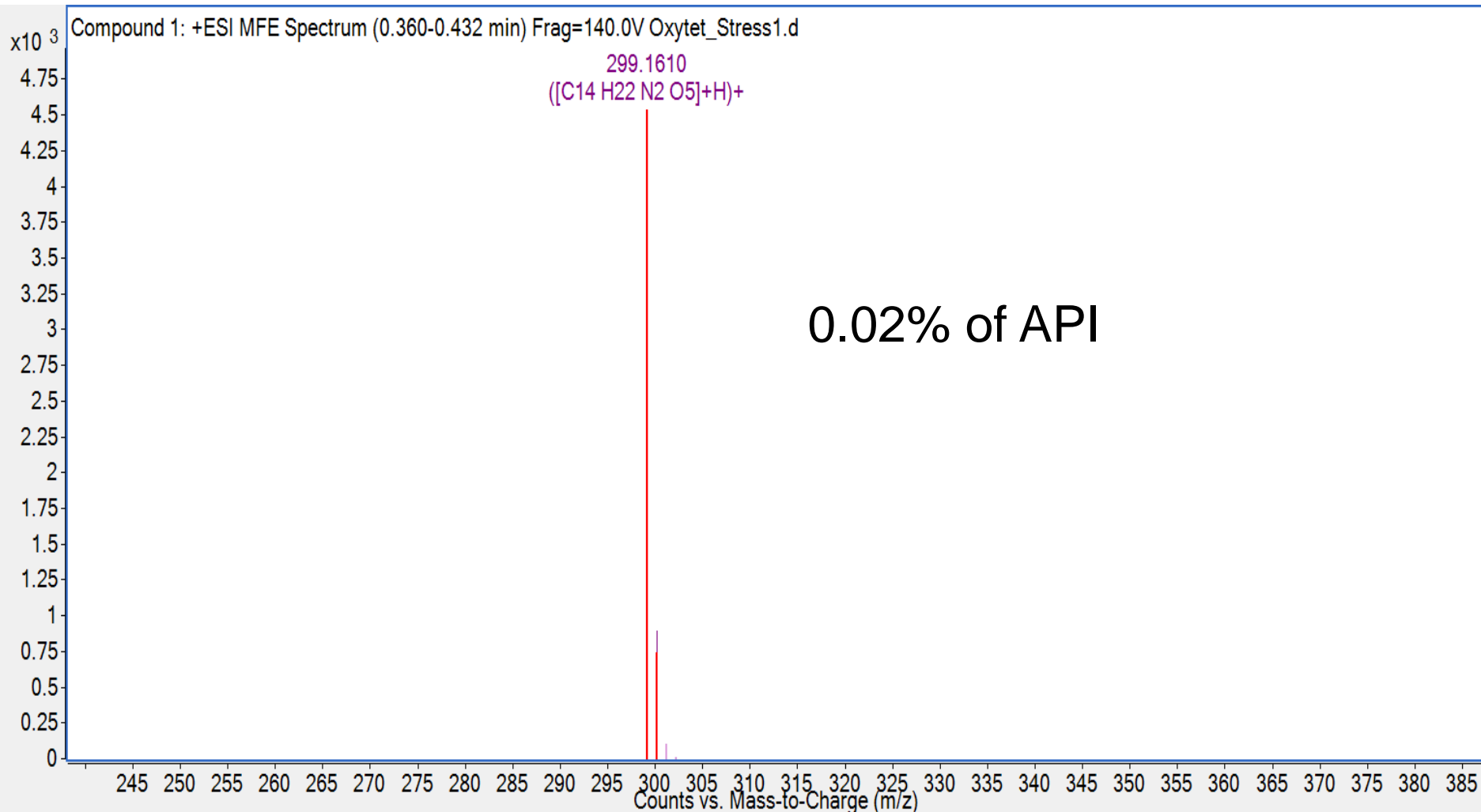
# Molecular Feature Extracted and Mass Defect Filtered Component Chromatograms for Oxytetracycline (H<sub>2</sub>O Stressed)



# Dynamic Range for API (oxytetracycline) in Stressed Sample: Area (V) = 32,008,508



# Dynamic Range for Degradation Component at 0.02% level in Stressed Sample: Area (V) = 7,057



# Formula Generation (MFG) shows the efficiency of extracting related components from H2O stressed sample:

Compound List															
Automatically Show Columns															
Show/Hide	Cpd	File	Label	Formula	RT	Sco	Mass	Avg Mass	Std Dev	Vol %	Vol	Width	Height	Base Peak	
<input checked="" type="checkbox"/>	30	Oxytet_Stress1.d	Compound 30	C22 H26 N2 O10	2.454	99.65	478.1591	478.4583		0.29	286141	0.054	65375	479.1664	
<input checked="" type="checkbox"/>	58	Oxytet_Stress1.d	Compound 58	C21 H26 N2 O8	3.349	99.52	434.1691	434.4346		8.43	8314507	0.078	1273518	435.1763	
<input checked="" type="checkbox"/>	19	Oxytet_Stress1.d	Compound 19	C21 H26 N2 O8	2.08	99.29	434.1692	434.4574	0.0009	11.31	11157941	0.229	927936	435.1764	
<input checked="" type="checkbox"/>	65	Oxytet_Stress1.d	Compound 65	C21 H24 N2 O7	3.583	99.2	416.1583	416.4043	0.0027	4.49	4424762	0.103	624789	417.1656	
<input checked="" type="checkbox"/>	60	Oxytet_Stress1.d	Compound 60	C21 H24 N2 O7	3.433	99.19	416.1585	416.4063		5.41	5341620	0.08	858938	417.1658	
<input checked="" type="checkbox"/>	61	Oxytet_Stress1.d	Compound 61	C22 H24 N2 O9	3.456	99.16	460.1485	460.4244	0.0009	4.89	4822337	0.088	643484	461.1557	
<input checked="" type="checkbox"/>	17	Oxytet_Stress1.d	Compound 17	C21 H26 N2 O8	1.699	99.1	434.1688	434.431		1.94	1909862	0.105	239508	435.1762	
<input checked="" type="checkbox"/>	74	Oxytet_Stress1.d	Compound 74	C22 H24 N2 O9	3.974	99.1	460.1481	460.4347		0.63	621419	0.08	96979	461.1556	
<input checked="" type="checkbox"/>	25	Oxytet_Stress1.d	Compound 25	C22 H24 N2 O9	2.356	99.05	460.1482	460.4251	0.0029	0.69	679638	0.047	158095	461.1557	
<input checked="" type="checkbox"/>	15	Oxytet_Stress1.d	Compound 15	C22 H24 N2 O9	1.458	98.95	460.1485	460.413	0.0016	1.89	1868410	0.061	416010	461.1559	
<input checked="" type="checkbox"/>	52	Oxytet_Stress1.d	Compound 52	C22 H24 N2 O9	3.247	98.93	460.1483	460.4076	0.0018	1.97	1945065	0.062	394815	461.1557	
<input checked="" type="checkbox"/>	43	Oxytet_Stress1.d	Compound 43	C22 H24 N2 O9	2.895	98.9	460.1476	460.4701	0.001	33.16	32713932	0.101	4358982	461.1562	
<input checked="" type="checkbox"/>	44	Oxytet_Stress1.d	Compound 44	C21 H24 N2 O9	2.939	98.88	448.1476	448.4248		0.2	199553	0.116	27658	449.1548	
<input checked="" type="checkbox"/>	37	Oxytet_Stress1.d	Compound 37	C22 H24 N2 O9	2.802	98.8	460.1485	460.4183		0.87	857048	0.034	282129	461.156	
<input checked="" type="checkbox"/>	34	Oxytet_Stress1.d	Compound 34	C22 H26 N2 O10	2.691	98.78	478.1591	478.4403		1.46	1442104	0.073	281843	479.1666	
<input checked="" type="checkbox"/>	29	Oxytet_Stress1.d	Compound 29	C22 H24 N2 O9	2.449	98.74	460.1488	460.4177	0.0011	4.29	4229494	0.076	748382	461.156	
<input checked="" type="checkbox"/>	13	Oxytet_Stress1.d	Compound 13	C21 H26 N2 O8	1.308	98.69	434.1692	434.4091		1.51	1487784	0.052	414266	435.1767	
<input checked="" type="checkbox"/>	12	Oxytet_Stress1.d	Compound 12	C21 H28 N2 O9	1.25	98.66	452.1788	452.4415		0.09	91620	0.063	19801	453.1861	
<input checked="" type="checkbox"/>	71	Oxytet_Stress1.d	Compound 71	C19 H22 N2 O8	3.788	98.62	406.1369	406.3924		0.22	212531	0.053	51814	407.1443	
<input checked="" type="checkbox"/>	54	Oxytet_Stress1.d	Compound 54	C22 H24 N2 O8	3.272	98.59	444.1532	444.4302	0.0012	0.75	736332	0.064	151385	445.1607	
<input checked="" type="checkbox"/>	50	Oxytet_Stress1.d	Compound 50	C22 H24 N2 O9	3.094	98.41	460.1483	460.4645	0.0025	0.25	248595	0.053	82532	461.1557	
<input checked="" type="checkbox"/>	39	Oxytet_Stress1.d	Compound 39	C21 H24 N2 O9	2.819	98.25	448.1474	448.4089		0.09	91546	0.057	19383	449.1546	
<input checked="" type="checkbox"/>	14	Oxytet_Stress1.d	Compound 14	C21 H28 N2 O9	1.362	97.9	452.1786	452.4552		0.34	332937	0.049	87778	453.1859	
<input checked="" type="checkbox"/>	16	Oxytet_Stress1.d	Compound 16	C21 H27 N O10	1.468	97.83	453.1628	453.4515		0.04	35294	0.048	9283	454.1702	
<input checked="" type="checkbox"/>	80	Oxytet_Stress1.d	Compound 80	C21 H24 N2 O6	4.618	97.78	400.1626	400.478		0.14	137115	0.083	22720	401.1699	
<input checked="" type="checkbox"/>	2	Oxytet_Stress1.d	Compound 2	C21 H26 N2 O8	0.695	97.66	434.1682	434.4514		0.05	46317	0.054	12766	435.1754	
<input checked="" type="checkbox"/>	45	Oxytet_Stress1.d	Compound 45	C20 H25 N O7	3.011	97.64	381.1624	381.4337		0.06	61662	0.047	17529	382.1607	

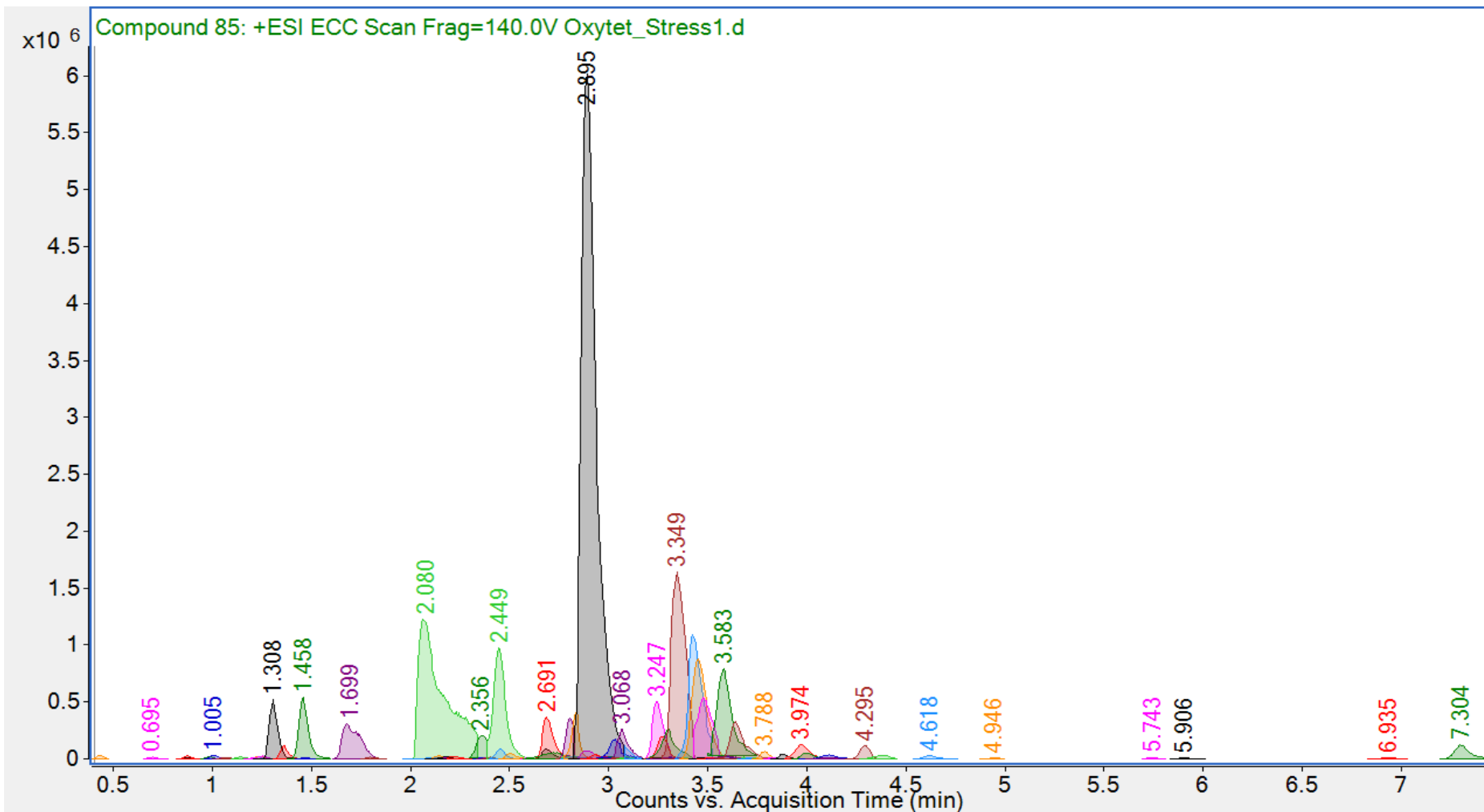


**4th Step – Now that a target list of impurities has been created, now acquire high-quality MS/MS spectra on just these compounds related to the API.**





# API related components in H2O stressed sample can direct the MS/MS experiments:



# Components are exported automatically by RT and mass for high efficiency MS/MS:

Export MS/MS Inclusion List Options

List of opened data files:

Oxy\_tet\_degrade\_2.d

Options Results Precursor Selection Charge State Ion Species

Export destination

One export file per data file:

☐ At the location of the data file

☒ At specified directory

C:\temp

If export file already exists

☒ Overwrite existing export file

☐ Auto-generate new export file name

Inclusion list format

☒ Auto MS/MS preferred list

☐ Auto MS/MS exclusion list

☐ Targeted MS/MS inclusion list

Precursor sort order

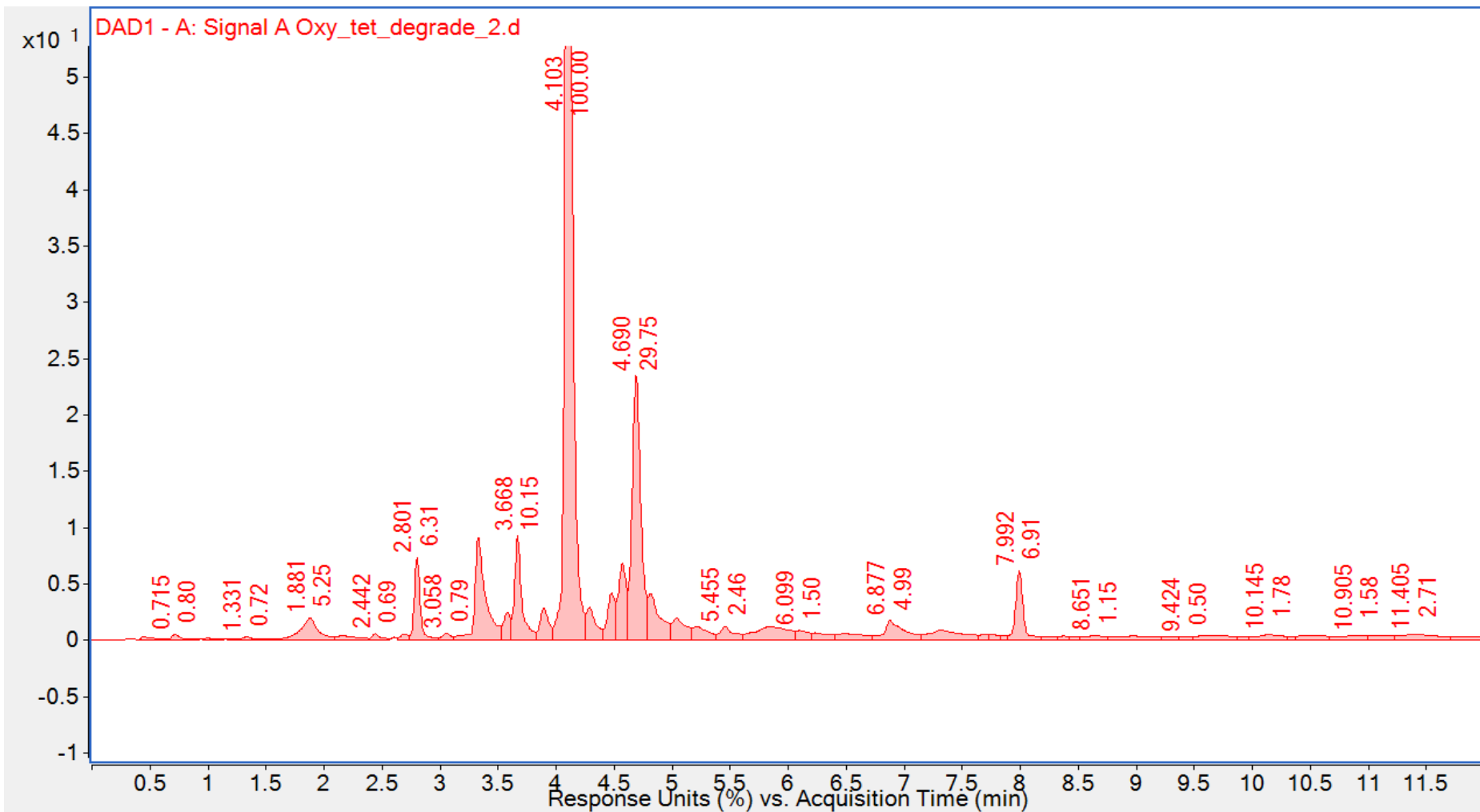
Sort by: Retention Time

Sort order: Increasing

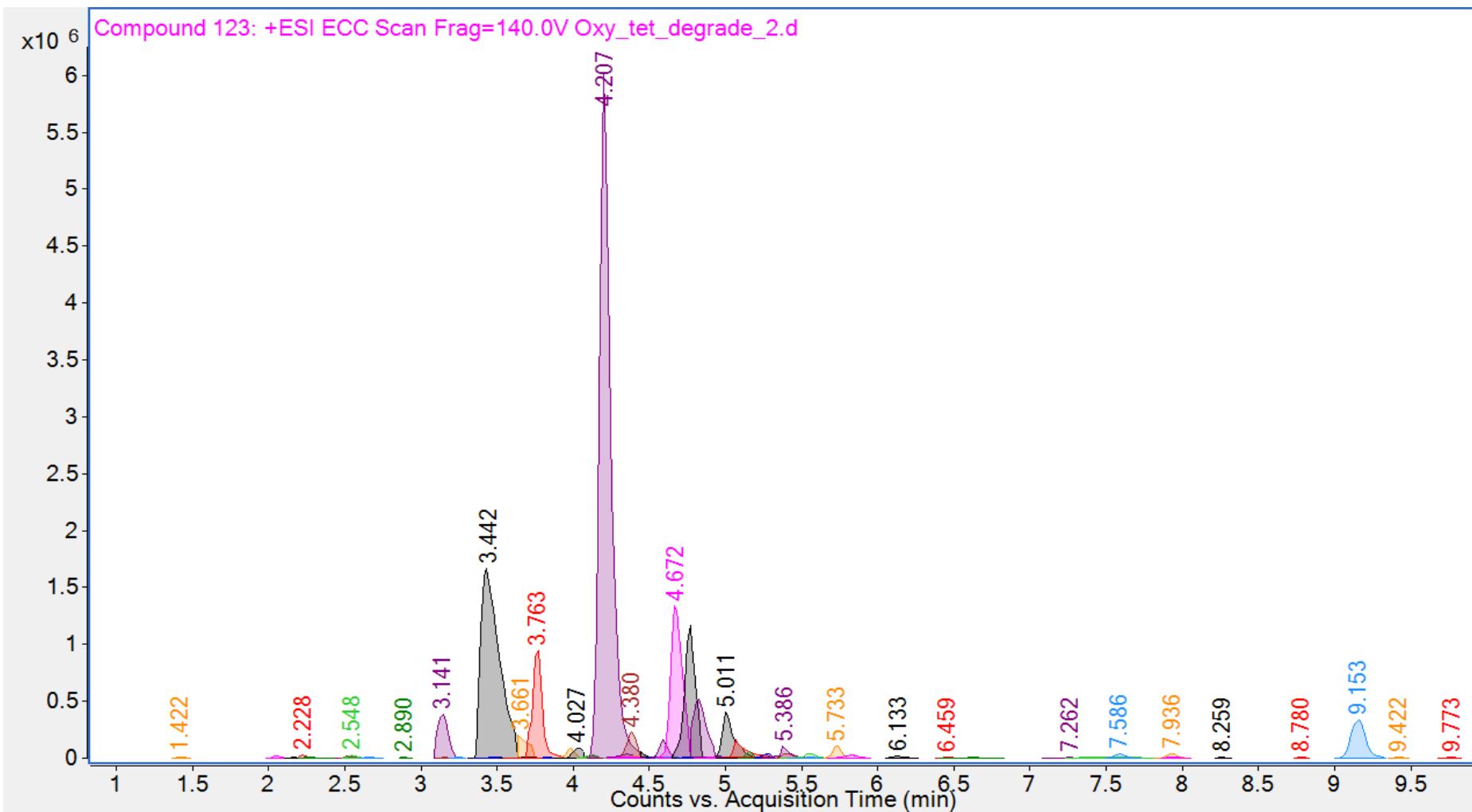
OK Cancel



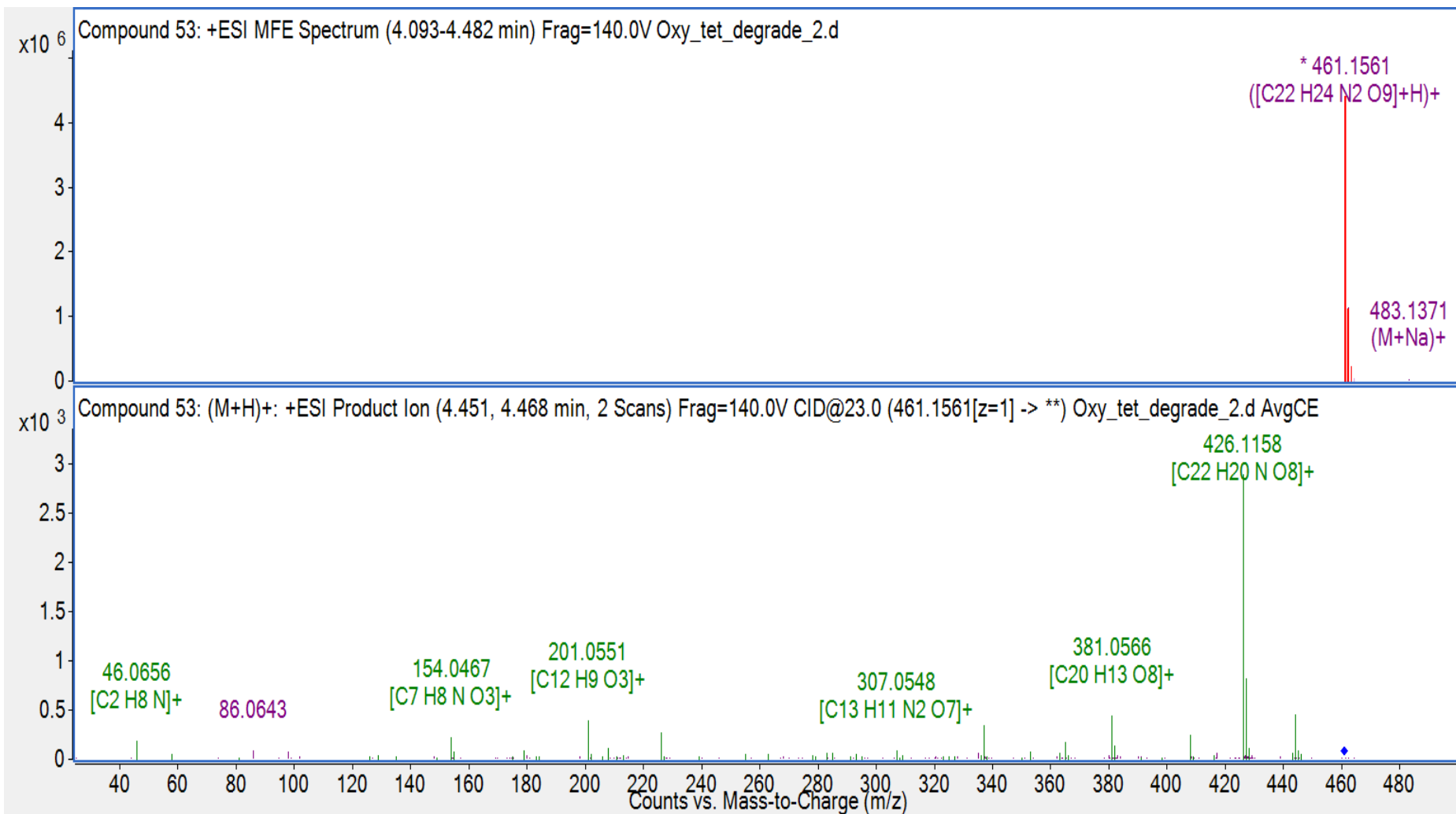
# UV Chromatogram for for Oxytetracycline H2O Stressed from Auto MS/MS experiment



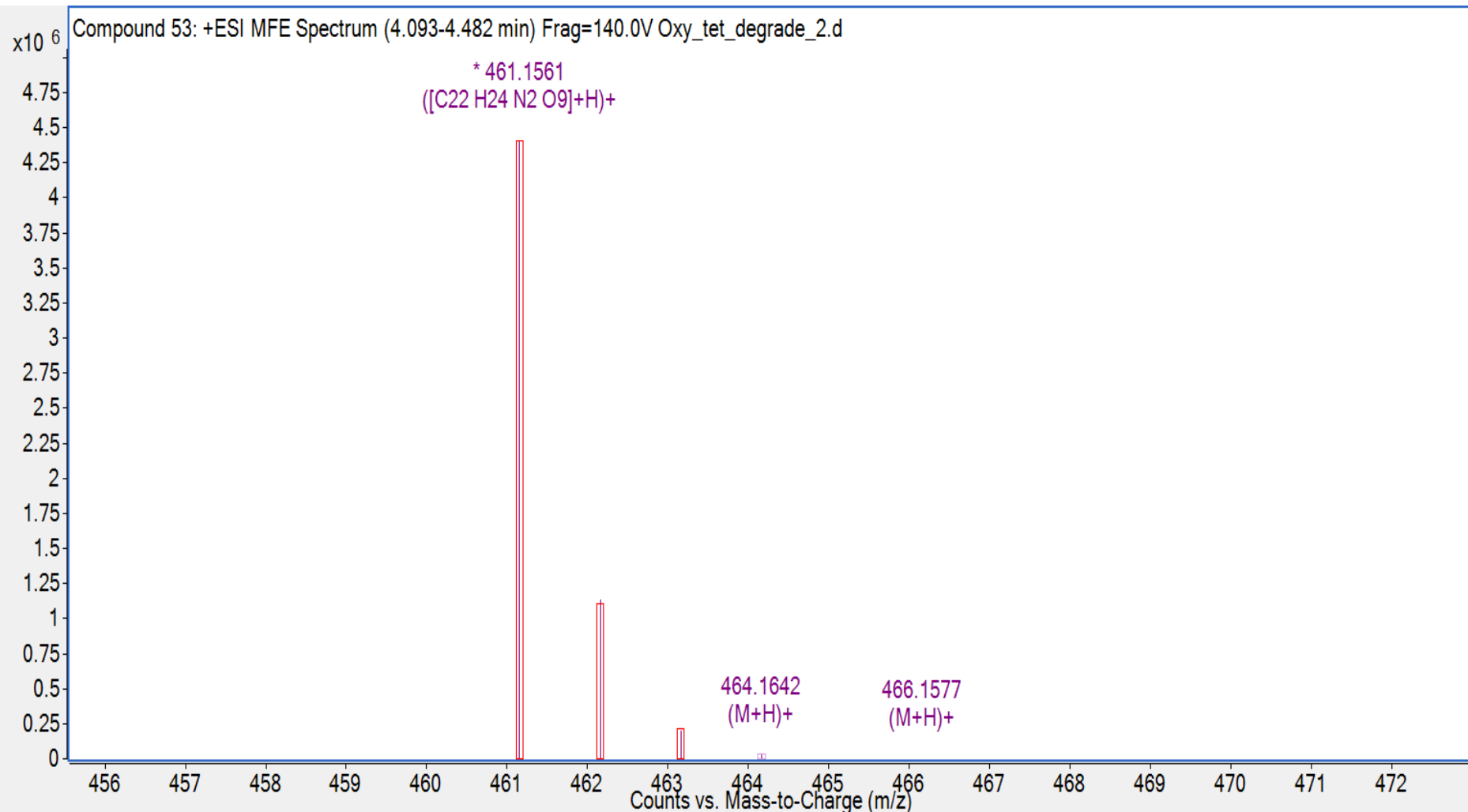
# Molecular Feature Extracted Component Chromatograms for Oxytetracycline H2O Stressed from Auto MS/MS experiment



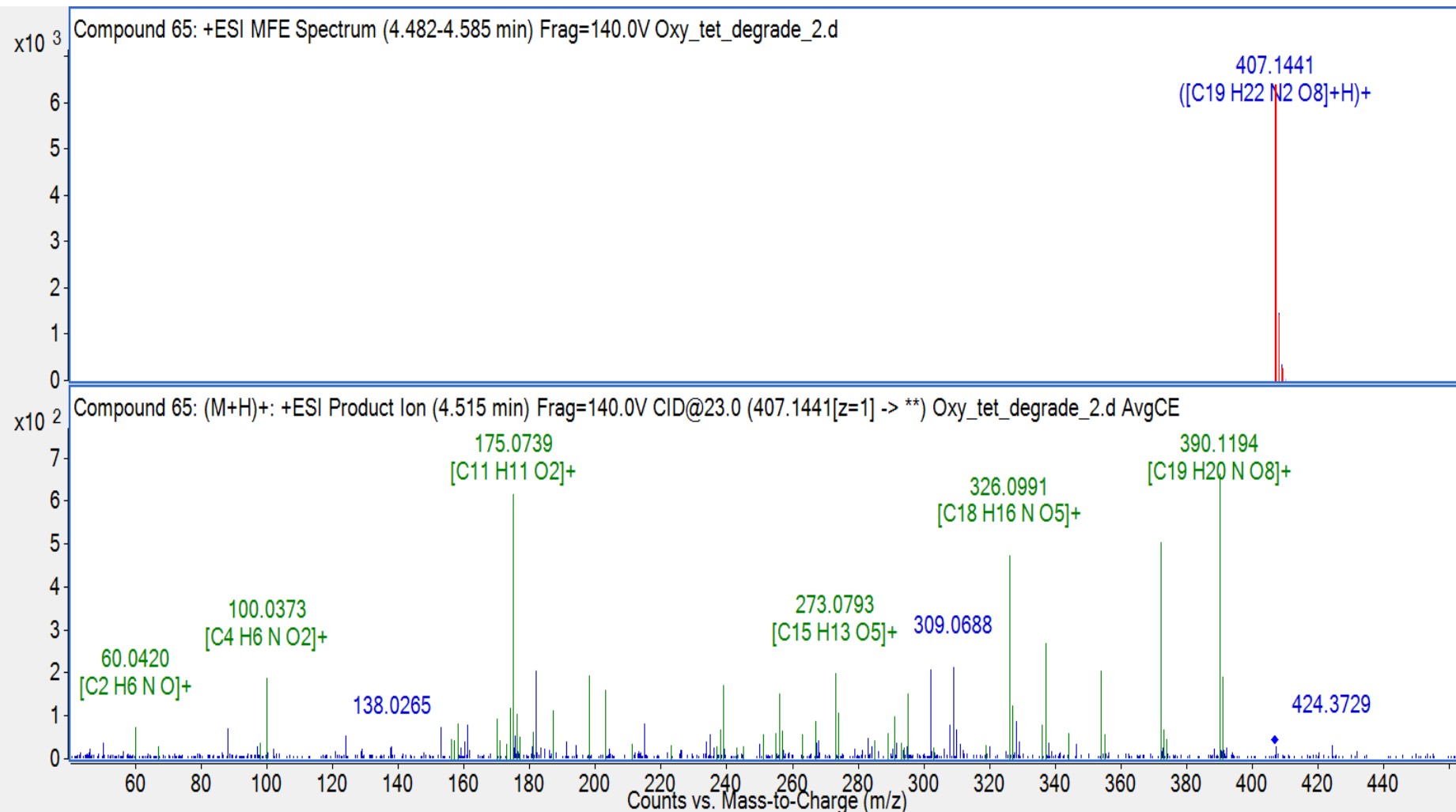
# MS/MS data packet for API in H2O stressed sample with formulas assigned for precursor and fragments



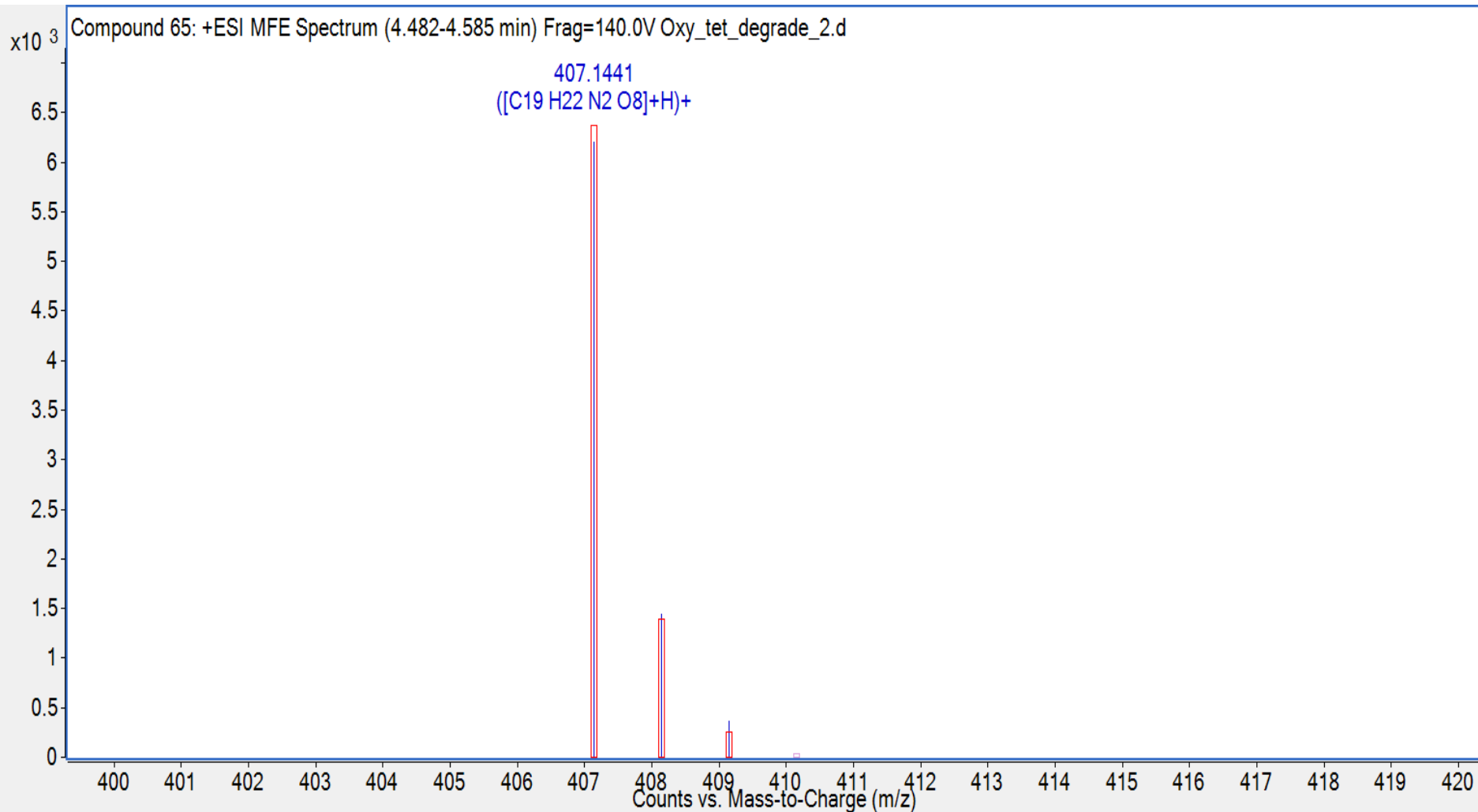
# Precursor mass and isotope match for API in H2O stressed sample



# MS/MS data packet for component at 0.03% rel area of API in H2O stressed sample with formulas assigned for precursor and fragments:



# Precursor mass and isotope match for component at 0.03% rel area of API in H2O stressed sample





# Find by AutoMSMS components related by common neutral loss and or common fragment ion

**Method Editor: Find Compounds by Auto MS/MS**

Find Compounds by Auto MS/MS | Method Items

**Processing** | Excluded Masses | Results

Compound identification

Retention time window: 0.250 min

Positive MS/MS TIC threshold: 1000

Negative MS/MS TIC threshold: 1000

Mass match tolerance: 0.0500 m/z

☐ Limit to the largest 50 compounds

Compound fragment filters

☒ Filter results by fragments

☐ Fragment mass filter 154.00 m/z

☒ Neutral loss filter 35.05 m/z

Match specified fragments:

☒ Any fragment

☐ All fragments

Mass match tolerance: Symmetric (m/z) ± 0.1001

Persistent background compounds

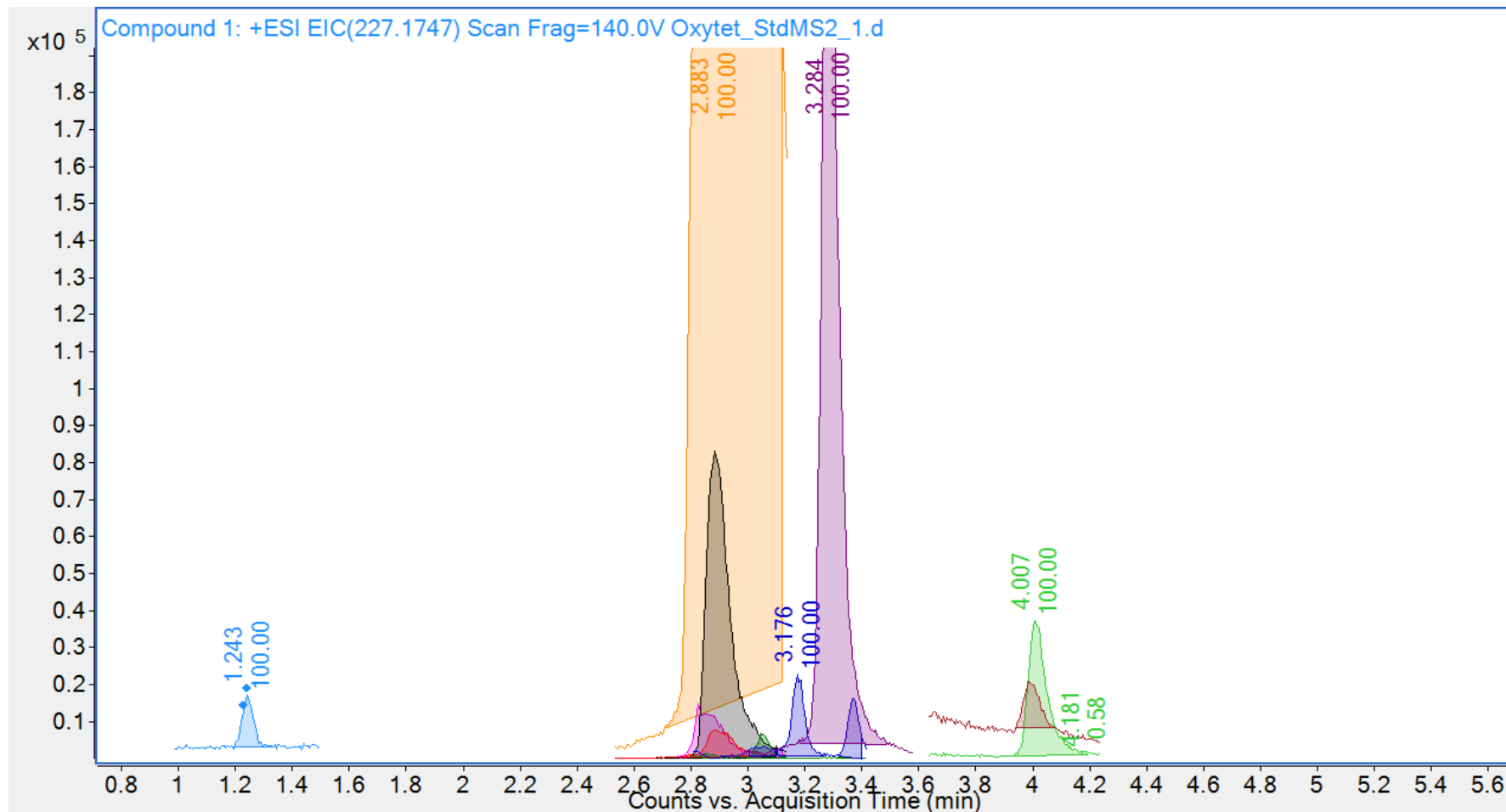
Compounds with same precursor m/z occurring at different times:

Remove if there are more than 5

Except when the TIC exceeds 100000.0



# Find by AutoMSMS components related by common neutral loss



**5th Step – Use MS/MS spectra and MassHunter Molecular Structure Correlation (MSC) tool to propose structures.**



# MS/MS structure assignment from MassHunter MSC (Molecular Structure Correlation) and Chemspider:

Agilent MassHunter Molecular Structure Correlator B.05.00 -- Oxy\_tet\_degrade\_2--M+H; ce=23

File Settings Help

Compound formula:  Add

M = 460.1488; 24 formula candidates from MFG

ID	Formula	Isomers	Taut. Grps	dM(ppm)	dM(ppm)	Probu
0	C22H24N2O9	101	45	-1.4	1.4	
1	C22H24N2O9	101	45	-1.4	1.4	
2	C23H20N6O5	60	51	1.5	1.5	
3	C19H16N12O3	0	0	-4.3	4.3	
4	C15H24N8O7S	0	0	0.1	0.1	
5	C18H20N8O7	3	3	-7.2	7.2	

Fragment formulas for C22H24N2O9

m/z	intensity	norm. intens.	formula	dM(ppm)
426.1158	3822.61	100.00	C22H20NO8	6.0
381.0566	601.98	15.75		
444.1241	586.93	15.35	C22H22NO9	10.8
201.0551	437.93	11.46	C12H9O3	-2.4
201.0551	437.93	11.46	C15H7N	10.9
337.0706	367.90	9.62	C19H13O6	0.2
337.0706	367.90	9.62	C14H13N2O8	-11.7
226.0758	292.48	7.65	C13H10N2O2	-9.4
226.0758	292.48	7.65	C6H14N2O7	16.6
226.0758	292.48	7.65	C10H12NO5	-21.2
408.1028	272.75	7.14		
154.0467	215.42	5.64	C4H10O6	3.2
154.0467	215.42	5.64	C7H8NO3	20.6
365.0651	213.40	5.58	C15H13N2O9	-9.7
46.0656	183.93	4.81	C2H8N	-10.3
307.0548	138.41	3.62	C13H11N2O7	4.2
208.0587	105.77	2.77	C7H12O7	-4.5
208.0587	105.77	2.77	C10H10NO4	8.3
208.0587	105.77	2.77	C13H8N2O	21.2
178.9956	88.27	2.31	C8H3O5	10.6
178.9956	88.27	2.31	C3H3N2O7	-11.9
178.9956	88.27	2.31	C11HNO2	25.6
86.0643	84.65	2.21	C4H8NO	-49.5
98.0633	78.09	2.04	C5H8NO	-33.2
155.0517	73.89	1.93	C11H7O	-16.5
353.0622	72.50	1.90	C14H13N2O9	-1.8

Structure Search

Parameters Competibles/Total: 9/46 Add Structure

ChemSpider (Web) Go Sort by Score Sort

Standard InChIKey: TUEWQXNXQFRUTK-KDEDHZJPSA-N  
Score: 76.06  
More Info ...  
MSC Save Delete  
ChemSpider: 8089428

Standard InChIKey: JLGOGFIHBRJQH-YUHHFAOYSA-N  
Score: 76.06  
More Info ...  
MSC Save Delete  
ChemSpider: 21926642

Standard InChIKey: OWFJMVZYSULZ-UHFFFAOYSA-N  
Score: 76.06  
More Info ...  
MSC Save Delete  
ChemSpider: 29786131

Compound formula: C22H24N2O9

Fragments of structure #1 -- elucidated: 93.3% ions, 99.5% Weight

#	Mass	Intensity	Weight(%)	No. of candid.	Best score
1	426.1158	3822.61	68.8	5	82.2
2	381.0566	601.98	5.5	3	62.0
3	444.1241	586.93	13.5	1	61.4
4	201.0551	437.93	0.1	6	82.8
5	337.0706	367.90	1.6	4	85.5
6	226.0758	292.48	0.1	3	45.9
7	408.1028	272.75	3.8	4	51.1
8	154.0467	215.42	0.0	5	64.6
9	365.0651	213.40	1.5	4	87.0
10	46.0656	183.93	0.0	3	99.1
11	307.0548	138.41	0.3	1	71.0
12	208.0587	105.77	0.0	7	76.0

Penalty=4.0 dM=6.0ppm Score=82.2 1 Of 1 C22H23NO8-3H

Penalty=4.0 dM=6.0ppm Score=82.2 1 Of 2 C22H23NO8-3H

Penalty=7.0 dM=6.0ppm Score=72.7 1 Of 4 C22H25NO8-5H

Penalty=7.0 dM=6.0ppm Score=72.7 1 Of 1 C22H25NO8-5H



# Structures can also be assigned to MS/MS spectra from User directed databases (compendium of “Learned” proprietary structures). PCDL database in MassHunter:

MassHunter PCDL Manager for Metabolomics - F:\cdb\_s\Metlin\_AMRT\_PCDL.cdb

File Edit View PCDL Links Help

Find Compounds

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

Mass

☐ [M+H]<sup>+</sup> ☒ Neutral ☐ [M-H]<sup>-</sup>

Mass tolerance:  10.0 ☒ ppm ☐ mDa

Retention time

☐ Require

RT tolerance:  0.1 min

Ion search mode

☒ Include neutrals  
☒ Include anions  
☒ Include cations

Formula:

Name:  Oxytetracycline

Notes:

IUPAC:

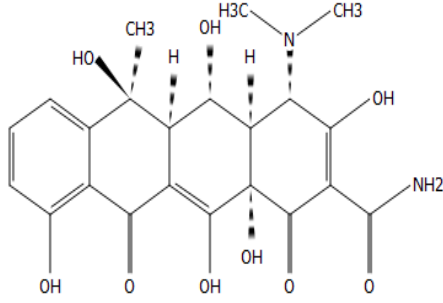
CAS:  KEGG:

ChemSpider:  HMP:

METLIN:  LMP:

Molecule:

Structure MOL Text



Notes: Antibiotic  
Drug

Single Search Results: 1 hit for

Compound Name	Formula	Mass	Anion	Cation	RT (min)	CAS	hemSpide	METLIN	HMP	KEGG	LMP	IUPAC Name	Num Spectra
Oxytetracycline	C22H2...	460.14...	<input type="checkbox"/>	<input type="checkbox"/>		<a href="#">79-57-2</a>		<a href="#">1690</a>		<a href="#">C066...</a>			6

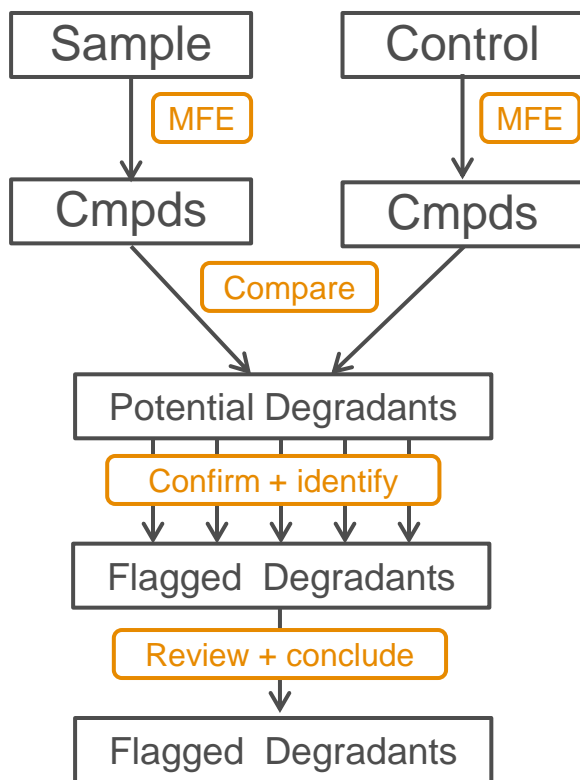


**6th Step – Use MassHunter Mass Profiler (MP) tool to compare any two LC/MS files.**



# Untargeted approach based on MFE comparison

Best approach for expected and unexpected metabolites, degradants, etc



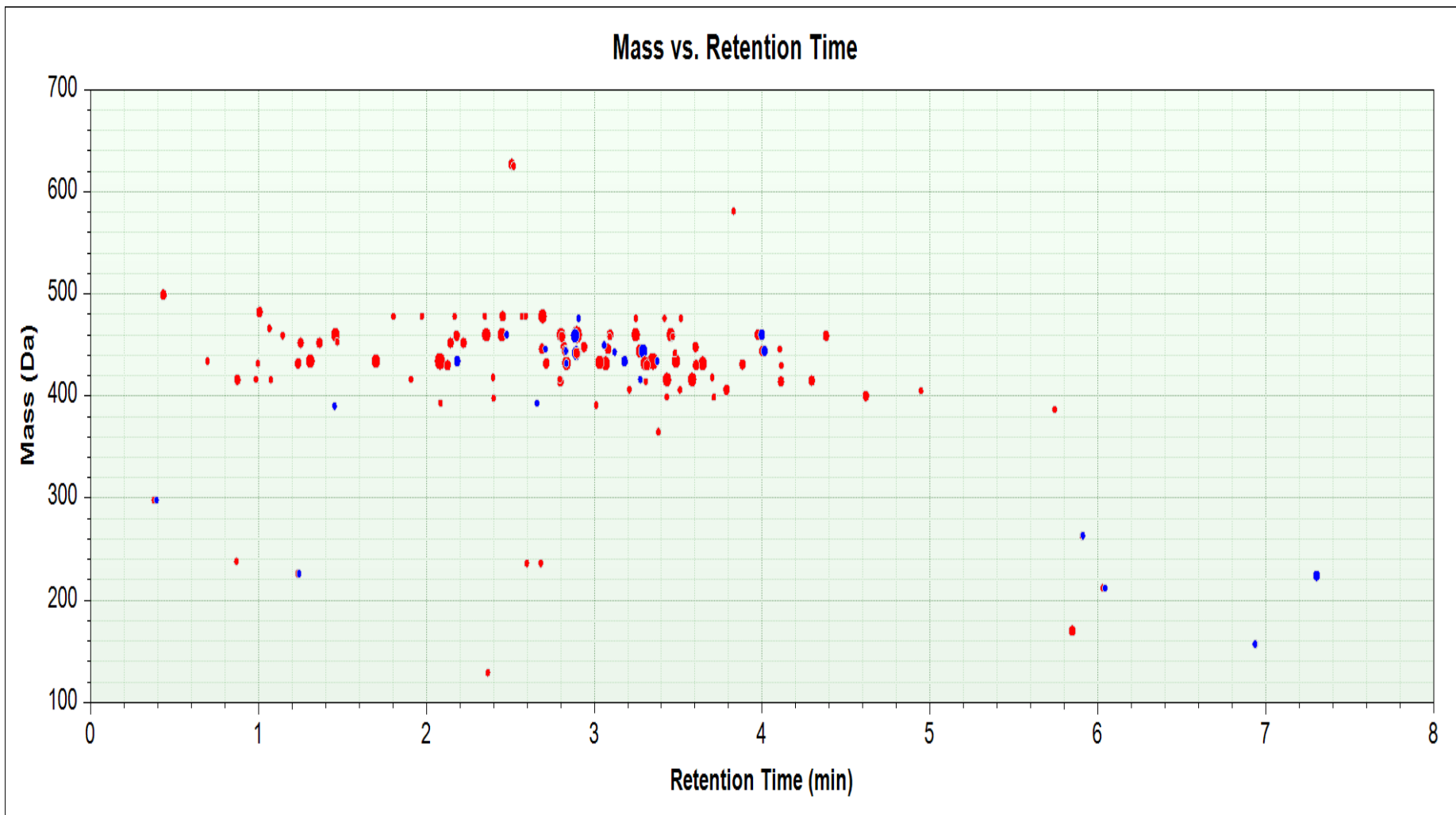
## Untargeted approach via comparison of MFE Compound lists

- Sophisticated Sample-Control Comparison Algorithm based on Molecular Feature Extraction (MFE) allows more comprehensive detection of differences via exploitation of mass and RT resolution.

Impurity Analysis: Mass Profiling Software

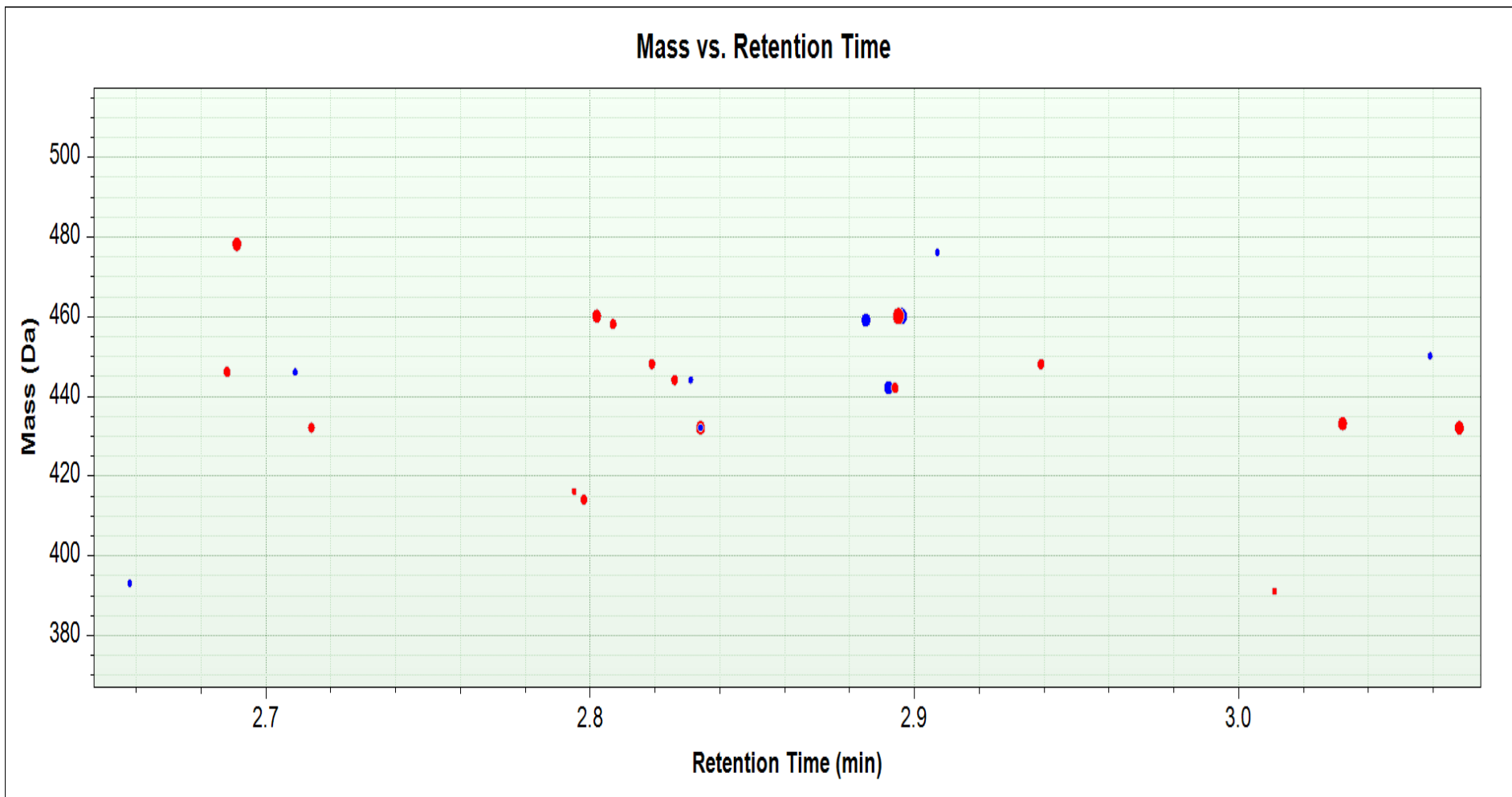


# Mass Profiler allows comparison of Std. (Blue) vs H2O stressed (Red) Oxytetracycline:

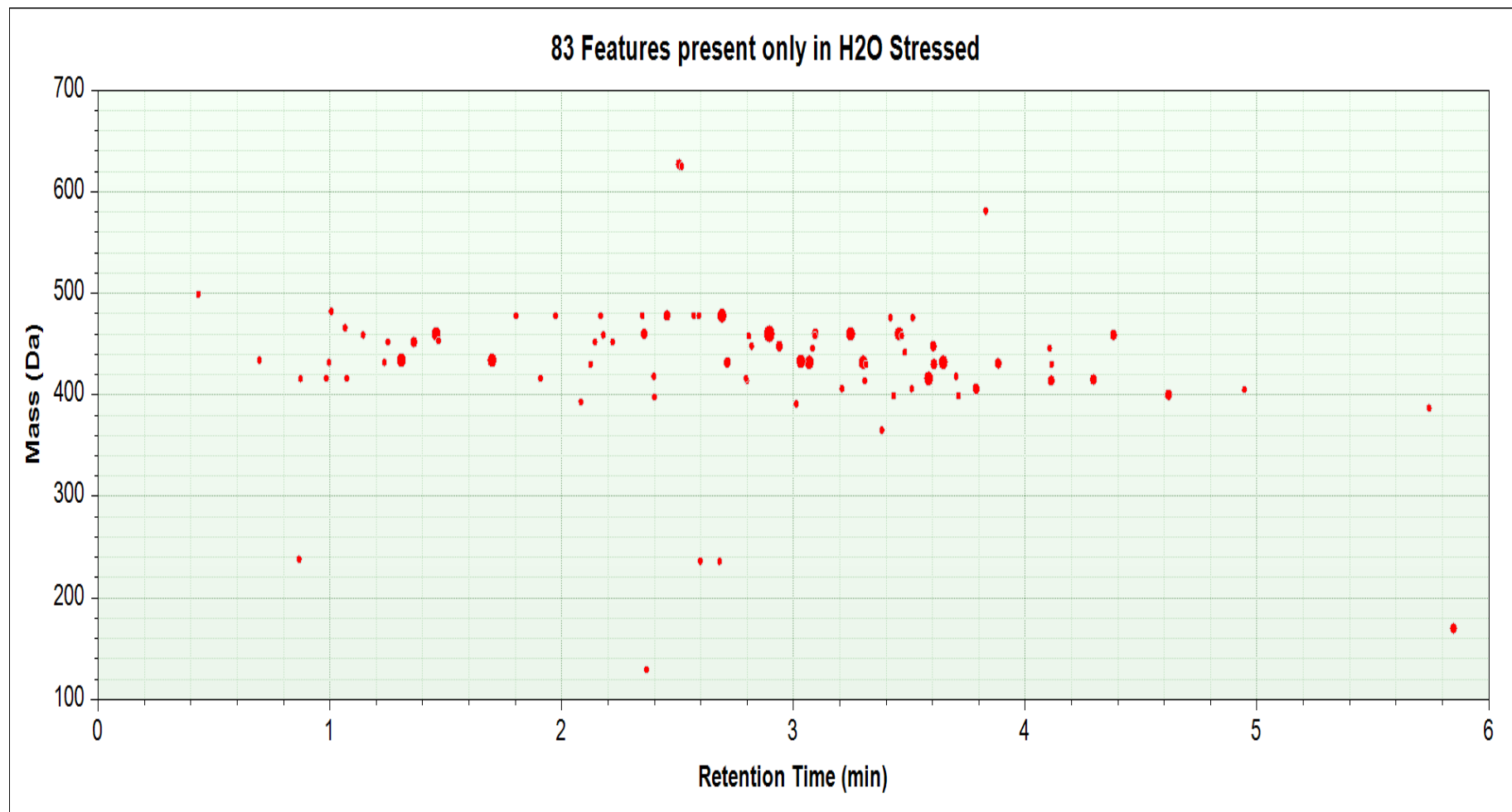




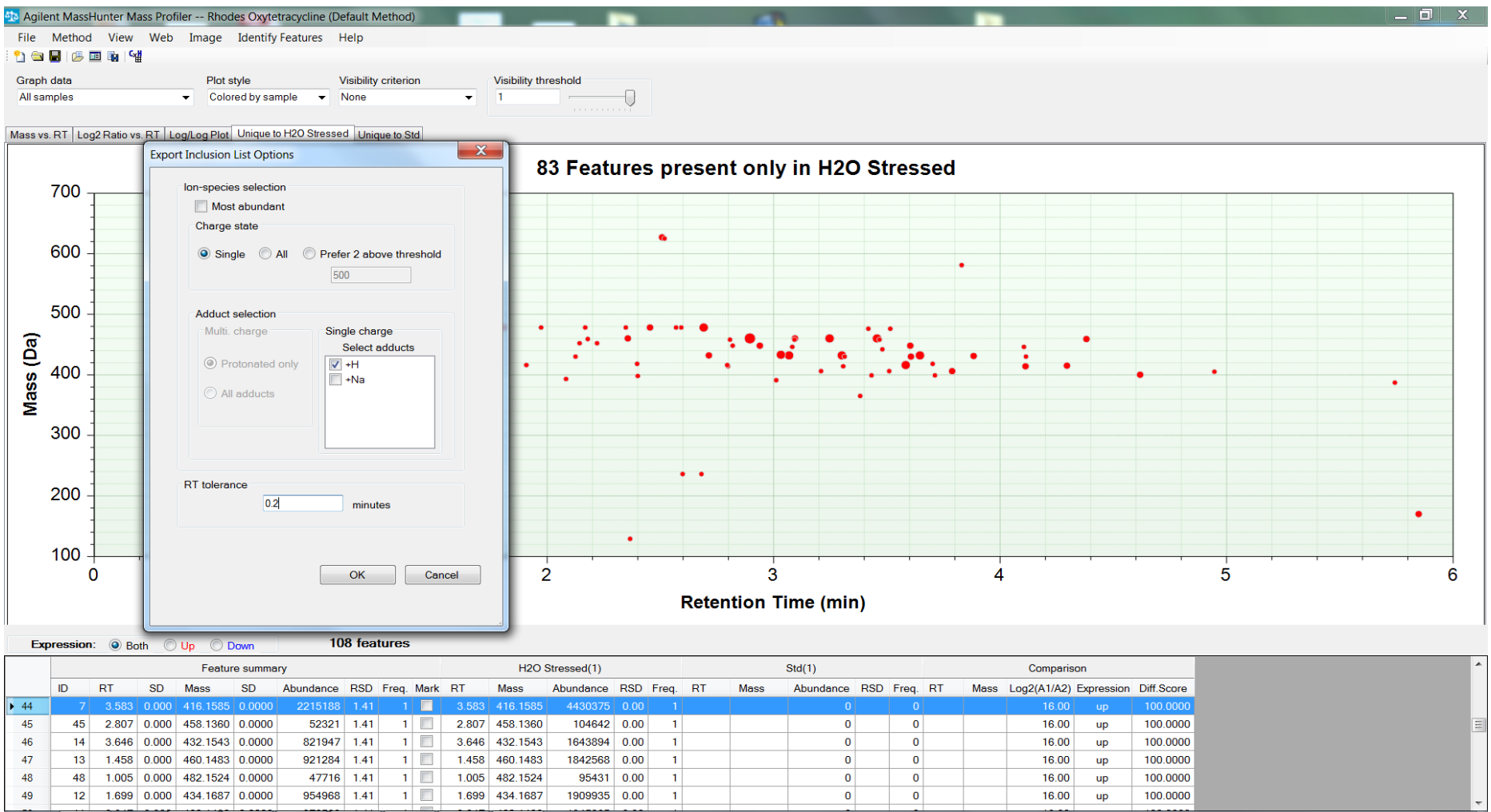
# Overlaps are components common to both samples:



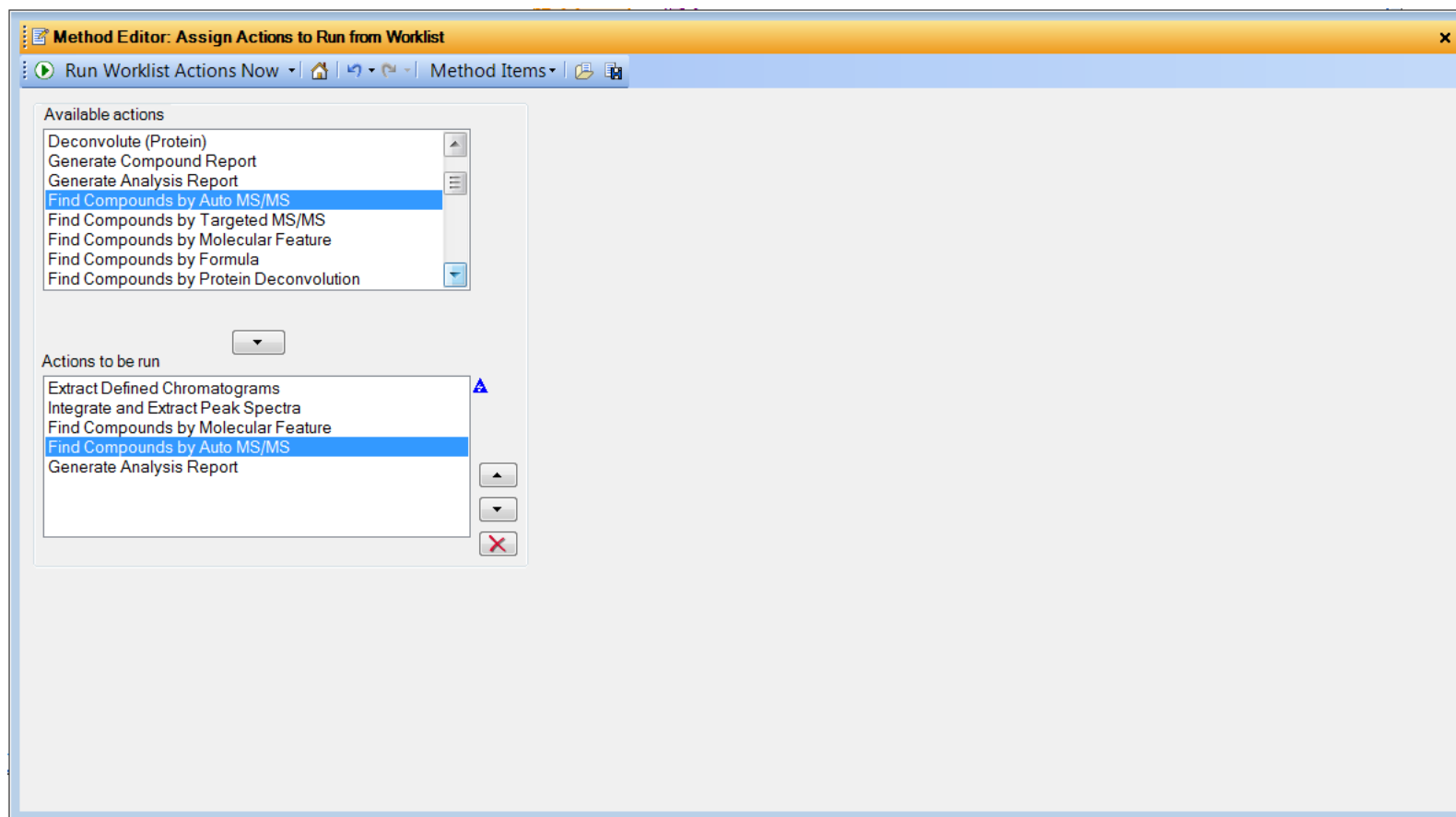
# Mass Profiler can find components only found in H2O stressed sample (Red, degradants from API):



# Mass Profiler can schedule further MS/MS studies if needed:



# Entire Workflow is Automated with Simple Instructions



## Summary:

- An example API was found to have 59 trace level impurities, of which 17 were related to the API structure.
- Degradation of the API produced approx 60 impurities related to the API.
- MassHunter software was utilized to find automatically the impurities (MFE), calculate elemental formulas (MFG), and possible structures (MSC).
- Using MFE, impurities can be found that are not resolved by the LC separation.
- Mass Profiler software allowed for easy sample comparisons.
- The individual software steps can be linked together in a simple MH work list to automate the complete workflow.



## **Additional Background Information:**



# Agilent Products for Pharmaceutical Research:

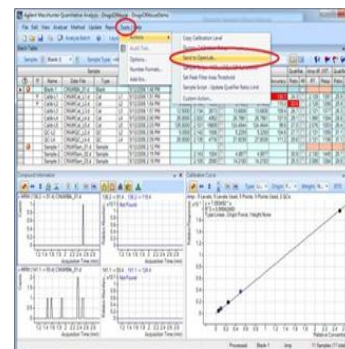


**7200 GC-Q-TOF**



**Bravo/  
Encore**

**Hi-DEF Q-TOF  
6500 series**



**MassHunter  
Software**



**7000 GC-QQQ,  
5977 GC-SQ**



**Infiniti  
1290 UHPLC**



**SQ 6100  
Series**



**QQQ  
6400 Series**



**Columns & Consumables**



**TOF  
6200 series**



**CE/MS**



**Agilent Technologies**

# 1200 Infinity Series HPLC, UHPLC, SFC for MS:

*More workflow-automation and application-based solutions*



Analytical  
SFC



Bio-inert  
HPLC



Nano LC  
& Cap LC



Preparative  
LC & LC/MS



GPC  
SEC



Isocratic  
LC



Multi-method and  
Method Development



High-throughput  
LC & LC/MS



Nanoflow  
HPLC-Chip MS

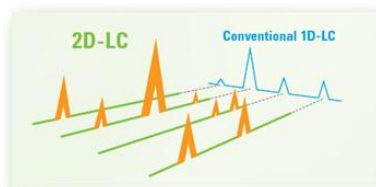




# Sample Introduction Choices for your Agilent LC/MS

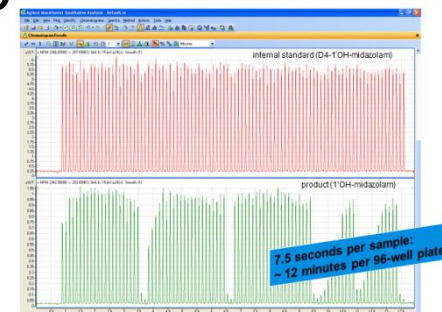
## – More than just HPLC/UHPLC:

New multi-dim LC choices with valve automation:



## HT SPE-MS Agilent RapidFire

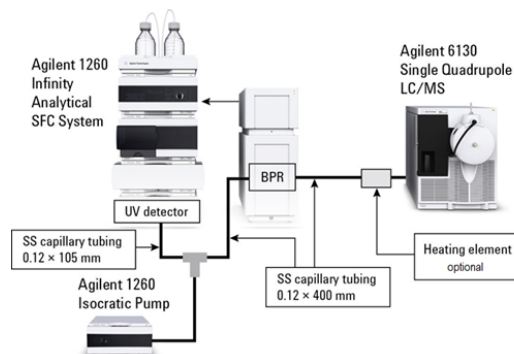
RapidFire High-Throughput Mass Spectrometry



## GC-APCI



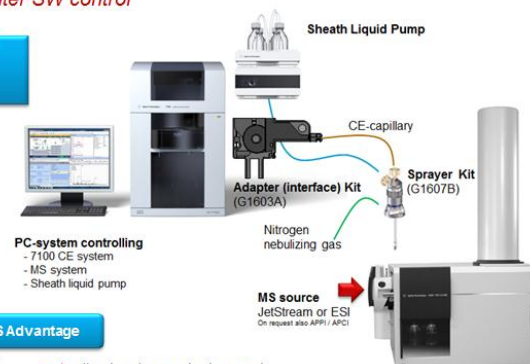
## SFC/MS: From SQ to QTOF



## Agilent CE/MS Setup

► New MassHunter SW control

Single point SW control  
- 7100 CE instrument  
- TOF, QTOF, QQQ  
- LC make-up flow



### The Agilent CE/MS Advantage

Single vendor solution: direct and competent support  
Sheath Liquid Interface: robust and reliable, offering efficient control on chemistry  
Capillary outlet on ground: no compromises on voltages for CE or ESI-MS

# LC/MS Ion Sources – Many Choices

- Widest choice of sources from any manufacturer
  - Electrospray (ESI)
  - Agilent JetStream ESI
  - Nanoelectrospray (nanoESI)
  - APCI
  - APPI
  - Dual ESI sources for TOF and Q-TOF
- Multimode Source
  - ESI and APCI combined in one source
  - Simultaneous operation
- HPLC-Chip/MS
  - Nanoelectrospray made easy
  - Reproducible results
  - Sample processing on chip
- Most sources are interchangeable between MS platforms

**MultiMode**



**Nano HPLC-Chip**



**APCI**



**ESI**



**NanoESI**



**APPI**



**AJS-ESI**

**Ionize Almost Anything!**



Agilent Technologies