Is Faster Always Better: Coupling UHPLC Chromatography with Multistage-Mass Spectrometry for Impurity Profiling Applications

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Faster.. Faster…Faster…
But at what cost?

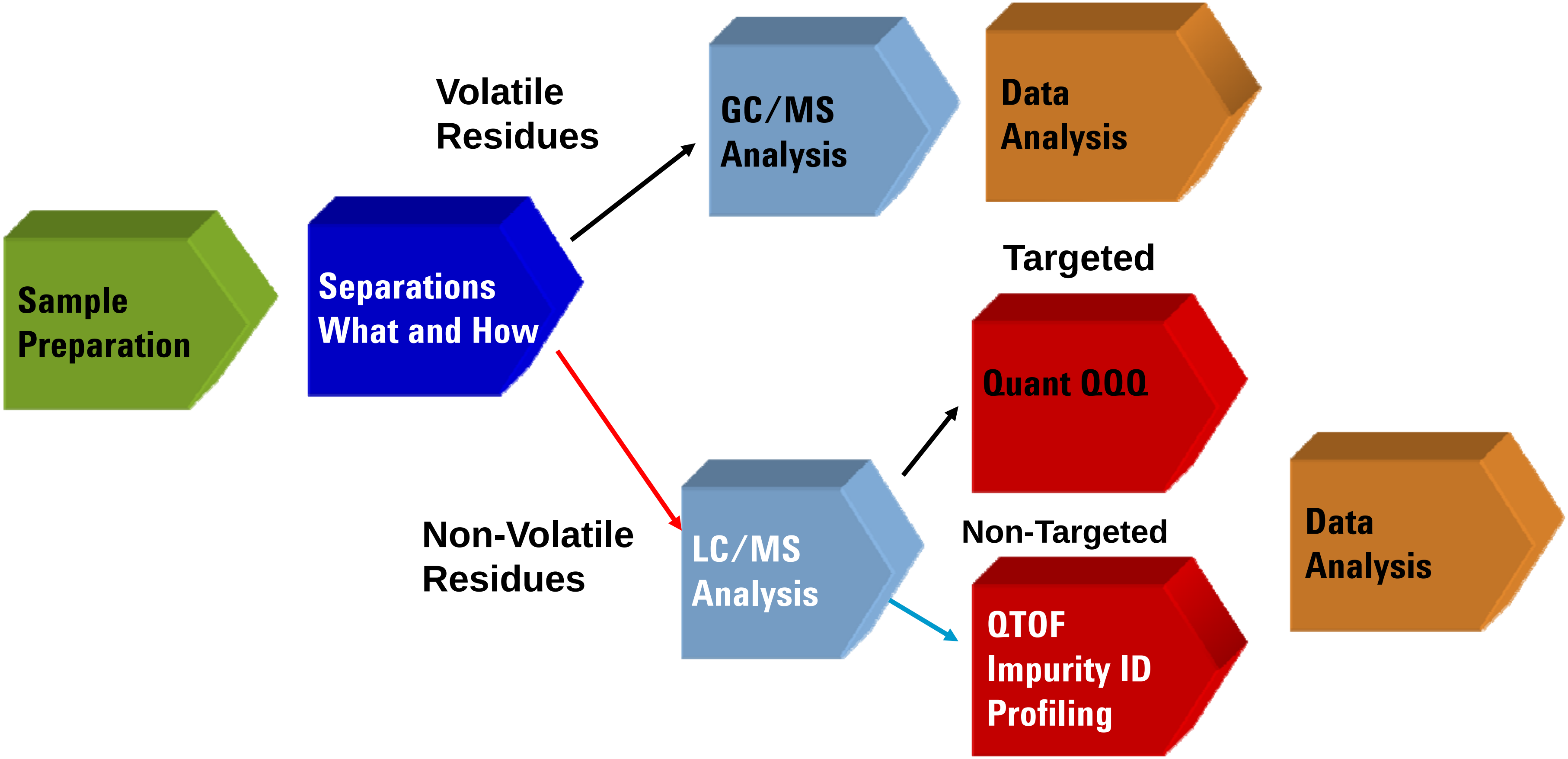
"Whistler Sliding Center"
Designed as "Worlds Fastest"

In Practice Multiple Crashes

Why → Speeds EXCEEDED Reaction Times

Challenges and Workflow for Impurity Profiling

Objective: Detection - ID low-level Impurities Pharmaceutical Products
Dynamic Range, Variety Compound Classes



The Impact of Going Faster …. In MS world

Ionization Efficiency

– Electrospray Ionization, Thermally Assisted Electrospray

– Produce Stable $[M+H]^+$

– Ion Transmission Efficiency

Faster Scanning Effect on Mass Spec Detection

– Scan Speed –10-20 Spectra/S MS and MSMS Modes (dead times)

– Dynamic Range (Detector Saturation)

– Mass Resolution – Time or Space

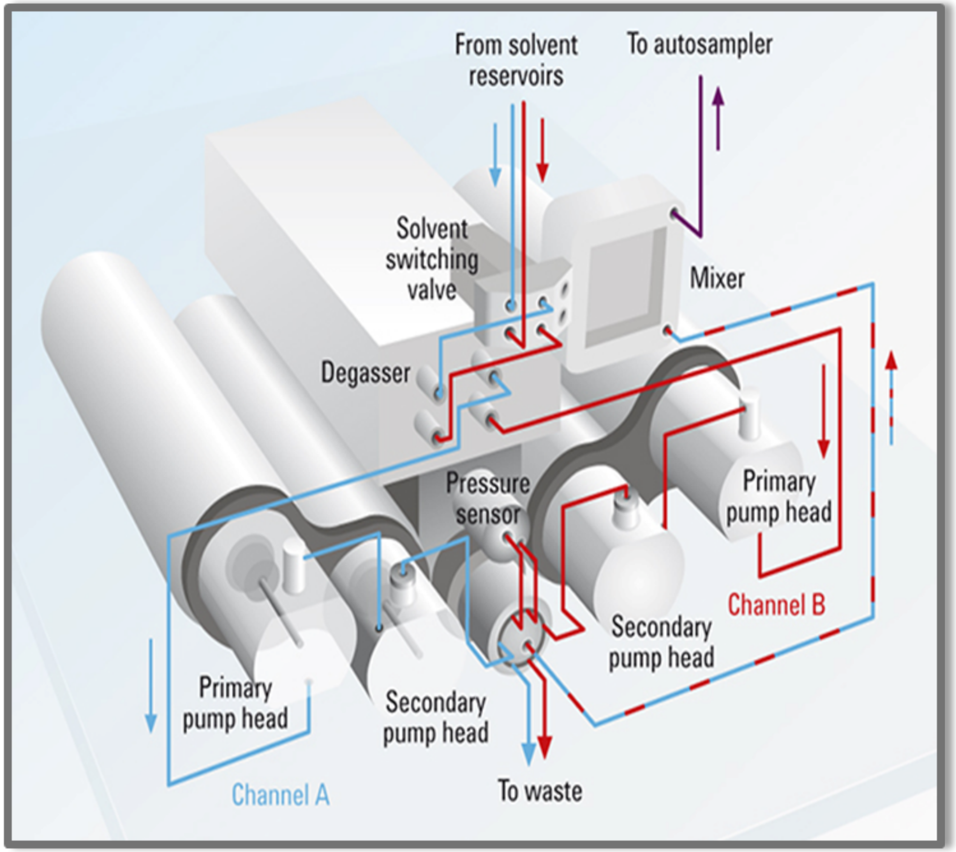
– Scan Speed vs Mass Accuracy

– Unknown Identification: Isotope Fidelity and Mass Accuracy

Do Narrower Peaks Result in Better Detection of Impurities

New 1290 Infinity UHPLC Binary Pump

Separations



„Next Generation“ Technology

- 1200 bar @ 2.0 mL/min
  800 bar @ 5.0 mL/min
- Low Dead volume: 10 µl
- Low Mixing Noise
- High RT precision
- Automatic purge
- Automatic correction of micro leaks

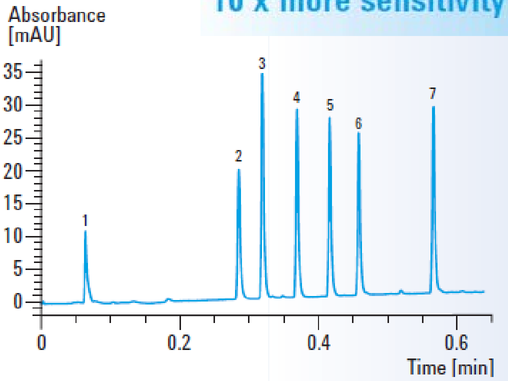
So What Does Higher Pressure Enable?

… Speed, Resolution, Sensitivity

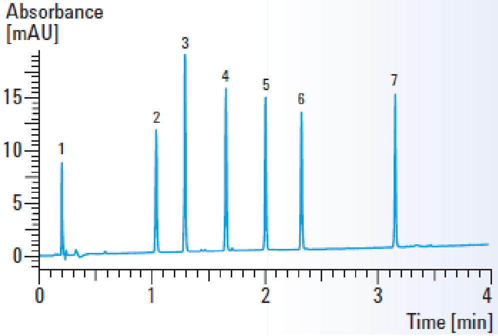
Separations

20 x more speed

10 x more sensitivity



Column: ZORBAX RRHD SB-C18, 2.1 x 50 mm, 1.8 µm
Injection volume: 2 µL
Run time: 0.6 min
Flow: 2 mL/min
Peak capacity: 119 W(1/2)
Signal/noise: 740
Pressure: 1030 bar



Column: ZORBAX RRHD SB-C18, 2.1 x 100 mm, 1.8 µm
Injection volume: 2 µL
Run time: 4 min
Flow: 1.2 mL/min
Peak capacity: 306 W(1/2)
Signal/noise: 861
Pressure: 1007 bar

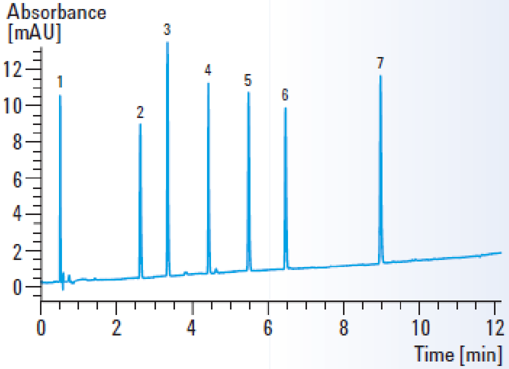
3 x more speed

1.4 x more peak capacity

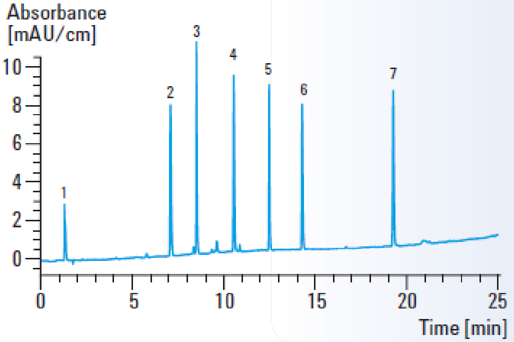
11 x more sensitivity

1.6 x more peak capacity

10 x more sensitivity



Column: ZORBAX RRHD SB-C18, 2.1 x 150 mm, 1.8 µm
Injection volume: 2 µL
Run time: 12 min
Flow: 0.7 mL/min
Peak capacity: 375 W(1/2)
Signal/noise: 784
Pressure: 965 bar

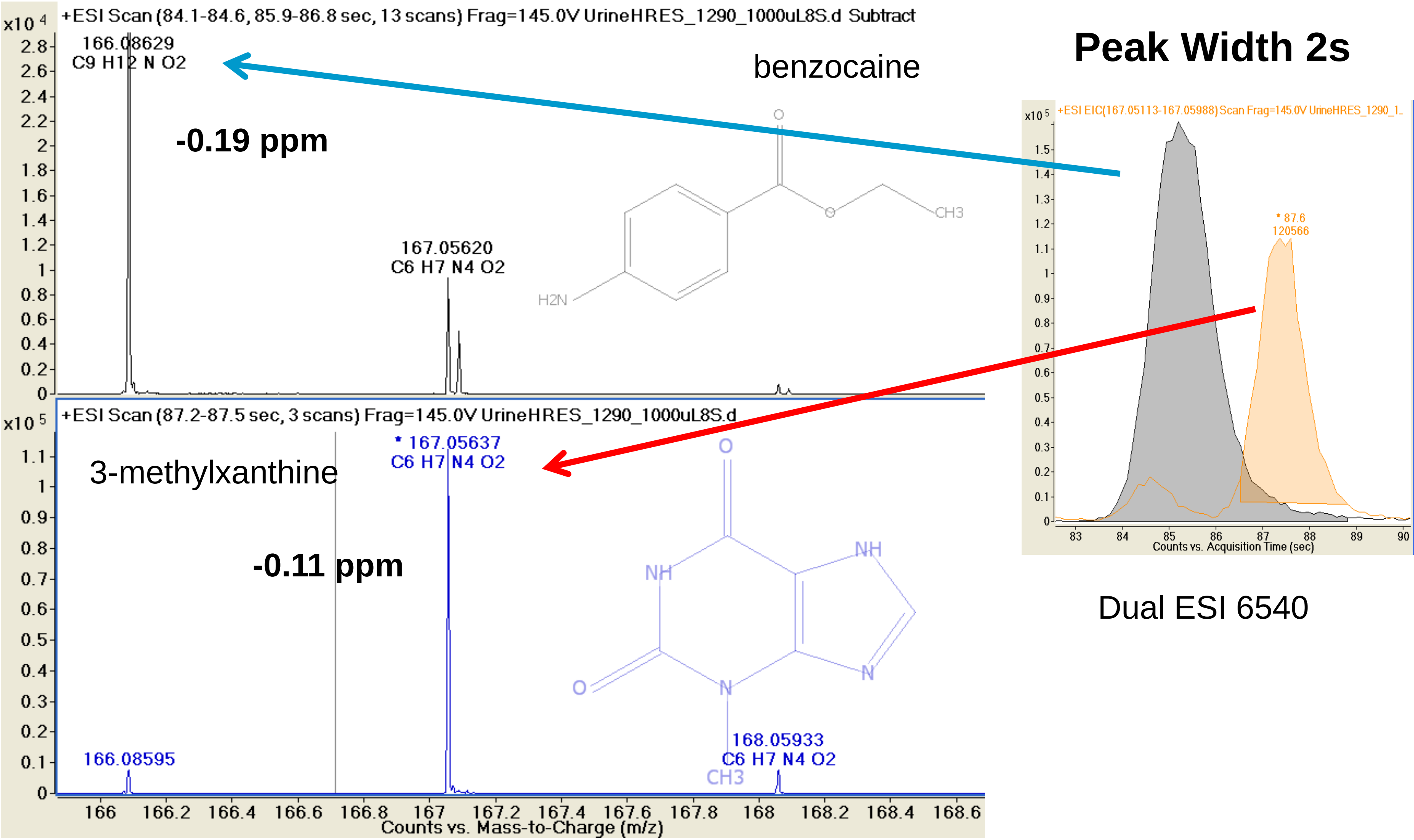


Column: ZORBAX RRHD SB-C18, 2.1 x 250 mm, 1.8 µm
Injection volume: 2 µL
Run time: 25 min
Flow: 0.4 mL/min
Peak capacity: 430 W(1/2)
Signal/noise: 2270
Pressure: 1020 bar

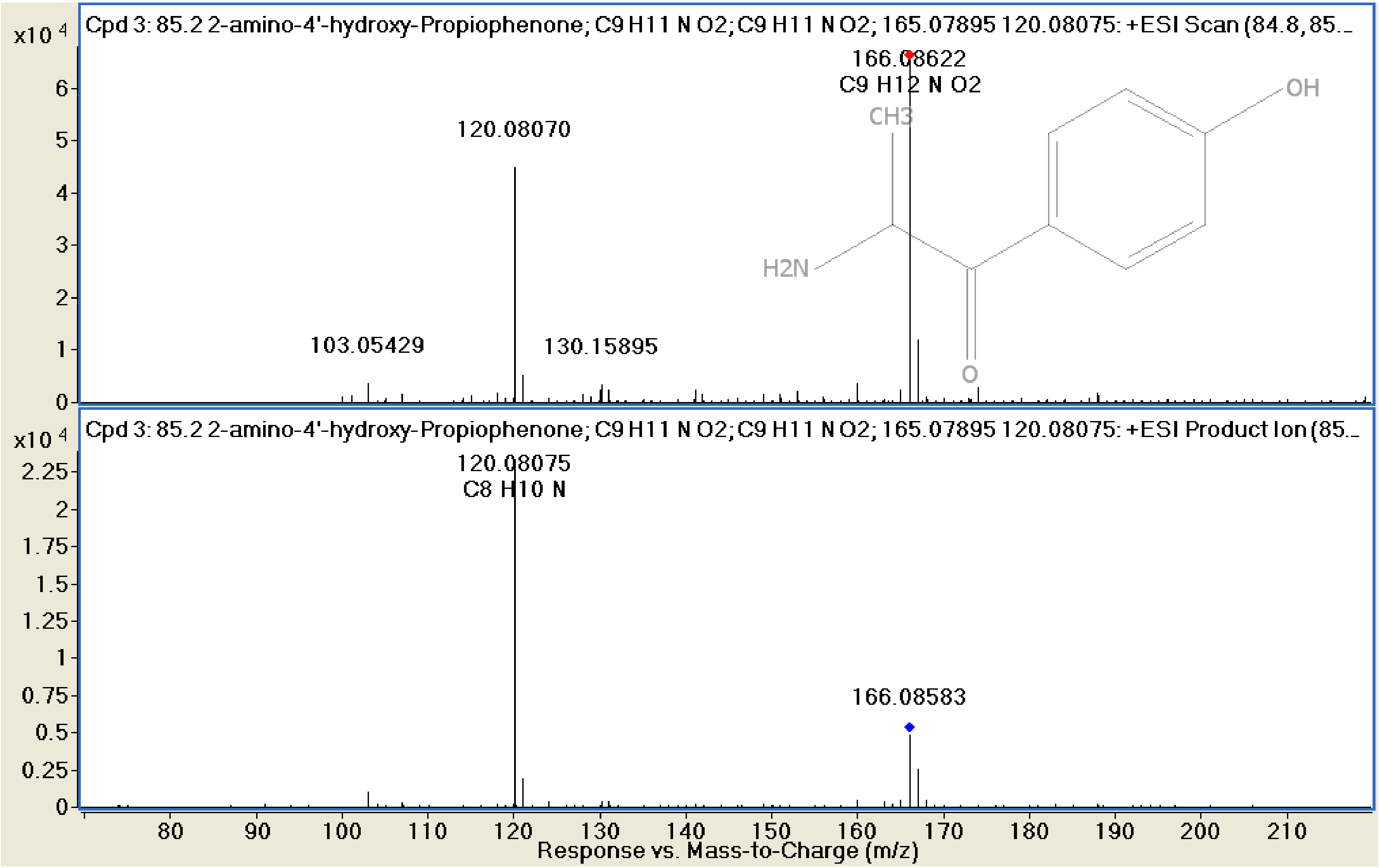
1.9 x more peak capacity

30 x more sensitivity

Resolving Coeluting Compounds 1mL/min 10Hz

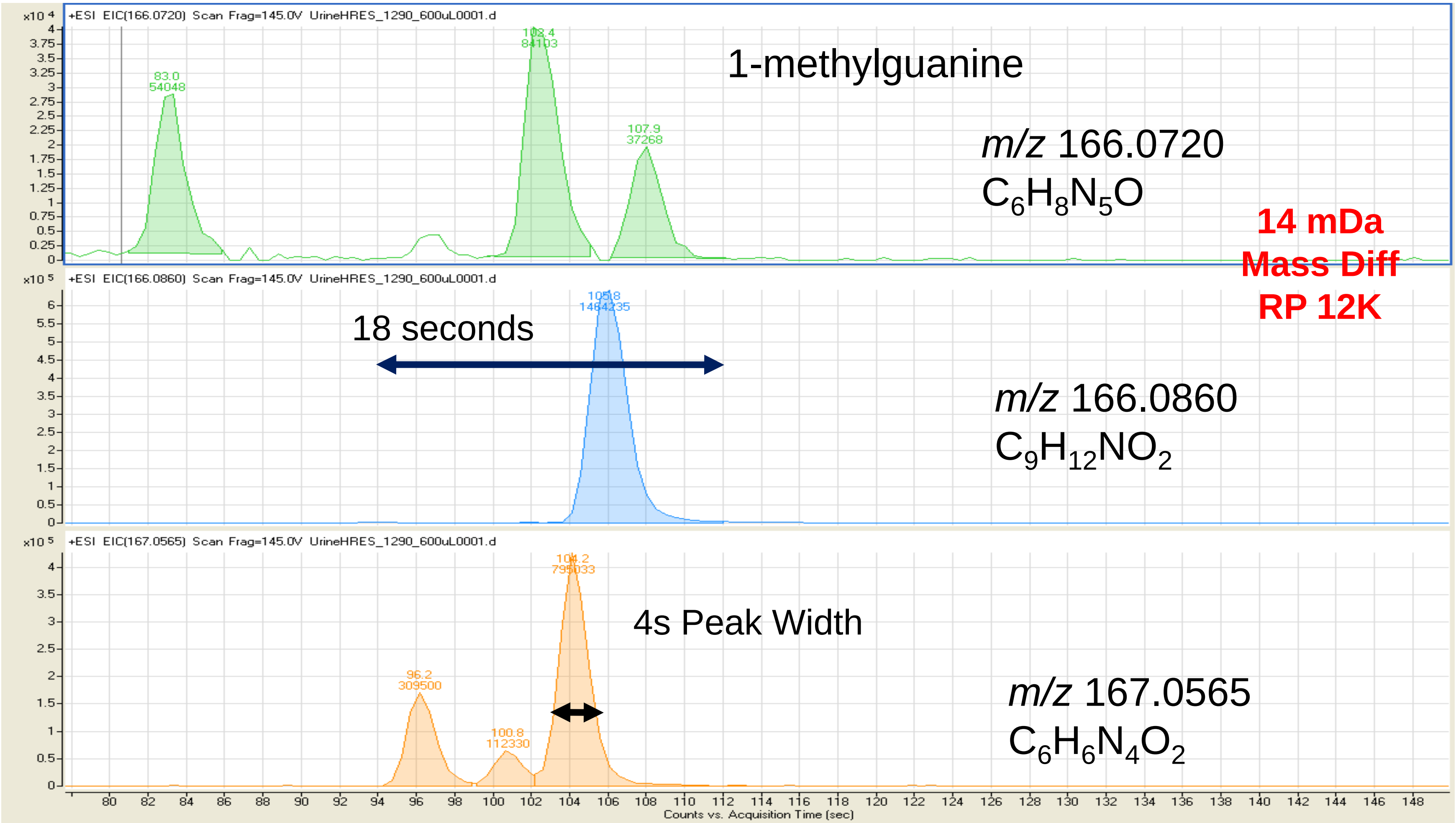


MS/MS Confirmation of $C_9H_{11}NO_2$ Compound



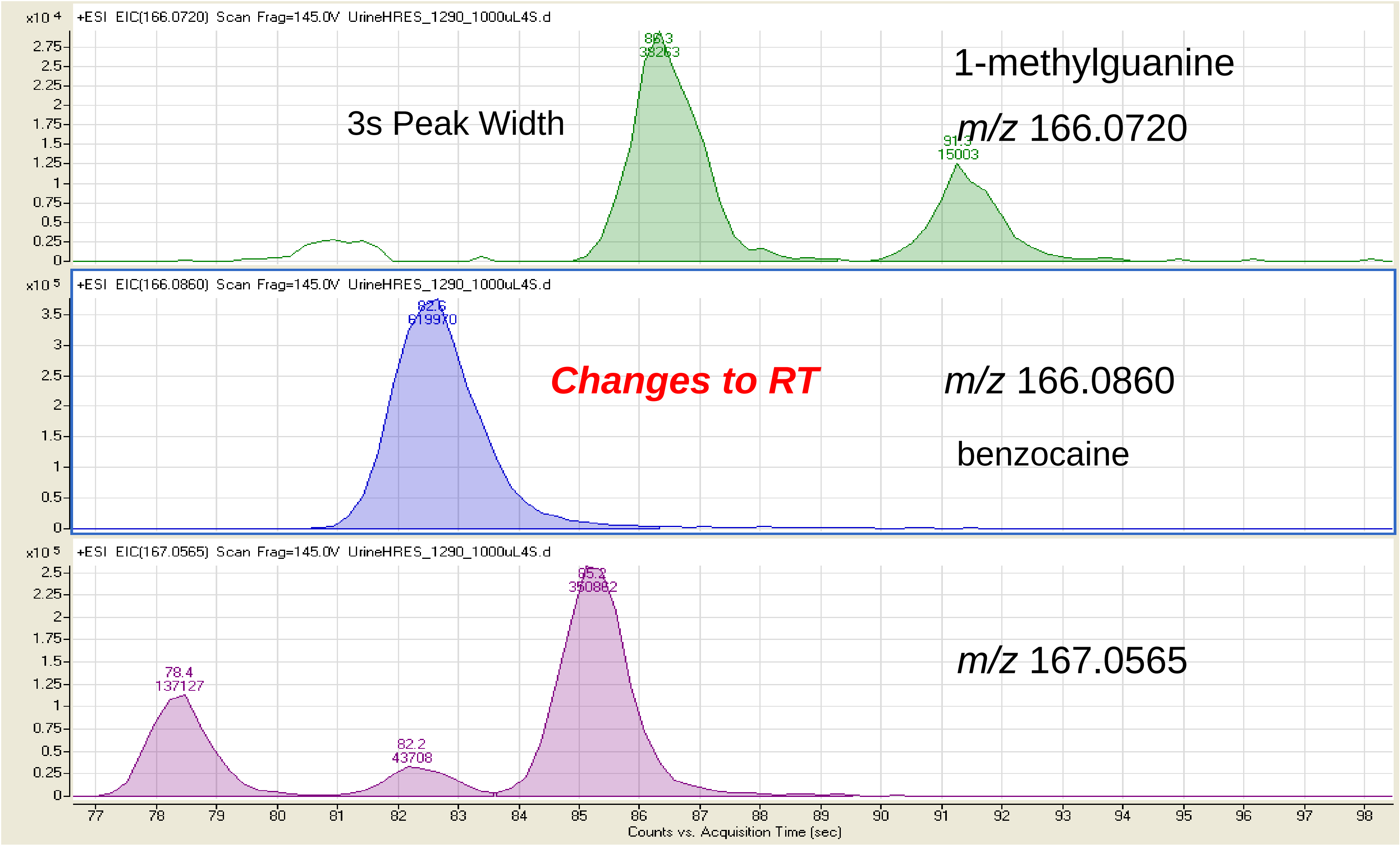
Separation of Coeluting Compounds

600 µL/min

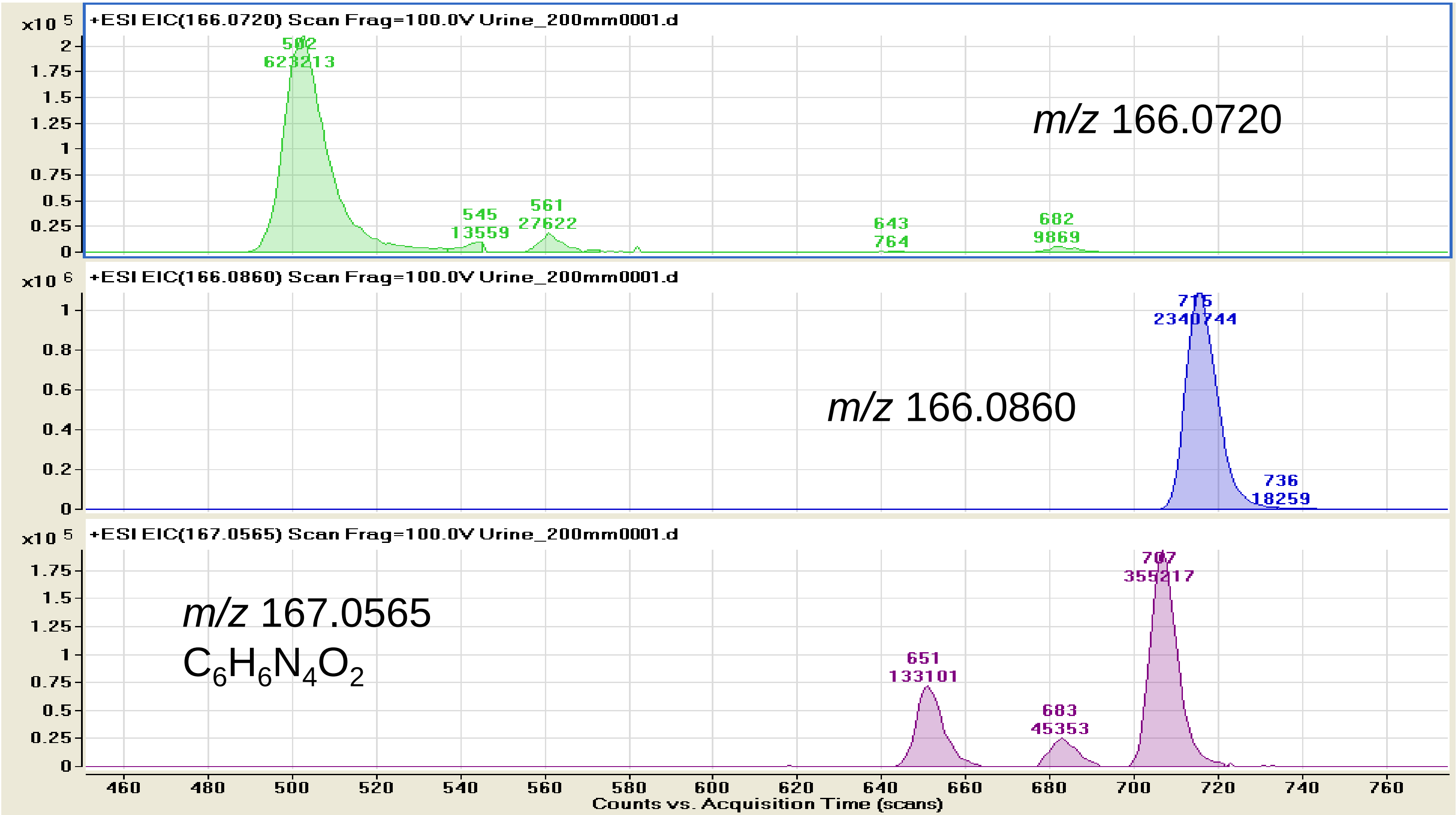


Separation of Coeluting Compounds

1000 µL/min



Separation of Coeluting Compounds 2.1 x 200 mm



Requirements for Optimal MS Detection

Time-of-Flight Technology Ideal Match for UHPLC

- **Efficient Ionization** – Stable Molecular Ions
- **Ultra fast detection rates**
  - 20 spectra per second in MS
  - 10 spectra per second in MS/MS mode
- **Mass resolution independent of Acquisition Rate**
- **Identification:** Sub-ppm mass accuracy Isotopes..
- **Wide dynamic range**

So How Can We Do IT????

Mass Resolution at What Cost?

Resolution - Function of Flight Time

6530 – 20,000 6540 – 40,000

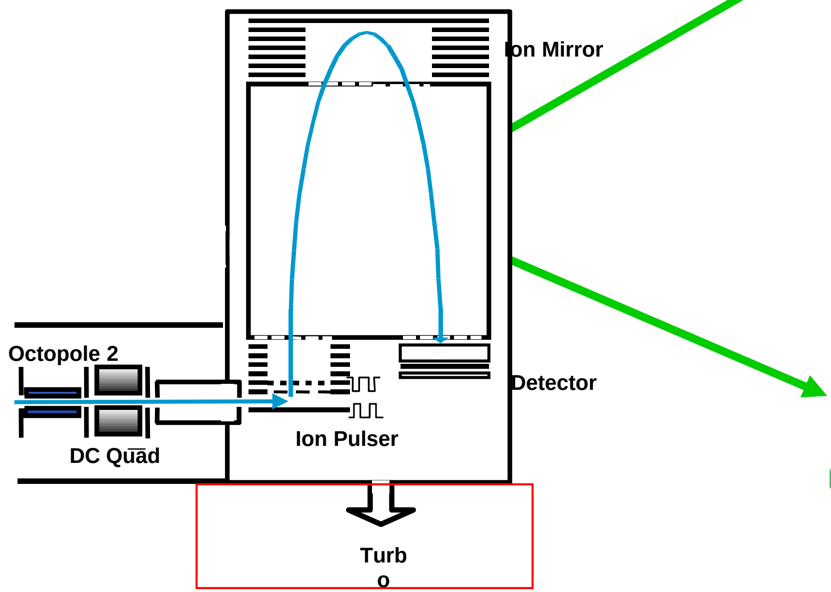
Sensitivity – 1pg 10:1 2pg 10:1

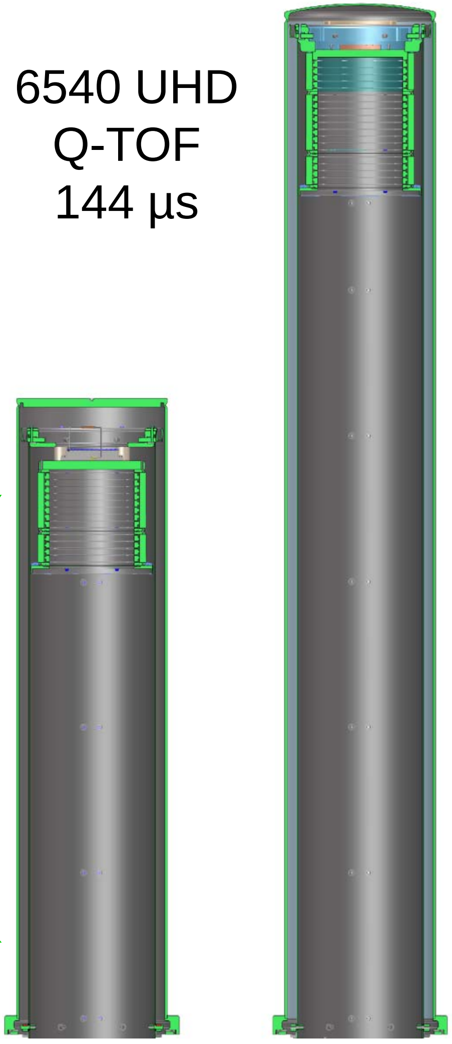
In Scan Dynamic Range

Transients/Scan 250-13,000

6540 UHD
Q-TOF
144 µs

6530
Q-TOF
90 µs

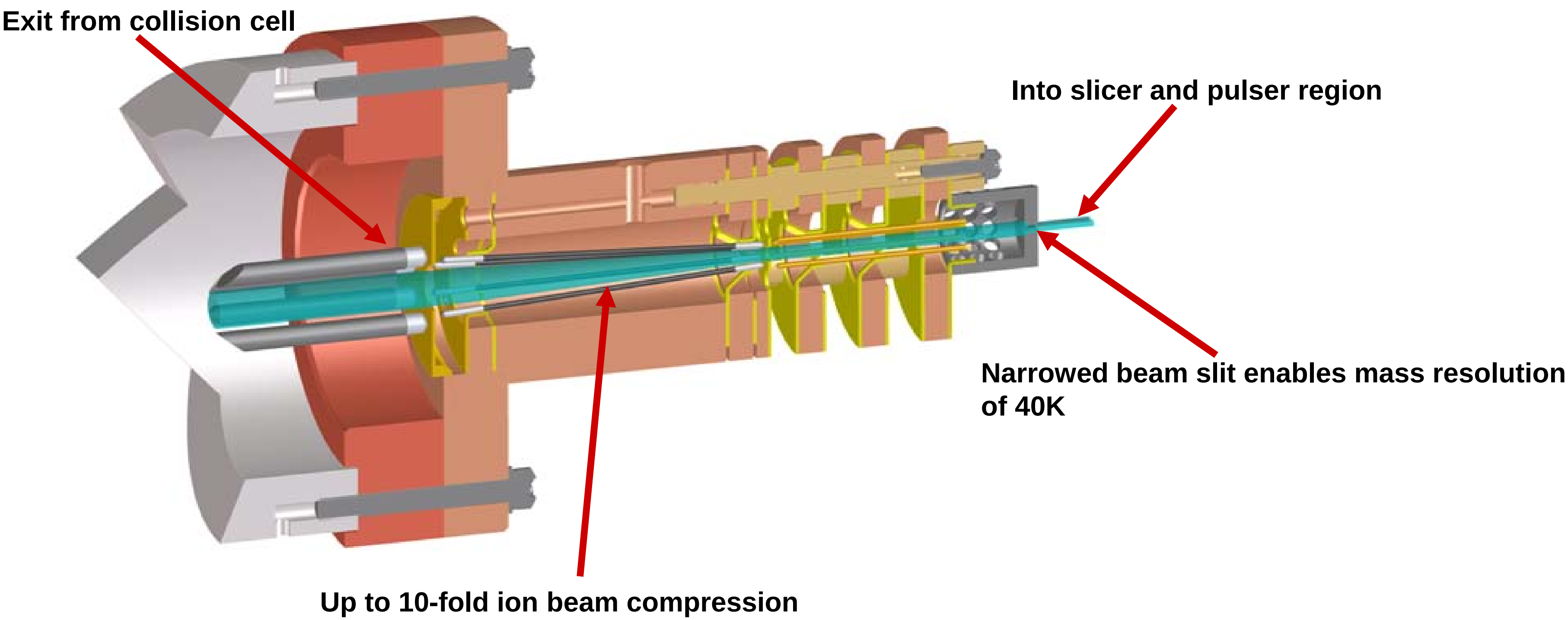




LC/MS
Analysis

Ion Beam Compression (IBC)* Technology Drives Higher Resolution

Compressed and cooled ion beam ensures the best sensitivity performance in high resolution mode



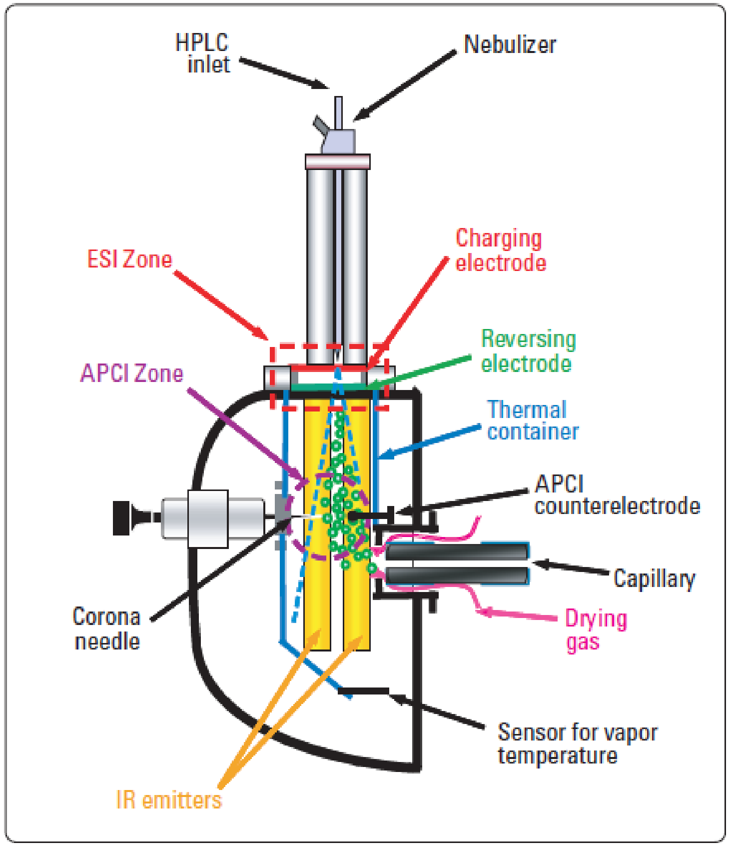
*** Patent pending**

Ionization At High LC Flow Rates (> 1mL/min)

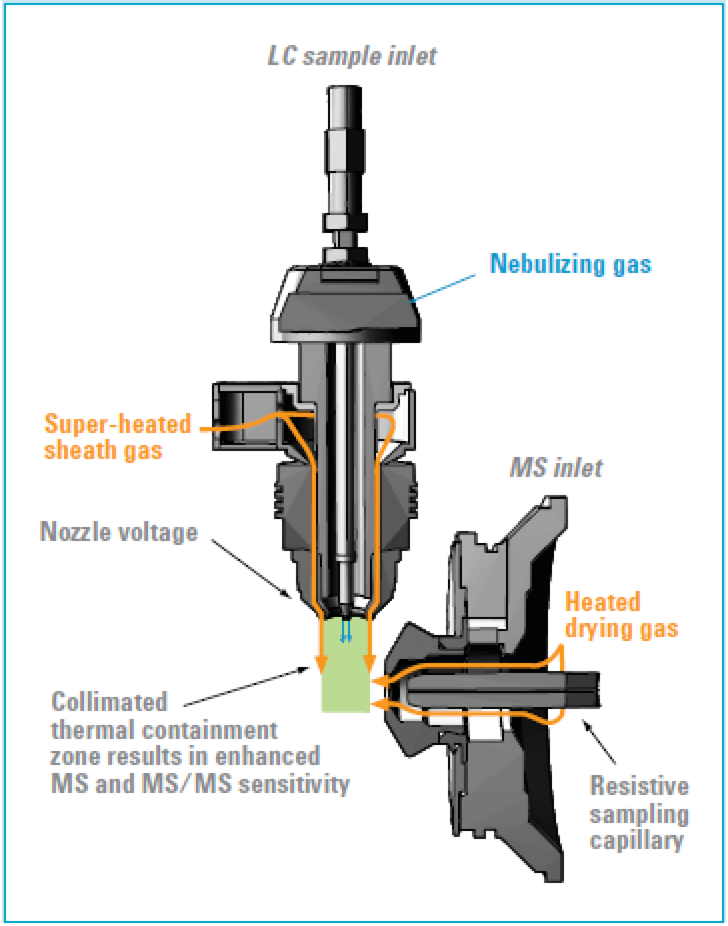
Ion Generation Critical Step

Split Flow ESI

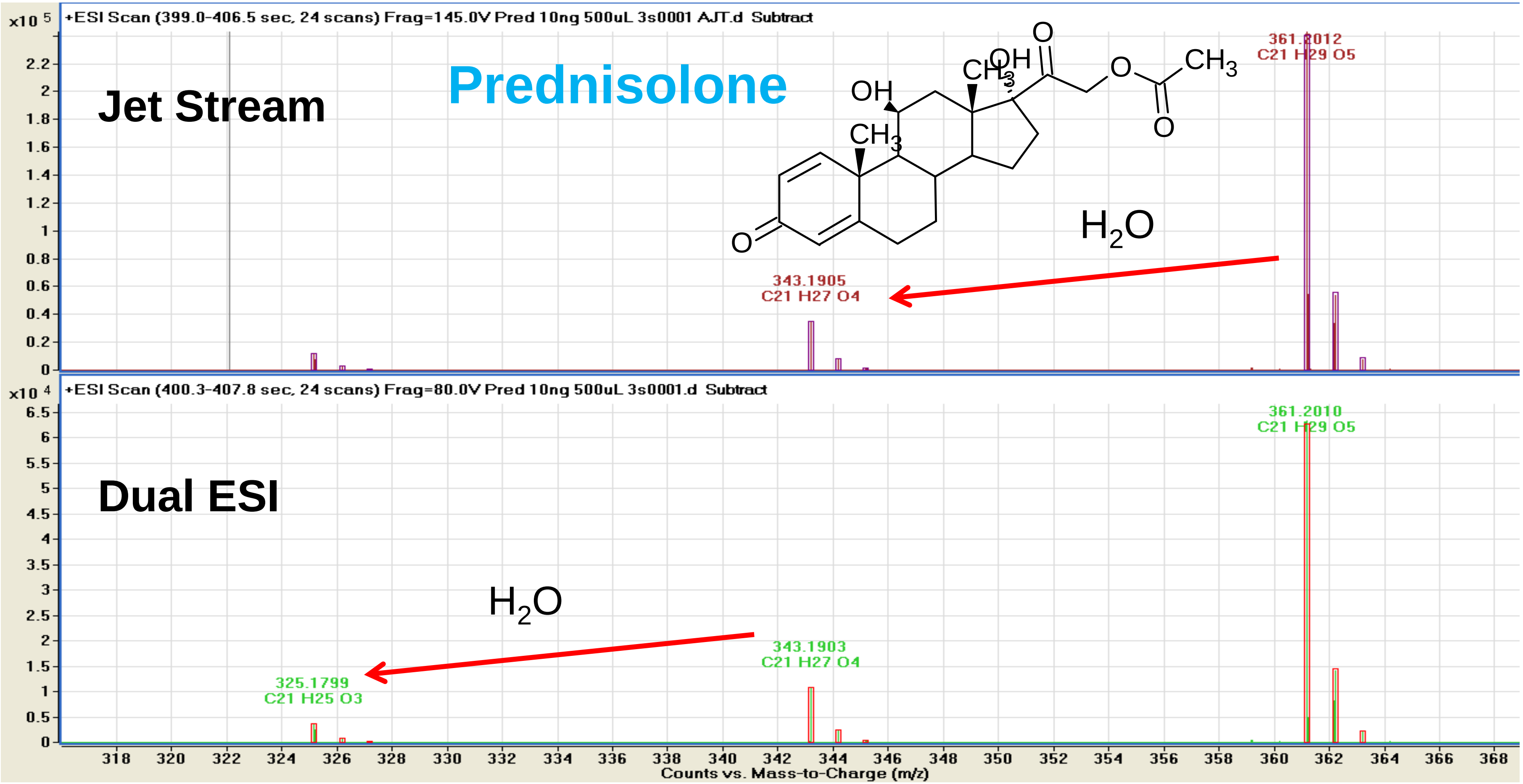
Multimode



Jet Stream

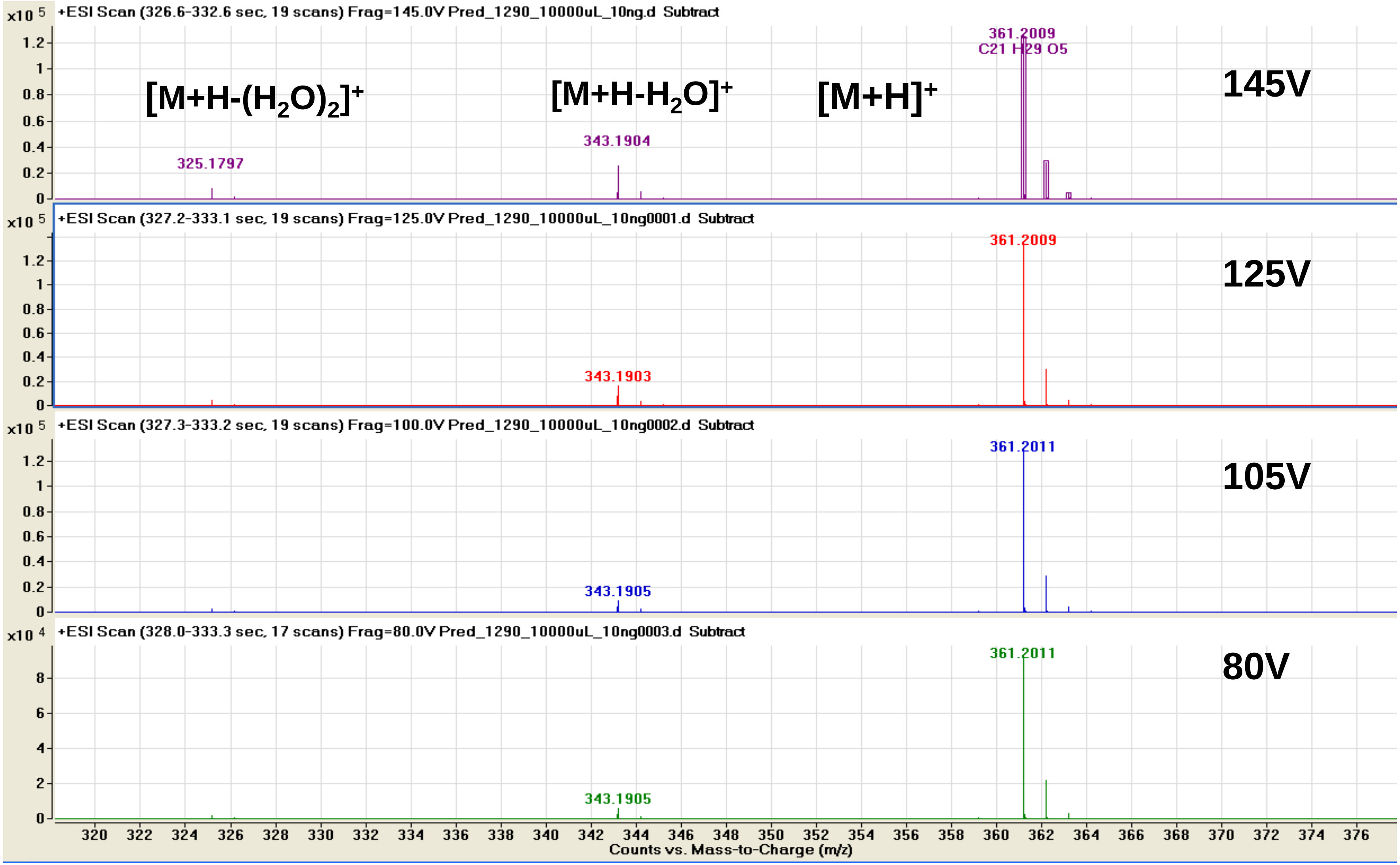


Comparison ESI and Jet Stream (500 µL/min)

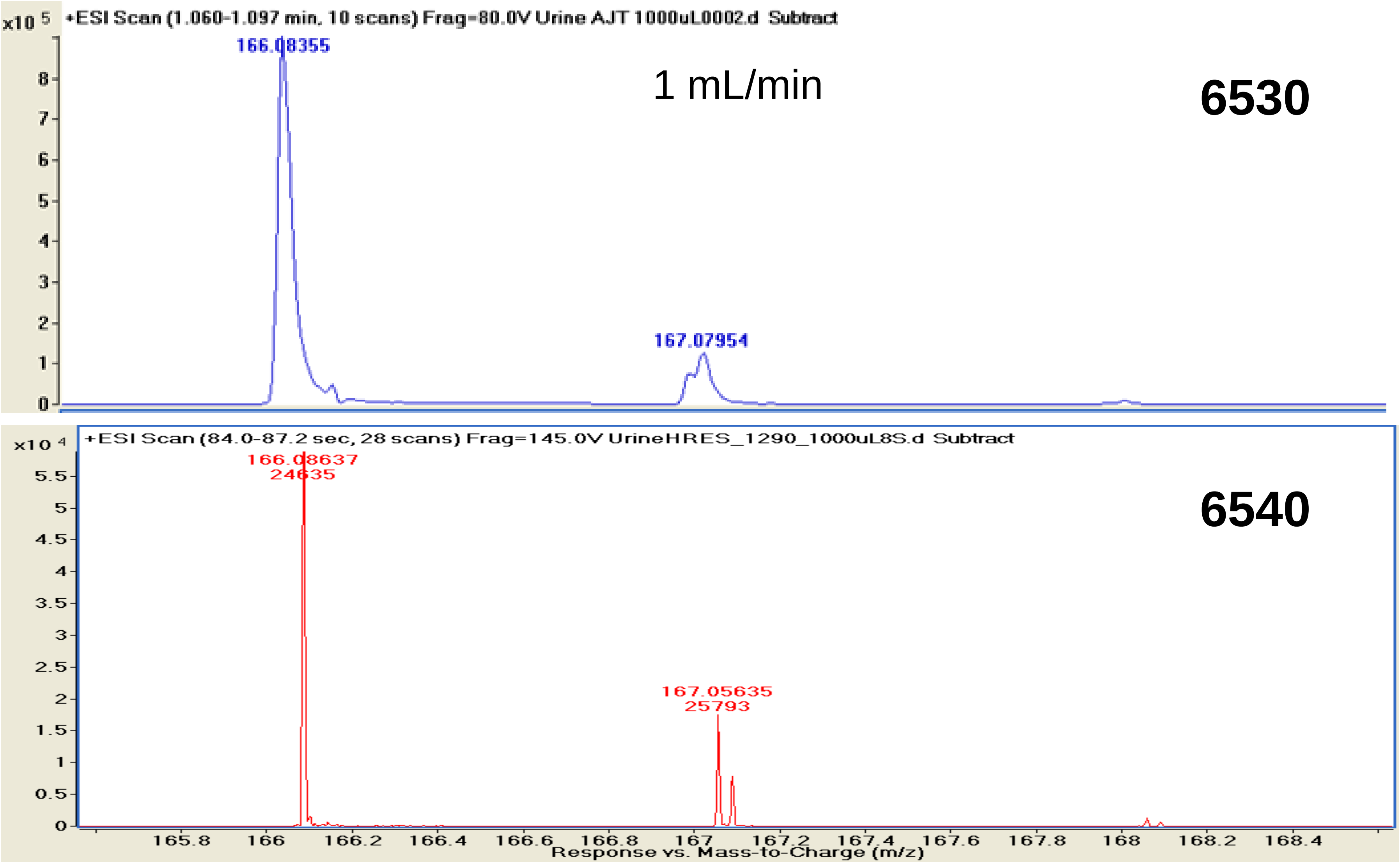


4 Fold increase in Signal with Jet Stream with Decrease in Dehydration

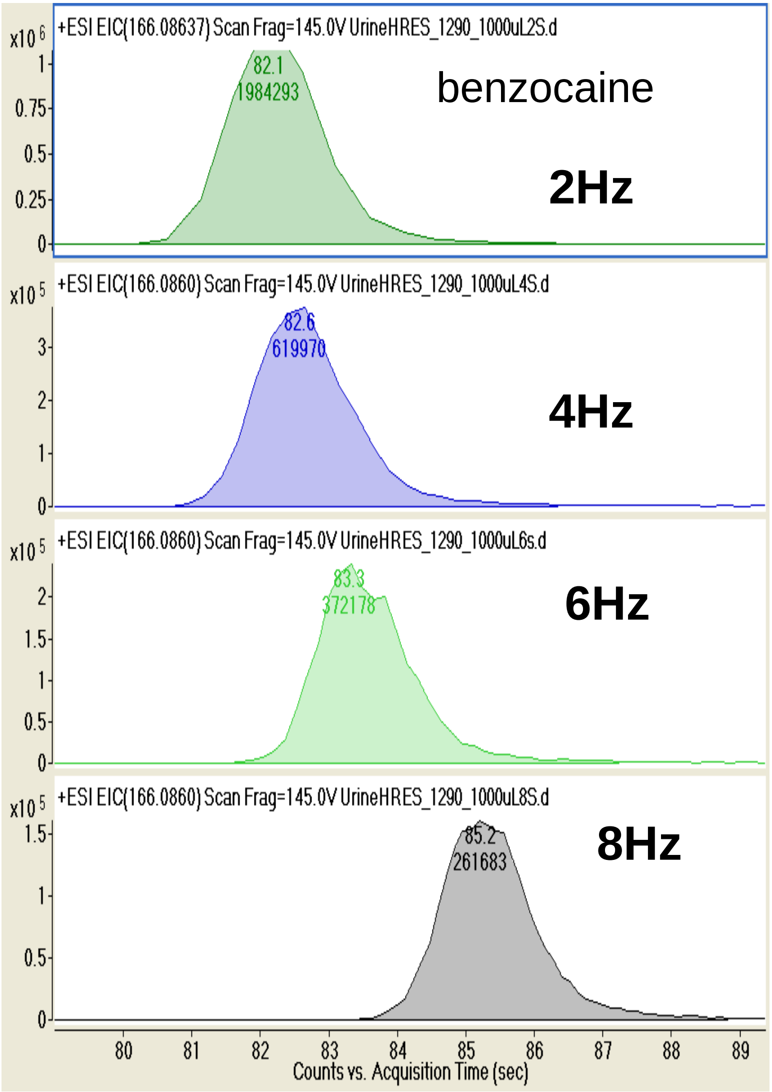
6540 In-source Fragmentation Prednisolone

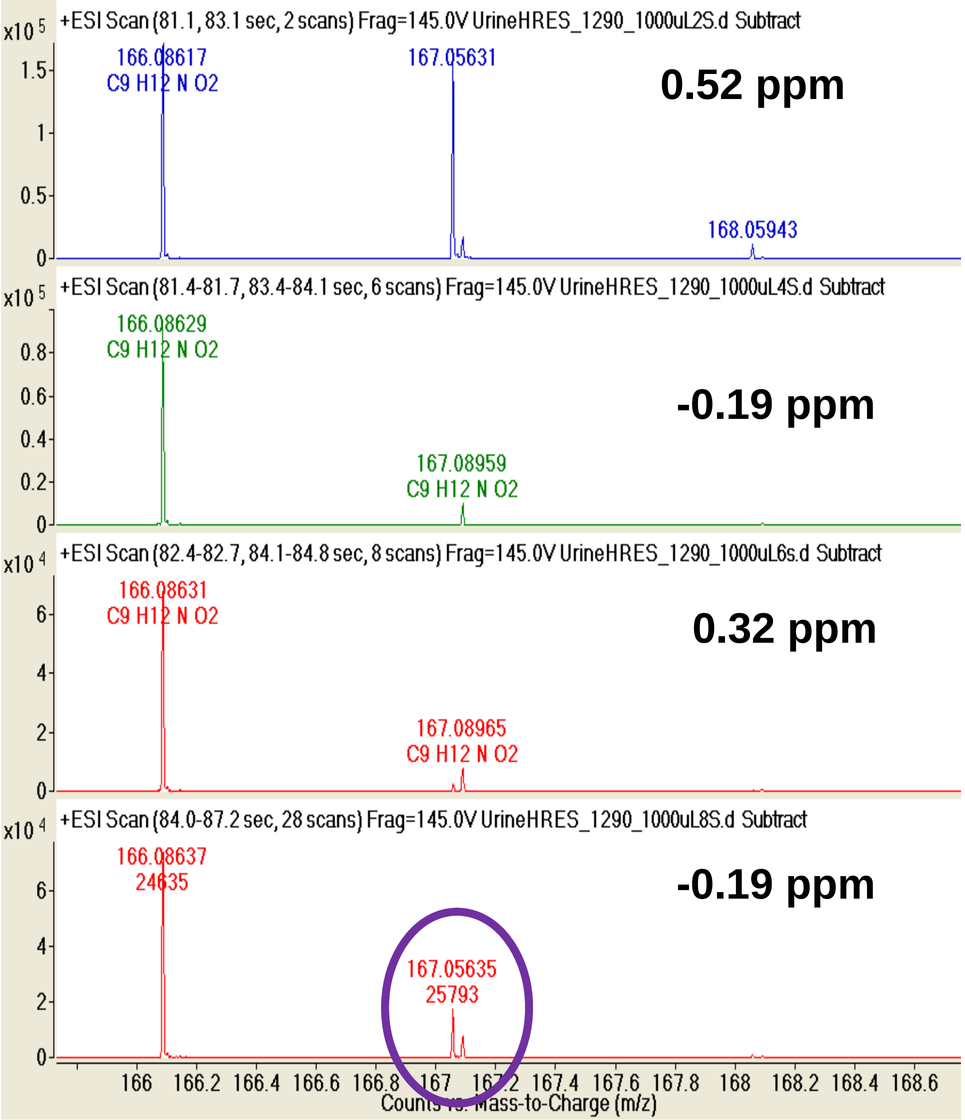


How Much Resolution is Enough Resolution?



Mass Accuracy vs Scan Rate Flow Rate 1 mL/min





Phytosterols Dietary Supplements in Headlines..

Natural Phytosterols and Phytostanols Clinically Proven to Reduce Cholesterol; A New JAMA Study...

Natural Phytosterols and Phytostanols Clinically Proven to Reduce Cholesterol; A New JAMA Study May Mislead Consumers Into Thinking All Natural Options Are Ineffective

Populations with a low incidence of coronary heart disease tend to have relatively low total blood cholesterol and LDL cholesterol levels. Our specialized formula exceeds strict quality standards for levels of beta sitosterol, stigmasterol, and campesterol - the active ingredients that help maintain cholesterol levels already within a normal range.*

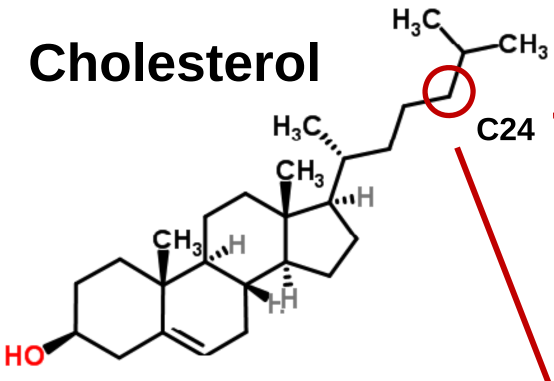
A healthy choice for daily cardiovascular maintenance, beta sitosterol is a powerful plant sterol that works naturally within the body. Our Beta Sitosterol features premium-grade plant sterols including beta sitosterol, campesterol and stigmasterol for broad-spectrum phytosterol nutrition

Got DATA and Results……. YES

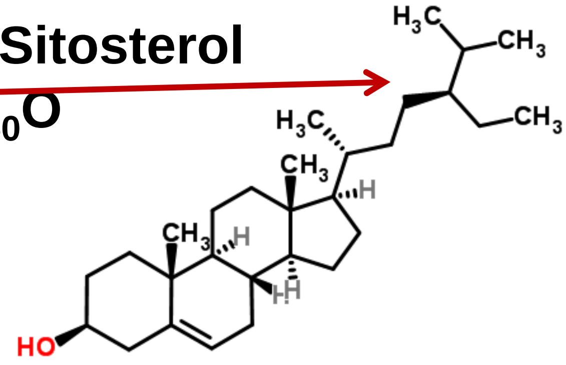
Going to Present Results…. NO

Phytosterols Modification of Cholesterol

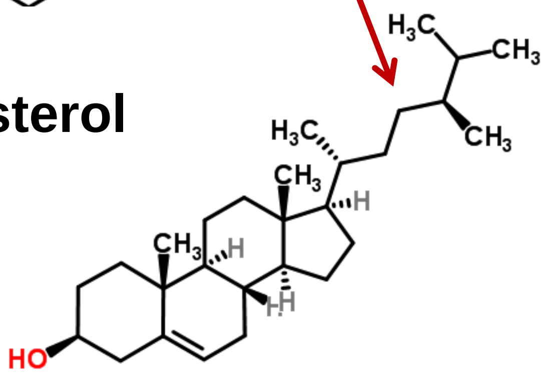
Cholesterol

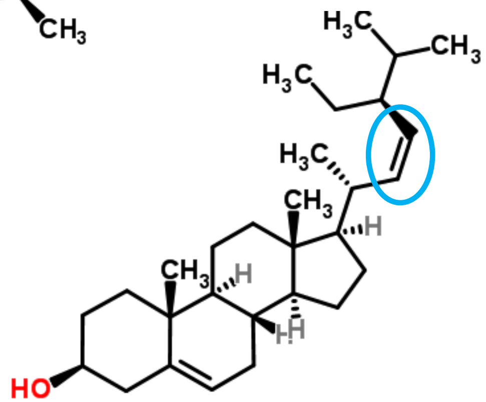


Beta-Sitosterol
$C_{29}H_{50}O$

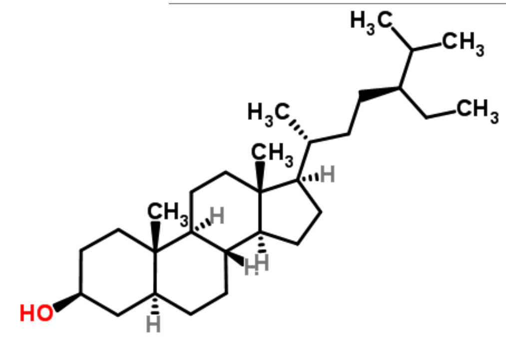


Campesterol
$C_{28}H_{48}O$





Stigmasterol
$C_{29}H_{48}O$



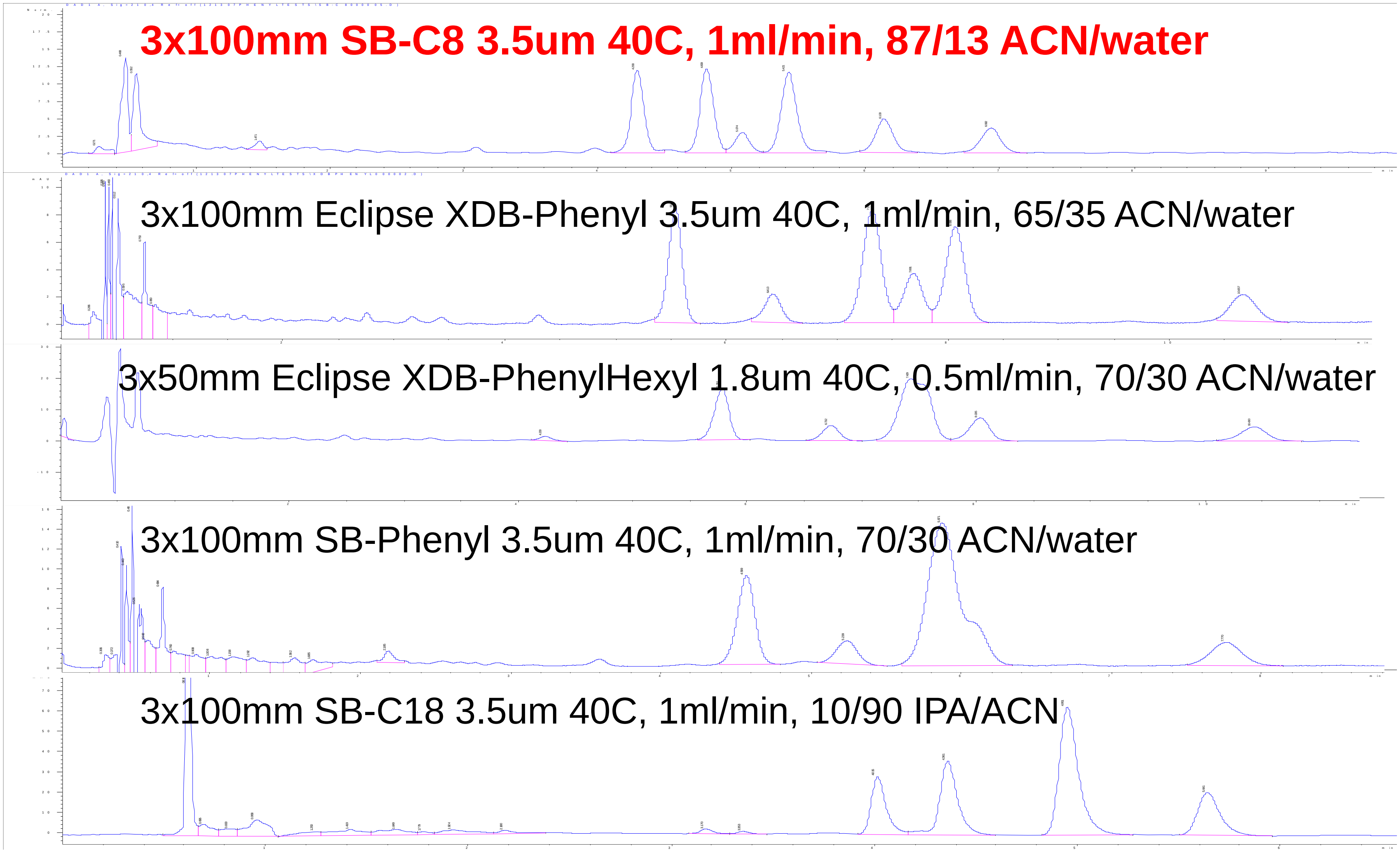
Dihydro Beta-Sitosterol
$C_{29}H_{52}O$

Phytosterols Commonly in Products

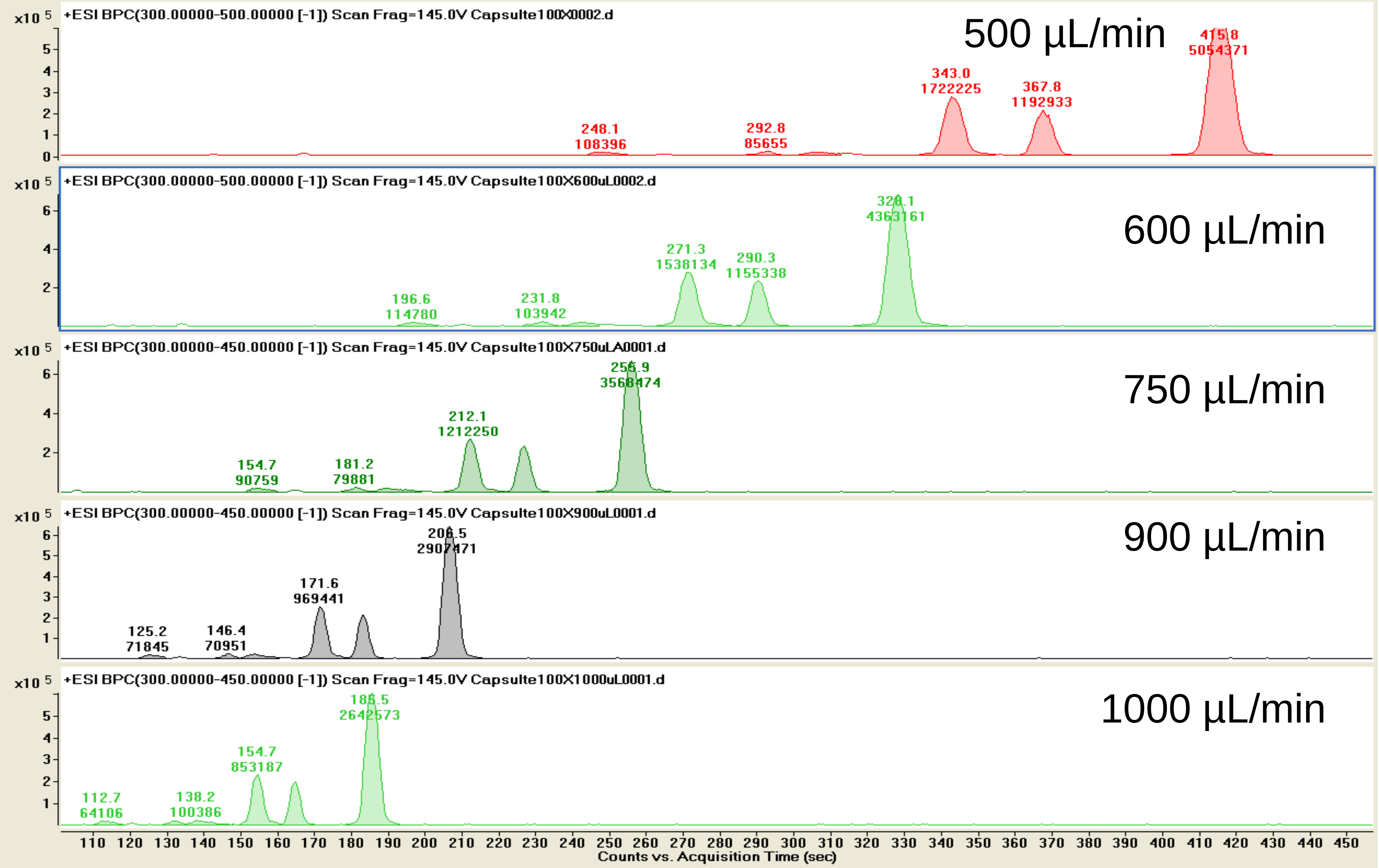
Name	M.form	# C=C bonds	Calc. Mwt
Cholesterol	$C_{27}H_{46}O$	1	386.366
ergosterol	$C_{28}H_{44}O$	(3) 2 conj.1 isol.	396.350
Brassicasterol	$C_{28}H_{46}O$	2	398.366
campesterol	$C_{28}H_{48}O$	1	400.382
campestAnol	$C_{28}H_{50}O$		402.398
Stigmasterol	$C_{29}H_{48}O$	2	412.382
delta-5-avenasterol	$C_{29}H_{48}O$		412.382
b-Sitosterol	$C_{29}H_{50}O$	1	414.398
sitostAnol	$C_{29}H_{52}O$		416.414
lanosterol	$C_{30}H_{50}O$	2	426.398

Column Selectivity Comparison

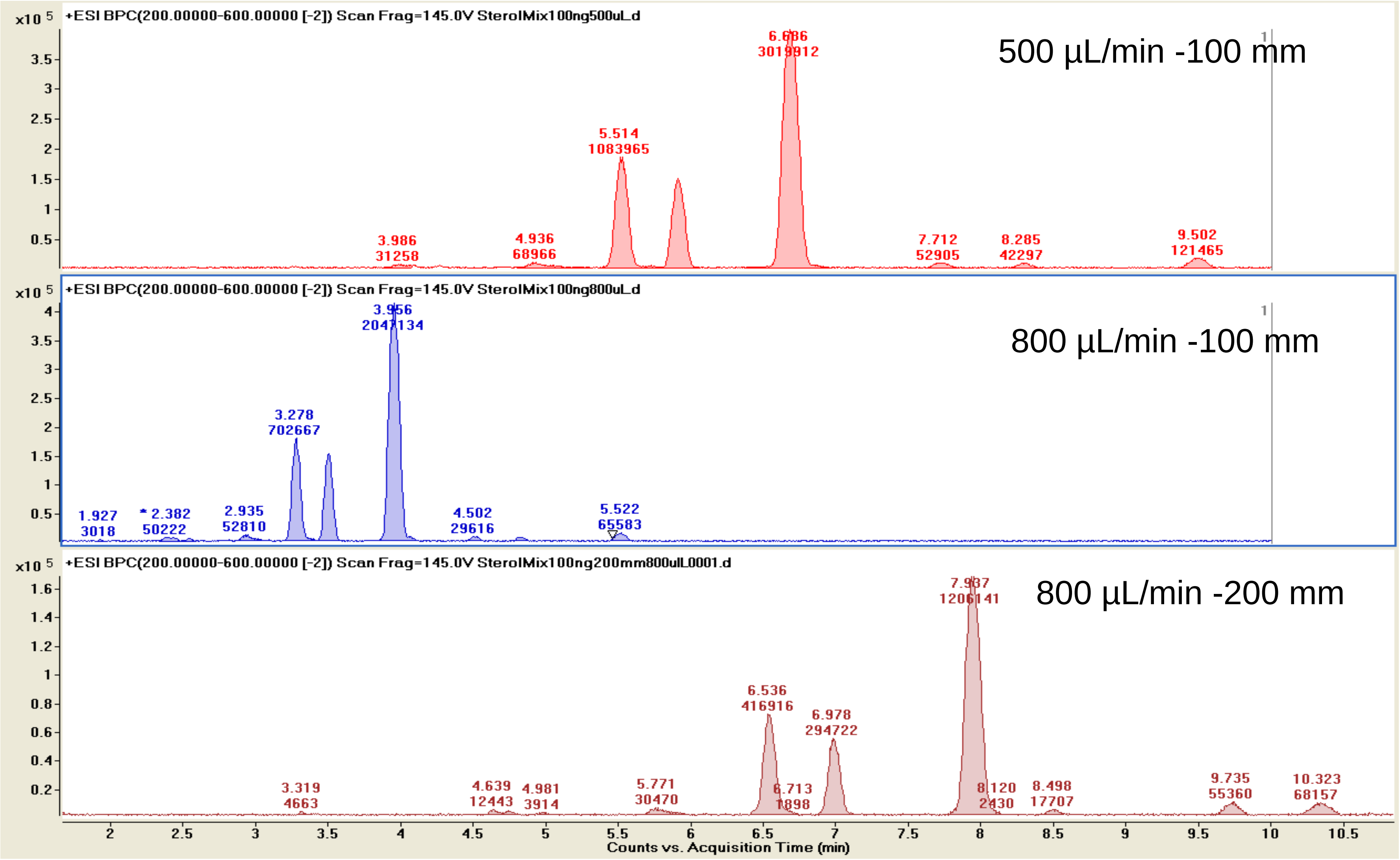
Separations



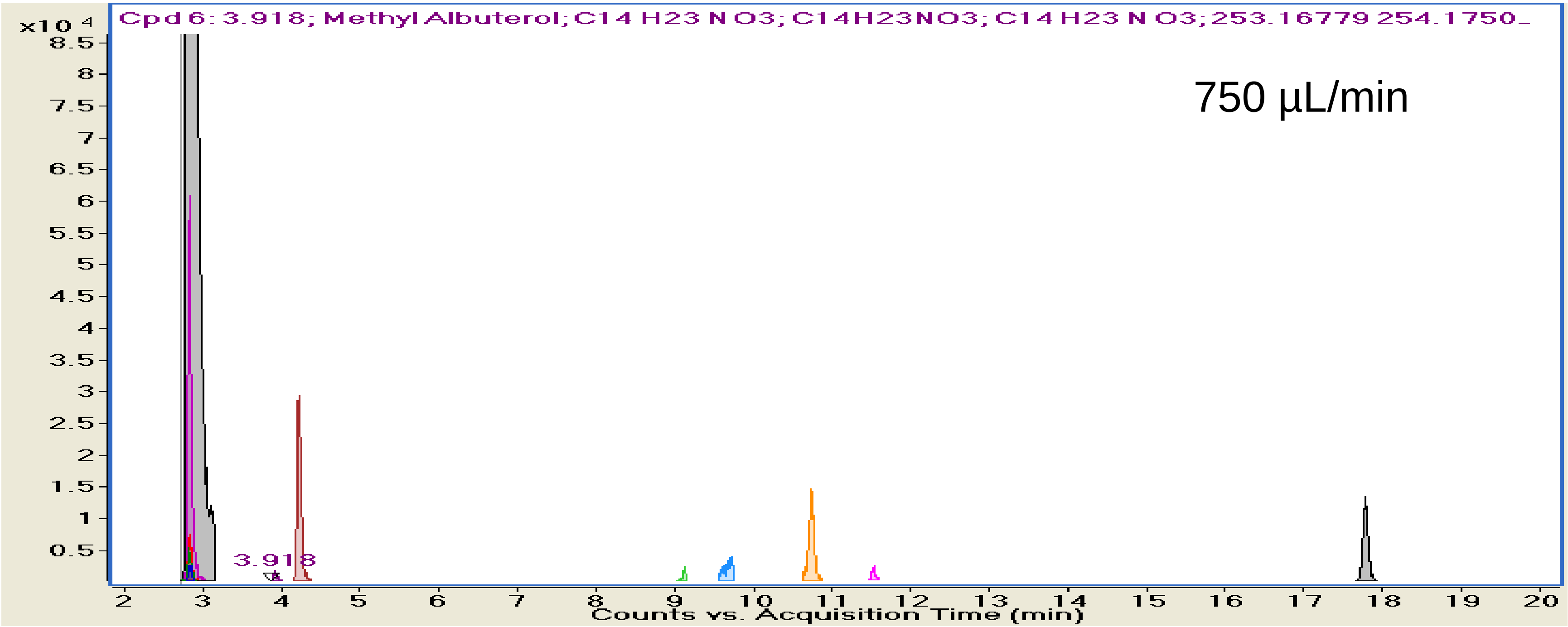
Cholestatin™ Sterol Separation 500 µL-1000 µL/min



Characterization of Sterol Mix – Flow Rate/Column

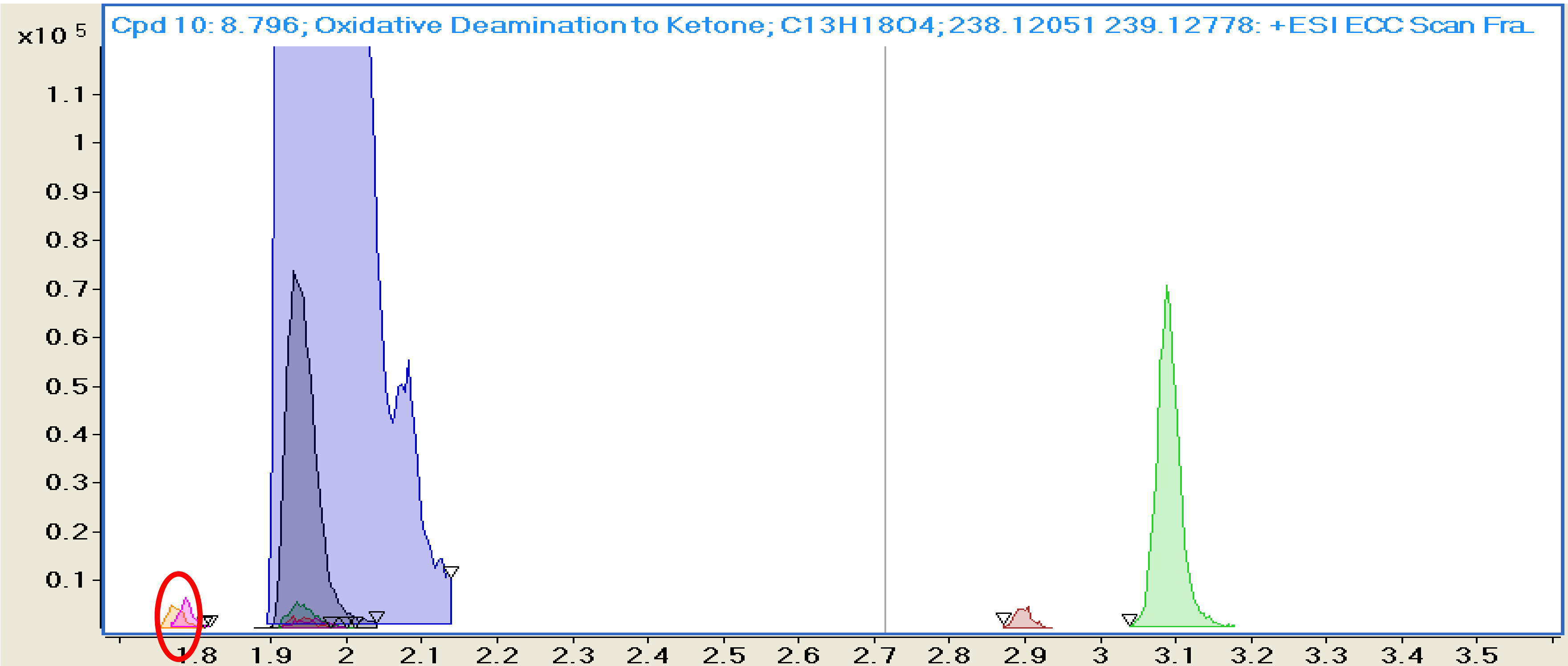


Degradation Products in Albuterol Sulfate



Name	RT	Mass	Height	Vol	Formula (MFG)	Diff (MF
Dehydration of Oximes	2.829	221.14171	51605	210293	C13 H19 N O2	-0.61
Albuterol	2.832	239.15226	681469	6759467	C13 H21 N O3	-0.49
2x Hydroxylation	2.832	271.14202	6627	29014	C13 H21 N O5	-0.18
Hydroxylation and Desaturation	2.833	253.13177	4167	19567	C13 H19 N O4	-1.43
3x Hydroxylation	2.835	287.13705	2228	9253	C13 H21 N O6	-0.56
Methyl Albuterol	3.918	253.16779	1600	5886	C14 H23 N O3	0.03
Albuterol Aldehyde	4.223	237.13659	25835	107334	C13 H19 N O3	-0.42
Decarbonylation	9.113	211.15732	2136	7308	C12 H21 N O2	-0.44
Hydrolysis of Aromatic Nitriles	9.688	257.16272	3463	26146	C13 H23 N O4	-0.05
Oxidative Deamination to Ketone	10.739	238.12072	12436	69224	C13 H18 O4	-0.88
Hydrolysis of Nitrate Esters	11.526	194.16706	2437	8555	C13 H22 O	0.05
Hydrolysis of Nitrate Esters	17.783	194.16706	11055	64058	C13 H22 O	0.02

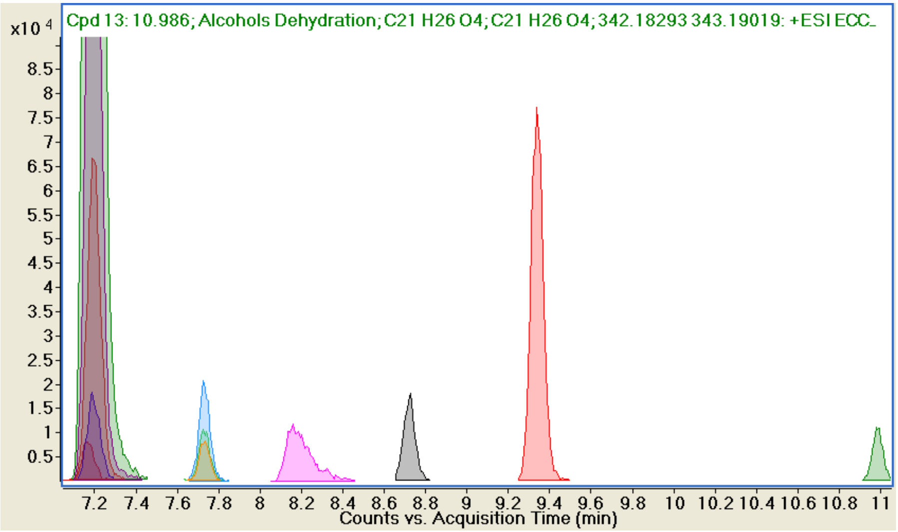
Albuterol Sulfate- Coeluting Impurities MW 225/255



Name	RT	Mass	Height	Vol	Formula (MFG)	Diff (MF
Aromatic Ring to Arene Oxide	1.773	255.14765	4251	7494	C13 H21 N O4	-2.31
Demethylation	1.787	225.1368	5646	8279	C12 H19 N O3	-1.38
Dehydration of Oximes	1.937	221.14161	63462	201571	C13 H19 N O2	-0.13
Albuterol	1.942	239.15236	504622	4692427	C13 H21 N O3	-0.92
Albuterol Aldehyde	1.955	237.137	1766	5668	C13 H19 N O3	-2.15
Methyl Albuterol	2.898	253.16835	3436	7706	C14 H23 N O3	-2.2
Albuterol Aldehyde	3.089	237.13685	61144	143761	C13 H19 N O3	-1.49
Oxidative Deamination to Ketone	8.796	238.12051	1790	5735	C13 H18 O4	0.01

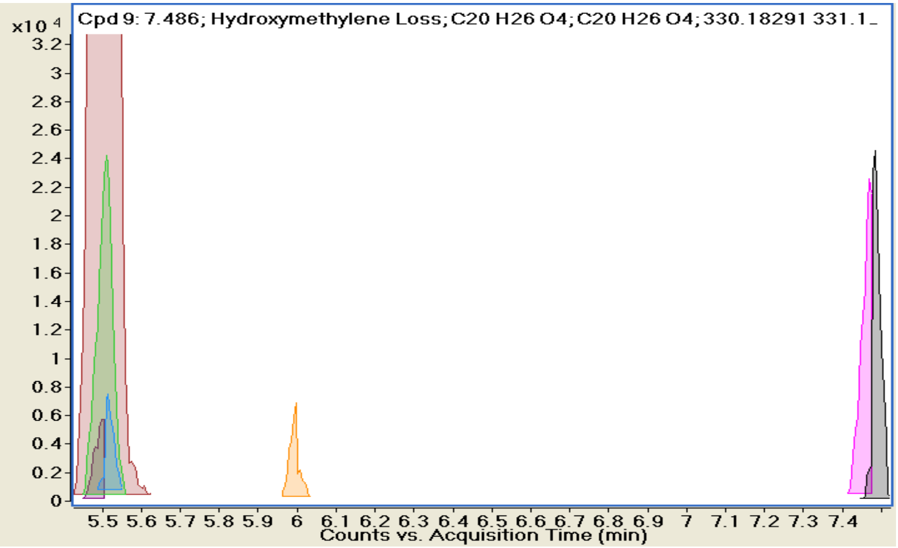
Prednisolone Impurities Function of Flow Rate

400 µL/min ESI 7 Compounds



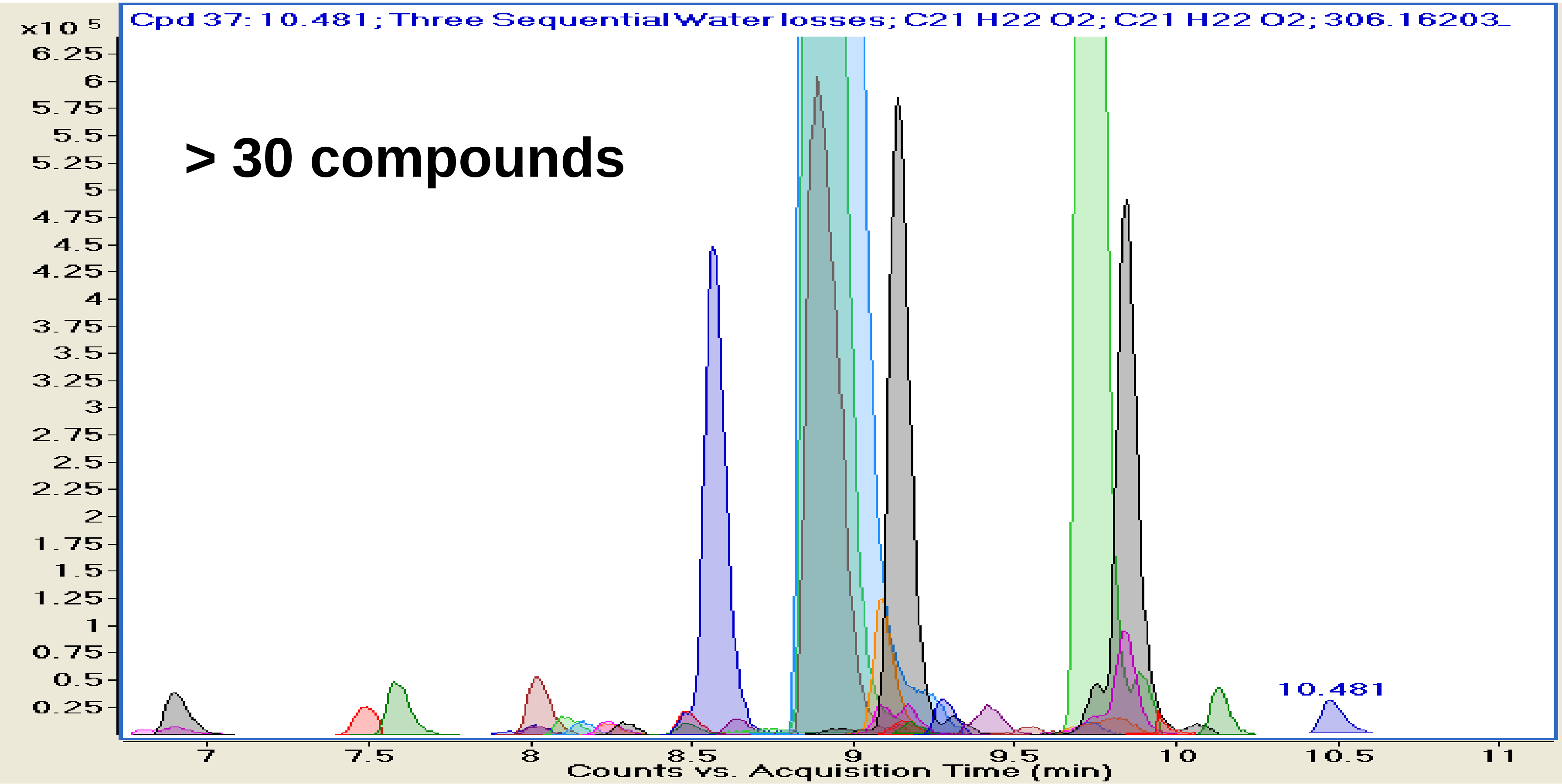
Name	RT	Mass	Height	Vol	Formula (MFG)	Diff (MFG, ppm)
Desaturation	7.168	358.17803	5085	37483	C21 H26 O5	-0.01
Prednisolone	7.194	360.19342	590243	3591682	C21 H28 O5	0.7
Three Sequential Water losses	7.194	306.16186	14969	82361	C21 H22 O2	0.41
Alcohols Dehydration	7.194	342.18299	161030	923857	C21 H26 O4	0.36
Two Sequential water Losses	7.195	324.17251	52642	316783	C21 H24 O3	0.11
Alcohols Dehydration	7.73	342.18297	8465	43261	C21 H26 O4	0.4
Prednisolone	7.73	360.19362	14576	78189	C21 H28 O5	0.15
Two Sequential water Losses	7.731	324.1726	6583	32437	C21 H24 O3	-0.19
Prednisolone	8.175	360.19329	8663	94080	C21 H28 O5	1.06
Dehydrogenation and two Decar...	8.721	300.17272	12865	71401	C19 H24 O3	-0.58
Hydroxymethylene Loss	9.339	330.18307	58546	328192	C20 H26 O4	0.11
Alcohols Dehydration	10.986	342.18293	8140	40929	C21 H26 O4	0.51

1000 µL/min ESI 3 Compounds



Name	RT	Mass	Height	Vol	Formula (MF	Diff (MFG,
Two Sequential water Losses	5.493	324.17264	5761	10397	C21 H24 O3	-0.29
Prednisolone	5.508	360.19372	271061	1081732	C21 H28 O5	-0.13
Alcohols Dehydration	5.509	342.18324	18680	68571	C21 H26 O4	-0.39
Two Sequential water Losses	5.515	324.17253	6336	13440	C21 H24 O3	0.04
Prednisolone	5.996	360.19382	5362	10863	C21 H28 O5	-0.41
Hydroxymethylene Loss	7.464	330.18308	18547	39569	C20 H26 O4	0.08
Hydroxymethylene Loss	7.486	330.18291	17995	37180	C20 H26 O4	0.6

Prednisolone Dual ESI, 275 µL/min, 10X Dilution



> 30 Compounds Identified

Name	RT	Mass	Height	Vol	Formula (DB)	Diff (DB,	Formula (MFG)	Diff (MFG,	Score (MFG)
Desaturation	6.888	358.17732	5588	57743	C21 H26 O5	1.96	C21 H26 O5	1.97	72.96
Hydroxylation	6.902	376.1885	14214	200080	C21 H28 O6	0.23	C21 H28 O6	0.23	72.22
Demethylation and two Hydroxylations	7.487	378.16757	14283	117564	C20 H26 O7	0.76	C20 H26 O7	0.76	96.29
Hydration, Hydrolysis (Internal)	7.588	378.20396	25037	240117	C21 H30 O6	0.74	C21 H30 O6	0.74	99.1
Prednisolone	8.015	360.19351	6557	51783	C21 H28 O5	0.45	C21 H28 O5	0.46	72.21
Alcohols Dehydration	8.021	342.183	5535	33681	C21 H26 O4	0.32	C21 H26 O4	0.32	78.7
Hydration, Hydrolysis (Internal)	8.023	378.20407	28479	263551	C21 H30 O6	0.45	C21 H30 O6	0.45	98.09
Desaturation	8.112	358.17779	9100	85596	C21 H26 O5	0.65	C21 H26 O5	0.66	83.89
Dehydration and Decarboxylation	8.16	328.16756	6146	55998	C20 H24 O4	-0.31	C20 H24 O4	-0.31	82.68
Two Sequential Desaturations	8.241	356.16208	8697	50821	C21 H24 O5	0.81	C21 H24 O5	0.81	83.33
Hydroxylation and Desaturation	8.243	374.1722	8696	62310	C21 H26 O6	1.96	C21 H26 O6	1.96	68.87
Prednisolone	8.292	360.19287	8968	49967	C21 H28 O5	2.23	C21 H28 O5	2.24	88.66
Hydroxylation	8.486	376.18811	16418	101100	C21 H28 O6	1.26	C21 H28 O6	1.26	92.49
Hydroxylation	8.488	376.1885	7623	47247	C21 H28 O6	0.23	C21 H28 O6	0.23	91.88
Desaturation	8.569	358.1779	224244	2198268	C21 H26 O5	0.32	C21 H26 O5	0.33	98.04
Ketone to Alcohol	8.641	362.2092	11076	71245	C21 H30 O5	0.33	C21 H30 O5	0.33	97.56
Alcohols Dehydration	8.9	342.18341	1182648	12286742	C21 H26 O4	-0.88	C21 H26 O4	-0.88	92.86
Prednisolone	8.91	360.19379	1246456	36847828	C21 H28 O5	-0.33	C21 H28 O5	-0.32	95.02
Demethylation	9.091	346.17806	63105	587269	C20 H26 O5	-0.11	C20 H26 O5	-0.11	97.9
Dehydration and Decarboxylation	9.129	328.1675	22689	231549	C20 H24 O4	-0.12	C20 H24 O4	-0.12	93.7
Dehydrogenation and two Decarboxyl...	9.141	300.17265	255211	2770656	C19 H24 O3	-0.36	C19 H24 O3	-0.35	94.52
Dehydration and Decarboxylation	9.178	328.16708	9649	48734	C20 H24 O4	1.15	C20 H24 O4	1.14	82.3
Hydroxylation and Desaturation	9.282	374.17295	16782	144924	C21 H26 O6	-0.02	C21 H26 O6	-0.02	98.62
Three Sequential Water losses	9.547	306.16246	5325	41890	C21 H22 O2	-1.55	C21 H22 O2	-1.55	63.44
Hydroxymethylene Loss	9.729	330.18326	886190	9555204	C20 H26 O4	-0.45	C20 H26 O4	-0.45	99.48
Dehydration and Decarboxylation	9.733	328.16759	5294	56322	C20 H24 O4	-0.39	C20 H24 O4	-0.4	75.99
Three Sequential Water losses	9.799	306.16204	12012	157330	C21 H22 O2	-0.19	C21 H22 O2	-0.19	77.6
Two Sequential water Losses	9.842	324.17261	77076	532528	C21 H24 O3	-0.21	C21 H24 O3	-0.2	99.22
Alcohols Dehydration	9.846	342.18336	203055	2448470	C21 H26 O4	-0.72	C21 H26 O4	-0.72	90.07
Hydroxymethylene Loss	9.948	330.18275	8608	53320	C20 H26 O4	1.08	C20 H26 O4	1.08	82.68
Hydroxymethylene Loss	10.133	330.18281	21197	176868	C20 H26 O4	0.9	C20 H26 O4	0.9	84.54
Three Sequential Water losses	10.481	306.16203	26378	164696	C21 H22 O2	-0.15	C21 H22 O2	-0.15	85.13

Is Going Faster and Faster Applicable to Impurity Profiling..

- Balance Choice between Speed and Resolution
- Ionization Compatible with High Flow Rates (Jet Stream)
- Resolution > 30,000 enables baseline separation
- Fast Scanning QTOF 4 to 10 Spectra/sec
- Mass Accuracy Independent of Scan Speed (< 0.50 ppm)
- Isotopic Fidelity key to confirm elemental compositions

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Thank you for your Attention