Relentless Innovation

the path to

Mass Spec/tacular

John Fjeldsted
Director, R&D
LC/MS Division

Agilent Technologies
Santa Clara, CA

Agilent Technologies

Our Agenda…

- Start with the Agilent R&D History
- Examine product goals and specifications.
- Learn about Agilent Jet Stream Technology
- Discover a new level of QTOF performance in the 6530 QTOF
- Find out how software innovation makes old workflows better and new workflows possible

Agilent Technologies

Agilent's 35+ Years in Mass Spectrometry

Nearly 40,000 systems – Applied to Chromatographic Analysis

Cutting Edge 6530 Q-TOF Performance

With…

Agilent Jet Stream Technology

True Hi-Def TOF Technology

Unmatched sensitivity, speed and mass accuracy

Unique data mining tools for faster compound ID

- ☑ Mass accuracy – approaching FTMS mass accuracy
- ☑ Sensitivity- high femtogram level detection
- ☑ Increased resolving power – 20,000
- ☑ Extended dynamic range – approaching 5 Decades in-Scan
- ☑ Acquisition speed – approaching 10 MS/MS per second

Leads the industry in all dimensions*

* For systems less than 0.5 Ton and 9 feet in height

Agilent QTOF Product Specifications

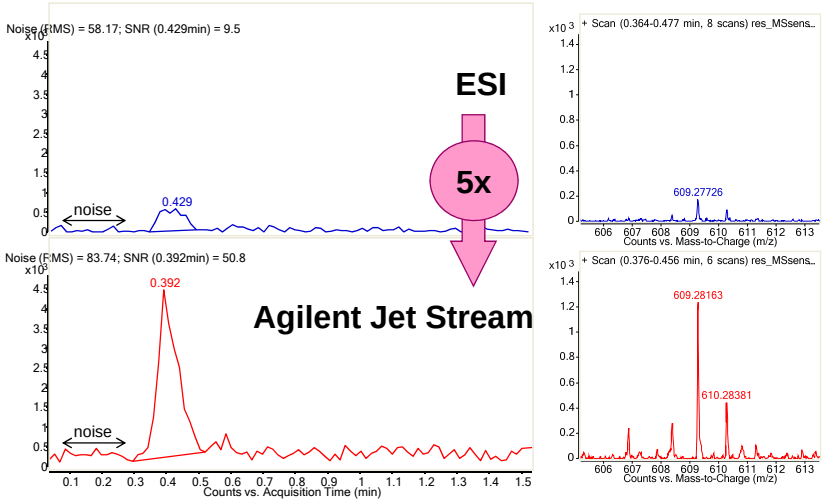
	6510	6520	6530
Sensitivity in MS mode; reserpine	5 pg; 10:1 S/N 1xRMS	5 pg; 10:1 S/N 1xRMS	**1 pg; 10:1 S/N 1xRMS**
Sensitivity in MS/MS mode; reserpine	5 pg; 50:1 S/N 1xRMS	5 pg; 50:1 S/N 1xRMS	**1 pg; 50:1 S/N 1xRMS**
Mass accuracy	<2 ppm- MS < 5ppm- MS/MS	< 2ppm- MS < 5ppm- MS/MS	<2 ppm- MS < 5 ppm MS/MS
Mass resolution	5,000 @ 118 m/z 12,000 @ 1522 m/z	10,000 @ 118 m/z 18000 @ 1522 m/z	10,000 @ 118 m/z **20,000 @ 1522 m/z**
Dynamic range	3.5 orders	5.0 orders	5.0 orders
Mass range	13,000 m/z TOF	4,000 m/z quad 20,000 m/z TOF	**8,000 m/z quad (opt)** 20,000 m/z TOF
Acquisition speed scan/sec MS	Up to 20 scan/sec	Up to 20 scan/sec	Up to 20 scan/sec
Acquisition speed scan/sec MS/MS	Up to 9 scan/sec	Up to 9 scan/sec	Up to 9 scan/sec

5x

5x

6530 QTOF Sensitivity with Agilent Jet Stream

1 pg Reserpine, MS S/N 10:1



These data were acquired with 75/25 MeOH/water on a 2.1x30mm 3.5um column. Final specs will use ACN/water and a 1.8um column and be common with the 6460 QQQ checkout

6530 QTOF Sensitivity with Agilent Jet Stream

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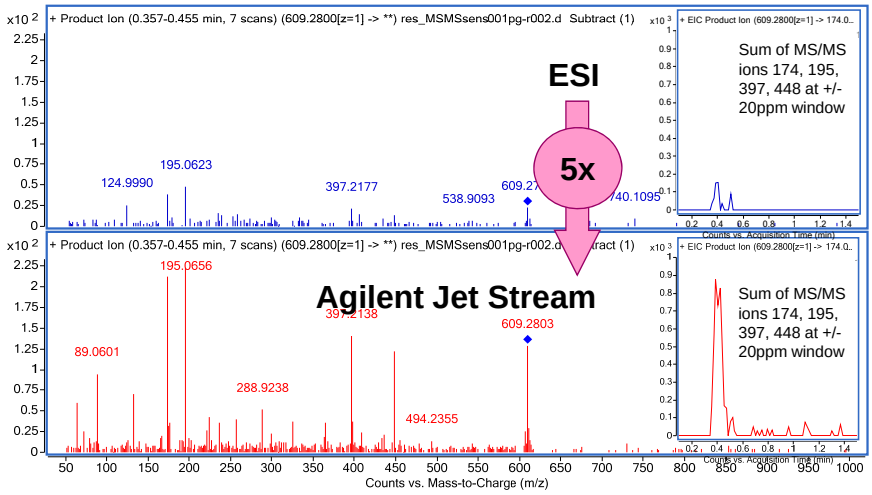

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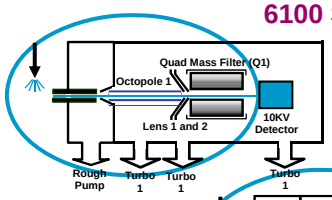
Agilent's LC/MS Systems – Innovation Inside

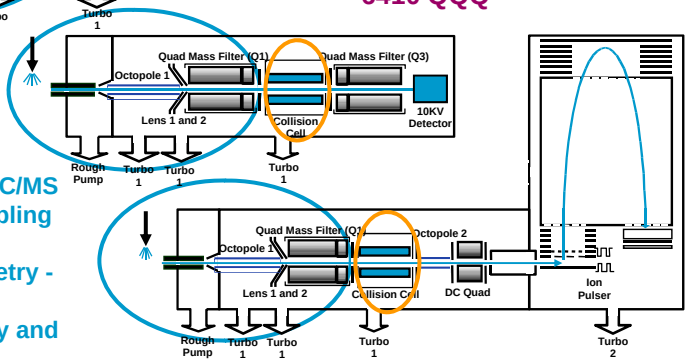
6100 Series SQ



New Collision Cell Incorporates Axial Acceleration for High Speed MS/MS Analysis

6410 QQQ

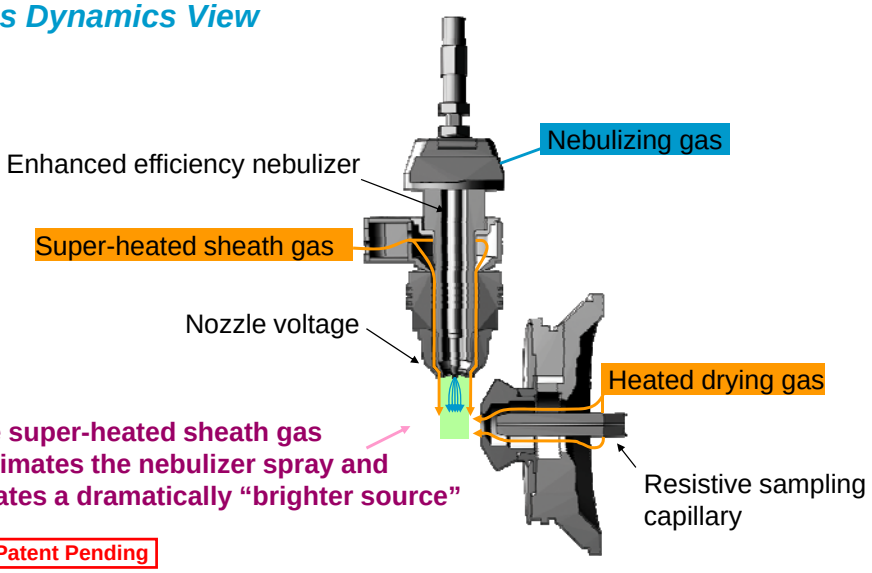
6510 Q-TOF



Over 10 Years in LC/MS Atmospheric Sampling and Patented Orthogonal Geometry - Result in Industry Leading Sensitivity and Robustness

Agilent Jet Stream Ion Generation

Gas Dynamics View



The super-heated sheath gas collimates the nebulizer spray and creates a dramatically "brighter source"

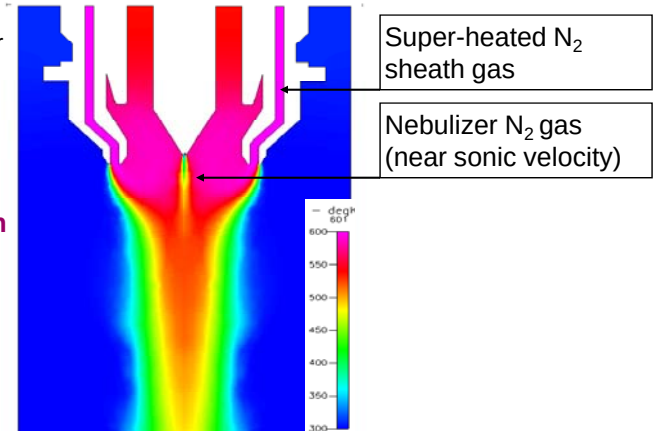
Patent Pending

Agilent Jet Stream Ion Generation
Thermal Dynamics View

This plot is a simulation showing the **thermal profile** of the Agilent Jet Stream Source

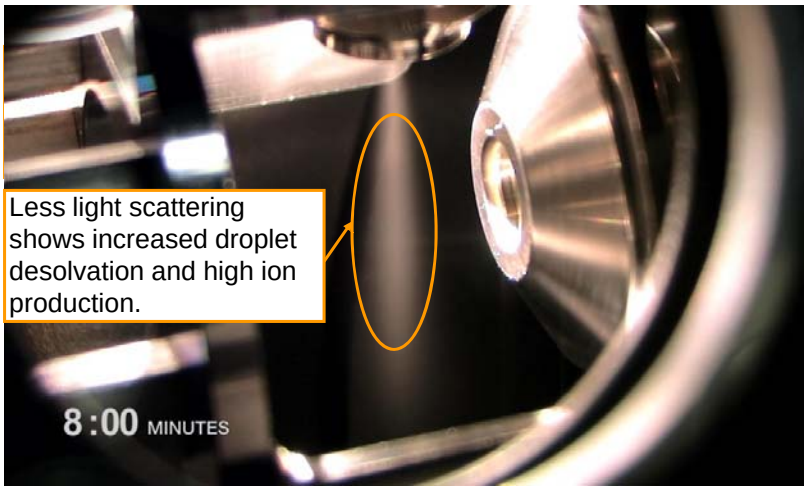
Thermal energy is focused to the nebulizer spray

Thermal focusing produces the most efficient desolvation and ion generation possible!



Patent Pending

Agilent Jet Stream In Action
Observing Thermal Focusing



Start temperature = 25 ºC

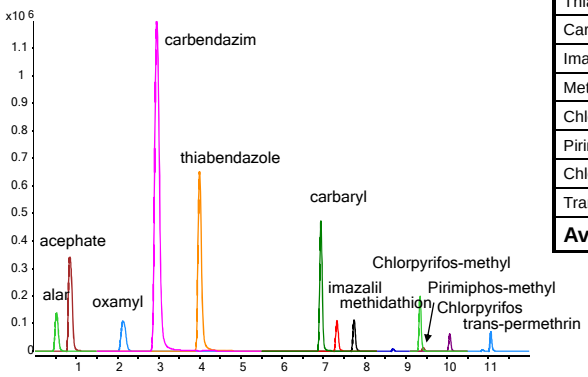
Stop temperature = 400 ºC

Agilent Jet Stream Performance

Signal Enhancement – 6460 QQQ

12 Pesticides

Relative increase in signal of
Agilent Jet Stream vs ESI = 5.8x

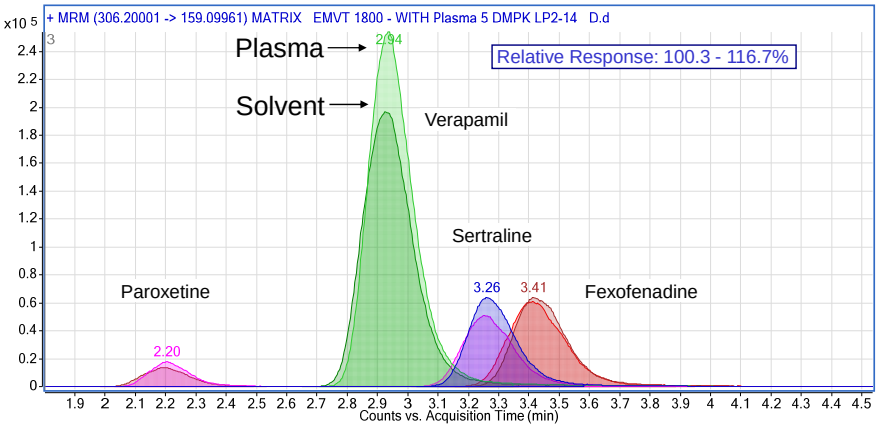


Pesticide	Optimal Temp [°C]	Signal Gain
Alar	380	5.60
Acephate	380	6.30
Oxamyl	250	5.10
Carbendazim	380	7.20
Thiabendazole	380	7.20
Carbaryl	380	6.60
Imazalil	380	8.10
Methidathion	250	5.00
Chlorpyrifos-methyl	100	6.40
Pirimiphos-methyl	380	5.50
Chlorpyrifos	380	4.00
Trans-permethrin	250	2.50
Average		**5.8x**

Agilent Jet Stream Performance

Effect of Matrix on Response – 6460 QQQ

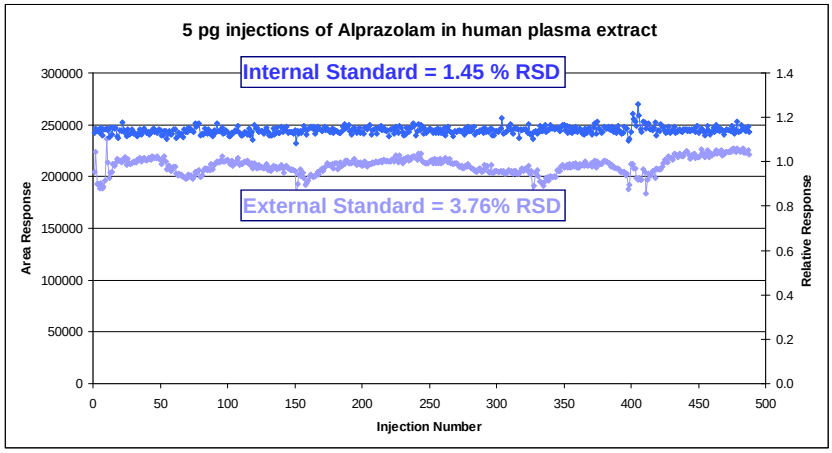
Results show 4 therapeutics in analyzed in solvent and plasma



Agilent Jet Stream Performance

Ruggedness & Reproducibility – 6460 QQQ

500 Injections of Alprazolam in Spiked Human Plasma Extract, ~ 10hrs.



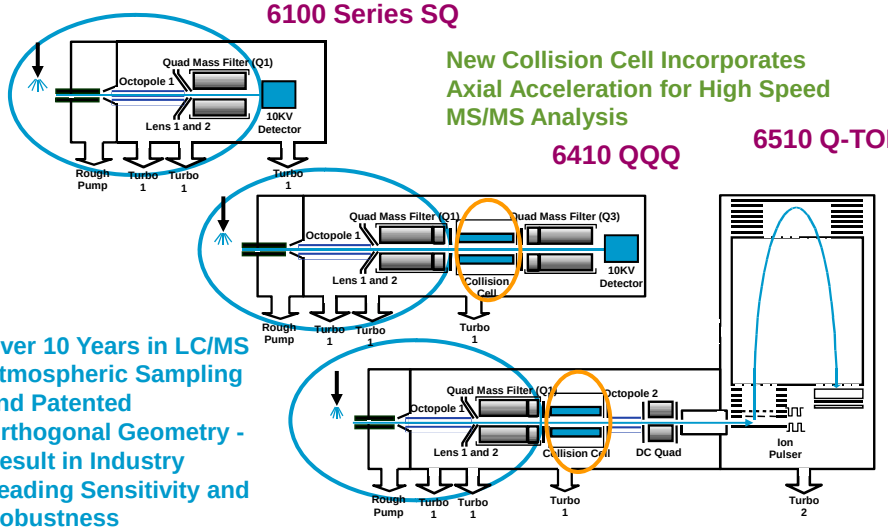
Agilent's LC/MS Systems – Innovation Inside

6100 Series SQ

New Collision Cell Incorporates Axial Acceleration for High Speed MS/MS Analysis

6410 QQQ

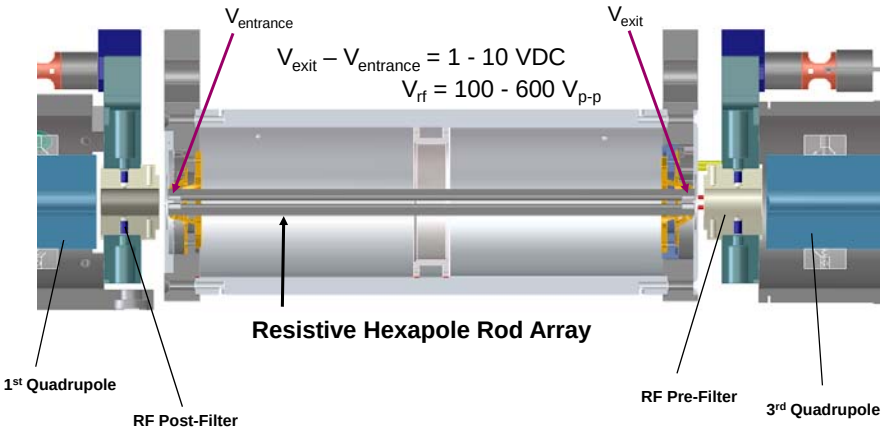
6510 Q-TOF



Over 10 Years in LC/MS Atmospheric Sampling and Patented Orthogonal Geometry - Result in Industry Leading Sensitivity and Robustness

Axial Acceleration Collision Cell

Designed for QQQ and QTOF Mass Spectrometers



Shown is the Agilent 6410 Triple Quad

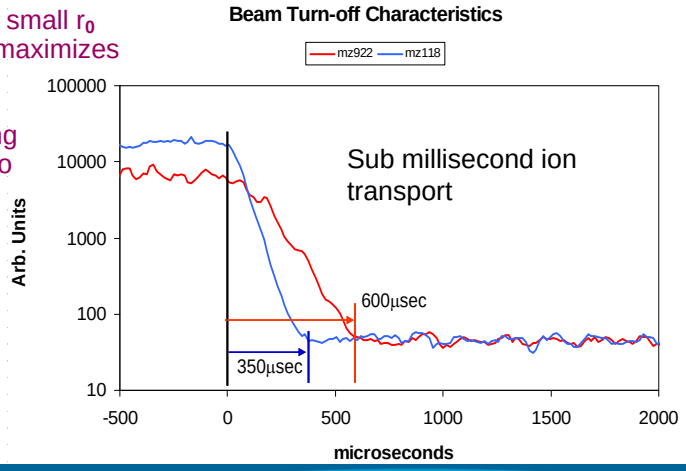
Axial Acceleration Collision Cell

Sub-millisecond ion transport times

Axial acceleration overcomes memory or cross-talk effects

Wide mass range small r_0 hexapole design maximizes sensitivity

Single accelerating potential is easy to operate.



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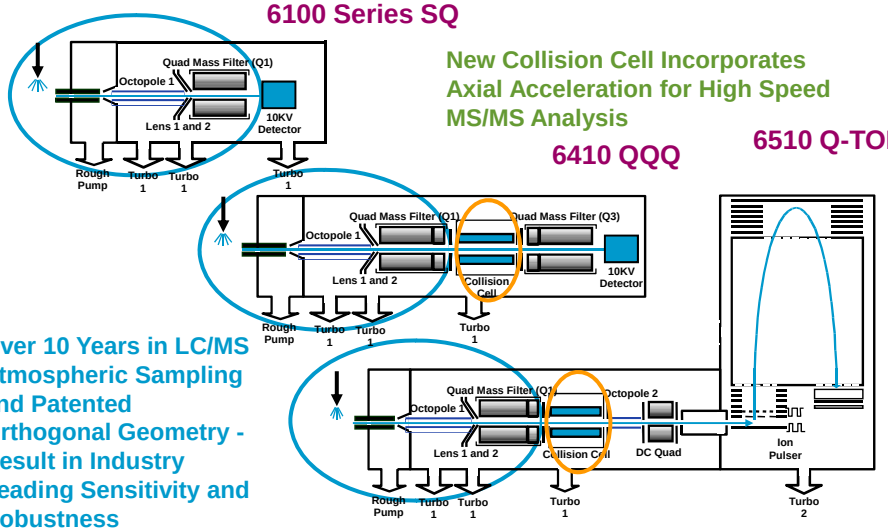


New Collision Cell Incorporates Axial Acceleration for High Speed MS/MS Analysis

6410 QQQ

6510 Q-TOF

Over 10 Years in LC/MS Atmospheric Sampling and Patented Orthogonal Geometry - Result in Industry Leading Sensitivity and Robustness

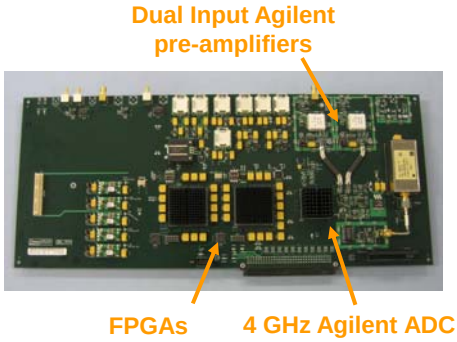
New Ultra High Speed Acquisition

Matching TOF/QTOF Analyzers and Electronics Performance

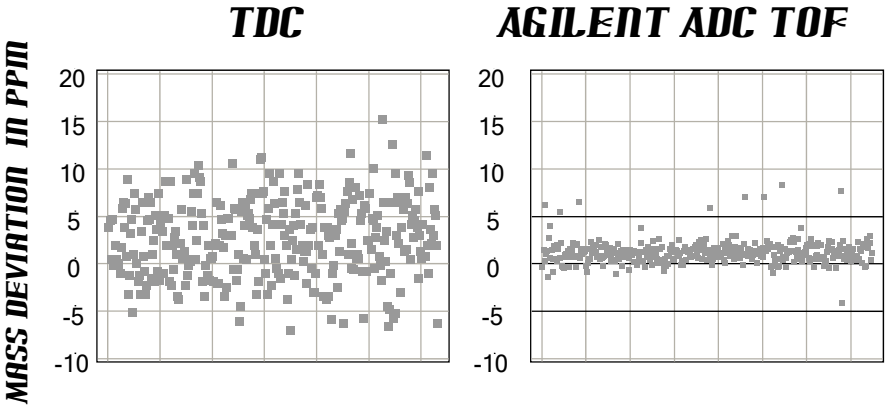
Two New Modes: **4 GHz for Enhanced Resolving Power**

2-Channel x 2 GHz Dual Gain for Extended Dynamic Range

- 4 GHz (8 bit) Analog-Digital-Converter **ADC**
  - Adapted from Agilent's High Speed Oscilloscope Systems
- Ultra High Speed **FPGA** process and store transients in real time
  - Up to 20,000 m/z depth

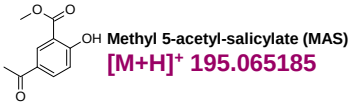


Mass Accuracy: Terfenadin

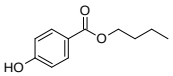


Enhanced Resolving Power

High Mass Accuracy for Isobars over Wide Dynamic Range

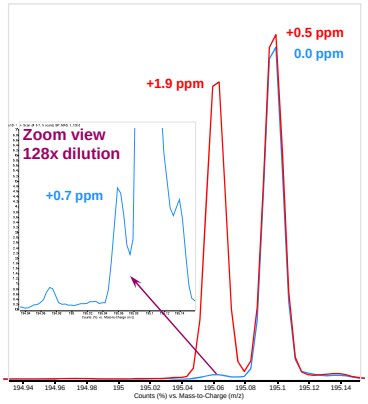


Methyl 5-acetyl-salicylate (MAS)
$[M+H]^+$ 195.065185

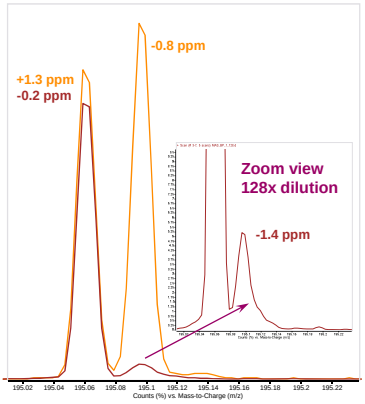


Butyl paraben (BP)
$[M+H]^+$ 195.101571

Dilute MAS, keep BP constant

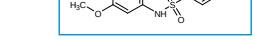


Keep MAS constant, dilute BP

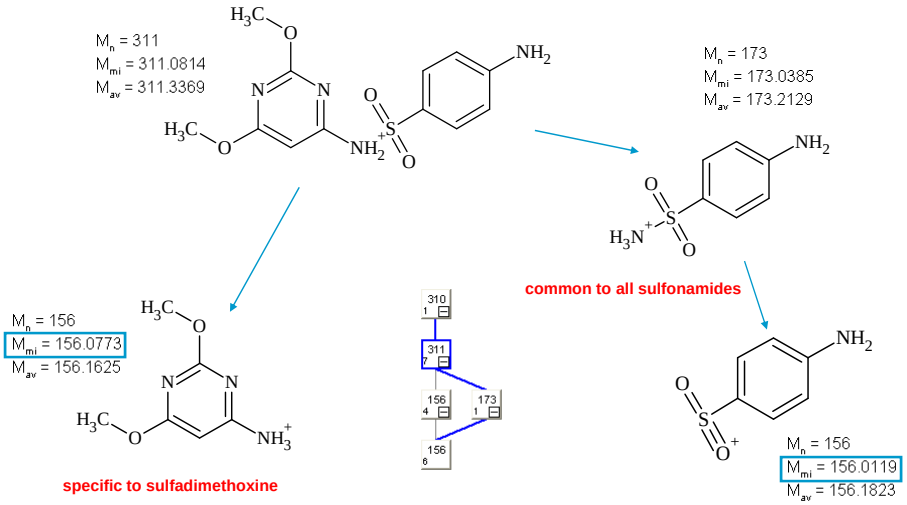


Molecular Formula Generation from Isotopic Pattern

Sulfadimethoxine

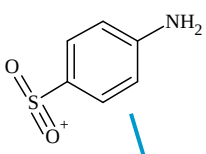


MS/MS Fragmentation Pathways

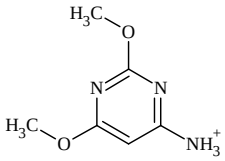


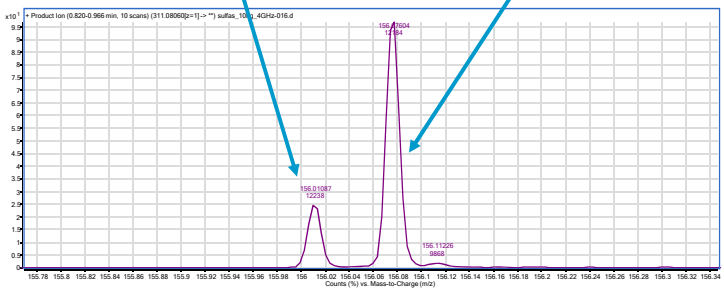
Sulfadimethoxine – MS/MS Fragmentation

m/z 156.0119



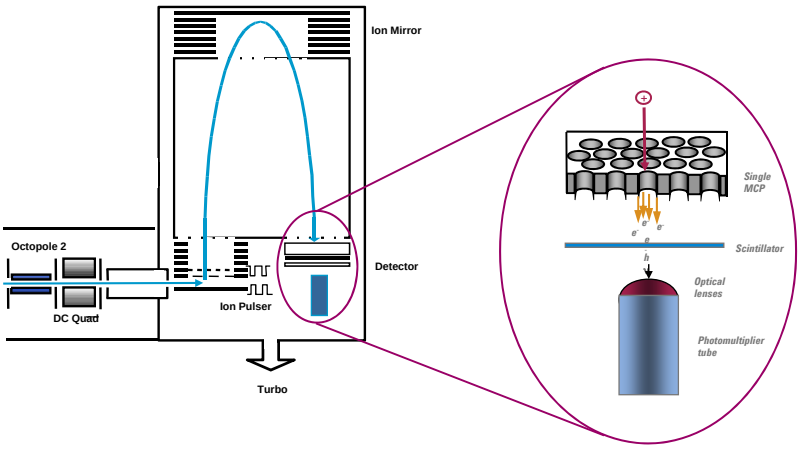
m/z 156.0773





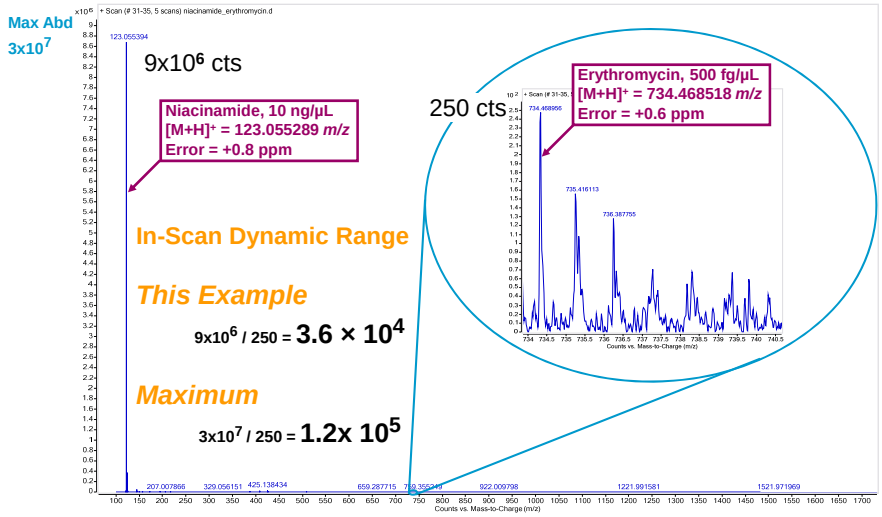
High Output PMT Based TOF Detector

PMT delivers needed output current for wide dynamic range



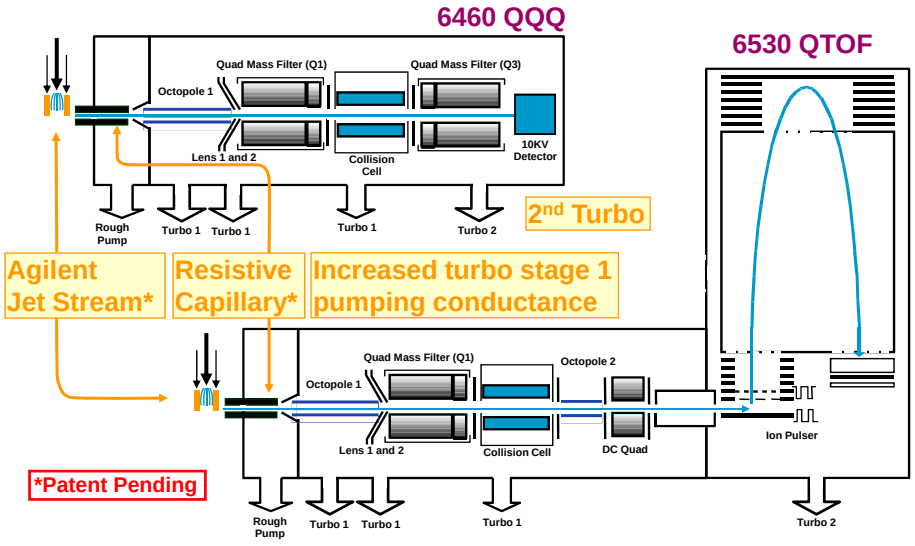
New 4GHz Acquisition System

up to 5 Decades of In-Spectrum Dynamic Range



Agilent 6460 QQQ and 6530 QTOF

What Else is New ?



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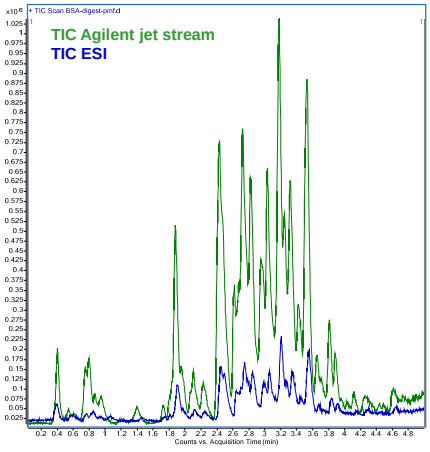
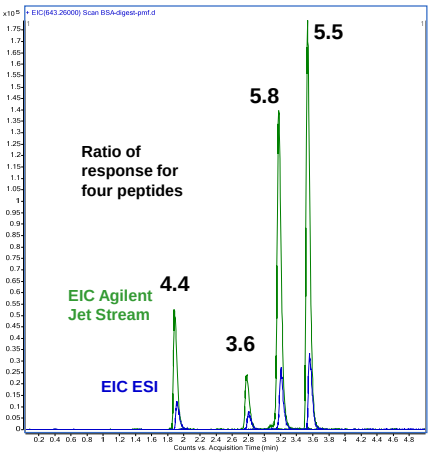
Agilent 6530 QTOF Performance

Agilent Jet Stream vs. ESI For 1 pmol BSA Digest – MS Mode Peptide Map





Protein Identification Summary

Sum Of Two Injections Per Condition, AutoMS/MS Mode

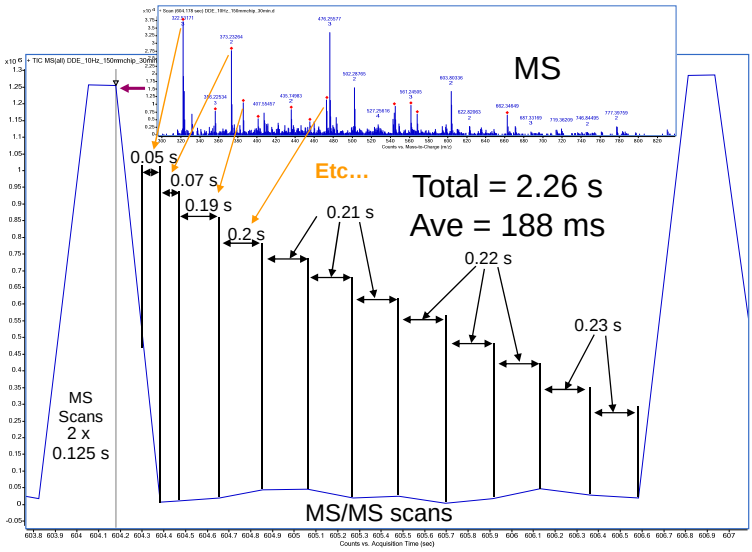
Significant gains at low concentrations

Amount Injected	# unique peptides		Protein Score		Sequence Coverage (%)	
	ESI	Agilent Jet Stream	ESI	Agilent Jet Stream	ESI	Agilent Jet Stream
100 fmol	9	21	111	282	15	45
200 fmol	17	28	209	399	31	51
500 fmol	26	31	374	476	44	57
1000 fmol	33	42	494	674	59	69

3x

Agilent QTOF – Accelerating Data Dependent MS/MS

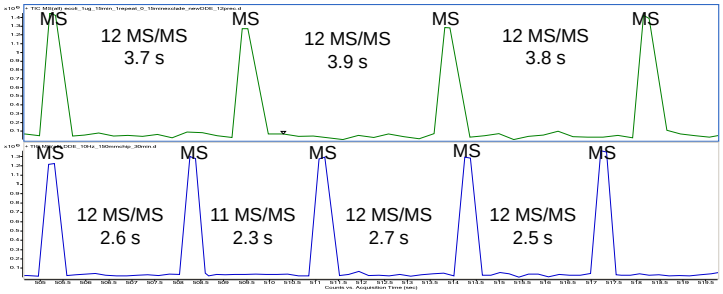
The intensity of the precursor ion is used to set the number of MS/MS transients



Agilent QTOF – Accelerating Data Dependent MS/MS

Initial
8 Hz MS
3 Hz MS/MS

Accelerated
8Hz MS
4.6 Hz MS/MS

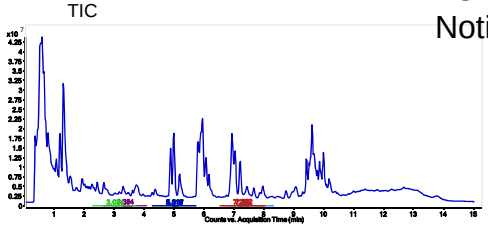


Auto MS/MS Parameter	Initial	Faster	Accelerated
MS/MS acquisition rate (spectra/s)	3	4	4.6
Excluded after __ spectra	2	1	1
Released after __ min	0.30	0.15	0.15
Valid ID's in a **20 min LC/MS/MS**	**693**	1069	**1162**
run of trypsin-digested *E. coli* proteins	**179**	234	**247**

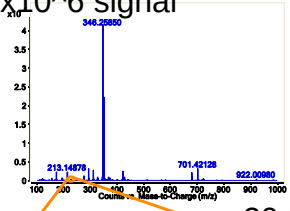
Triazines Spiked Into Tomato Matrix, 500 ppt
6530 QTOF, MS Mode, Extended Dynamic Range

2GHz Extended Dynamic range
Notice 4x10^6 signal

TIC

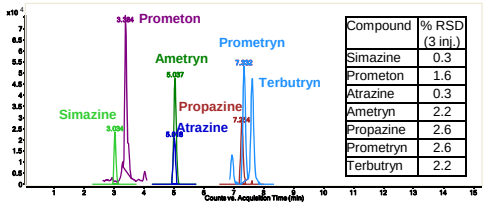


Spectrum at 5.018 min



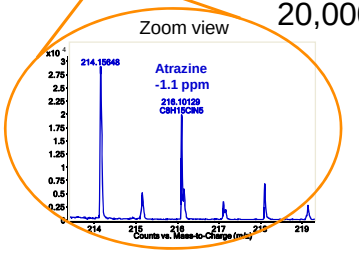
20,000 c

EICs



Compound	% RSD (3 inj.)
Simazine	0.3
Prometon	1.6
Atrazine	0.3
Ametryn	2.2
Propazine	2.6
Prometryn	2.6
Terbutryn	2.2

Zoom view

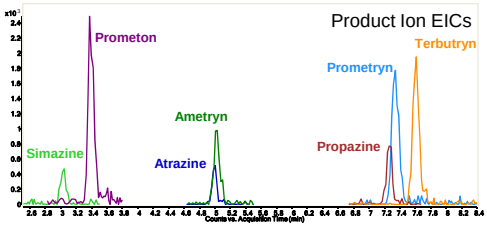


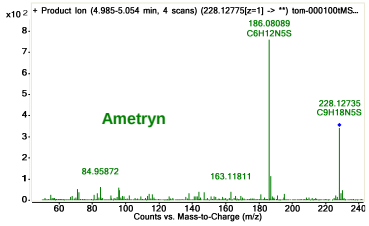
Note: on-column amount is 5 pg (10 µL x 500 ppt).
Identification done by database searching

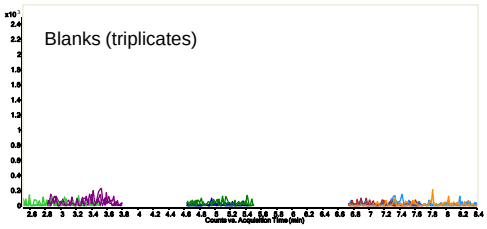
Triazines Spiked Into Tomato Matrix, 100 ppt
6530 QTOF, Targeted MS/MS Mode

On-column amount is 1 pg (10 µL x 100 ppt)

Compound	Precursor	Product Ion(s)	Collision energy, V	% RSD (3 inj.)
Simazine	202.0849	124.0868+132.0317	20	13.2
Prometon	226.1662	142.0717+184.1189	20	1.5
Atrazine	216.1010	174.0539	20	16.0
Ametryn	228.1277	186.0803	20	10.1
Propazine	242.1435	158.0487+200.0962	20	17.2
Prometryn	230.1177	146.0226+188.0692	25	14.2
Terbutryn	242.1435	186.0803	20	12.6

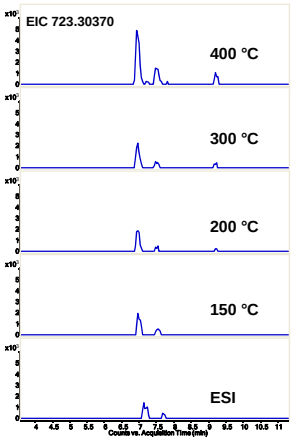




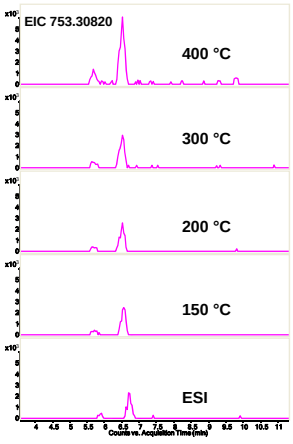


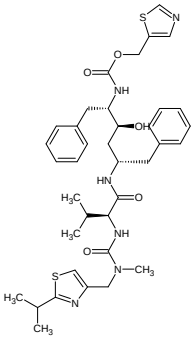
Ritonavir Microsomal Incubation Minor Metabolites
Effect Of Agilent jet stream Sheath Gas Temperature

Demethyl hydroxy metabolites



Dihydroxy metabolites



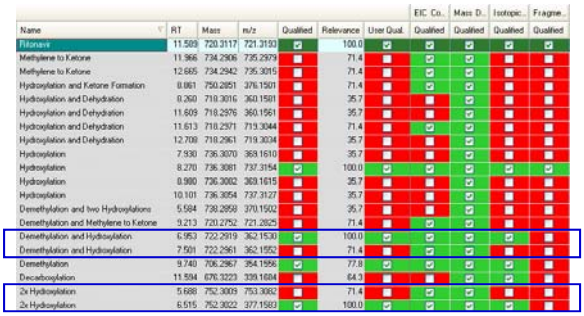


10 µL injection for all samples

Ritonavir Microsomal Incubation Minor Metabolites Metabolite ID Results

Agilent jet stream

These are the minor metabolites shown on the previous slide



Name	RT	Mass	m/z	Qualified	Relevance	User Qual.	EIC Co.. Qualified	Mass D.. Qualified	Isotopic.. Qualified	Fragme.. Qualified
Ritonavir	11.589	720.3117	721.3193	☑	100.0	☑	☑	☑	☑	☑
Methylene to Ketone	11.366	734.2906	735.2979	☐	71.4	☐	☑	☑	☐	☐
Methylene to Ketone	12.665	734.2942	735.3015	☐	71.4	☐	☑	☑	☐	☐
Hydroxylation and Ketone Formation	8.861	750.2851	751.2924	☐	71.4	☐	☑	☑	☐	☐
Hydroxylation and Dehydration	9.260	718.3016	719.3089	☐	35.7	☐	☐	☑	☐	☐
Hydroxylation and Dehydration	11.609	718.2976	719.3049	☐	35.7	☐	☐	☑	☐	☐
Hydroxylation and Dehydration	11.613	718.2971	719.3044	☐	71.4	☐	☑	☑	☐	☐
Hydroxylation and Dehydration	12.709	718.2961	719.3034	☐	35.7	☐	☐	☑	☐	☐
Hydroxylation	7.930	736.3070	737.3143	☐	35.7	☐	☐	☑	☐	☐
Hydroxylation	8.270	736.3081	737.3154	☑	100.0	☑	☑	☑	☑	☑
Hydroxylation	8.980	736.3082	737.3155	☐	35.7	☐	☐	☑	☐	☐
Hydroxylation	10.101	736.3054	737.3127	☐	35.7	☐	☐	☑	☐	☐
Demethylation and two Hydroxylations	5.584	738.2958	739.3031	☐	35.7	☐	☐	☑	☐	☐
Demethylation and Methylene to Ketone	9.213	720.2752	721.2825	☐	71.4	☐	☑	☑	☐	☐
Demethylation and Hydroxylation	6.953	722.2919	723.2992	☑	100.0	☑	☑	☑	☑	☐
Demethylation and Hydroxylation	7.501	722.2961	723.3034	☐	71.4	☐	☑	☑	☐	☐
Demethylation	9.740	706.2967	707.3040	☑	77.8	☑	☑	☑	☑	☐
Decarboxylation	11.594	676.3223	677.3296	☐	64.3	☐	☐	☑	☑	☐
2x Hydroxylation	5.688	752.3009	753.3082	☐	71.4	☐	☑	☑	☐	☐
2x Hydroxylation	6.515	752.3022	753.3095	☑	100.0	☑	☑	☑	☑	☐

Note! The literature reference shows 11 metabolites using 2X the injection volume and 3X the gradient length.

ESI

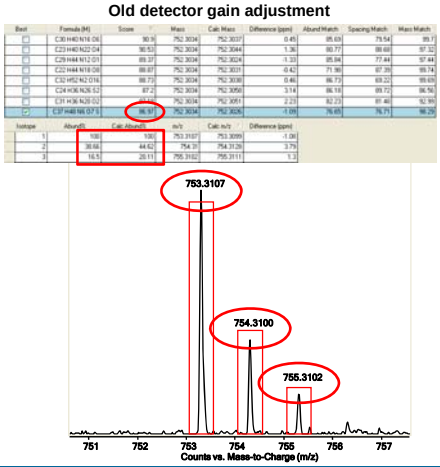
No other minor metabolites detected

Name	RT	Mass	m/z	Qualified	Relevance	User Qual.	EIC Co.. Qualified	Mass D.. Qualified	Isotopic.. Qualified	Fragme.. Qualified
Ritonavir	11.626	720.3140	721.3213	☑	100.0	☑	☑	☑	☑	☑
Hydroxylation and Dehydration	11.644	718.2974	719.3046	☐	71.4	☐	☑	☑	☐	☐
Hydroxylation	8.317	736.3084	737.3159	☑	100.0	☑	☑	☑	☑	☑
Demethylation	9.789	706.2956	707.3035	☐	71.4	☐	☑	☑	☐	☐

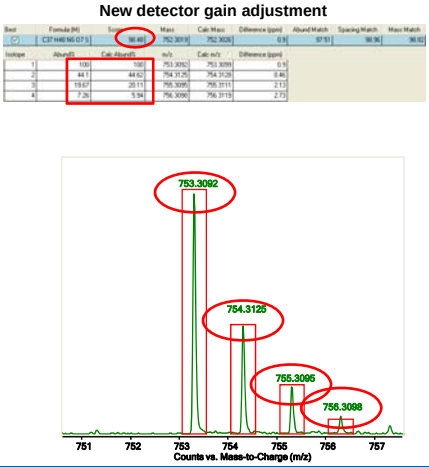
Ritonavir Minor Metabolite—MFG Improvements

- Modified detector gain adjustment and optimized MFG scoring algorithm
- [A+1]/[A] ratio improved from 87% of theoretical to 99%
- MFG score improved from 87 to 98
- MFG ranking improved from 8th place to 1st place on hit list

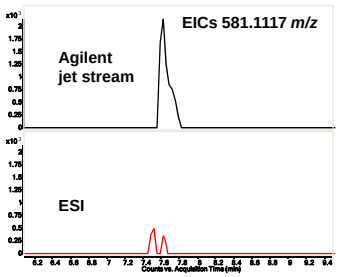
Old detector gain adjustment

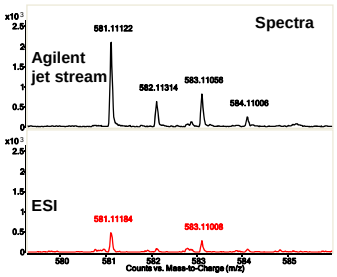


New detector gain adjustment



Diclofenac Microsomal Incubation
Minor Metabolites Trapped With Glutathione





Metabolite ID results

Agilent jet stream

Two glutathione conjugates detected

Name	RT	Mass	m/z	Qualified	Relevance	User Qual	EIC Co.. Qualified	Mass D.. Qualified	Isotopic.. Qualified
Hydroxylation	11.726	311.0129	310.0056	✓	100.0	✓	✓	✓	✓
Glutathione Conjugation, hydroxylation -HCl	7.617	582.1190	581.1117	✓	100.0	✓	✓	✓	✓
Glutathione Conjugation and hydroxylation	8.960	616.0901	615.0729		66.7		✓	✓	
Diclofenac	14.674	295.0146	294.0073	✓	100.0	✓	✓	✓	✓

ESI

No glutathione conjugates detected

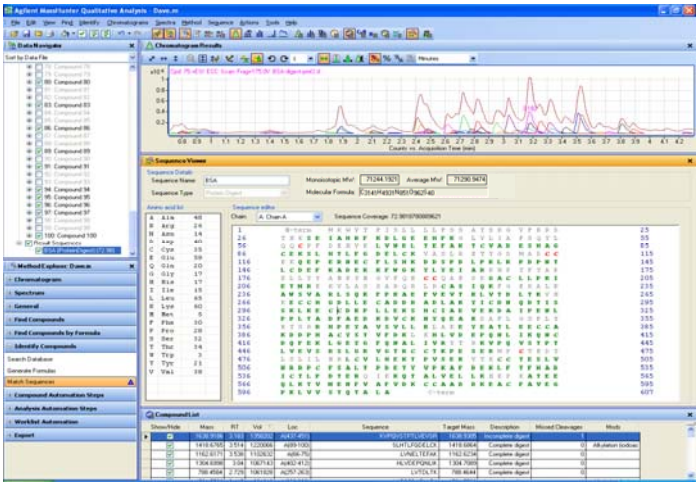
Name	RT	Mass	m/z	Qualified	Relevance	User Qual	EIC Co.. Qualified	Mass D.. Qualified	Isotopic.. Qualified
Hydroxylation	11.576	311.0121	310.0048	✓	100.0	✓	✓	✓	✓
Diclofenac	14.710	295.0176	294.0103	✓	100.0	✓	✓	✓	✓

Our Agenda…

- Start with the Agilent R&D History
- New product specifications. They're fantastic!
- Learn about Agilent Jet Stream Technology
- Discover a new level of QTOF performance in the 6530 QTOF
- Find out how software innovation makes old workflows better and new workflows possible

BioConfirm B.02.00 – Integrated into Mass Hunter

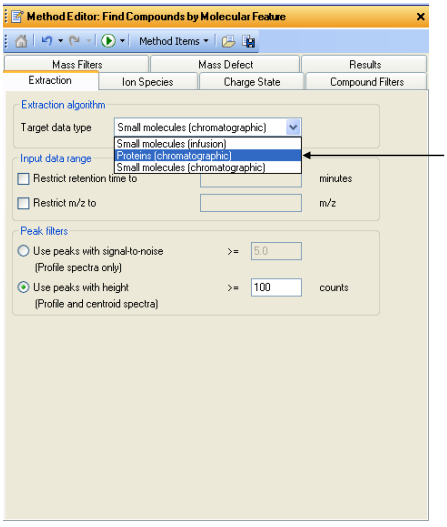
Sample: BSA digest, 6530 Accurate Mass QTOF
2.1mm,1.8um Column



Sequence Editor-Matcher now embedded in Qualitative Analysis

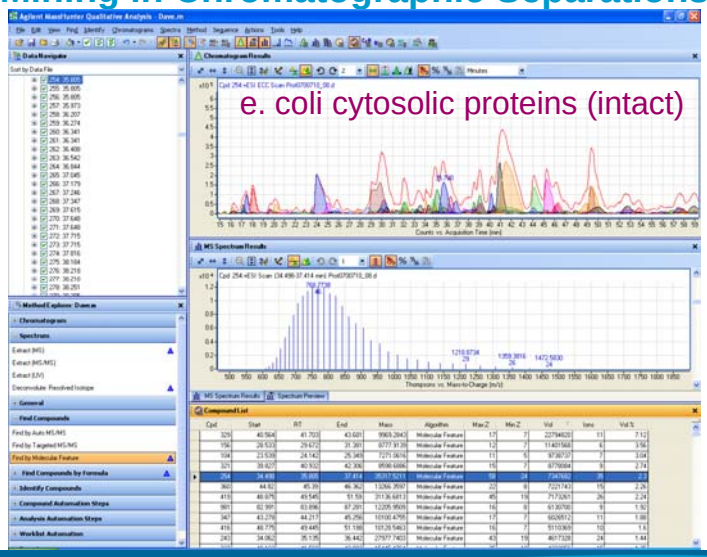
Matched peptides in compound list

Introducing Molecular Feature Extractor for Intact Proteins



- This algorithm is completely unique to Agilent
- LC/MS-based method vs. mass spectrum-based method for deconvolution
- 10-50x faster than Maximum Entropy Deconvolution for complex LC/MS data
- No previous knowledge of protein mass is required
- Works like “small molecule” MFE, but instead of grouping co-eluting isotopes, co-eluting charge states are grouped into a compound

Intact Protein MFE for Comprehensive Protein Mining in Chromatographic Separations



Protein compound EIC's and TCC

Charge states for protein compound

Protein masses in compound list

MFE Results of Keller Mix in Mass Profiler

Currently under development, supported in future release

This should prove to be a powerful tool for analyzing batch to batch variations in the production of Biologics.