Evaluation of the Importance of Accurate Mass, Mass Resolution and Dynamic Range for Impurity Profiling Applications with Multistage Mass Spectrometry

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Impurity Profiling..

- **Identification of Impurities in Active Pharmaceutical Ingredients (API) Arising from Manufacturing/Processing**
- **Differentiation from other Pharma Applications**
  – Chromatography, Quantitation, Identification
- **Source of Impurities …**
  – Residual Solvents, Intermediates, Catalysts, Processing Aids
- **Techniques**
  – GC/MS, LC/UV, ICP/MS, LC/MS and LC/MS/MS

Traditional Method for Impurity Analysis

Plant is Shutdown… Product has Failed..

Why, When, Where, How

Easy.. Just have Ph.D. Analytical Chemists Acquire Lots and Lot of GCMS and LCMS Data

Dump data onto Coworkers Desk ID the Differences..

Manual, Biased Approach

Look for Known Things First

High/Low Major Differences

Is the Change Real?

Although Small a Needle in Hay Can Cause One Pain

Manually Compare LC/UV and MS Chromatograms from "Control" versus "Contaminated" Samples

API Degradation by Aging

Pathways

- Hydrolysis, Oxidation, Dehydration, Ring Cleavage, Photolysis

Challenge

- Identify low-level degradation products in the presence of the major API component

Dream Solution – All Components ID and Separated

Chromatography – Infinite Resolving Power and Ultra Fast Separation
Mass Spec – Infinite Scan Speed, Dynamic Range, Resolving Power with sub-ppm Mass Accuracy

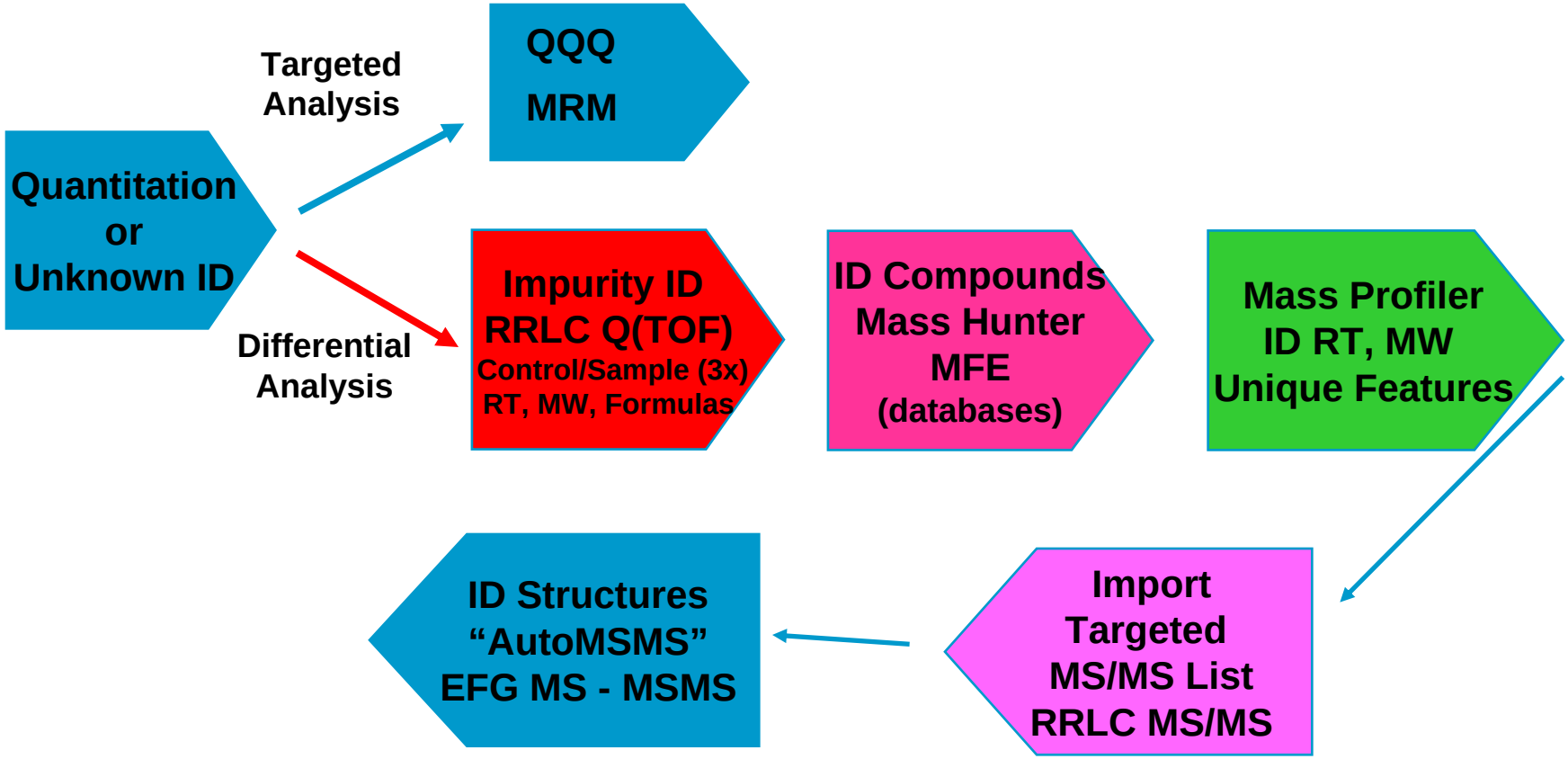
Reality:

Coalition, ion suppression, limited dynamic range, accurate mass

New Analog/Digital Technology Evaluate the Importance of Resolution, Accurate Mass, Dynamic Range with Automated Data Mining Tools for Impurity Profiling

Impurity Profiling Workflow:

- **Objective:** Detection - ID low-level Impurities in Active Pharmaceutical Ingredients.



Sensitivity versus Unknown Identification

Ease of Use	Scan speed	Scan sensitivity	Qualitative (identification, structural info)	Sensitivity (specific detection)	Quantitation (accuracy, precision)
Single quad	TOF	TOF	Q-TOF (MS/MS w/ accurate mass)	QQQ esp. dirty matrix, < 5 pg on-col.	QQQ
TOF	Q-TOF	Q-TOF	TOF unknowns (acc. mass CID)	Single quad (SIM)	Single quad
QQQ	Single quad	Single quad	QQQ (MS/MS)	TOF (accurate mass EICs)	TOF
Q-TOF	QQQ	QQQ	Single quad (CID)	Q-TOF *	Q-TOF *

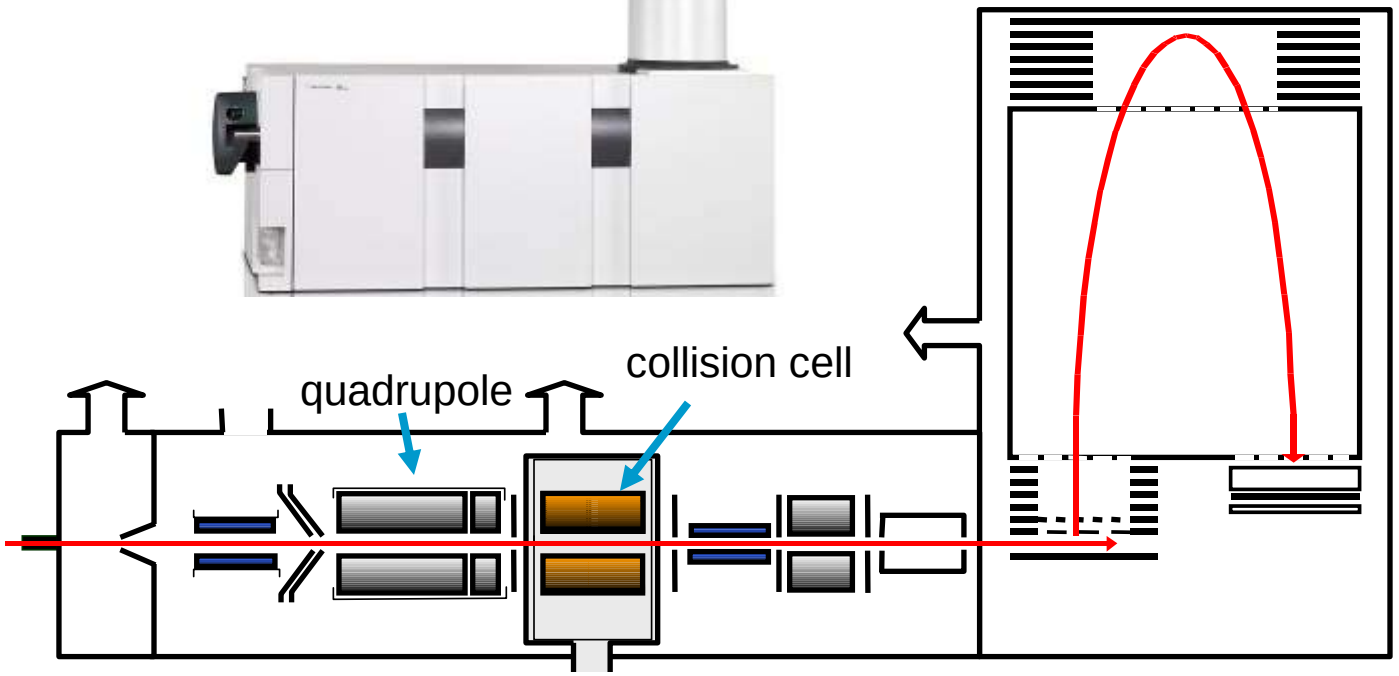
Better

Agilent LC/MS/MS Solution

1200 RRLC

6520 QTOF
Accurate Mass

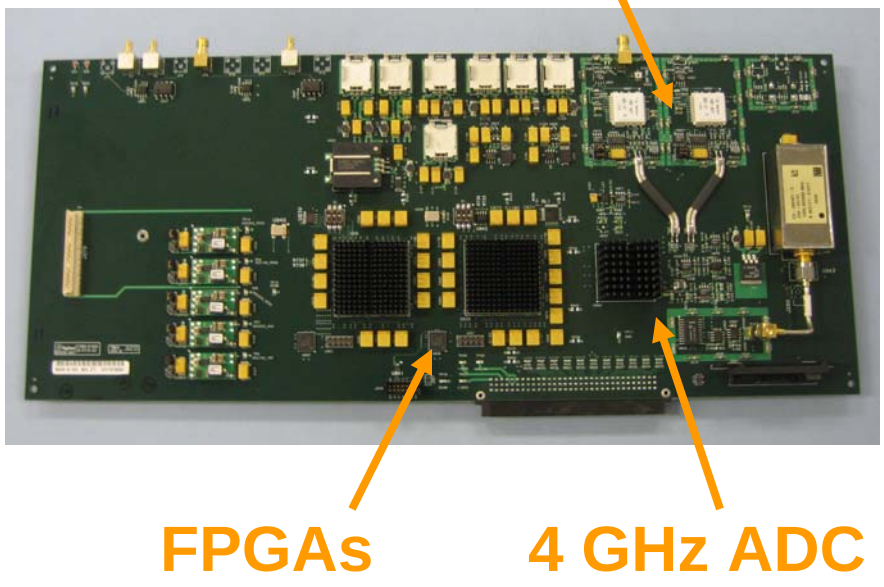
time-of-flight
analyzer



New Ultra High Speed Acquisition

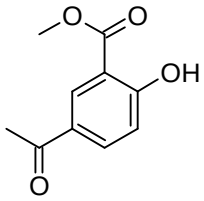
Intelligence at the Molecular Level

- 4 GHz (8 bit) Analog-Digital-Converter **ADC**
  - Adapted from Agilent High Speed Oscilloscope Systems
- Ultra High Speed **FPGA** Processors and Memory
  - 4Ghz peak detection
  - 4Ghz gain scaling
  - Up to 20,000 m/z depth
- Dual Input / Dual Gain High Bandwidth Input **Amplifier** for Extended Dynamic Range



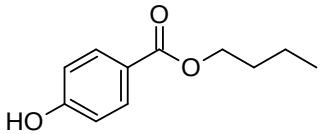
Enhancements in Time-of-Flight and QTOF

New Acquisition Technology Increasing Resolving Power



Methyl 5-acetylsalicylate

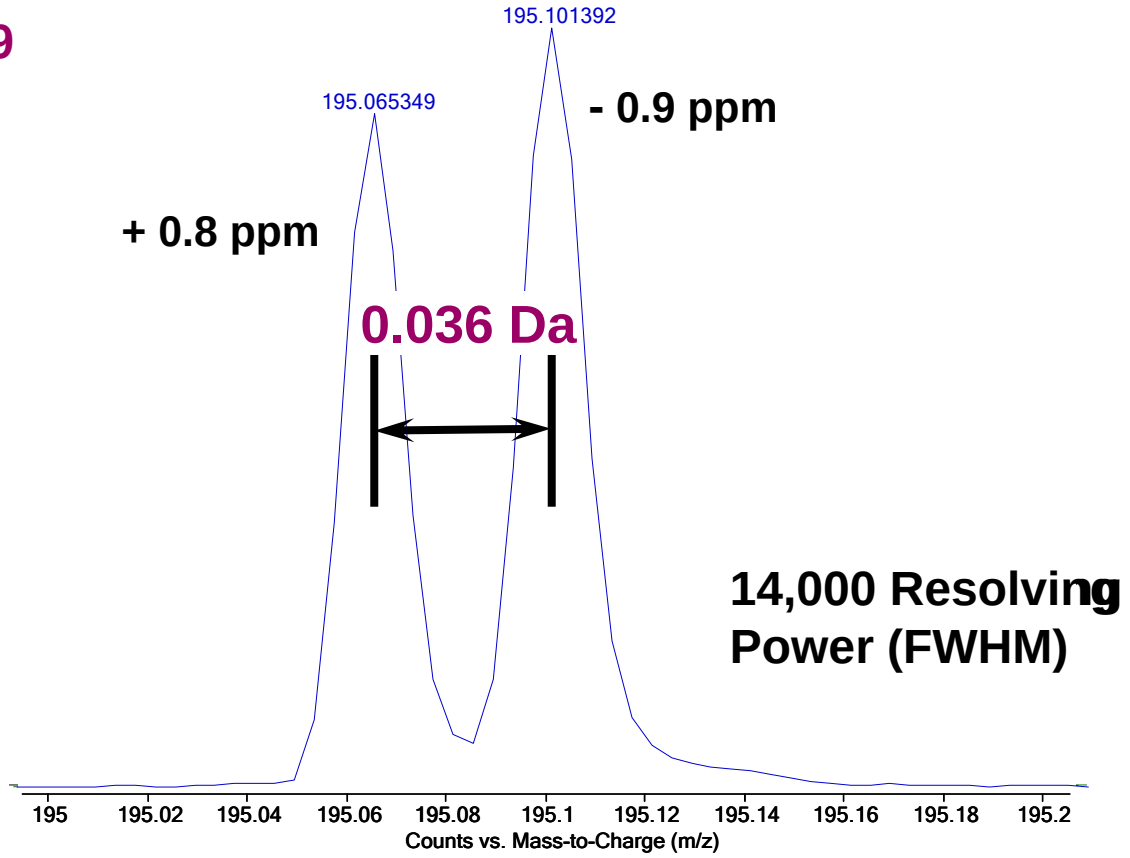
$[M+H]^+$ 195.06519



Butyl paraben

$[M+H]^+$ 195.10157

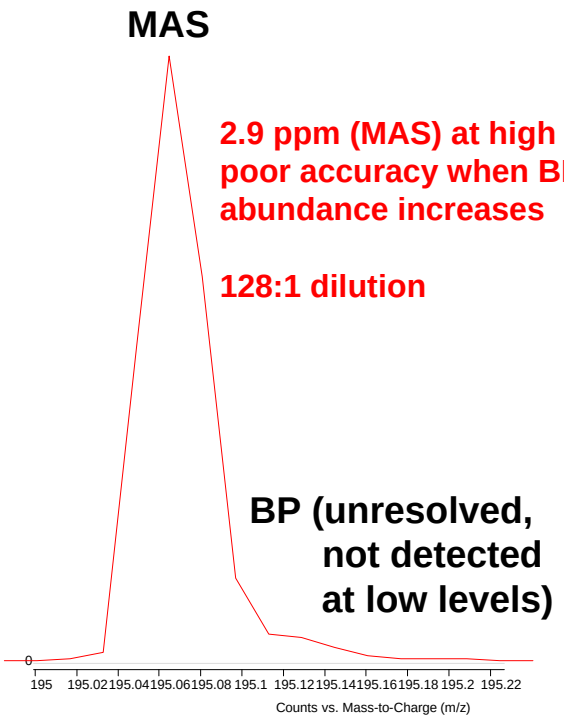
8 bit – 4 GHz
Analog Digital Converter
Transient Peak Apex Picking

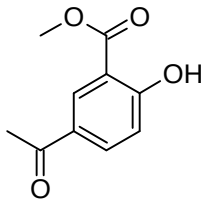


FIA, 2 scans/sec (6713 transients/scan)
2 IRM averaged over five scans

Enhanced Resolving Power
Improved Mass Accuracy for Isobars

Previous 1 GHz Sampling

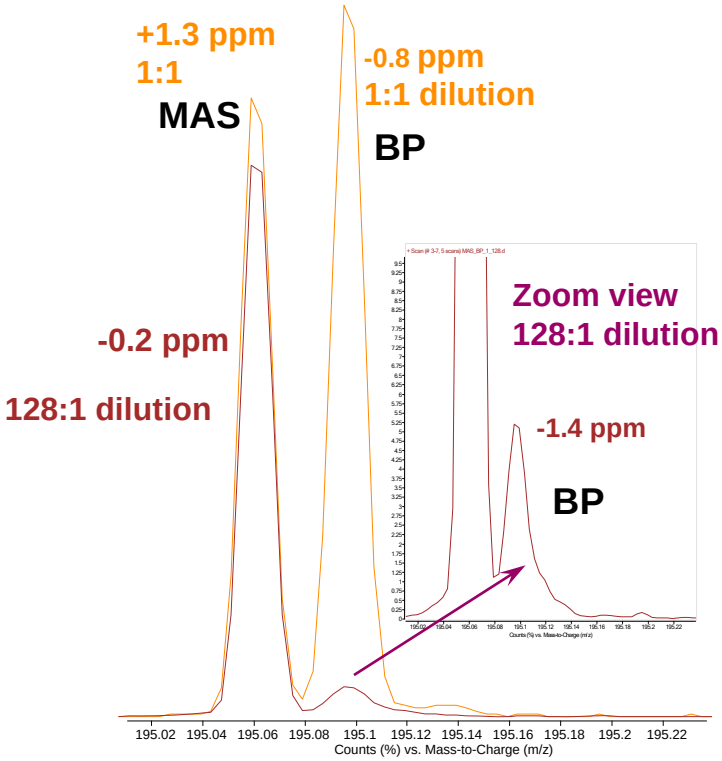


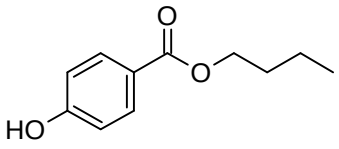


Methyl 5-acetyl-salicylate (MAS)

$[M+H]^+$ 195.065185

New 4 GHz Sampling

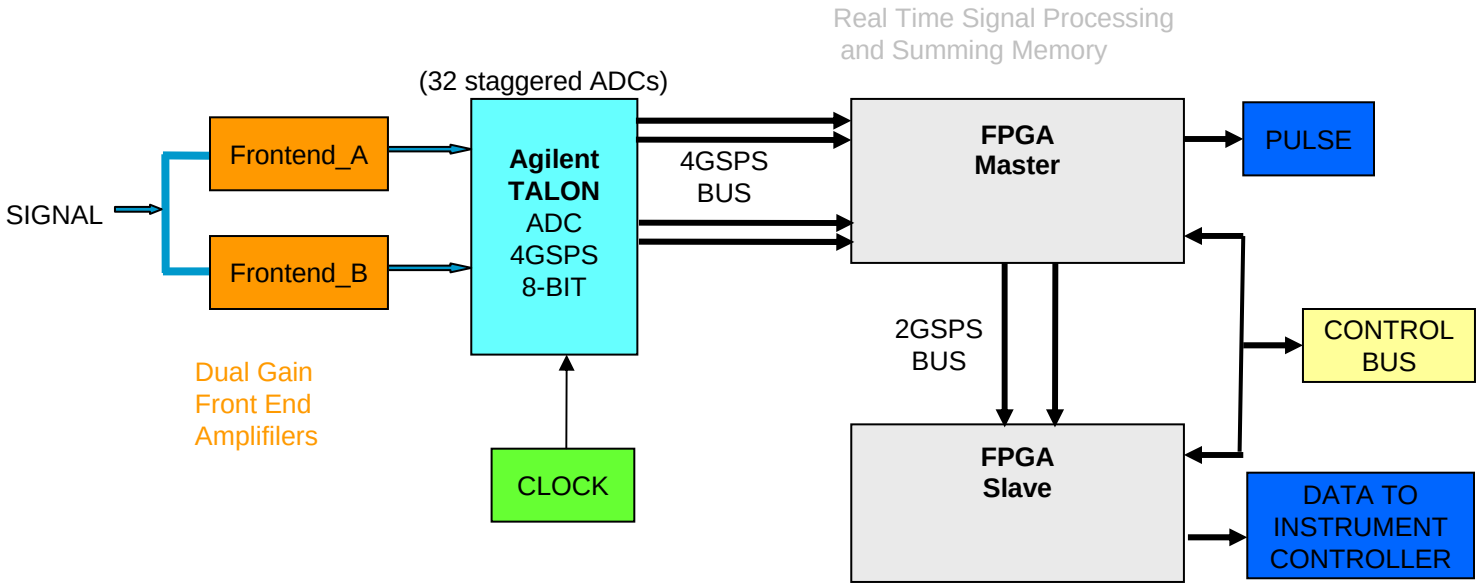




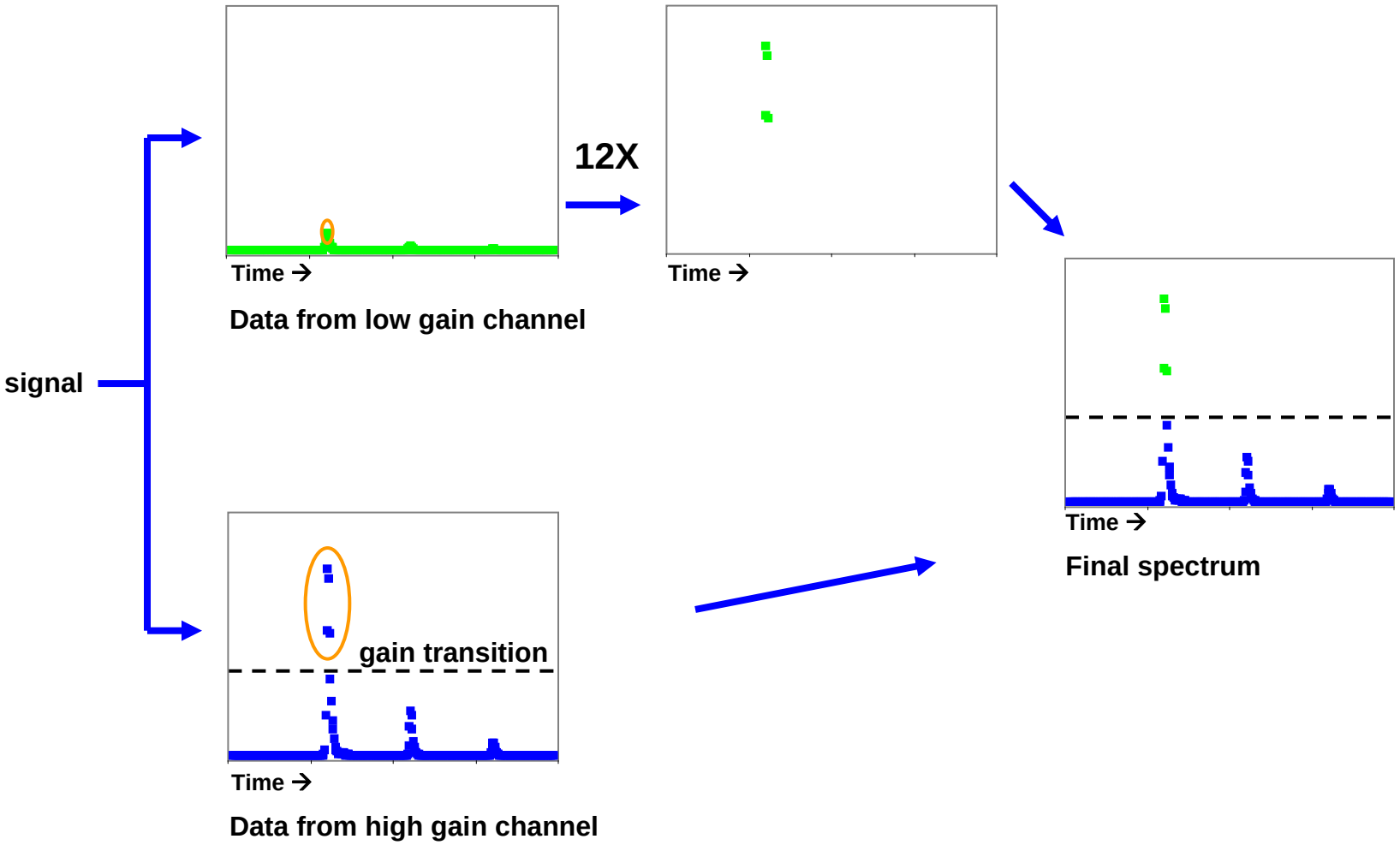
Butyl paraben (BP)

$[M+H]^+$ 195.101571

Extended Dynamic Range Mode Hardware



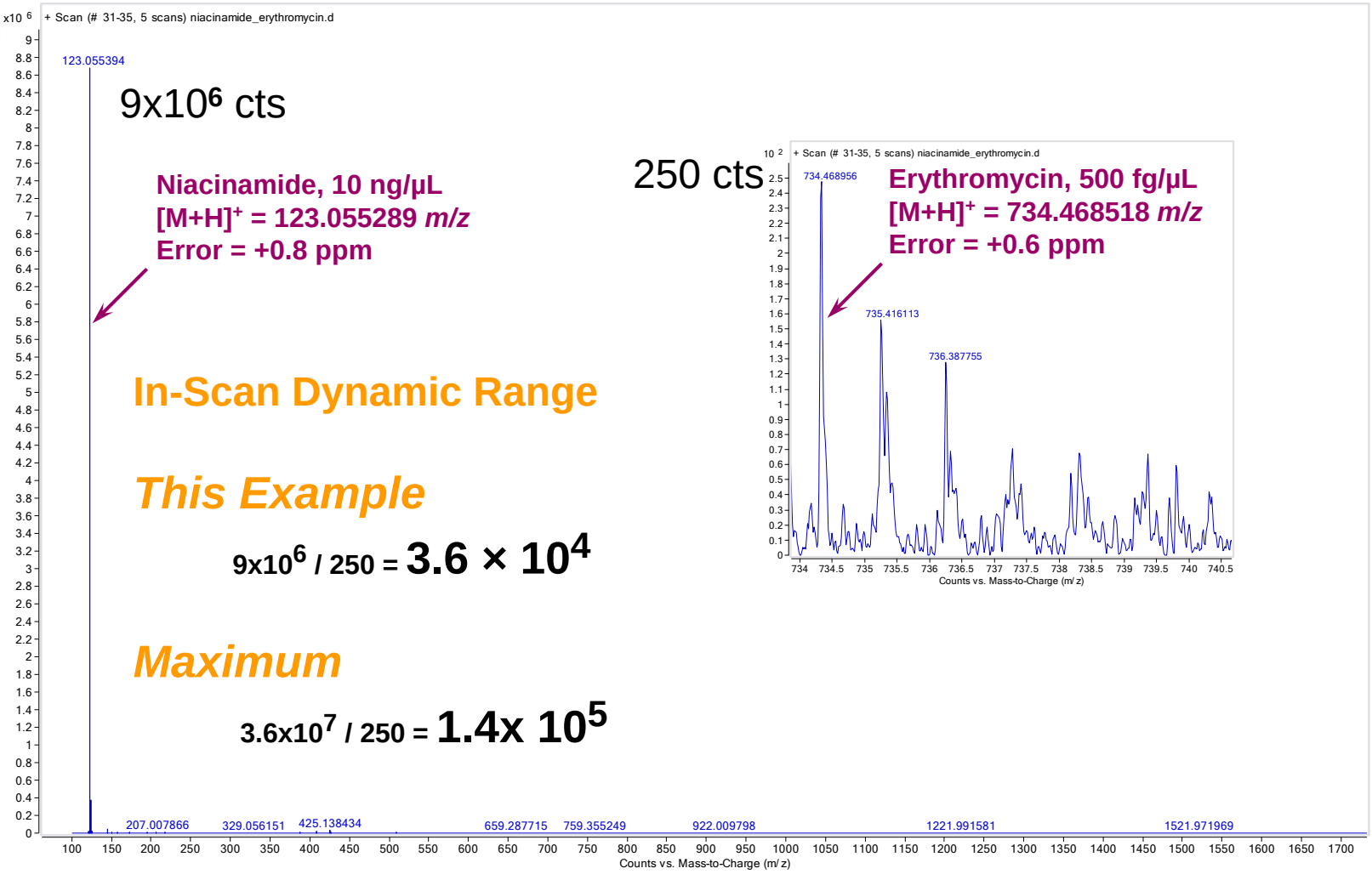
Extended Dynamic Range Mode



Extended Dynamic Range

Up To 5 Decades Of In-Spectrum Dynamic Range

Max Abd
3.6×10^7



9×10^6 cts

Niacinamide, 10 ng/µL
$[M+H]^+ = 123.055289$ *m/z*
Error = +0.8 ppm

250 cts

Erythromycin, 500 fg/µL
$[M+H]^+ = 734.468518$ *m/z*
Error = +0.6 ppm

In-Scan Dynamic Range

This Example

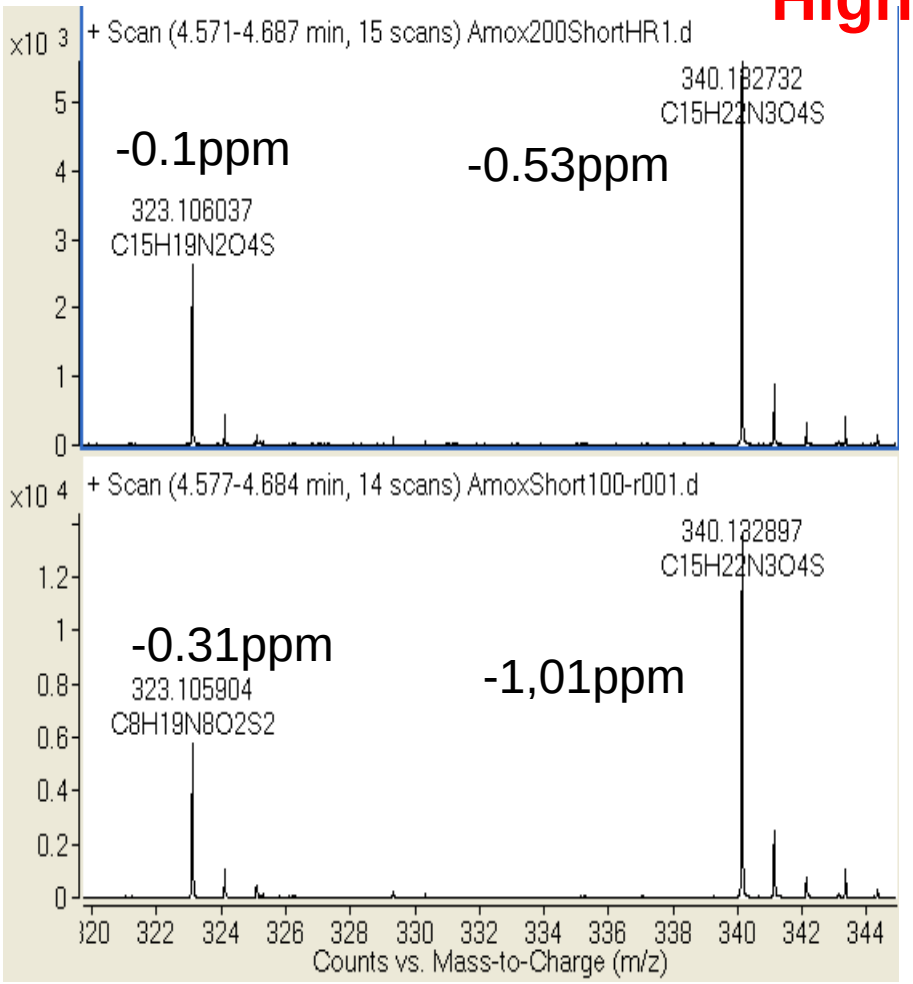
$9 \times 10^6 / 250 = 3.6 \times 10^4$

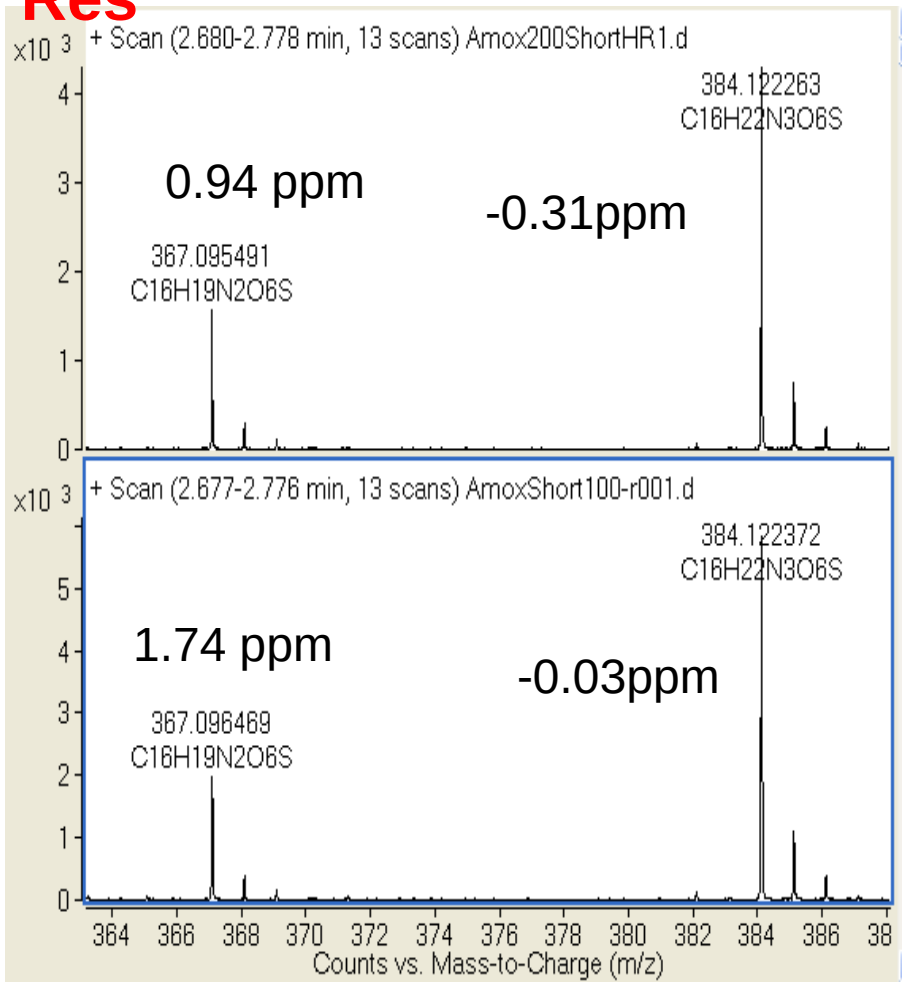
Maximum

$3.6 \times 10^7 / 250 = 1.4 \times 10^5$

Mass Accuracy Function of Acquisition State

High Res

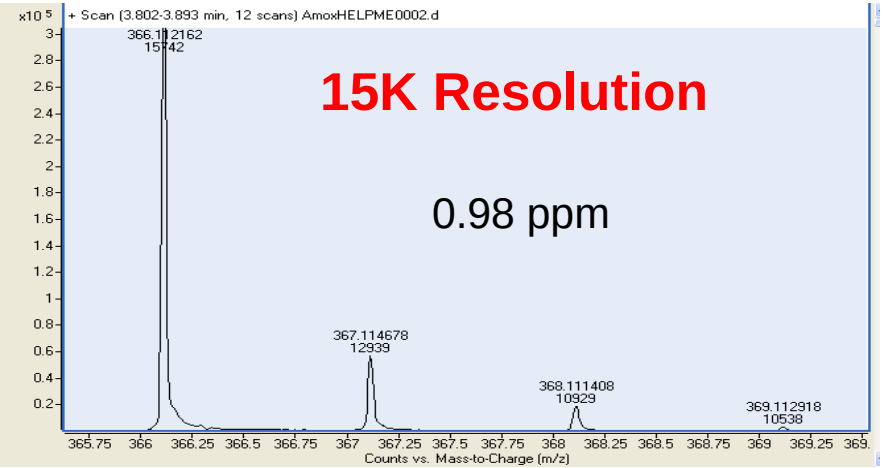




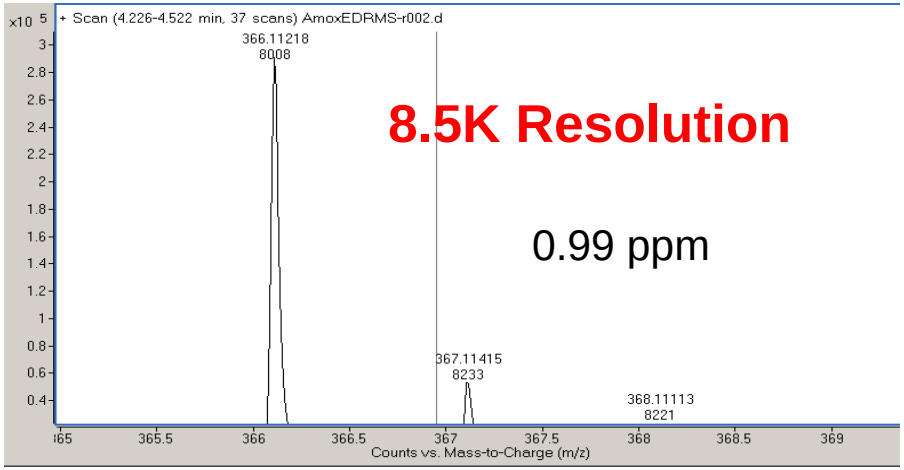
Extended DR

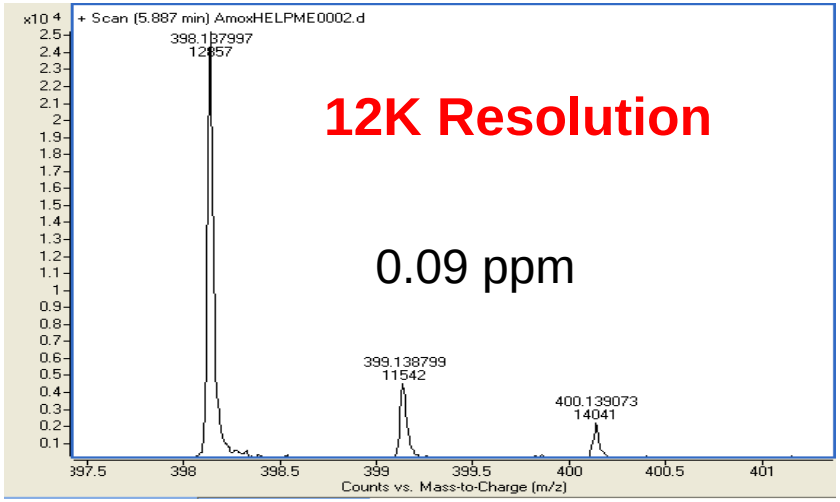
Resolution Comparison

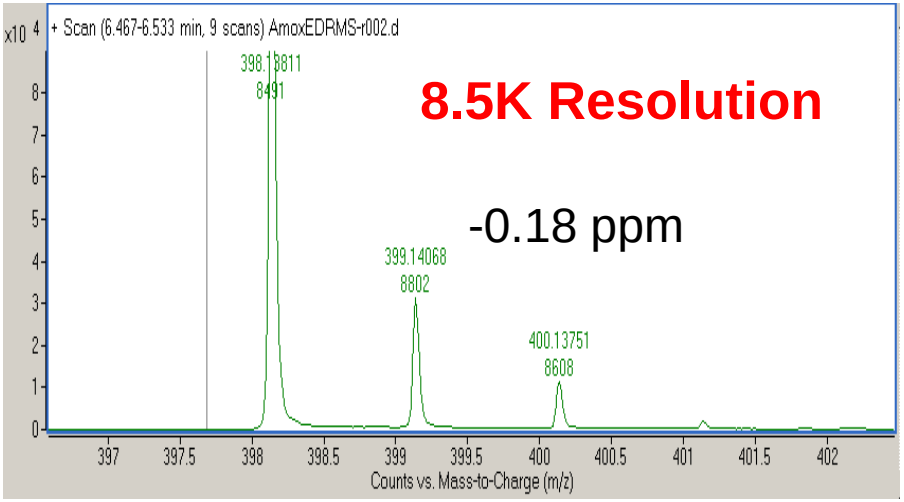
High Resolution Mode



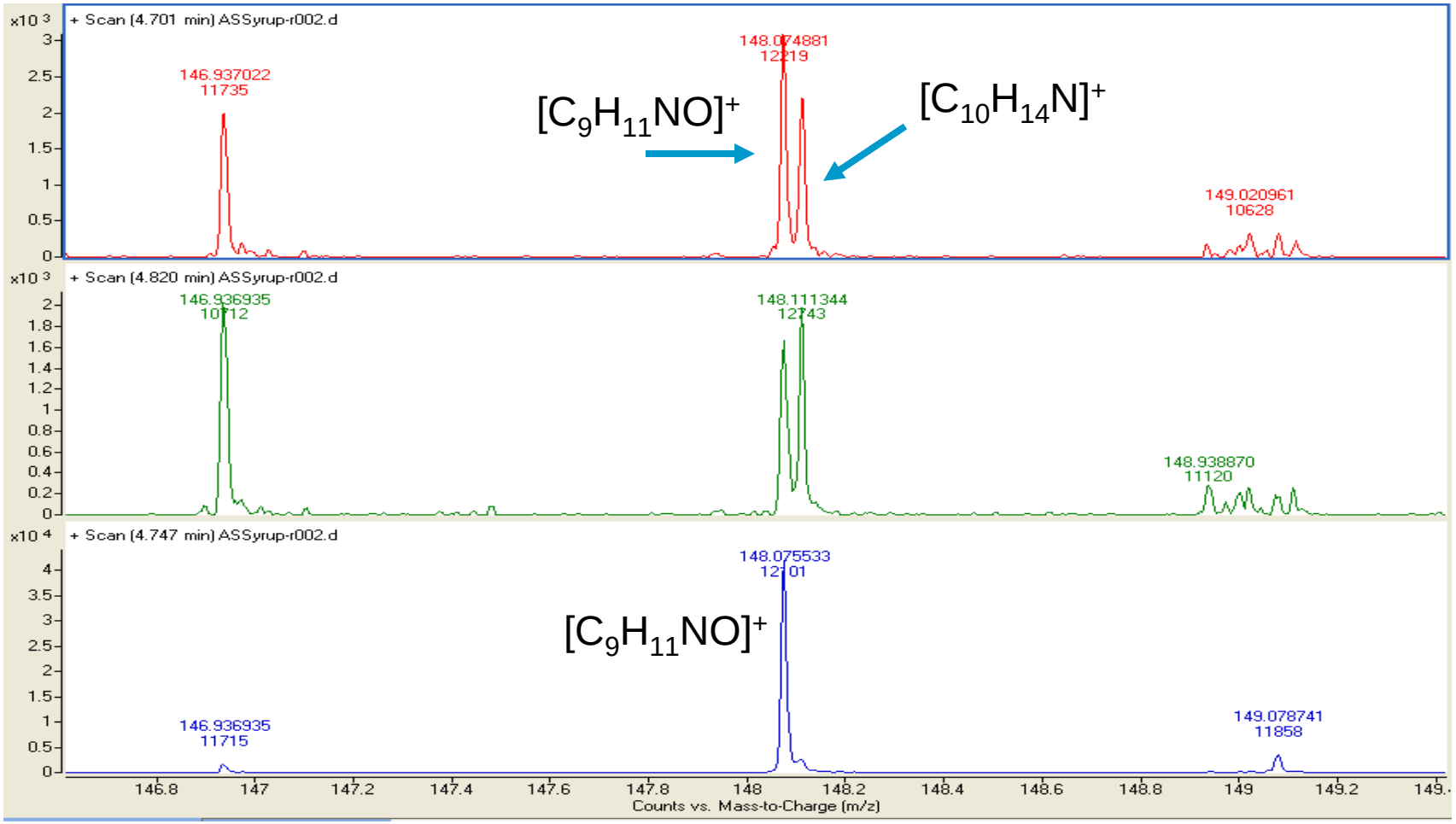
Extended Dynamic Range







Application of Enhanced Resolving Power $C_9H_{10}NO$ Product from Albuterol from $C_{10}H_{13}N$

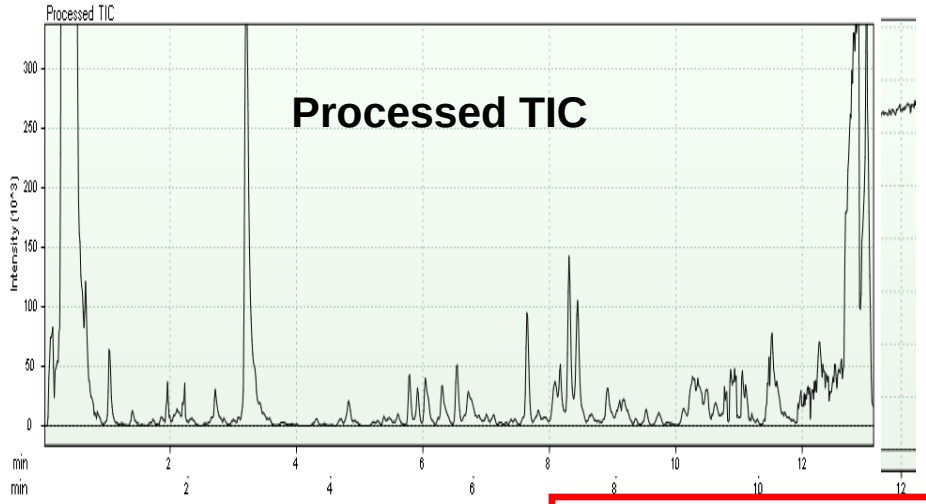


Automated Data Analysis – Show me the Data

- Find Compounds from Background – MFE Algorithm
- Differential Analysis – Mass Profiler Software
- Identification: Molecular Formula Generation, Databases
- Automation: MetID for Impurity Profiling

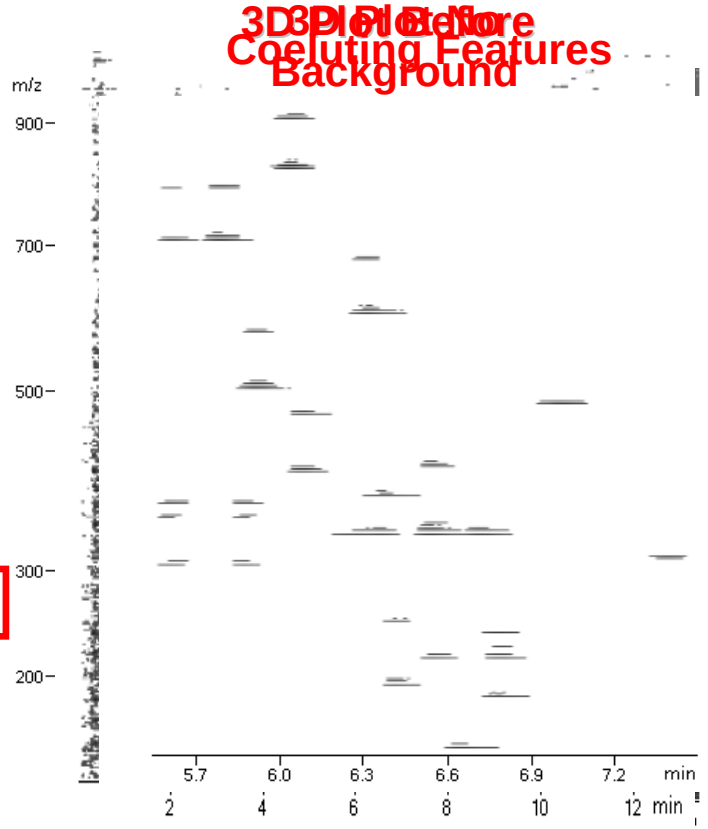
Molecular Feature Extraction (MFE)
Automated Data Reduction Software

Finds Features in TOF/QTOF Data



species	RT	m/z	mass	abund.
M	8.162		342.1467	130643
M+H	8.165	343.1547	342.1474	11889
M+H+1	8.162	344.1581		2290
M+H+2	8.157	345.1748		369
M+H4N	8.164	360.1807	342.1469	8420
M+H4N+1	8.156	361.1893		1227
M+Na	8.162	365.1359	342.1466	75678
M+Na+1	8.163	366.1394		15324
M+Na+2	8.162	367.1429		1901
2M+Na	8.164	707.2810	342.1459	4629
2M+Na+1	8.162	708.2860		1808
2M+Na+2	8.173	709.2895		336

Data Reduced sum intensities of isotopes, adducts, clusters and multiply charges ions together.



Molecular Feature Extraction (MFE)

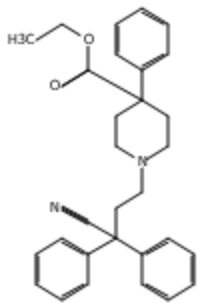
Generate Empirical Formulas

Group #1(RT=10.19)--4 Compounds

	Species	RT	m/z	Mass	Abundance	Width	Satur.	Q Score
1	M	10.193		454.0047	50500	0.071		72.6
2	M+H	10.198	455.0120	454.0047	34232	0.058		60.8
3	M+H+1	10.177	456.0209		12572	0.084		51.0
4	M+H+2	10.175	456.9993		3695	0.048		53.7
5								
6	M	10.195		452.2466	35303036	0.114	x	100.0
7	M+H	10.204	453.2538	452.2466	16689319	0.128	x	95.1
8	M+H+1	10.195	454.2574		13454754	0.105	x	97.7
9	M+H+2	10.182	455.2607		4301315	0.081	x	100.0
10	M+H+3	10.181	456.2636		386489	0.082		100.0
11	M+H+4	10.182	457.2667		31758	0.082		100.0
12	M+H+5	10.205	458.2607		2874	0.067		86.9
13								
14	M+Na	10.185	475.2360	452.2468	316119	0.098		100.0
15	M+Na+1	10.185	476.2390		95815	0.101		100.0
16	M+Na+2	10.187	477.2425		17223	0.106		100.0
17	M+Na+3	10.196	478.2454		2227	0.113		0.0
18								
19	M+K	10.182	491.2122	452.2491	3428	0.103		0.0
20	M+K+1	10.221	492.2180		1711	0.096		0.0

Structure Infomation

Structure | MOL Text



Notes

meperidine derivative used for treatment of diarrhea

drug

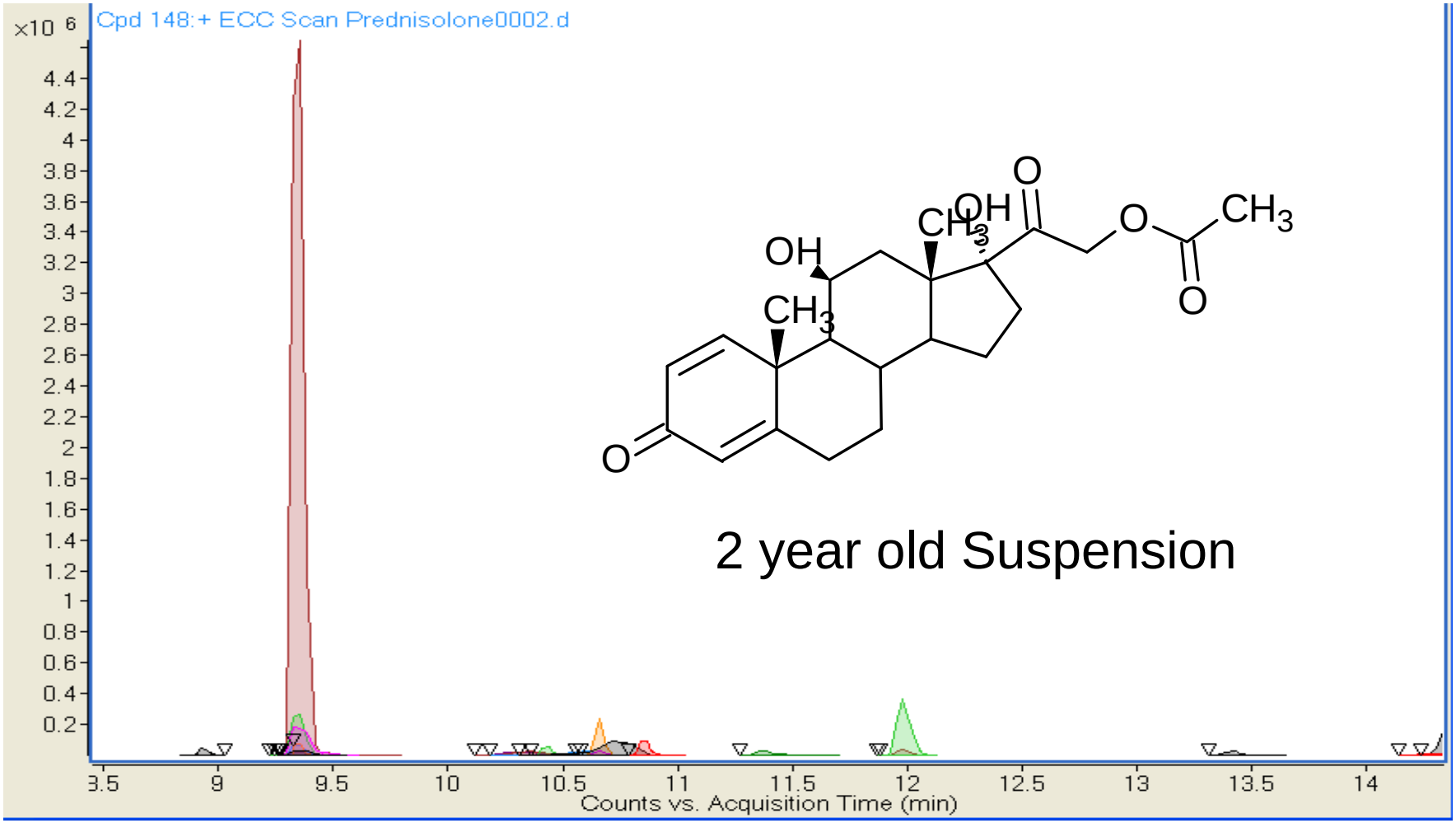
Dollery, Colin Therapeutic Drugs, 2nd Ed. 1999 p. D156

Mass = 452.2466, Time = 10.195

	Obs. Mass	Obs. RT	Name	Formula	ΔMass(ppm)	ΔRT	MFG Score
1	452.2466	10.195	Diphenoxylate	C30H32N2O2	-0.4		100.0
2	452.2466	10.195		C18H36N4O9	3.7		85.8
3	452.2466	10.195		C15H28N14O3	0.7		81.1
4	452.2466	10.195		C23H32N8S	1.1		78.1

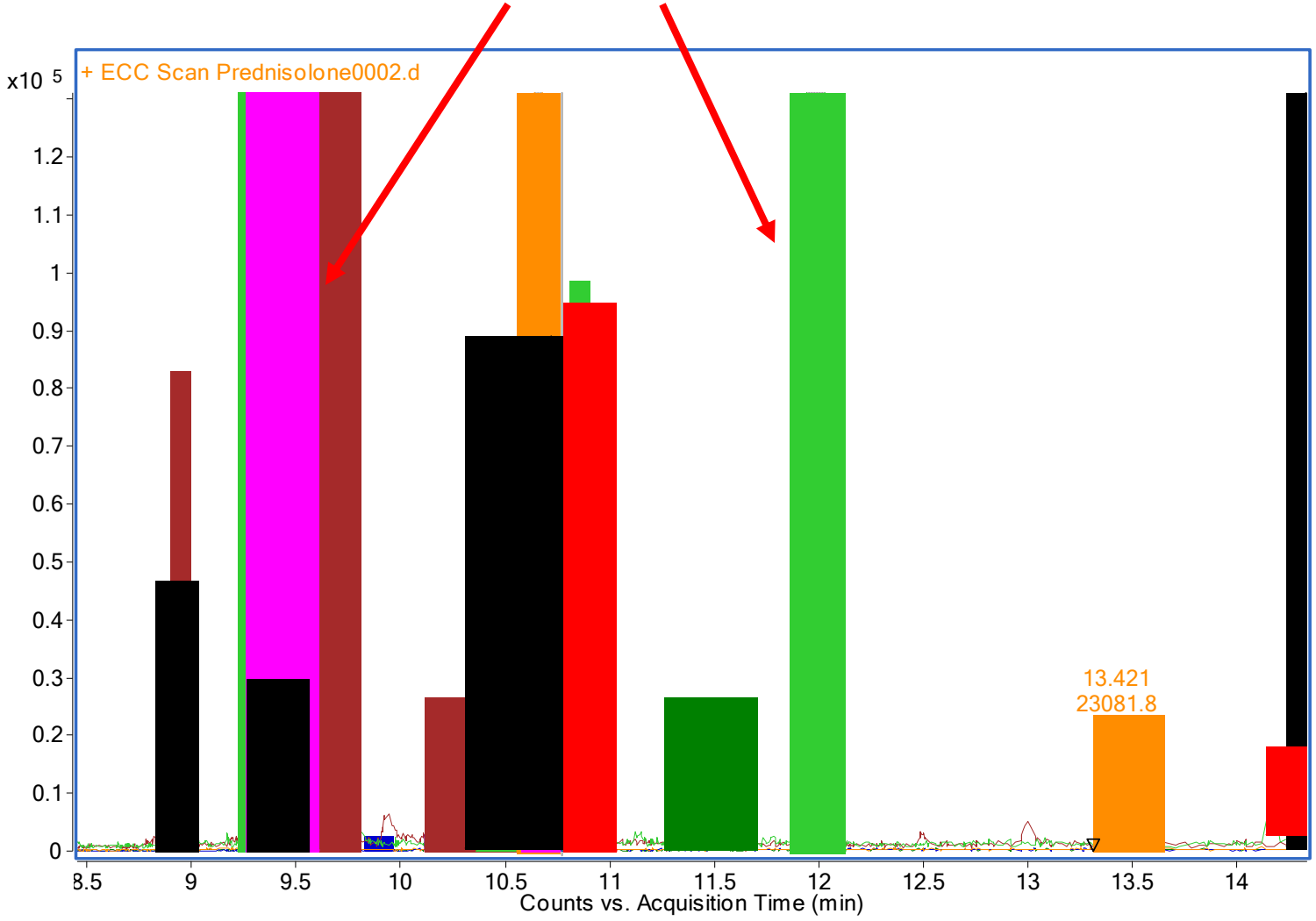
Molecular Formula Generation and Database Search

Prednisolone: $C_{21}H_{28}O_5$ 360.193674

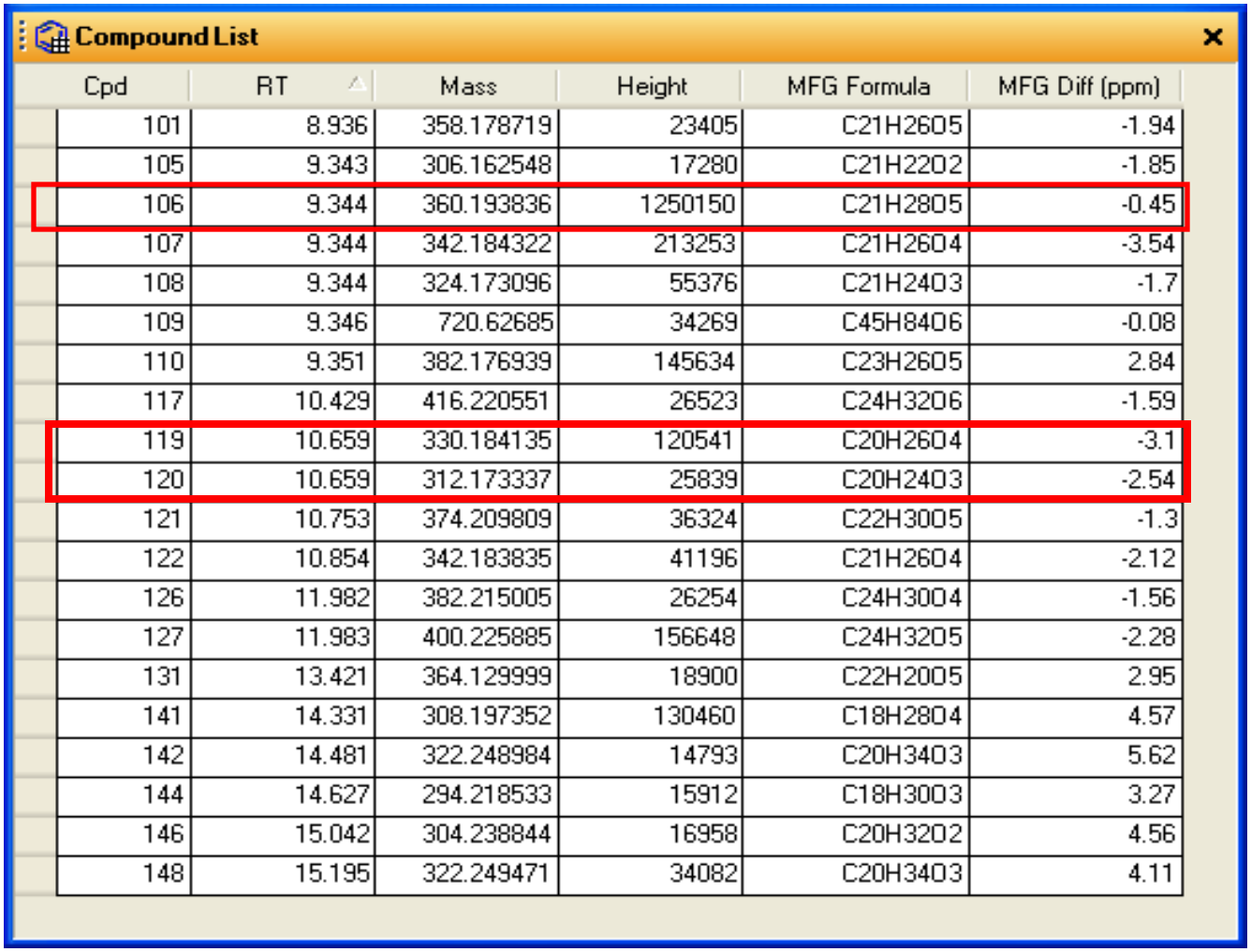


2 year old Suspension

Sequential Neutral Water Losses Detected



12 Potential Degradation Products in Prenisolone



Compound List

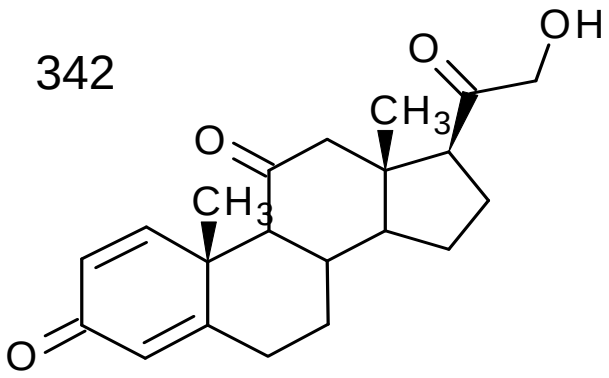
Cpd	RT	Mass	Height	MFG Formula	MFG Diff (ppm)
101	8.936	358.178719	23405	C21H2605	-1.94
105	9.343	306.162548	17280	C21H2202	-1.85
106	9.344	360.193836	1250150	C21H2805	-0.45
107	9.344	342.184322	213253	C21H2604	-3.54
108	9.344	324.173096	55376	C21H2403	-1.7
109	9.346	720.62685	34269	C45H8406	-0.08
110	9.351	382.176939	145634	C23H2605	2.84
117	10.429	416.220551	26523	C24H3206	-1.59
119	10.659	330.184135	120541	C20H2604	-3.1
120	10.659	312.173337	25839	C20H2403	-2.54
121	10.753	374.209809	36324	C22H3005	-1.3
122	10.854	342.183835	41196	C21H2604	-2.12
126	11.982	382.215005	26254	C24H3004	-1.56
127	11.983	400.225885	156648	C24H3205	-2.28
131	13.421	364.129999	18900	C22H2005	2.95
141	14.331	308.197352	130460	C18H2804	4.57
142	14.481	322.248984	14793	C20H3403	5.62
144	14.627	294.218533	15912	C18H3003	3.27
146	15.042	304.238844	16958	C20H3202	4.56
148	15.195	322.249471	34082	C20H3403	4.11

CH_2O Loss

$-H_2$, $-H_2O$, $-CO_2H_2$, $+H_2$, $+CH_4+H_2-O$,

Prednisolone

342



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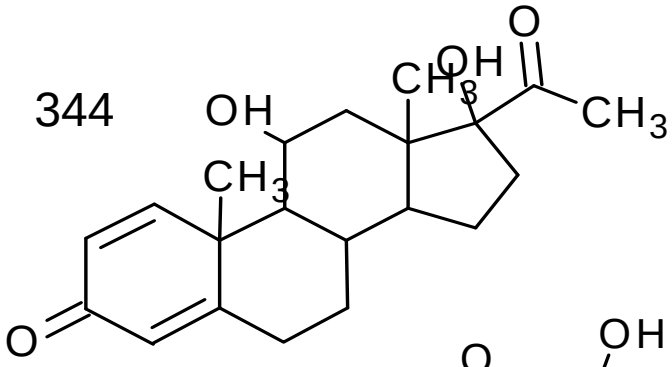

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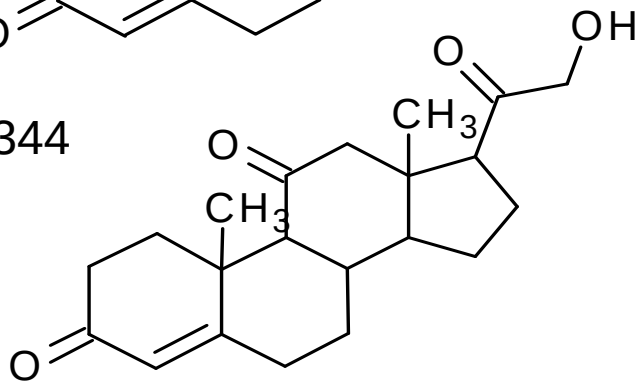

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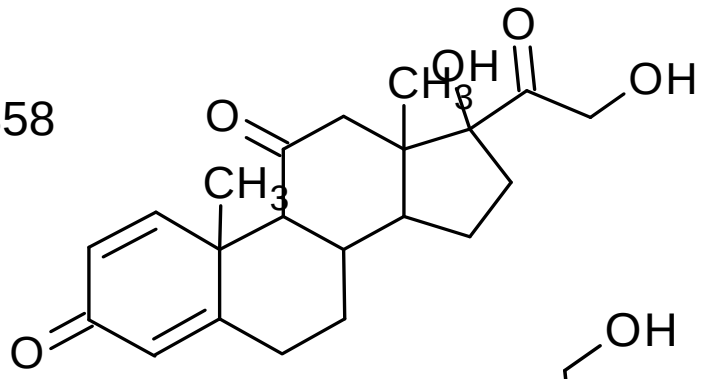

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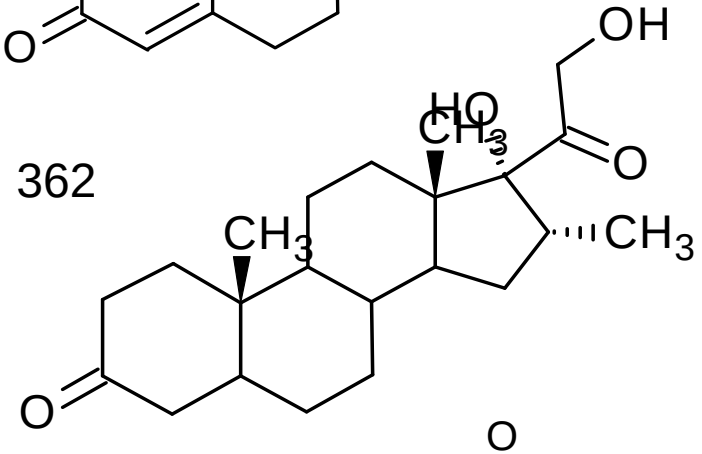

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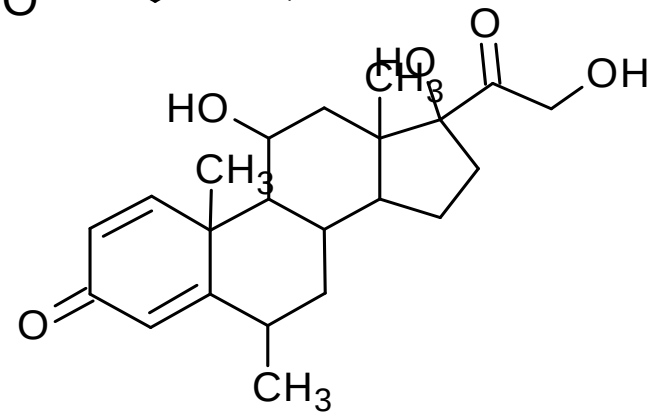
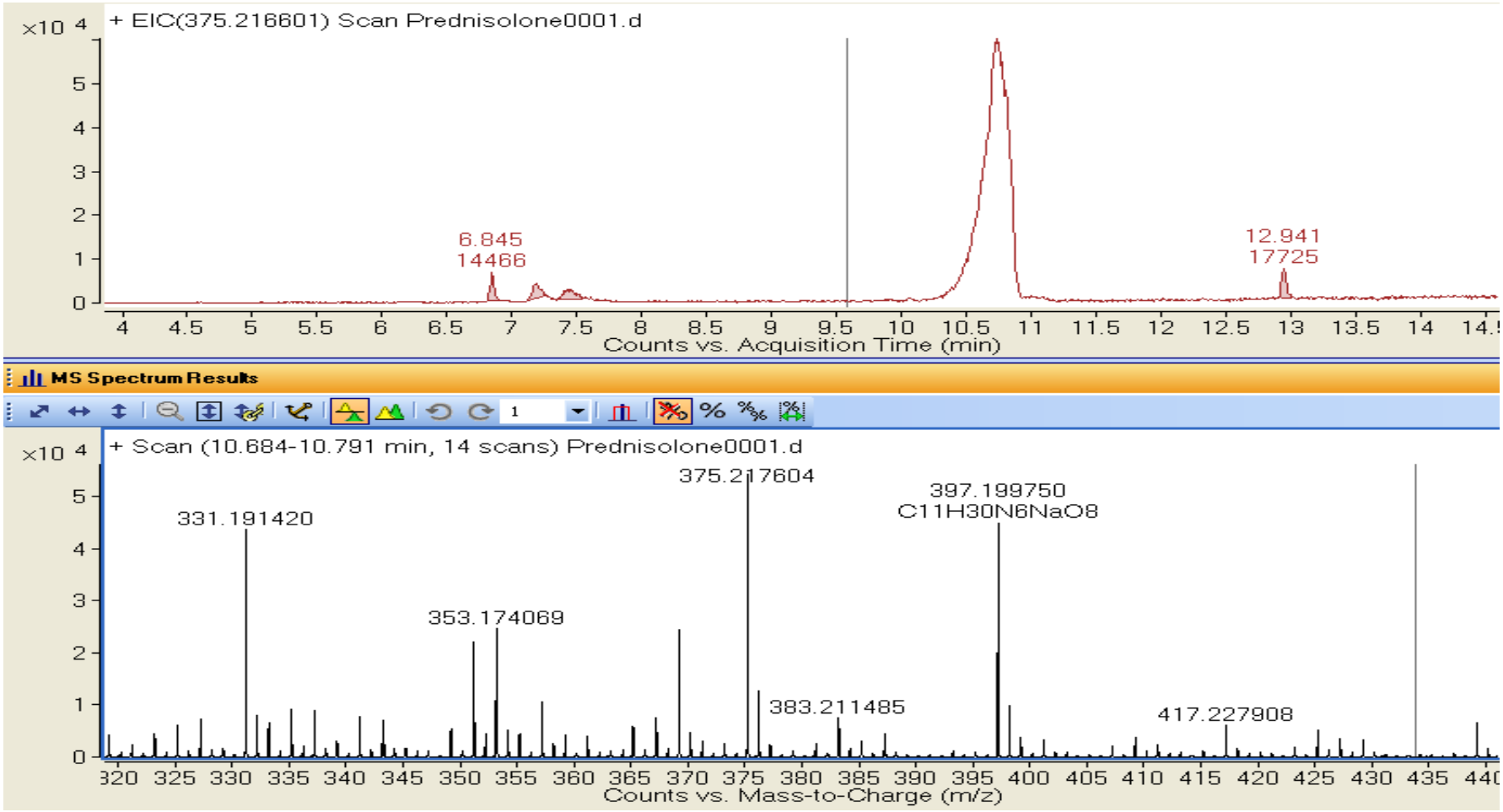


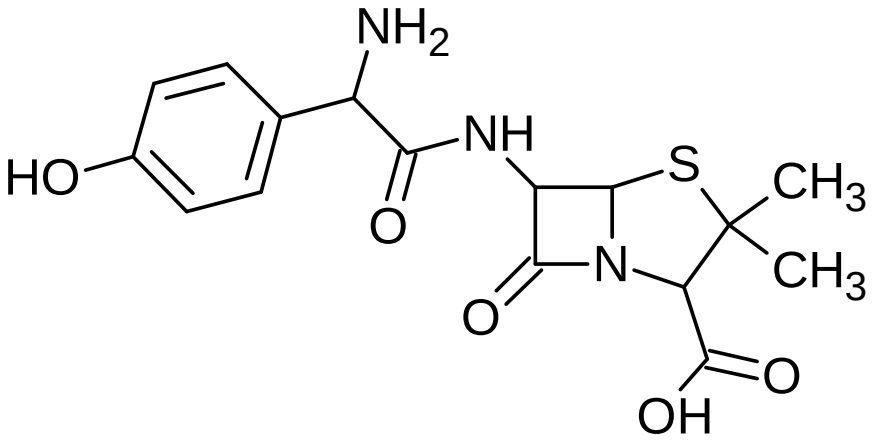
Is 6-methyl Prednisolone Present? [M+H]+ 375



Degradation Products of Amoxicillin and AmoxClavin

$C_{16}H_{19}N_3SO_5$

MW 365.10454



Source

- Tablet 875mg New
- Tablet 500mg 09/07

Sample Preparation

- Tablets Dissolved in 40mL Methanol Water with 0.1% Formic Acid
- Syndicated 20 minutes
- Spin 5 minutes 14000 RPM
- Dilute 100 and 1000 fold with water

Experimental Conditions

Chromatography

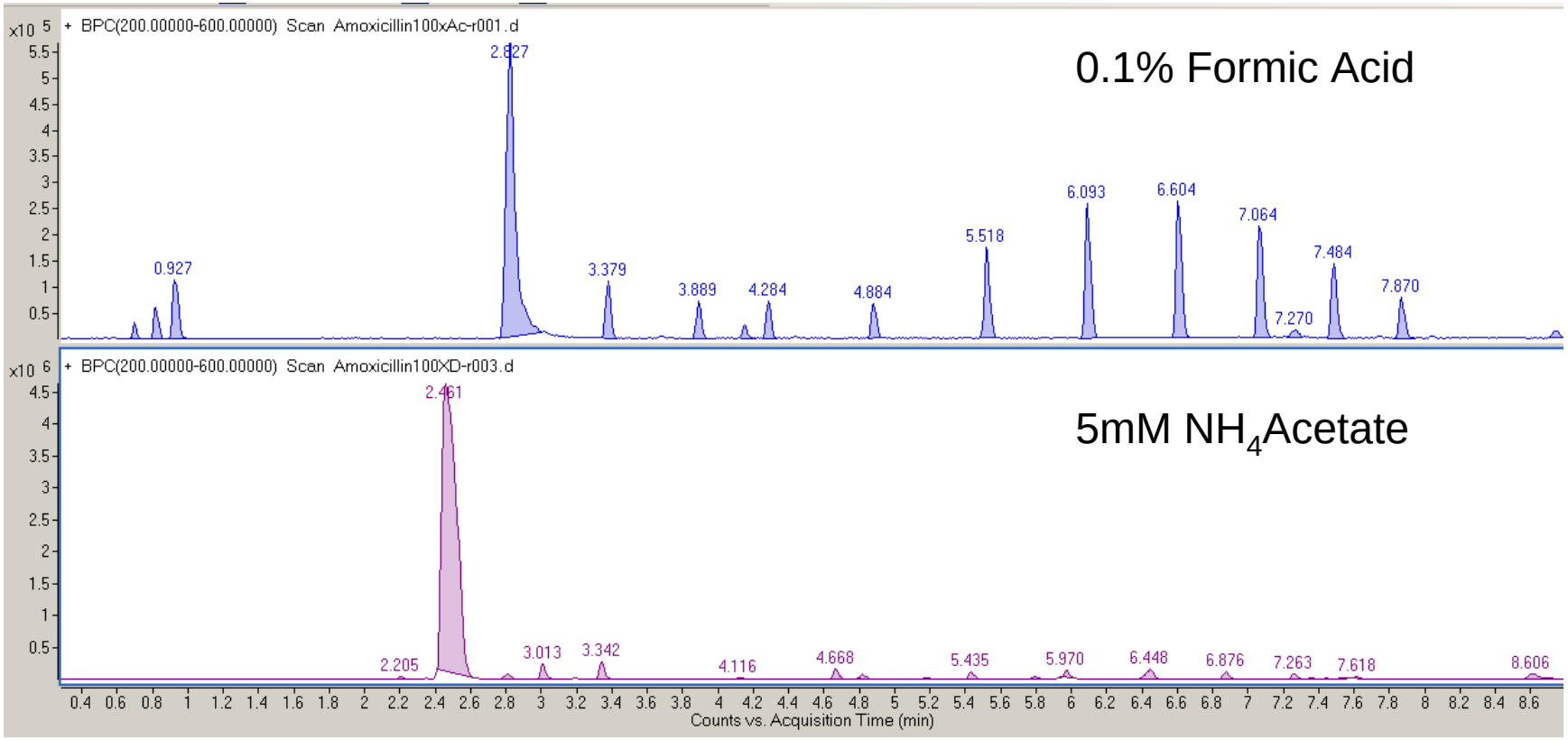
- Agilent 1200 RRLC
- Ecilpse Plus C18 2.1x100mm 1.8µm heated to 50^{o}C
- Mobile Phase:
  - Water/Methanol 0.1% FA
  - Water/Methanol 5mM NH4Ac
- Multiple Gradients Tested
  - 0 to 95% in 16 Minutes
  - 0 to 95% in 13 Minutes
- Flow Rate: 400-500 µL/min

Mass Spectrometry

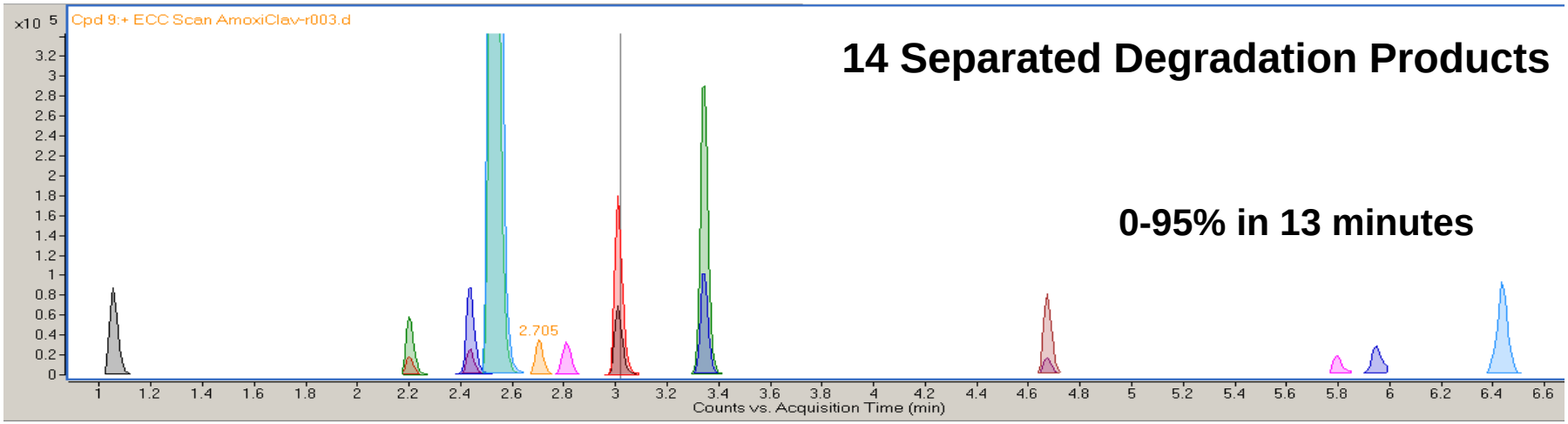
- Agilent 6520 QTOF
- Positive ion ESI
- Mass Range m/z 100-1000
- Acquisition Rate: 2 spectra/s
- Drying Gas: 250^{o}C
- Internal Reference Mass
  - m/z 121 and 922
- Fragmenter: 165C
- Enhanced and High Resolution Modes

Buffer in Mobile Phase

10X Enhancement with NH_4Acetate

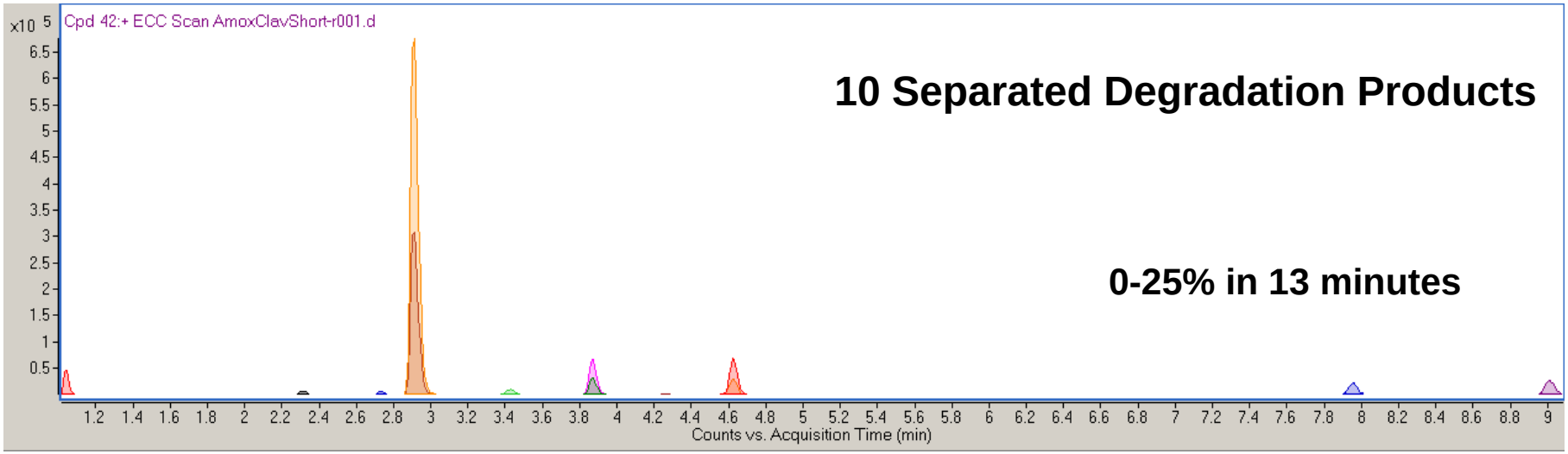


Effect of Gradient



14 Separated Degradation Products

0-95% in 13 minutes

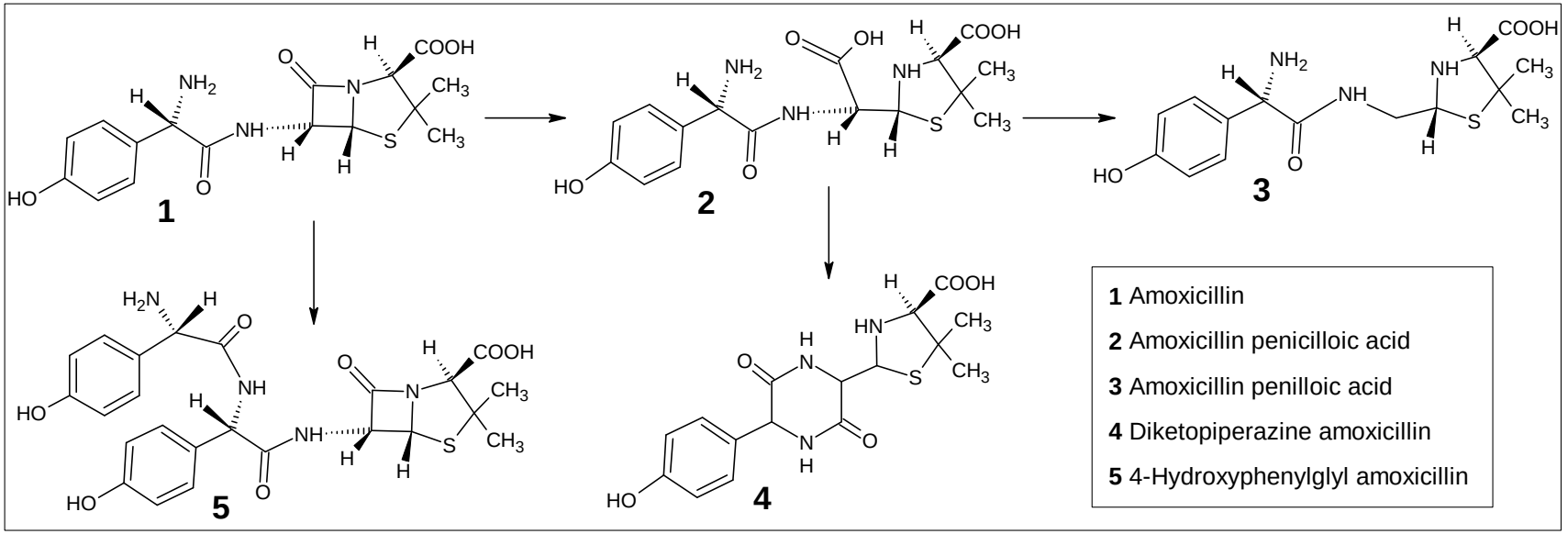


10 Separated Degradation Products

0-25% in 13 minutes

Degradation Pathway of Amoxicillin

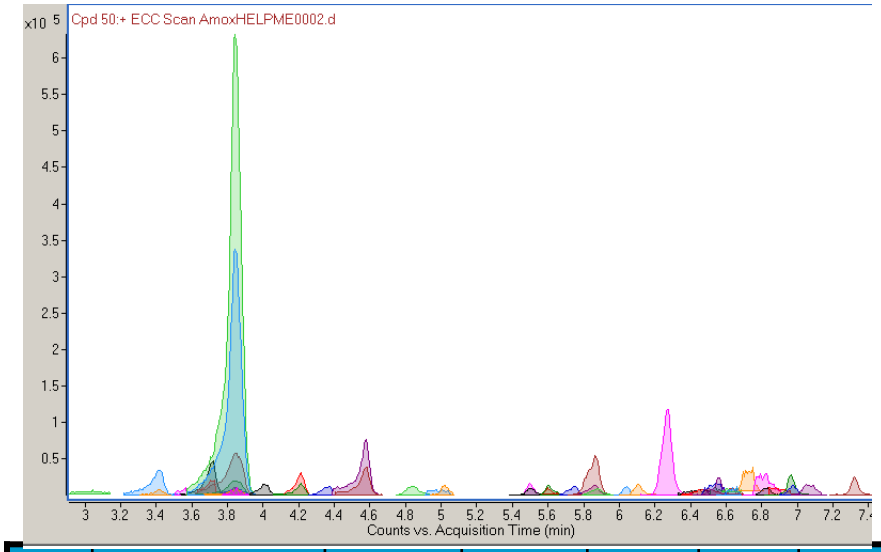
(previous TOF/Trap work)



E. Naegele, R. Moritt, J. Am. Soc. Mass Spectrom. 2005, 16, 1670-1676

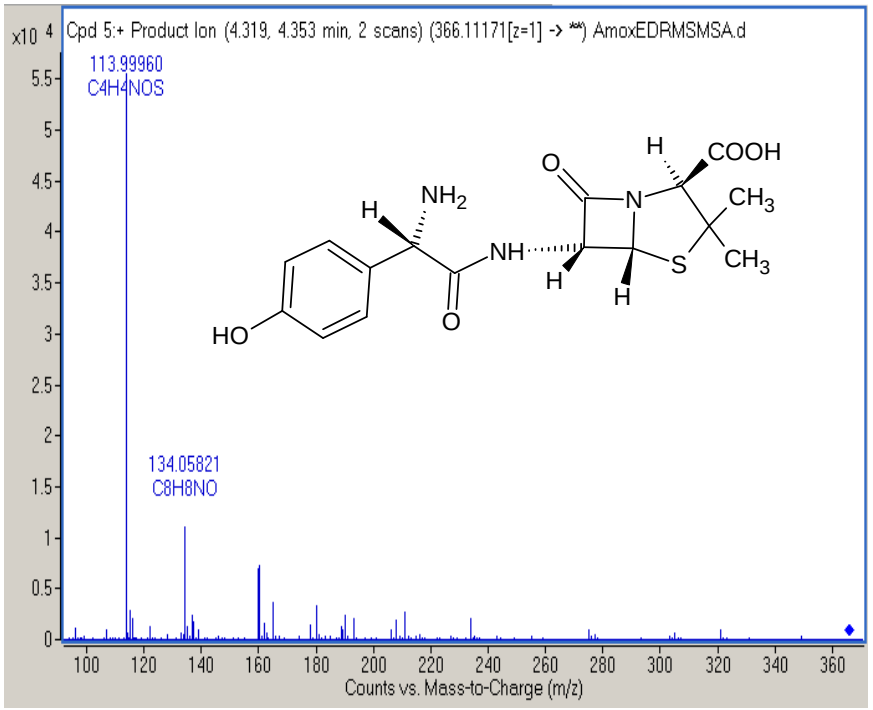
Accurate Mass:

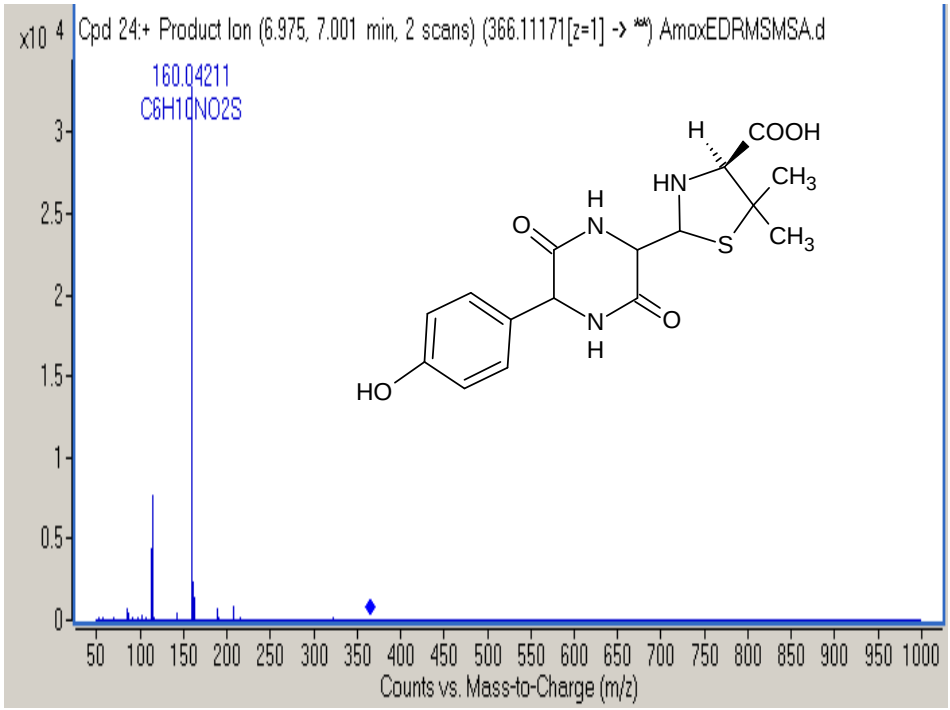
High Resolution Mode



RT [min]	Compound name	Formula	Calculated mass	Measured Mass	Mass accuracy [mDa]	Mass accuracy [ppm]
3.71	amoxicillin penicilloic acid 2	$C_{16}H_{22}N_3O_6S$	384.1229	384.12274	-0.93	-0.36
3.84	amoxicillin 1	$C_{16}H_{20}N_3O_5S$	366.1124	366.11222	1.09	-0.4
4.57	amoxicillin penilloic acid I and II 3	$C_{15}H_{22}N_3O_4S$	340.1331	340.13352	-2.84	-0.96
5.954	Amoxicillin Acid	$C_{17}H_{23}N_3SO_6$	398.13803	398.13826	-0.58	-0.23
6.269	diketopiperazine amoxicillin 5	$C_{16}H_{20}N_3O_5S$	366.1124	366.11286	-2.85	-1.04

Structural Information from MS/MS $C_{16}H_{19}N_3SO_5$





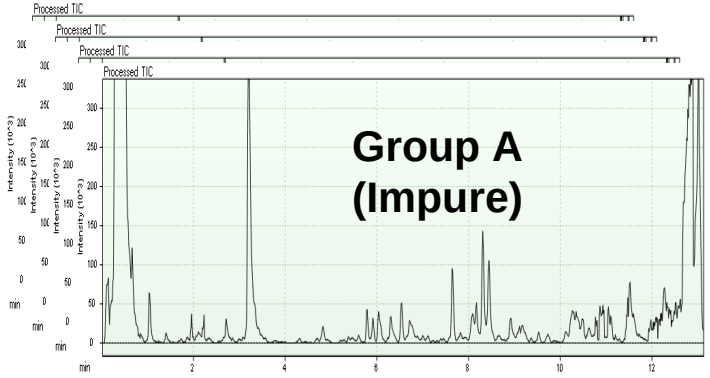
m/z	Formula	Abund%	Difference (ppm)	Loss Mass	Loss Formula
70.06483	C4H8N	6.29	4.16	296.04669	C12H12N2O5S
113.9996	C4H4NOS	66.02	10.6	252.11101	C12H16N2O4
134.05821	C8H8NO	12.06	13.69	232.05178	C8H12N2O4S
134.05821	C3H8N3O3	12.06	-16.32	232.0558	C13H12O2S
160.04098	C9H6NO2	7.97	-10.44	206.07251	C7H14N2O3S
160.04098	C6H10NO2S	7.97	10.62	206.06914	C10H10N2O3
165.06415	C8H9N2O2	4.01	10.33	201.04596	C8H11NO3S
180.04691	C9H10NOS	3.66	4.7	186.06406	C7H10N2O4
180.04691	C12H6NO	3.66	-14.02	186.06743	C4H14N2O4S

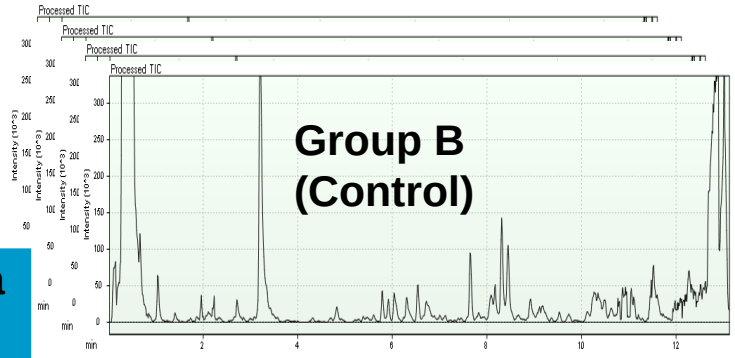
m/z	Formula	Abund%	Difference (ppm)	Loss Mass	Loss Formula
113.0343	C4H5N2O2	9.28	2.22	253.07726	C12H15NO3S
114.03705	C5H8NS	16.55	1.27	252.07462	C11H12N2O5
160.04211	C6H10NO2S	74.18	3.53	206.06914	C10H10N2O3

$C_6H_{10}NO_2S$ 3.53 ppm Error

Differential Analysis Using Mass Profiler

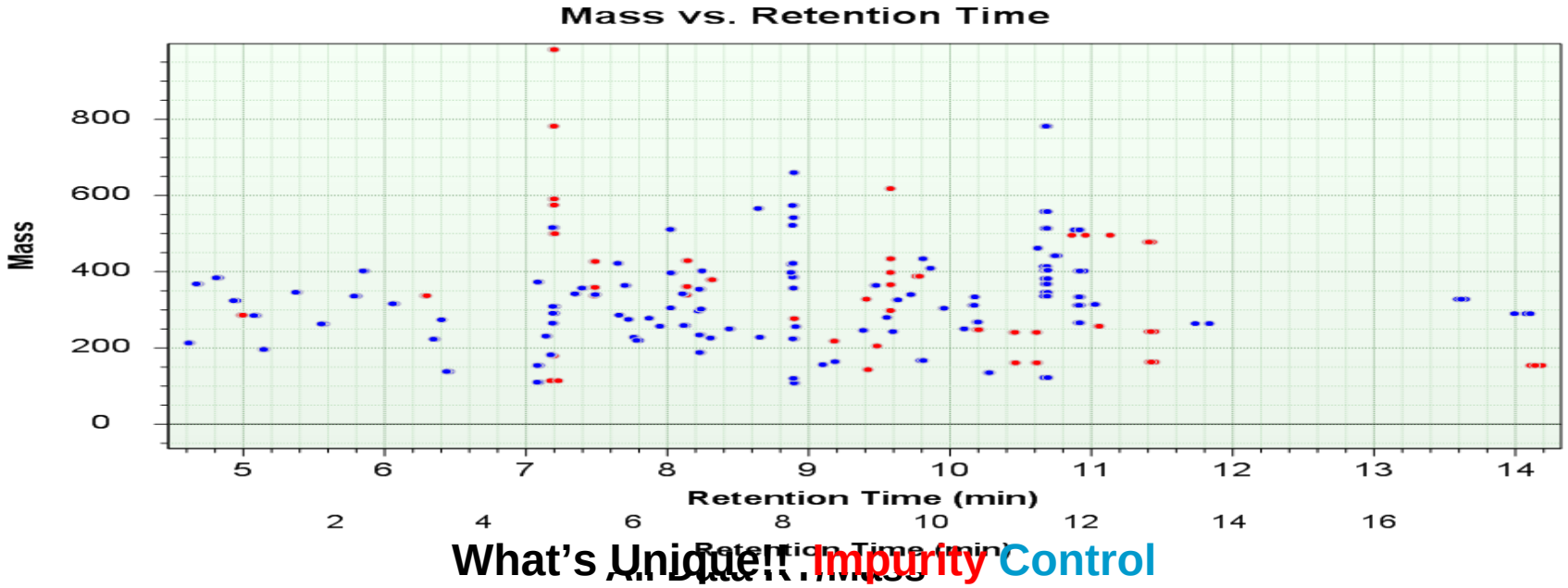
Impurity Analysis: Mass Profiling Software





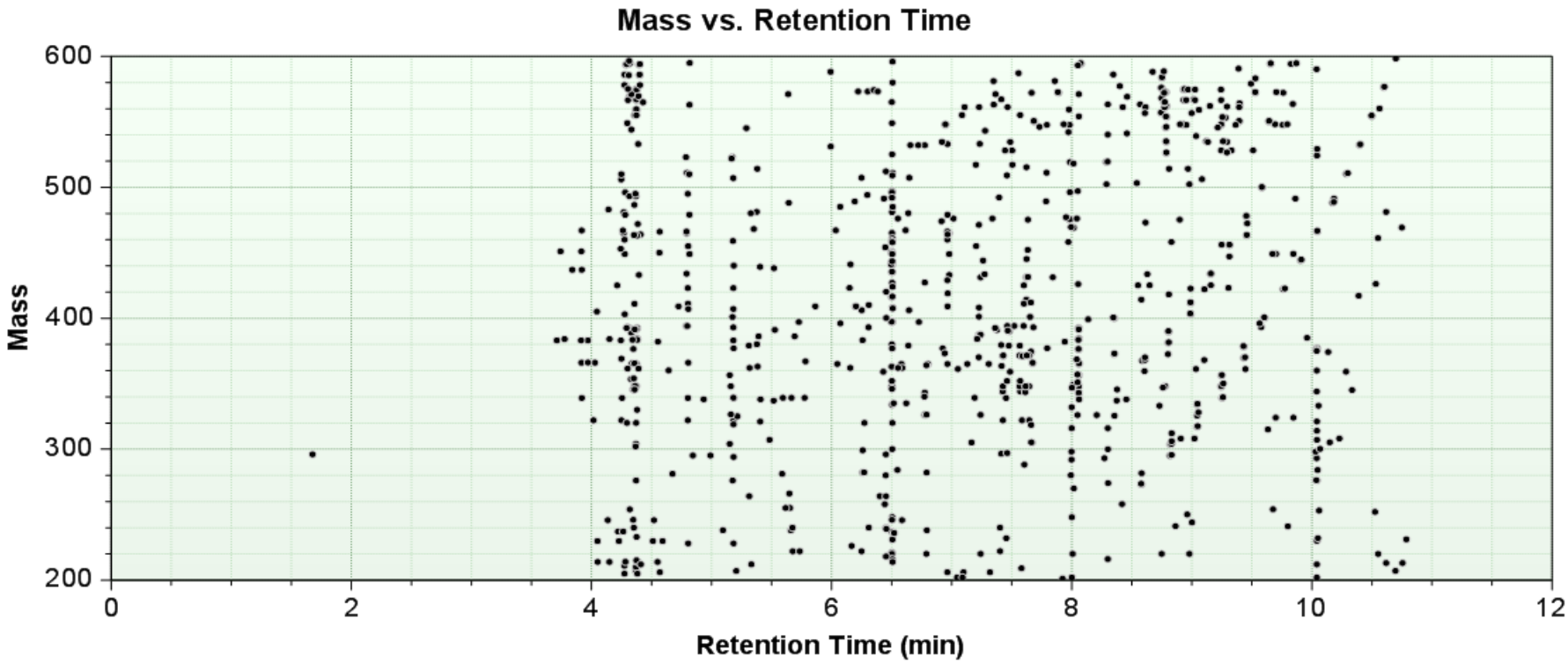
Aligns Data RT, Mass, Abundance

What's Changed? Mass/RT >2 Fold Change



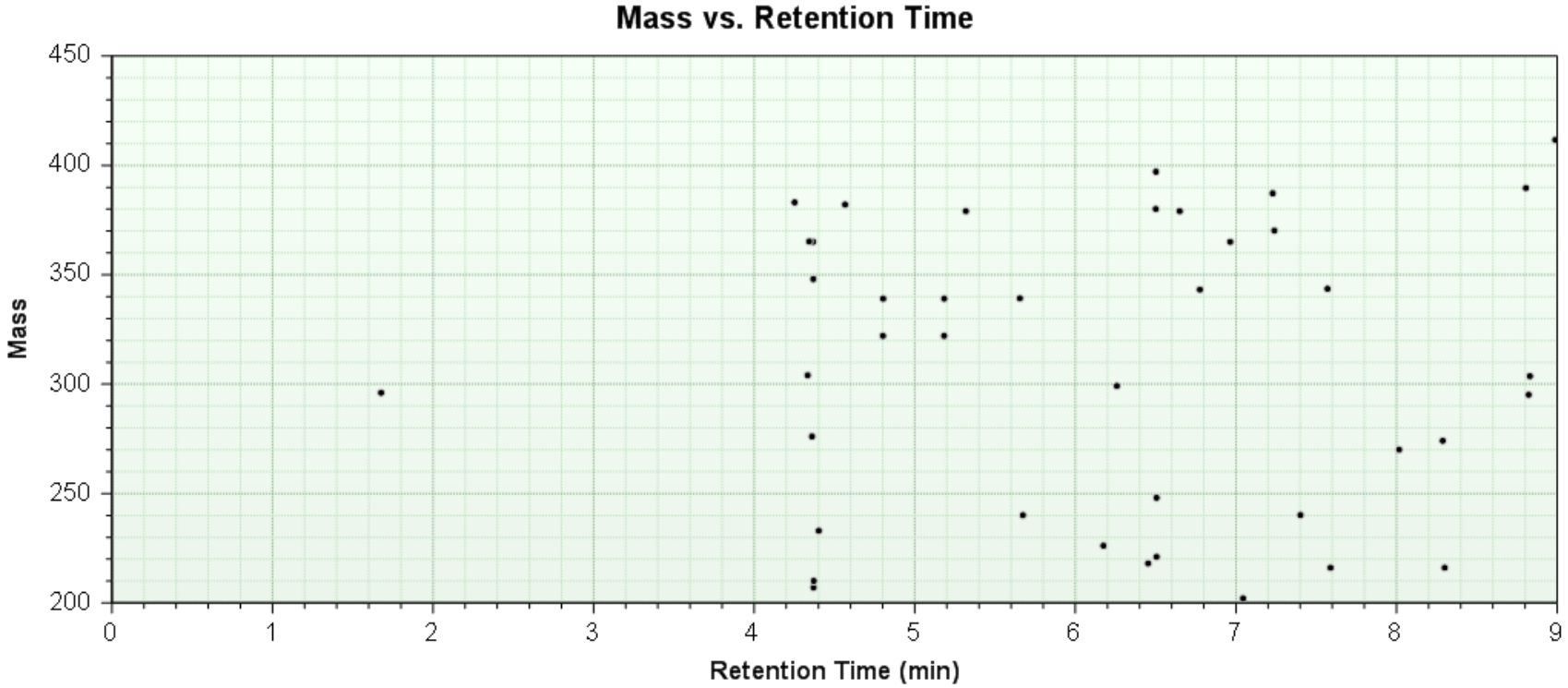
What's Unique!? Impurity Control

S/N 5:1, RI > 0.01%, Mass 200-600, RT 1-11 Minutes 835 Features

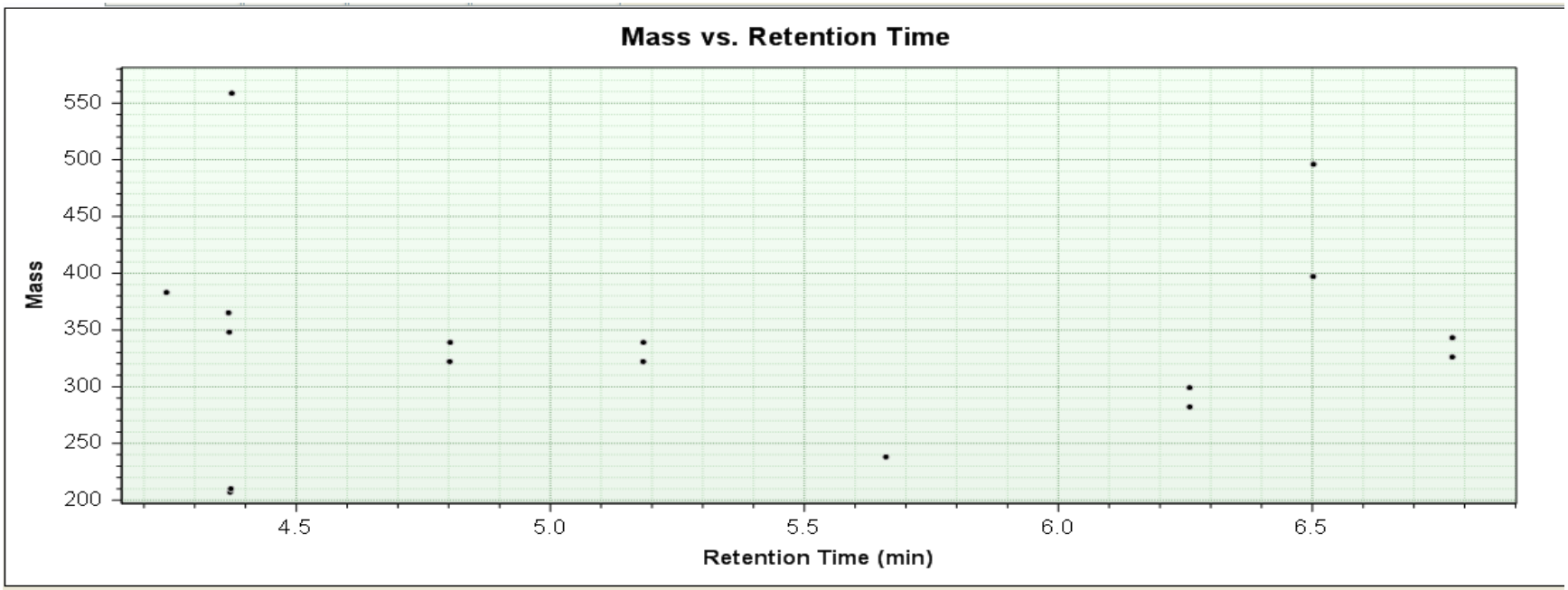


36 Features after Filtering

Isotope Pattern, Background Subtraction, # Ions



Detected 17 Degradation Products



Expression: (•) Both ( ) Up ( ) Down 17 Features

	ID	RT	SD	Mass	SD	Abundance	RSD	Freq.	Mark	RT	Mass	Abundance	RSD	Freq.	RT	Mass	Abundance	RSD	Freq.	RT	Mass	Log2(A1/A2)
1	13	4.244	0.009	383.1152	0.0005	267975	0.29	6		4.252	383.1152	336921	0.03	3	4.237	383.1153	199030	0.13	3	-0.015	0.0001	0.76
2	1	4.367	0.027	365.1048	0.0004	13924040	0.55	6		4.391	365.1052	6891901	0.02	3	4.342	365.1045	20956180	0.01	3	-0.049	-0.0007	-1.60
3	2	4.368	0.027	348.0785	0.0003	9272090	0.56	6		4.392	348.0787	4610367	0.03	3	4.344	348.0782	13933810	0.11	3	-0.049	-0.0005	-1.60
4	4	4.369	0.030	207.0352	0.0002	1071072	0.62	6		4.396	207.0350	463589	0.18	3	4.343	207.0353	1678555	0.00	3	-0.054	0.0002	-1.86
5	15	4.371	0.031	210.0634	0.0002	193118	0.63	6		4.398	210.0634	82008	0.16	3	4.344	210.0634	304229	0.01	3	-0.054	0.0000	-1.89
6	16	4.373	0.032	558.6432	0.0009	47625	0.71	6		4.402	558.6435	16814	0.03	3	4.344	558.6430	78435	0.02	3	-0.058	-0.0005	-2.22
7	9	4.802	0.009	322.0988	0.0003	465558	0.14	6		4.796	322.0987	406343	0.00	3	4.808	322.0989	524774	0.01	3	0.012	0.0001	-0.37
8	7	4.803	0.009	339.1254	0.0003	633992	0.04	6		4.796	339.1254	655426	0.01	3	4.809	339.1255	612558	0.01	3	0.013	0.0002	0.10
9	8	5.183	0.011	322.0989	0.0003	538439	0.20	6		5.174	322.0988	638707	0.00	3	5.191	322.0989	438171	0.01	3	0.016	0.0001	0.54
10	5	5.183	0.011	339.1256	0.0003	1036877	0.21	6		5.175	339.1255	1232619	0.02	3	5.192	339.1256	841134	0.01	3	0.017	0.0001	0.55
11	12	5.661	0.010	238.1414	0.0003	299491	0.44	6		5.654	238.1413	182178	0.26	3	5.667	238.1415	416804	0.01	3	0.012	0.0002	-1.19
12	14	6.258	0.016	299.1945	0.0003	253315	0.79	6		6.245	299.1943	71245	0.04	3	6.271	299.1947	435385	0.01	3	0.026	0.0004	-2.61
13	10	6.259	0.016	282.1679	0.0003	397361	0.78	6		6.245	282.1677	113254	0.02	3	6.272	282.1682	681468	0.01	3	0.026	0.0004	-2.59
14	3	6.502	0.007	397.1312	0.0005	3293522	0.72	6		6.498	397.1312	1120785	0.03	3	6.505	397.1313	5466259	0.01	3	0.007	0.0001	-2.29
15	17	6.502	0.008	496.0561	0.0013	18941	0.58	6		6.498	496.0555	9037	0.13	3	6.506	496.0568	28845	0.08	3	0.008	0.0013	-1.67
16	11	6.776	0.018	326.1942	0.0005	351970	0.97	6		6.762	326.1940	41734	0.04	3	6.789	326.1945	662205	0.01	3	0.027	0.0005	-3.99
17	6	6.776	0.017	343.2211	0.0006	883377	0.97	6		6.762	343.2208	103229	0.02	3	6.789	343.2214	1663524	0.01	3	0.027	0.0007	-4.01

Composition and Database Searching

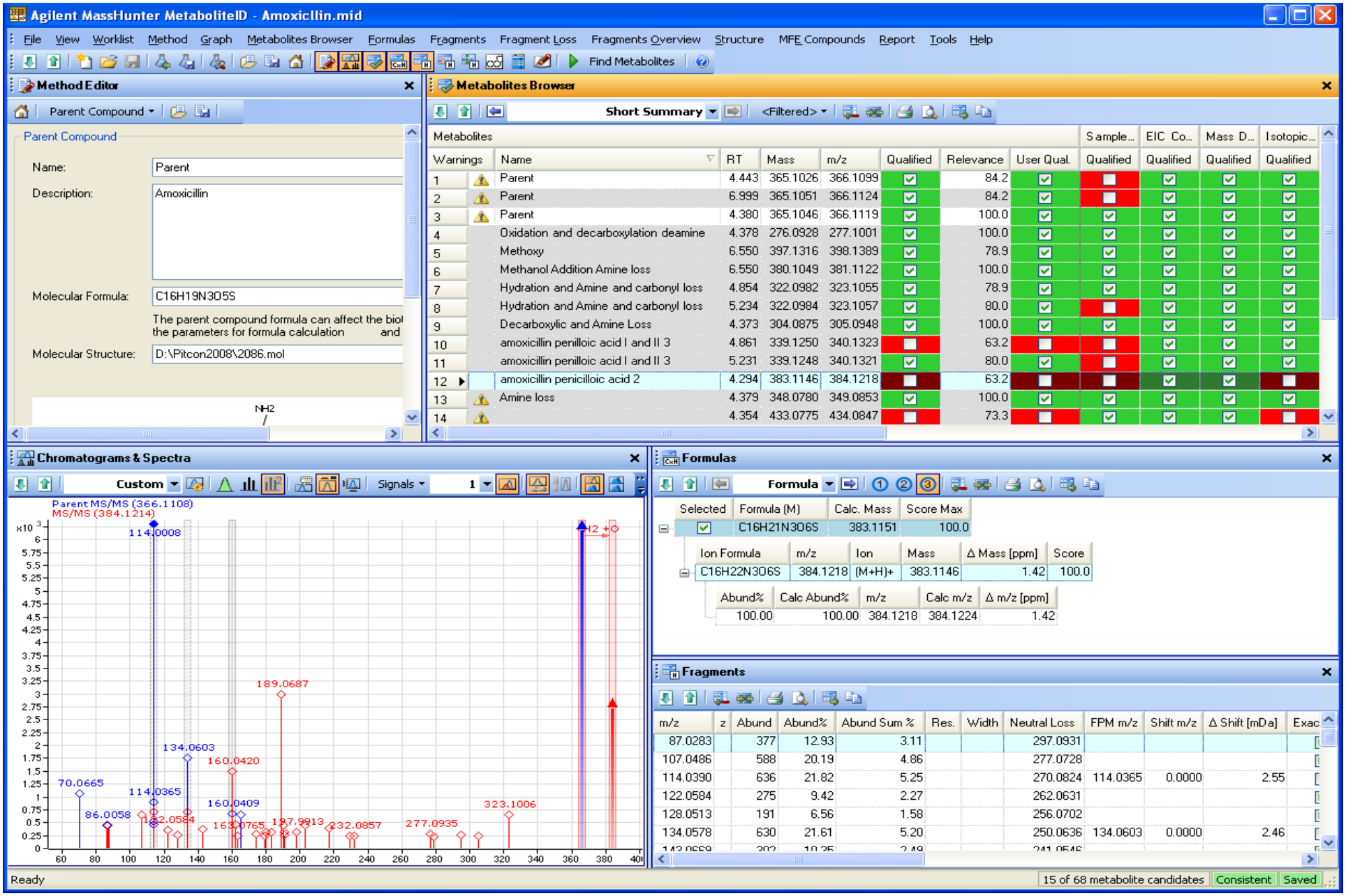
Composition Summary: 36 Features

	Mass	RT	Feature ID	Abundance	log2(A1/A2)	Name	Formula	ΔMass(ppm)
2	383.1152	4.252	17	168460	16.00	Amoxicillin Hydrolysis	C16H21N3O6S	-1.9
3	304.0884	4.333	22	72261	-3.11	Sulfametoyl	C15H16N2O3S	-0.3
4	365.2759	4.342	18	156997	-16.00			
5	276.0932	4.360	23	43922	-3.28		C14H16N2O2S	0.8
6	365.1048	4.367	1	13924040	-1.60	Amoxicillin	C16H19N3O5S	0.6
7	348.0785	4.368	2	9272090	-1.60	Amoxicillin Amine Loss	C16H16N2O5S	-0.3
8	207.0352	4.369	5	1071072	-1.86		C10H9NO2S	1.1
9	210.0634	4.371	16	193118	-1.89		C9H10N2O4	2.2
10	233.0143	4.402	39	9642	16.00		C11H7NO3S	2.2
11	382.1308	4.566	21	79152	16.00		C16H22N4O5S	0.4
12	322.0988	4.802	11	465558	-0.37	Amoxicillin Degrad Product B	C15H18N2O4S	-0.3
13	339.1254	4.803	9	633992	0.10	Amoxicillin Decarbonyl	C15H21N3O4S	0.3
14	322.0989	5.183	10	538439	0.54	Amoxicillin Degrad Product B	C15H18N2O4S	0.1
15	339.1256	5.183	6	1036877	0.55	Amoxicillin Decarbonyl	C15H21N3O4S	-0.5
16	379.1200	5.318	38	11471	-16.00	Amoxicillin Degrad Product E	C17H21N3O5S	-0.2
17	339.2619	5.654	19	146888	-2.32		C20H37NOS	-5.7
18	240.1112	5.673	29	23537	16.00		C11H16N2O4	-0.5
19	226.1681	6.175	34	16770	-16.00	Crotethamide	C12H22N2O2	1.3
20	299.1945	6.258	13	253315	-2.61		C16H29NO2S	-8.8
21	218.1030	6.453	41	4889	16.00	Primidone	C12H14N2O2	8.9
22	380.1049	6.501	8	698499	-2.85	Amoxicillin Degrad Product F	C17H20N2O6S	-2.6
23	397.1312	6.502	3	3293522	-2.29	Amoxicillin Degrad Product E	C17H23N3O6S	-1.7

MetID Software For Impurity Profiling

Overview MetID

1. Parent Compound: MW, Formula and Structure
2. Transformations: List Proposed Degradation Products
3. Identification Criteria: Vary Importance of Tests
4. Find Compounds by MFE: Molecular Feature Extraction
5. Find Compounds by AutoMSMS: For MSMS data
6. Sample Comparison: Mass Profiler What is Different
7. Isotope Pattern Filtering: Best for Halogenated Species
8. Mass Defect Filtering: From Proposed Compounds
9. EIC Generation: Confirms Presence of Compounds


Agilent MassHunter MetaboliteID - Amoxicllin.mid
File View Worklist Method Graph Metabolites Browser Formulas Fragments Fragment Loss Fragments Overview Structure MFE Compounds Report Tools Help
Find Metabolites
Method Editor
Parent Compound
Parent Compound
Name: Parent
Description: Amoxicillin
Molecular Formula: C16H19N3O5S
The parent compound formula can affect the biol
the parameters for formula calculation and
Molecular Structure: D:\Pitcon2008\2086.mol
NH2
Metabolites Browser
Short Summary
<Filtered>
Metabolites
Warnings Name RT Mass m/z Qualified Relevance User Qual. Sample... EIC Co... Mass D... Isotopic...
1 Parent 4.443 365.1026 366.1099 84.2
2 Parent 6.999 365.1051 366.1124 84.2
3 Parent 4.380 365.1046 366.1119 100.0
4 Oxidation and decarboxylation deamine 4.378 276.0928 277.1001 100.0
5 Methoxy 6.550 397.1316 398.1389 78.9
6 Methanol Addition Amine loss 6.550 380.1049 381.1122 100.0
7 Hydration and Amine and carbonyl loss 4.854 322.0982 323.1055 78.9
8 Hydration and Amine and carbonyl loss 5.234 322.0984 323.1057 80.0
9 Decarboxylic and Amine Loss 4.373 304.0875 305.0948 100.0
10 amoxicillin penilloic acid I and II 3 4.861 339.1250 340.1323 63.2
11 amoxicillin penilloic acid I and II 3 5.231 339.1248 340.1321 80.0
12 amoxicillin penicilloic acid 2 4.294 383.1146 384.1218 63.2
13 Amine loss 4.379 348.0780 349.0853 100.0
14 4.354 433.0775 434.0847 73.3
Chromatograms & Spectra
Custom
Signals
1
Parent MS/MS (366.1108)
MS/MS (384.1214)
x10 3
114.0008
189.0687
134.0603
160.0420
70.0665
114.0365
160.0409
86.0058
323.1006
277.0935
232.0857
Formulas
Formula
Selected Formula (M) Calc. Mass Score Max
C16H21N3O6S 383.1151 100.0
Ion Formula m/z Ion Mass Δ Mass [ppm] Score
C16H22N3O6S 384.1218 (M+H)+ 383.1146 1.42 100.0
Abund% Calc Abund% m/z Calc m/z Δ m/z [ppm]
100.00 100.00 384.1218 384.1224 1.42
Fragments
m/z z Abund Abund% Abund Sum % Res. Width Neutral Loss FPM m/z Shift m/z Δ Shift [mDa] Exac
87.0283 377 12.93 3.11 297.0931
107.0486 588 20.19 4.86 277.0728
114.0390 636 21.82 5.25 270.0824 114.0365 0.0000 2.55
122.0584 275 9.42 2.27 262.0631
128.0513 191 6.56 1.58 256.0702
134.0578 630 21.61 5.20 250.0636 134.0603 0.0000 2.46
Ready
15 of 68 metabolite candidates
Consistent
Saved

Method Input

Parent Compound

Parent Compound

Name: Parent

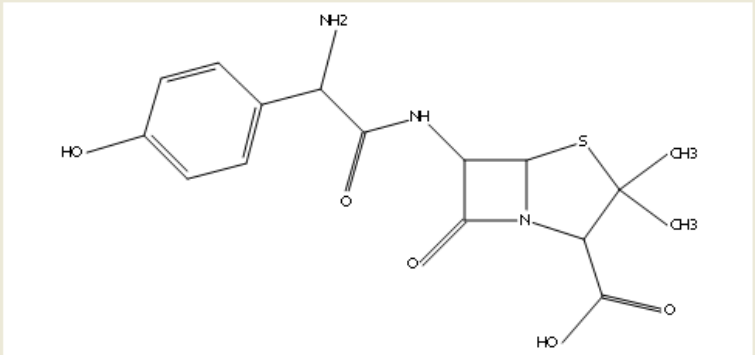
Description: Amoxicillin

Molecular Formula: C16H19N3O5S 365.1045 Da

The parent compound formula can affect the biotransformation list, the parameters for formula calculation and mass defect filter.

Molecular Structure: D:\Pitcon2008\2086.mol

Browse...



Propose Degradation Products

Biotransformations

Biotransformation Repository

Use	Name	Phase	Formula	Mass	Req.	Result Formula	Result Mass
☑	Parent	I		0.0000		C16H19N3O5S	365.1045
☑	diketopiperazine	I		0.0000		C16H19N3O5S	365.1045
☑	amoxicillin penicilloic acid 2	I	+H2 +O	18.0106		C16H21N3O6S	383.1151
☑	amoxicillin penilloic acid I and II 3	I	+H2-C-O	-25.9793		C15H21N3O4S	339.1253
☑	4-hydroxyphenylglycyl amoxicillin 4	I	+C8+H7+O2+N	149.0477		C24H26N4O7S	514.1522
☑	Methoxy	I	+C+H4+O	32.0262		C17H23N3O6S	397.1308
☑	Dimethoxy Addition	I	+C2+O2+H4	60.0211		C18H23N3O7S	425.1257
☑	Amine loss	I	-N-H3	-17.0265		C16H16N2O5S	348.0780
☑	Decarboxylic and Amine Loss	I	-N-H3-C-O2	-61.0164		C15H16N2O3S	304.0882
☑	Oxidation	I	+O	15.9949		C16H19N3O6S	381.0995
☑	Dioxidation	I	+O2	31.9898		C16H19N3O7S	397.0944
☑	Hydration decarboynl	I	+H2+O-C-O	-9.9843		C15H21N3O5S	355.1202
☑	Hydration and Amine and carbonyl loss	I	-N-H-C-O	-43.0058		C15H18N2O4S	322.0987
☑	Methoxy for Hydroxyl	I	+C+H2	14.0157		C17H21N3O5S	379.1202
☑	Acetic Acid Addition	I	+C+H3+O2	47.0133		C17H22N3O7S	412.1178
☑	Methanol Addition Amine loss	I	+C+H3+O-N-H2	14.9997		C17H20N2O6S	380.1042
☑	Oxidation and decarboxylation deamine	I	+O-C2-O4-N-H3	-89.0113		C14H16N2O2S	276.0932
☐							

Proposed Degradation Products
Qualified by Mass Defect and Isotope Pattern

Metabolites								Sample...	EIC Co...	Mass D...	Isotopic...	Fragme...	Biotran...	Formulas
Warnings	Name	RT	Mass	m/z	Qualified	Relevance	User Qual.	Qualified	Qualified	Qualified	Qualified	Qualified	Assigned	Assigned
1	amoxicillin penicilloic acid 2	3.909	383.1152	384.1225	☑	80.0	☑	☐	☑	☑	☑	☐	☑	☑
2	⚠	4.265	387.0867	388.0940	☑	100.0	☑	☑	☑	☑	☑	☐	☐	☐
3	Decarboxylic and Amine Loss	4.337	304.0882	305.0955	☑	100.0	☑	☑	☑	☑	☑	☐	☑	☑
4	Parent	4.340	365.1043	366.1116	☑	100.0	☑	☑	☑	☑	☑	☐	☑	☑
5	Oxidation and decarboxylation deamine	4.341	276.0930	277.1003	☑	100.0	☑	☑	☑	☑	☑	☐	☑	☑
6	Amine loss	4.345	348.0780	349.0853	☑	100.0	☑	☑	☑	☑	☑	☐	☑	☑
7		4.359	384.0791	385.0863	☑	100.0	☑	☑	☑	☑	☑	☐	☐	☑
8	⚠ Parent	4.396	365.1049	366.1123	☑	80.0	☑	☐	☑	☑	☑	☐	☑	☑
9	Hydration and Amine and carbonyl loss	4.807	322.0988	323.1061	☑	80.0	☑	☐	☑	☑	☑	☐	☑	☑
10		4.808	411.1101	412.1175	☑	100.0	☑	☑	☑	☑	☑	☐	☐	☑
11	amoxicillin penilloic acid I and II 3	4.808	339.1256	340.1328	☑	80.0	☑	☐	☑	☑	☑	☐	☑	☑
12	Hydration and Amine and carbonyl loss	5.191	322.0985	323.1058	☑	80.0	☑	☐	☑	☑	☑	☐	☑	☑
13	amoxicillin penilloic acid I and II 3	5.192	339.1252	340.1326	☑	80.0	☑	☐	☑	☑	☑	☐	☑	☑
14	⚠	6.509	435.0840	436.0912	☑	100.0	☑	☑	☑	☑	☑	☐	☐	☐
15	Methanol Addition Amine loss	6.511	380.1047	381.1120	☑	100.0	☑	☑	☑	☑	☑	☐	☑	☑
16 ▶	Methoxy	6.511	397.1308	398.1381	☑	100.0	☑	☑	☑	☑	☑	☐	☑	☑
17	Amine loss	6.511	348.0777	349.0850	☐	73.3	☐	☑	☐	☑	☑	☐	☑	☑
18	⚠	6.513	481.0834	482.0909	☑	100.0	☑	☑	☑	☑	☑	☐	☐	☐
19	⚠ Parent	6.965	365.1058	366.1131	☑	80.0	☑	☐	☑	☑	☑	☐	☑	☑
20	Parent	7.002	365.1044	366.1118	☐	53.3	☐	☐	☐	☑	☑	☐	☑	☑
21	⚠ Parent	7.265	365.1040	366.1115	☐	53.3	☐	☐	☐	☑	☑	☐	☑	☑

Let's Determine Composition of MW 411

Accurate Mass Generates Composition
Acid Hydrolysis (H_2O+CO_2H-OH)

Selected	Formula (M)	Calc. Mass	Score Max	Score Mass Max	Score Abund. Max	Score Spacing Max	|Δ Mass| [ppm] Max
☑	C17H21N3O7S	411.1100	100.0	99.8	99.1	98.2	0.41

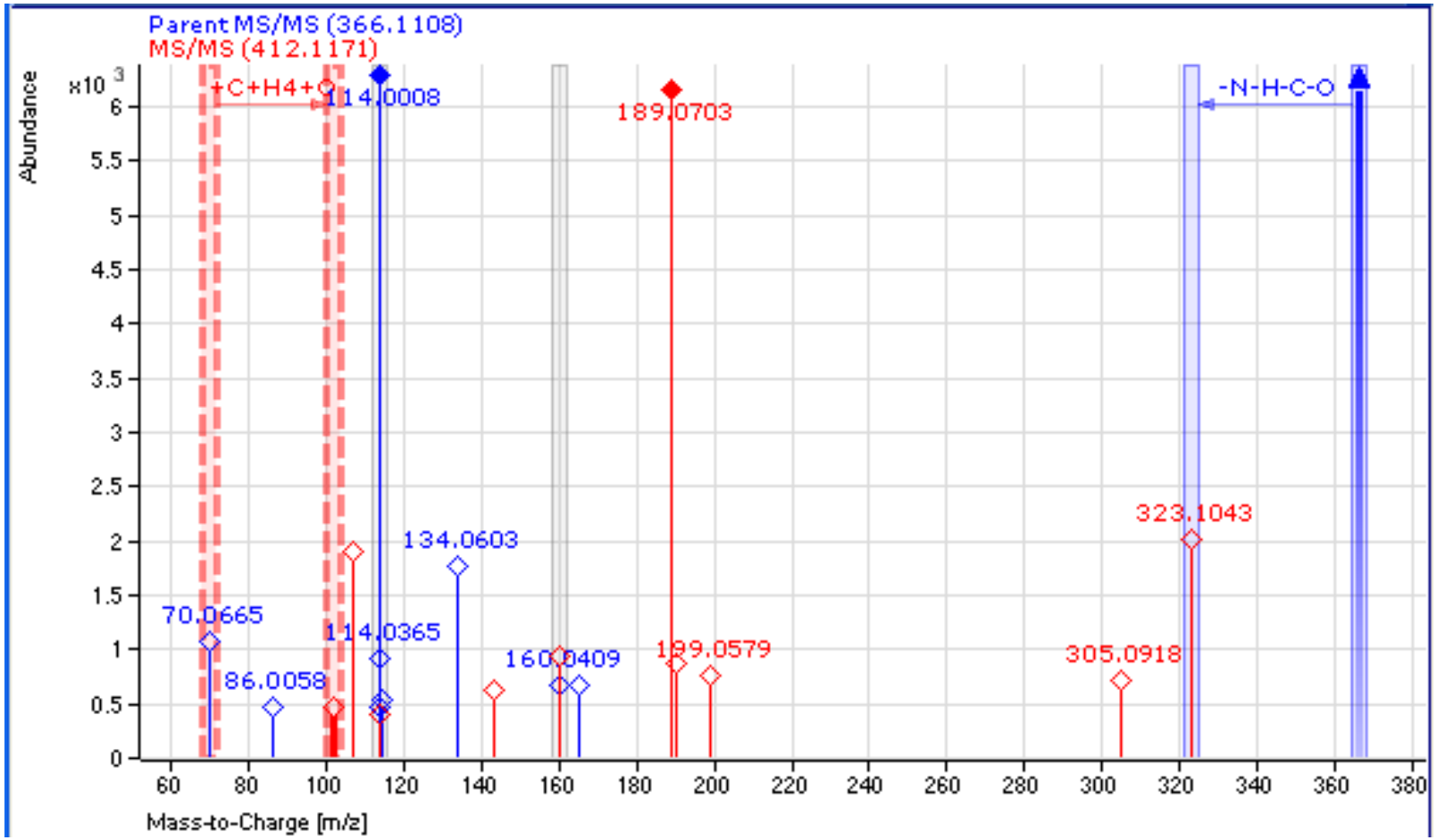
Ion Formula	m/z	Ion	Mass	Δ Mass [ppm]	Score	Mass Score	Abund. Score	Spacing Score	|Δ Mass| [ppm]
C17H22N3O7S	412.1175	(M+H)+	411.1102	-0.41	100.0	99.8	99.1	98.2	0.41

Abund%	Calc Abund%	m/z	Calc m/z	Δ m/z [ppm]
100.00	100.00	412.1175	412.1173	-0.41
21.77	20.79	413.1205	413.1202	-0.73
8.39	7.97	414.1166	414.1172	1.46
1.89	1.36	415.1183	415.1188	1.21

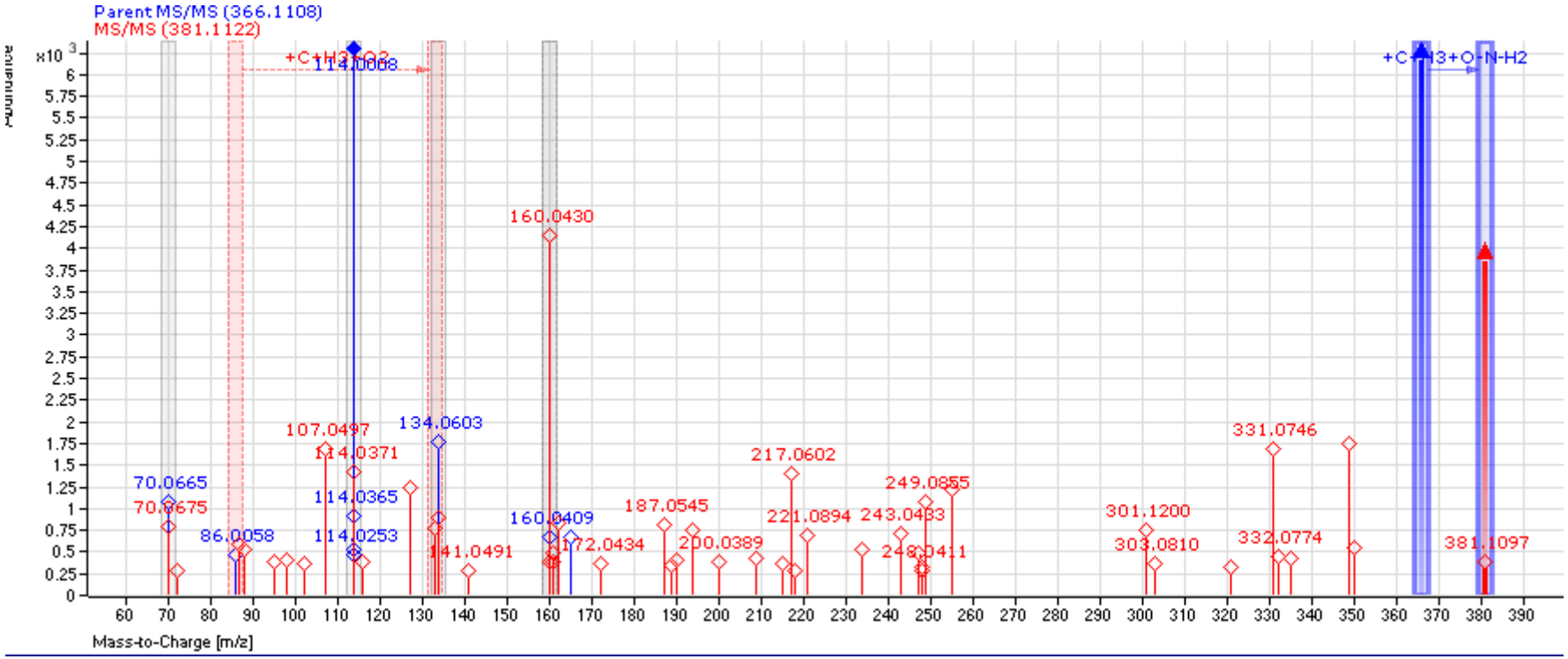
Accurate Mass of Fragments Ions

m/z	Abund	Abund%	Abun...	Neutral Loss	FPM m/z	Shift m/z	Shift Formula	Ion Formula	Loss Formula	Calc m/z	Δ m/z [ppm]	Δ m/...	Loss Mass
323.1043	1915	30.68	13.54	89.0128	366.1108	-43.0058	-N-H-C-O	C15H19N2O4S	C2H3NO3	323.1060	5.39	1.74	89.0113
305.0918	626	10.03	4.43	107.0253				C15H17N2O3S	C2H5NO4	305.0954	11.92	3.64	107.0219
199.0579	674	10.79	4.76	213.0592				C13H11S	C4H11N3O7	199.0576	-1.52	-0.30	213.0597
190.0738	795	12.73	5.62	222.0433									
189.0703	6242	100.00	44.14	223.0468				C7H13N2O2S	C10H9NO5	189.0692	-5.59	-1.06	223.0481
160.0406	850	13.61	6.01	252.0765	160.0409	0.0000		C9H6NO2	C8H16N2O5S	160.0393	-8.16	-1.31	252.0780
143.0673	540	8.65	3.82	269.0497				C7H11O3	C10H11N3O4S	143.0703	20.44	2.92	269.0470
114.0379	319	5.11	2.26	298.0791	114.0365	0.0000		C5H8NS	C12H14N2O7	114.0372	-6.60	-0.75	298.0801
107.0485	1805	28.91	12.76	305.0686				C7H7O	C10H15N3O6S	107.0491	6.21	0.66	305.0682

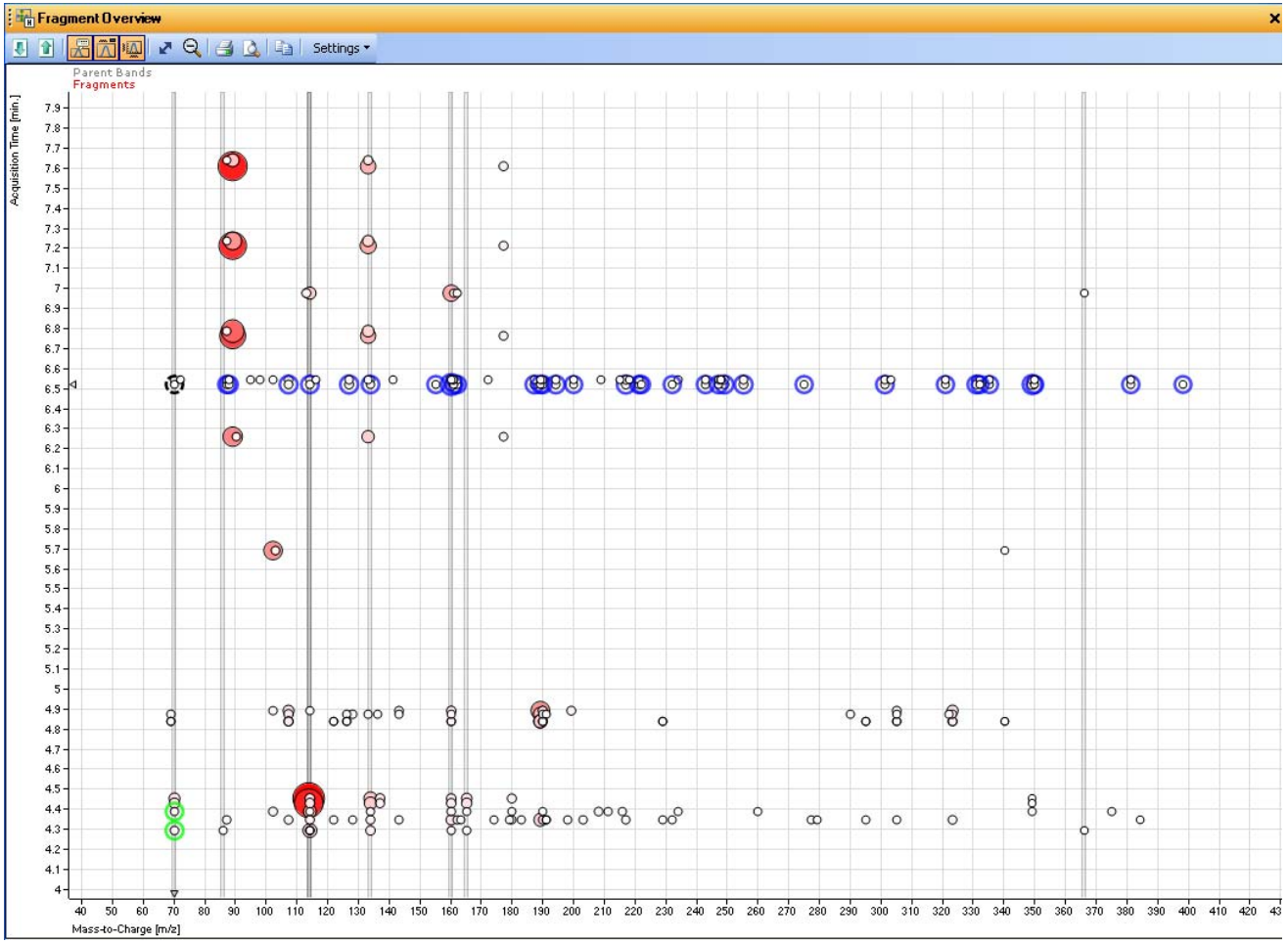
Overlay MSMS Spectrum Parent/Product



MSMS of MW 380 Compound



3D of MSMS Spectra Fragmentation Pattern



Summary:

1. New 4GHz A/D Electronics, Resolution > 10,000: *m/z* > 100, Which Enables Resolving Trace Level Degradation Products from Chemical Background
2. Automated Data Analysis: Molecular Feature Extraction
3. Differential Analysis: MassProfiler
4. Identification: Personal Metlin Database
5. Impurity Profiling: MetID Software with MS/MS Data

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- Mike Woodman Agilent Technologies, Schaumburg, IL
- Dawn Kasper, RDH Buffalo Grove, IL

- Thank you .. Kentucky Fried Chicken Boulder, CO.. For being open at 10:50pm ….
- Thank you.. For your Attention..

References:

- J. Roy, AAPS PharmSciTech, 2002, 3,(2)
- L. Zhou, B. Mao, R. Reamer, T. Novak, Z. Ge, J Pharm. Biomed. Analysis, 44(2007) 421-429
- S. Erram; C. Fanska, M. Asif; J Pharm. Biomed. Analysis; 40(2006)864-874
- Y. Wu; Biomed Chromatography; 14(2000) 384-396

Albuterol Sulfate/Levosalbutamol

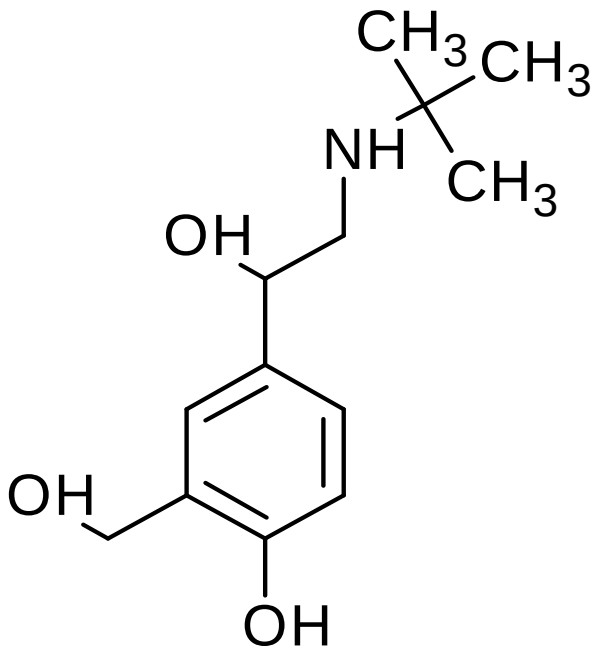
$C_{13}H_{21}NO_3$

MW 239.15214

Sources

- Syrup 2MG/ML 10/26/99
- Aerosol 90ug/dose New
- Suspension 0.65mg/3mL New

Add reference article



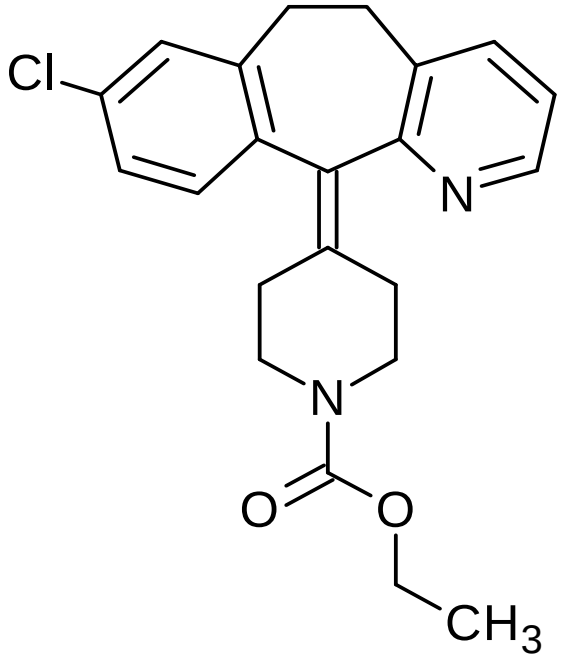
Loratadine

$C_{22}H_{23}N_2O_2Cl$

MW 383.15257

Source

- Syrup 10mg/mL 05/02
- Claritan D 07/02



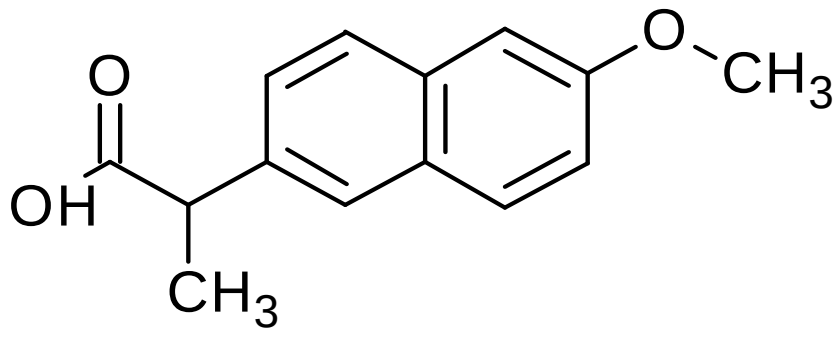
Naproxen

$C_{14}H_{14}O_3$

MW 230.094294

Source

- Tablet 500mg 08/97
- Tablet 220mg New



Diphenoxylate

Diphenoxylate

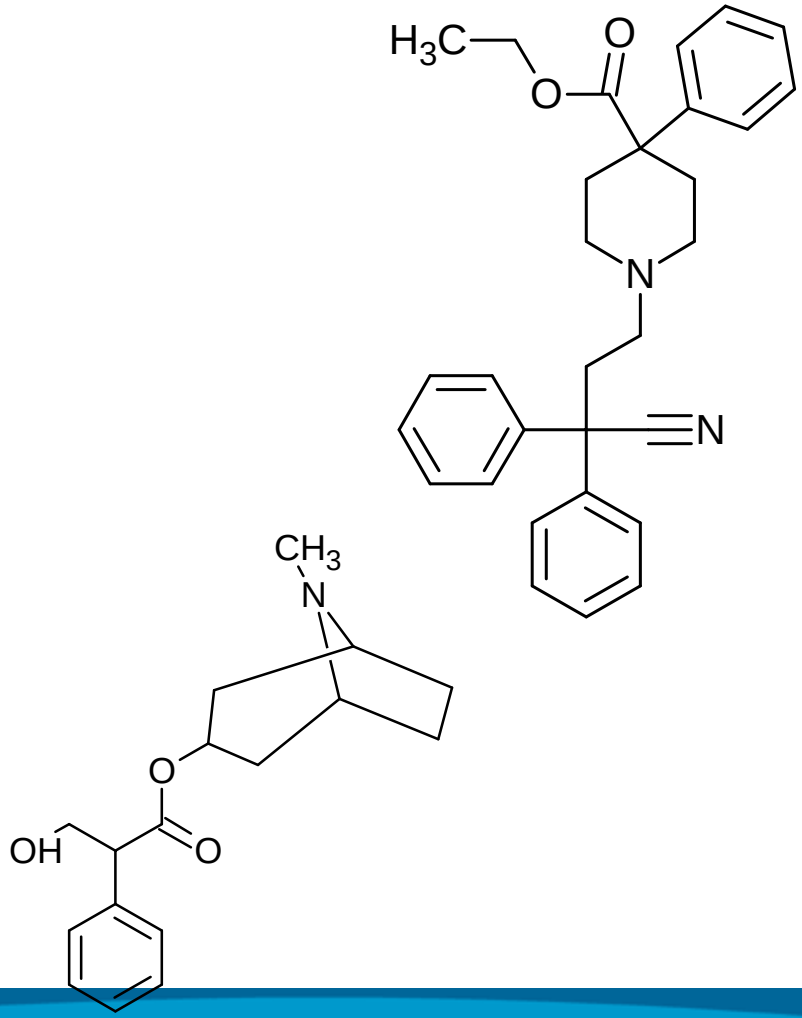
- $C_{30}H_{32}N_2O_2$
- MW 452.246378

Atropine

- $C_{17}H_{23}NO_3$
- ME 289.16

Source

- Tablet 5/1994



Hyosophen-Donnatal

Phenobarbital

- $C_{12}H_{12}N_2O_3$
- MW 232.084794

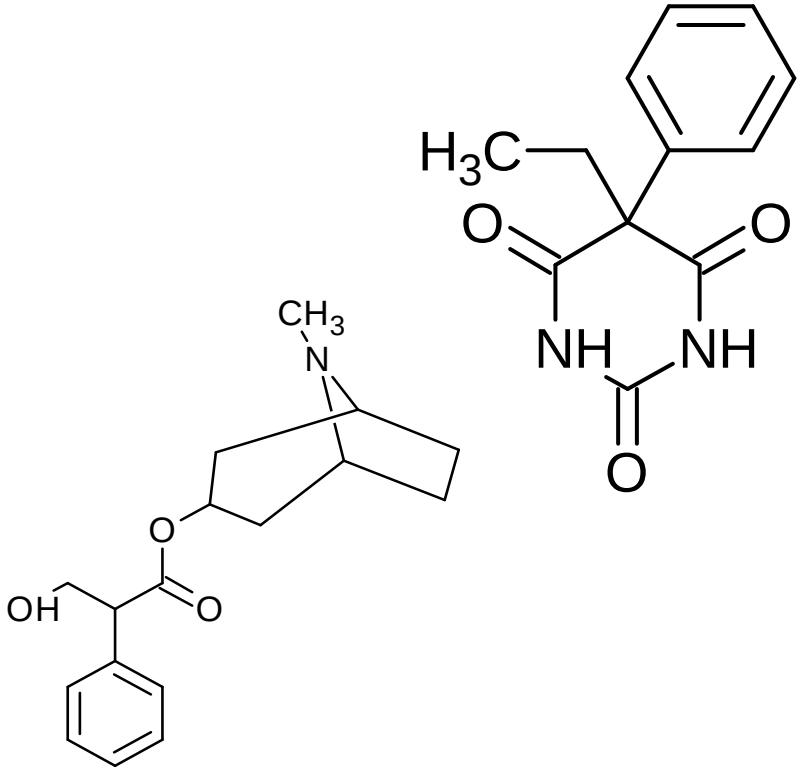
Atropine

- $C_{17}H_{23}NO_3$
- MW 289.16

Scopolamine

- $C_{17}H_{21}NO_4$
- MW 303.147058

Tablets 90mg 1/97



Atarax

Atarax

$C_{21}H_{27}N_2O_2Cl$

MW

Source

- Tablet 10mg 7/2002