Ultra-High Sensitivity in Triple Quadrupole LC/MS/MS Performance

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with contributions from
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The Agilent 6460: Cutting Edge QQQ Performance

With…

Agilent Jet Stream Technology

Unmatched sensitivity

Workflow improvements

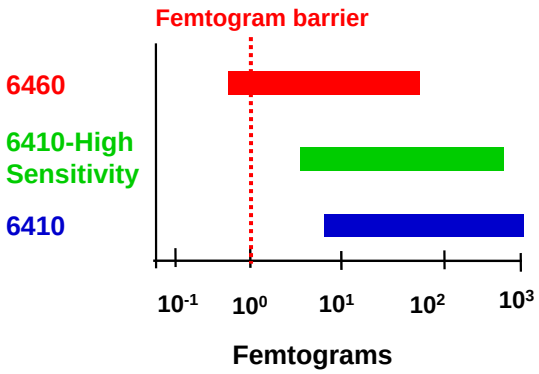
Faster method development

The industry's most sensitive QQQ

- ☑ 100fg reserpine sensitivity with less than 10% RSD !
- ☑ Fast Pos/Neg switching
- ☑ Faster MRMs and more MRMs per time segment
- ☑ New Optimizer software enables faster MS/MS method development

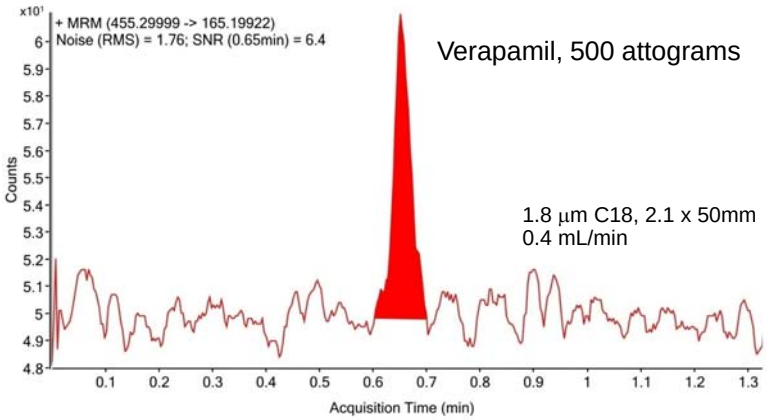
Breaking the “fg barrier” with the new 6460A Triple Quad LC/MS/MS

Performance of Agilent QQQ models



Agilent 6460 QQQ Performance

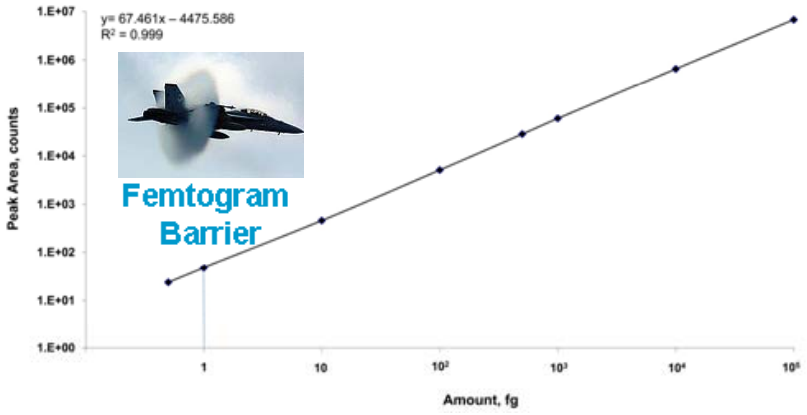
Shattering the Femtogram Barrier – 500 Attograms



Breakthrough Sensitivity

6460 triple quadrupole with Agilent Jet Stream technology breaks the femtogram barrier, shown here with 500 attograms of verapamil injected on-column, using unit resolution for both Q1 and Q3.

Agilent 6460 QQQ Performance –
500 attograms verapamil and extended dynamic linear range



Five Decades of Linearity
6460 triple quad with Agilent Jet Stream technology exhibits outstanding performance with 5 decades of linearity from sub-femtogram to 100 picograms of verapamil injected on-column.

Why focus on improving the efficiency of ESI?

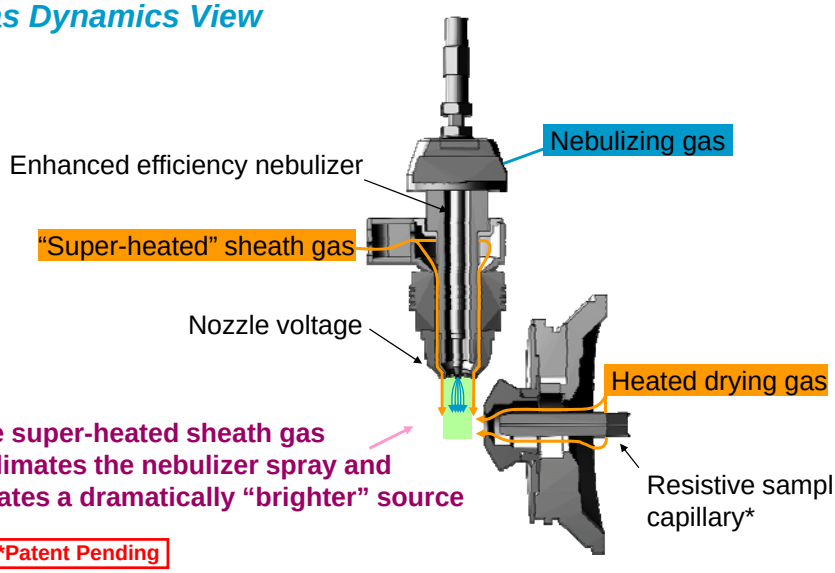
Ionization and Transmission Efficiency in an Electrospray Ionization–Mass Spectrometry Interface
Jason S. Pagea, Ryan T. Kellya, Keqi Tanga and Richard D. Smith,
Biological Sciences Division, Pacific Northwest National Laboratory, Richland, Washington, USA

Journal of the American Society for Mass Spectrometry
Volume 18, Issue 9, September 2007, pages 1582-1590

"Ion transmission efficiency, also defined as the fraction of ES current that enters the mass analyzer, has traditionally been limited by losses at the mass spectrometer inlet and at the skimmer [[7] and [21]]. It has been estimated that only about **one out of every 10^3–10^5** analyte ions generated by ESI at atmospheric pressure is actually detected using present instrument designs [[7], [10] and [22]]."

Agilent Jet Stream* Ion Generation

Gas Dynamics View



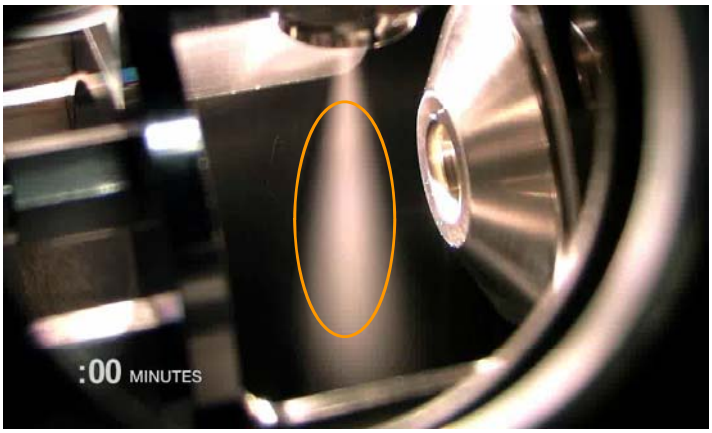
The super-heated sheath gas collimates the nebulizer spray and creates a dramatically "brighter" source

***Patent Pending**

Agilent Jet Stream animation

Agilent Jet Stream In Action

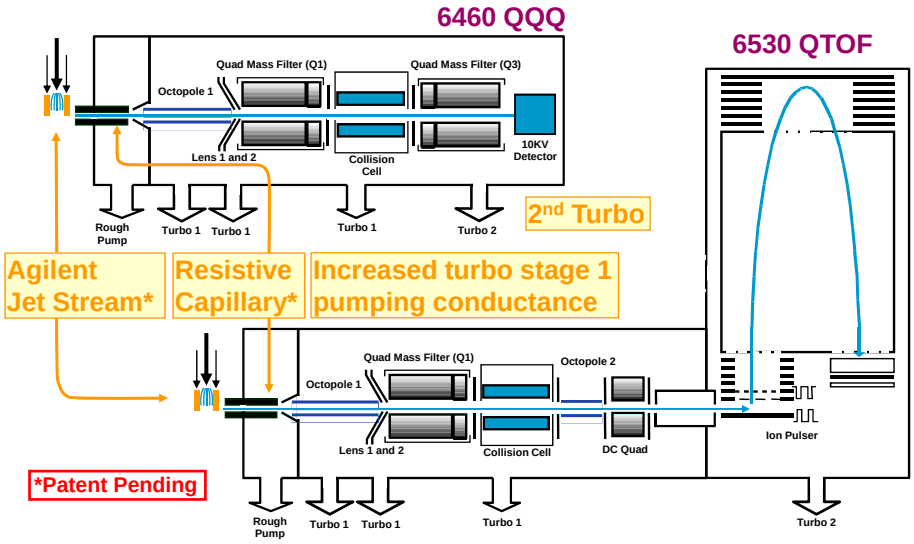
Observing Thermal Gradient Focusing



Fast-forward video of sheath gas heating from ambient to 400°C over 8 minutes

Agilent Jet Stream available on 6460 QQQ, 6x30 TOF/QTOF

What else is new for these systems?



Reserpine sensitivity comparisons

6460 QQQ: > >> Signal/Noise vs. 6410 QQQ for 500 femtogram injections on column
Noise = 3x RMS noise

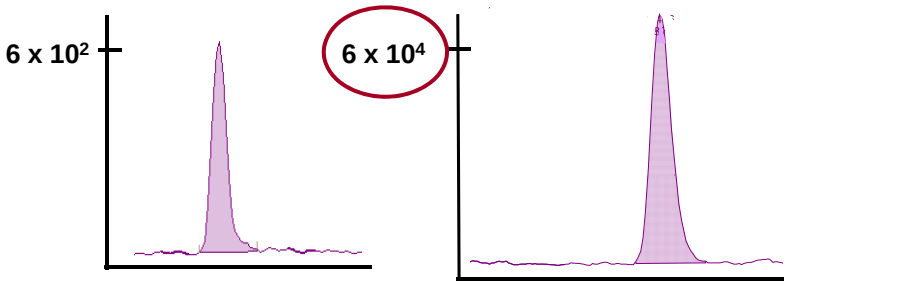
6410 QQQ

SNR = 36:1

New 6460 QQQ

SNR = 354:1

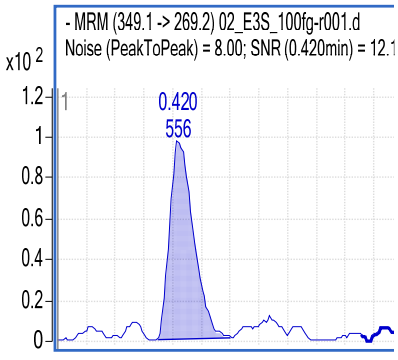
10x improvement



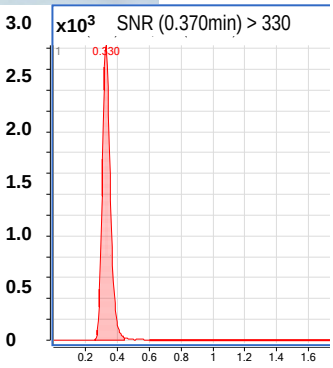
Limits of Detection (Estrone 3- sulfate) in Negative Ion Mode

100 fg on-column
LOD 25 femtogram

100 fg on-column
LOD 0.9 femtogram!



6410

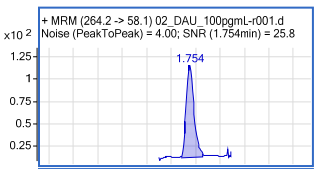


6460 **Sub-Femtogram LOD!**

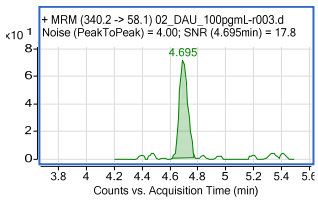
6460 Triple Quad compared to 6410 Triple Quad

6410 Triple Quad:

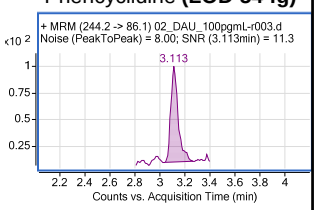
Tramadol **(LOD 23 fg)**



Propoxyphene **(LOD 33 fg)**

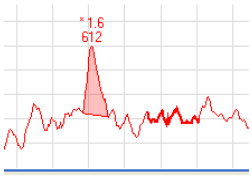


Phencyclidine **(LOD 54 fg)**



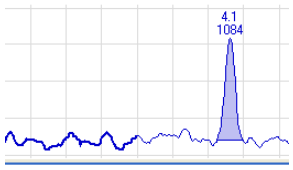
6460 Triple Quad:

Tramadol **(LOD 3 fg)**



6460 8 x more S/N

Propoxyphene **(LOD 2 fg)**



6460 16 x more S/N

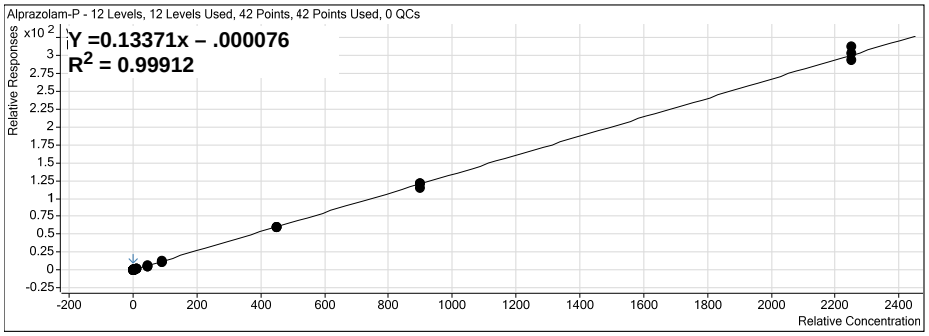
Phencyclidine **(LOD 3.5 fg)**

6460 15 x more S/N

Five Orders of Linearity with new 6460 Triple Quad

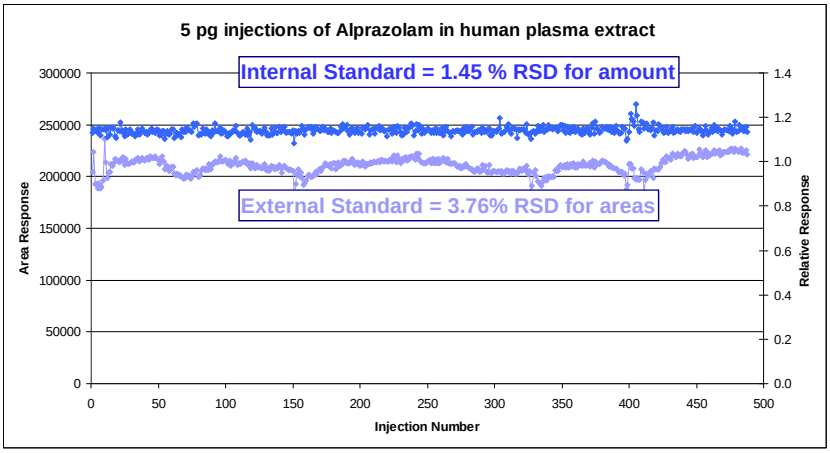
Alprazolam 22 femtograms to 22 nanograms!

12 calibration levels



Agilent Jet Stream Performance
Ruggedness & Reproducibility – 6460 QQQ

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

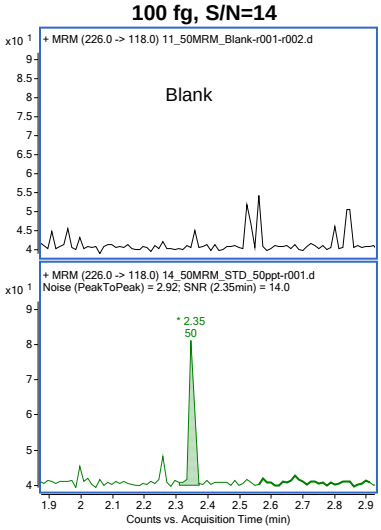


6460 QQQ performance: some additional application examples

Cyprodinil: LOD 21 femtograms
with 100 msec +/- switching and 5 msec dwell times

Positive ion

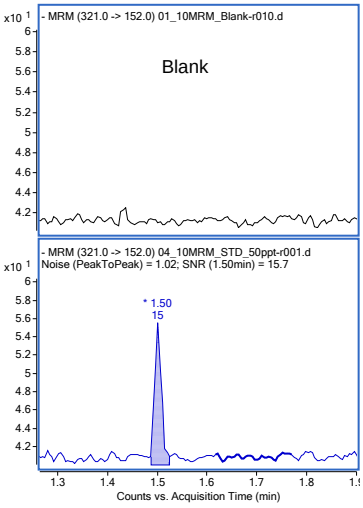
100 fg, S/N=14



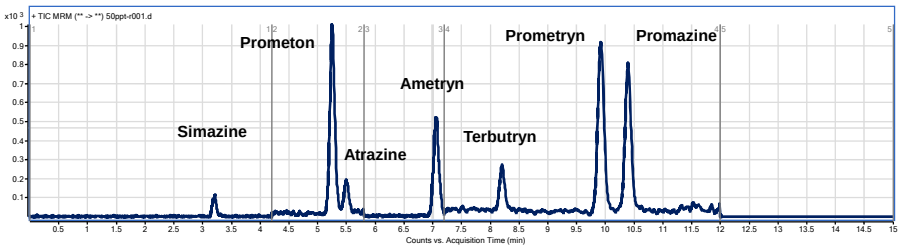
Chloramphenicol: LOD 50 femtograms
with 100 msec +/- switching and 5 msec dwell times

Negative ion

250 fg, S/N=15.7

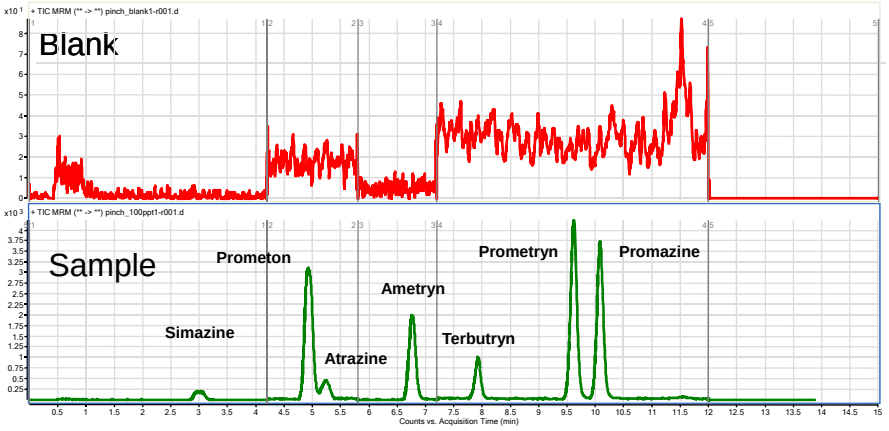


Agilent 6460 QQQ
Trace analysis of pesticides



Solvent Std, 1uL injection, 50ppt (50 fg each on-column)

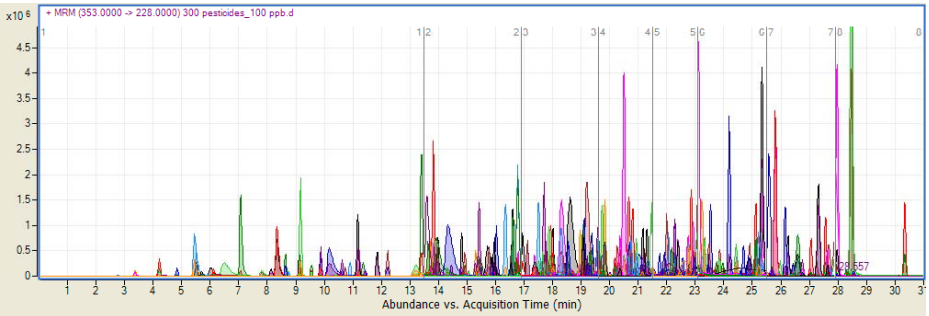
Agilent 6460 QQQ
Trace analysis of pesticides



Herbicides in Spinach Matrix -1uL injection, 100ppt level
(100 fg each on-column)

LC/QQQ - MRM EICs of 301 Pesticides for analysis of water or foods

Agilent 1200 HPLC With 1.8 micron ZORBAX SB C-18 Column.

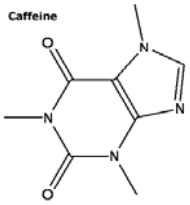


Application note 5989-8614EN
lists precursor, two product ions, and MS parameters for all **301** compounds

Identification of PPCPs in Water The Use of LC/MS/MS

Pharmaceuticals and Personal Care Products (PPCPs) refer, in general, to any product used by individuals for personal health or cosmetic reasons or used by agribusiness to enhance growth or health of livestock.

PPCPs comprise a diverse collection of thousands of chemical substances, including prescription and over-the-counter therapeutic drugs, veterinary drugs, fragrances, and cosmetics.



EPA 1694 - Identification of PPCPs in Water

Antibiotics

Pain Killers

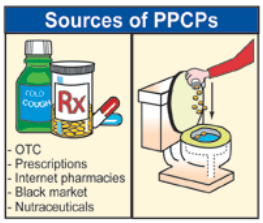
Birth Control Steroids

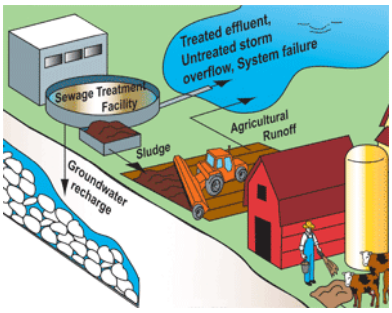
Anti-Depressants

Acetaminophen
Albuterol
Ampicillin
Anhydrochlortetracycline (ACTC)
Anhydrotetracycline (ATC)
Azithromycin
Caffeine
Carbadox
Carbamazepine
Cefotaxime
Chlortetracycline (CTC)
Cimetidine
Ciprofloxacin
Clarithromycin
Clinafloxacin
Cloxacillin
Codeine
Cotinine
Dehydronifedipine
Demeclocycline
Digoxigenin
Digoxin
Diltiazem
1,7-Dimethylxanthine
Diphenhydramine
Doxycycline
Enrofloxacin
4-Epianhydrochlortetracycline (EACTC)
4-Epianhydrotetracycline (EATC)
4-Epichlortetracycline (ECTC)
4-Epioxytetracycline (EOTC)
4-Epitetracycline (ETC)
Erythromycin
17α-Ethinyl estradiol
Flumequine
Fluoxetine
Gemfibrozil
Ibuprofen
Isochlortetracycline (ICTC)
Lincomycin
Lomefloxacin
Meclofenamic
Miconazole
Minocycline
Naproxen
Norfloxacin
Norgestimate
Ofloxacin
Ormetoprim
Oxacillin
Oxolinic acid
Oxytetracycline (OTC)
Penicillin V
Penicillin G
Ranitidine
Roxithromycin
Sarafloxacin
Sulfachloropyridazine
Sulfadiazine
Sulfadimethoxine
Sulfamerazine
Sulfamethazine
Sulfamethizole
Sulfamethoxazole
Sulfanilamide
Sulfathiazole
Tetracycline (TC)
Thiabendazole
Triclocarban
Triclosan
Trimethoprim
Tylosin
Virginiamycin
Warfarin
Other standards

Where are PPCPs found in the environment?

PPCPs in the environment are frequently found in aquatic environments because PPCPs dissolve easily and don't evaporate at normal temperature and pressure. Practices such as the use of sewage sludge ("biosolids") and reclaimed water for irrigation bring PPCPs into contact with the soil (landfill also).





Issues with PPCPs

- Large quantities enter the environment
- Sewage systems are not equipped for PPCP removal
- Risks are unknown
  - Resistance to antibiotics
  - Disruption of aquatic endocrine systems
- Effects on aquatic life are a major concern. Exposure risks for aquatic organisms are much larger than those for humans. Aquatic organisms have:
  - continual exposures
  - multi-generational exposures
  - exposure to higher concentrations of PPCPs in untreated water
  - possible low dose effects (subtle effects on ecological receptors)

http://www.epa.gov/ppcp

EPA Method 1694: Pharmaceuticals and Personal Care Products in Water Soil, Sediment, and Bio-solids by LC/MS/MS

Implentation on the Agilent 6410B with improvements

Imma Ferrer[1], E. Michael Thurman[1], and Jerry Zweigenbaum[2]

[1]Center for Environmental Mass Spectrometry, University of Colorado, Boulder, CO and [2]Agilent Technologies, Inc. Wilmington, DE

Agilent Method with Additional Confirmation

To provide additional confirmation for the EPA method, a second MRM transition was added for 60 of the 65 analytes analyzed. This gives higher confidence of correct identification and quantification than prescribed by EPA.

EU identification points:

- 1 Precursor Ion — 1 point
- 2 Product Ions — 3 points
- Retention Time — 1 point

Total = 5 points (satisfies EU identification requirements - similar to degree of confirmation required by spectral searching)

List of Group 1 Compounds EPA 1694: 46 Analytes

Acetaminophen	Codeine	Lincomycin	Sarafloxacin	Sulfanilamide
Ampicillin	Cotinine	Lomefloxacin	Sulfachloro-pyridazine	Thiabendazole
Azithromycin	Dehydronifedipine	Miconazole	Sulfadiazine	Trimethoprim
Caffeine	Digoxigenin	Norfloxacin	Sulfadi-methoxine	Tylosin
Carbadox	Diltiazem	Ofloxacin	Sulfadiazine	Virginiamycin
Carbamazepine	1,7-Dimethyl-xanthine	Oxacillin	Sulfadi-methoxine	
Cefotaxime	Diphenhydramine	Oxolinic acid	Sulfamerazine	
Ciprofloxacin	Enrofloxacin Erythromycin	Penicillin G	Sulfamethazine	
Clarithromycin	Flumequine	Penicillin V	Sulfamethizole	
Cloxacillin	Fluoxetine	Roxithromycin	Sulfa-methoxazole	

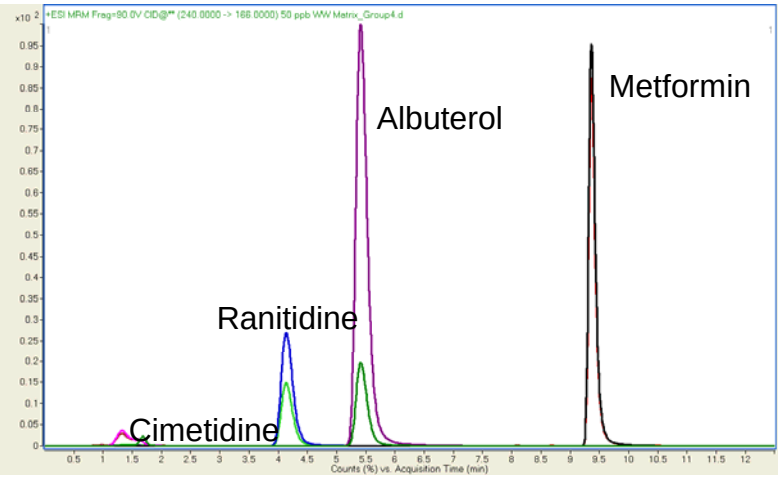
List of Group 2, 3 and 4 Compounds: EPA 1694: 19 Analytes

Anhydrotetracycline	Doxycycline	Minocycline	Triclocarban Triclosan Warfarin
Chlorotetracycline	4-Epianhydrotetra-cycline	Tetracycline Meclocycline	Albuterol Cimetidine Metformin
Demeclocycline	4-Epitetracycline	Gemfibrozil Ibuprofen Naproxen	Ranitidine

List of Labeled Internal Standards

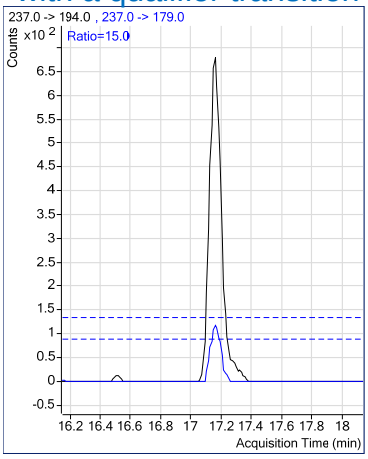
13C-15N-Acetaminophen	13C2-Erythromycin	13C6-Sulfamethazine	13C3-Trimethoprim
13C3-Atrazine	Fluoxetine-d6	13C6-Sulfamethoxazole	Warfarin-d5
13C3-Caffeine	Gemfibrozil-d6	13C6-2,4,5-Tricloro-phenoxyacetic acid	Carbamazepine-d10 (Extra compound, not EPA list)
13C3-15N-Cipro-floxacin	13C3-Ibuprofen	13C6-Triclocarban	
Cotinine-d3	13C-Naproxen-d3	13C12-Triclosan	

Overlaid EICs: Group 4 Compounds using HILIC

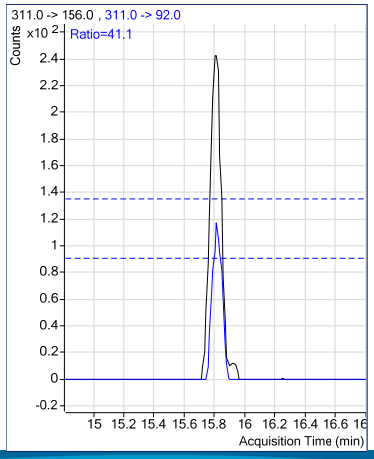


High-Confidence Identification/Confirmation Using Qualifier Ions

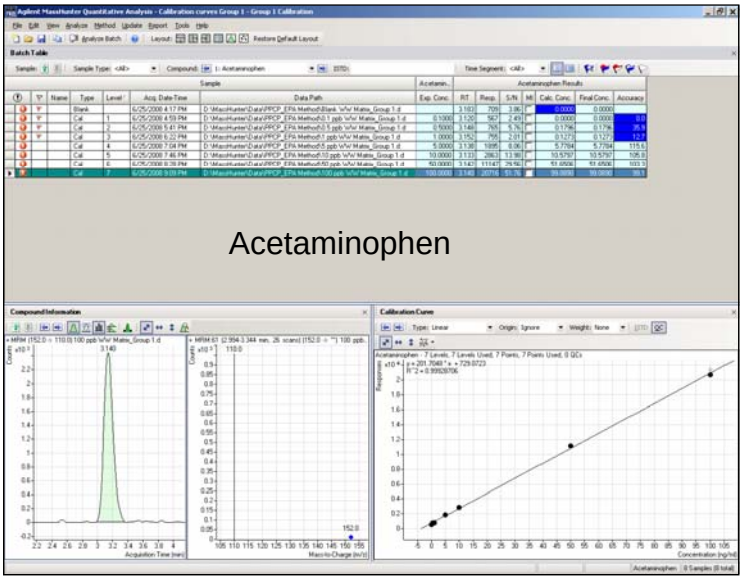
Carbamazepine
with a qualifier transition



Sulfadimethoxine
with a qualifier transition



Calibration Curves in Wastewater Matrix



Agilent MassHunter Workstation

More Innovations in Mass Spectrometry Software

MassHunter Optimizer
Automated MRM Method Development Software

Traditional MS/MS method development:

- Manual optimization of even three parameters, for dozens of compounds
  => MANY Days to WEEKS of tedious, interactive work

WITH Optimizer:

- Optimization can be fully automated for multiple compounds
  => One or a few DAYS of unattended work!

Compound-specific optimization for MRM experiments

- Selection and optimization of precursor and product ions
- Supports optimization with multiple methods (e.g. pos then neg)
- Creation of a compound database with optimized parameters for re-use

MassHunter Optimizer
Advantages over previous solutions

Utmost flexibility via support of all common optimization modes:

- Manual infusion (syringe pump)
- Automatic infusion (via loop injection at lower flows)
- Flow injection analysis without column (FIA)
- Analysis with column (multiple compounds per run)

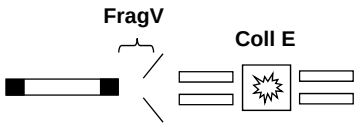
Optimization WITH column for highest success:

- Infusion or FIA can result in 20% of cmpds not optimizing
- Optimizer includes analysis WITH column using fast LC.

Version 2, early 2009: Automatic creation of LC/MS method for large numbers of compounds from Compound Database, using “Scheduled MRM”

MassHunter Optimizer

Step 5 – Execute Automatic Optimization



Run 1: The precursor ion is selected based on user criteria (adduct ions, charge states) and Fragmentor voltage is optimized

(optional): *fine adjustment of Fragmentor voltage*

Run 2: Coarse product ion scan finds the largest 4 product ions with corresponding Collision Energy (default CE 0, 10, 20, 30, 40).

(optional) *fine adjustment of Collision Energy*

Post Run: Populates Compound Database with the 4 best transitions w/ optimum Fragmentor Voltages and Collision Energies
Prints Optimization Report

MassHunter "Scheduled MRM"

Increased Utility and Performance

New applications require quantitation of 100 – 1000 compounds in one MRM method !

- Food and environmental analysis (e.g. pesticides)
- Targeted quantitation of proteins via peptides (proteomics)

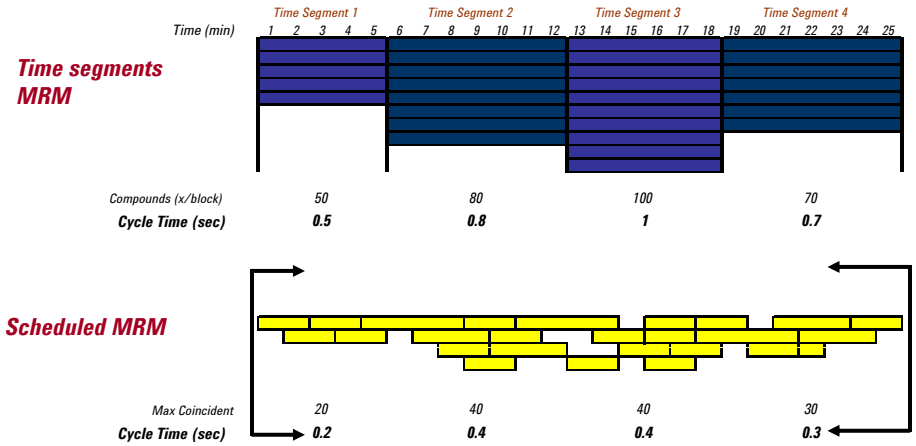
WITHOUT Scheduled MRM:

- Need to manually set up multiple time segments to maximize dwell times
- Tedious to set up; problematic if changes in retention times

WITH Scheduled MRM:

- Automatic setup of overlapping time segments without user intervention
- Fewer MRMs per unit time results in longer dwell time => incr sensitivity
- Unaffected by chromatographic time shifts

Scheduled MRM
Increased Utility and Performance



2x shorter cycle times better for narrow chromatographic peaks, more analytes, longer dwell time per analyte.

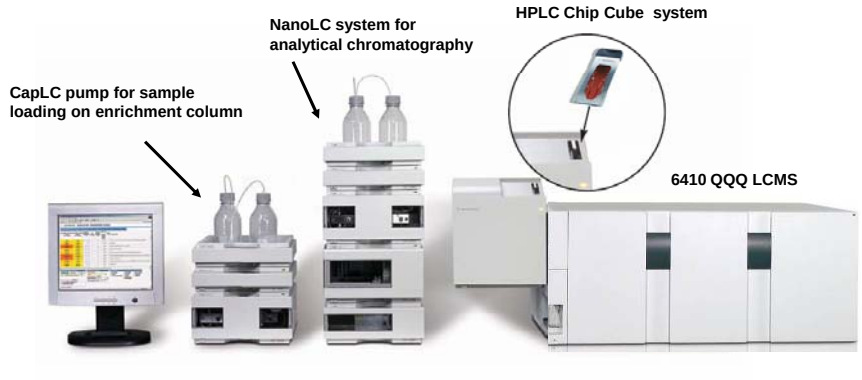
Application example for new software features – Optimizer and Scheduled MRM

Peptide quantitation from protein digests for protein biomarker studies

- SpectrumMill Peptide Selector to choose useful peptides
- QQQ Optimizer to create MRM methods for quantitation
- HPLC-Chip MS/MS for rapid and reliable quant with small sample quantities
- MassHunter Quantitative Analysis for efficient data processing

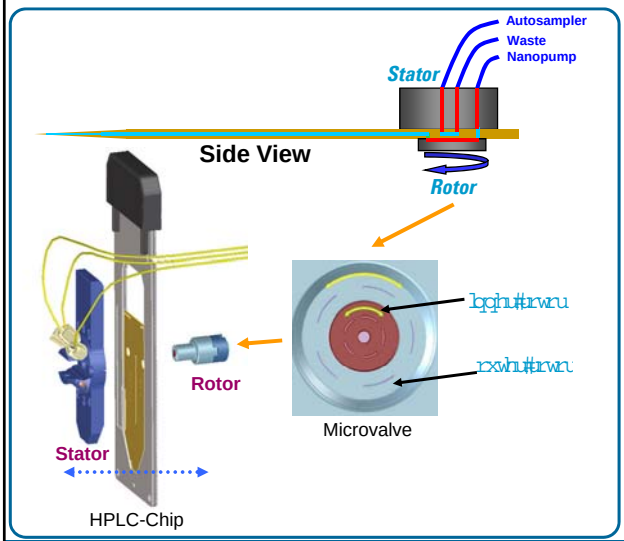
Agilent HPLC-Chip/QQQ LCMS Technology

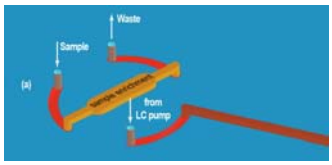
Nanospray chip configuration brings new era in high sensitivity quantitation

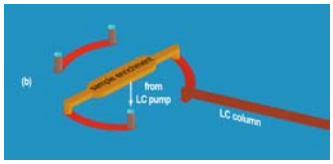


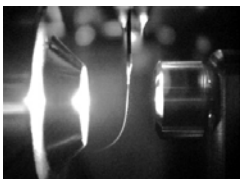
Sensitivity: down to low amol
Dynamic range: up to 10^5

HPLC-Chip/MS Interface: making nanoESI almost routine

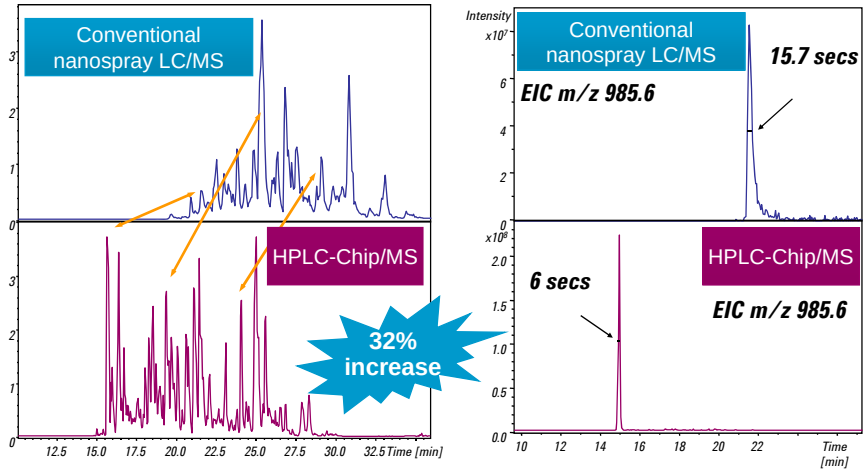






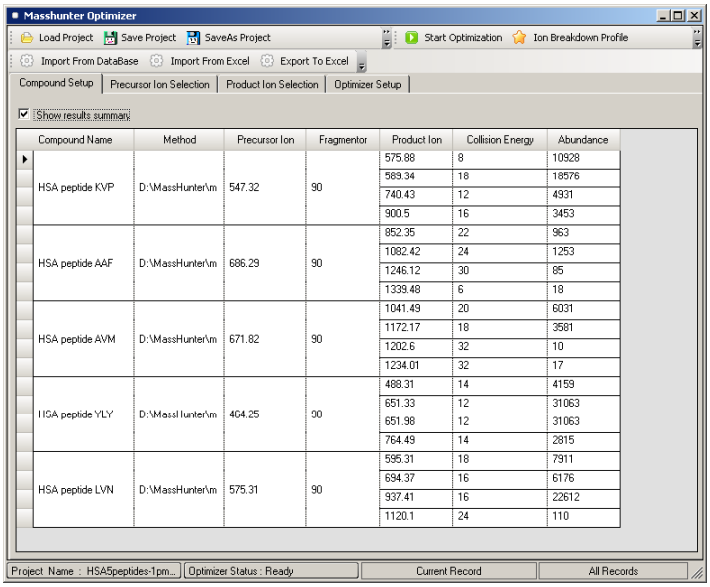


Chromatographic Performance
Protein Digest Mixture



Reduced MS complexity + reduced ionization competition = improved ID

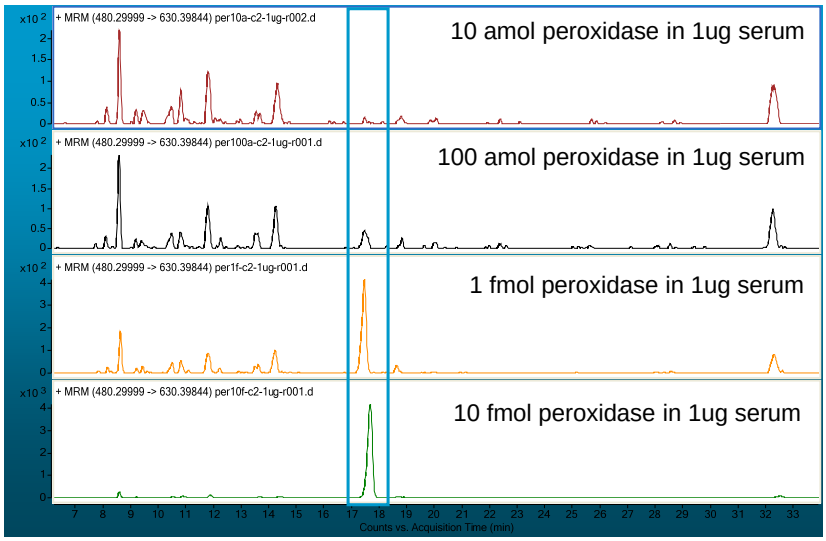
MassHunter Optimizer – optimization results for peptides



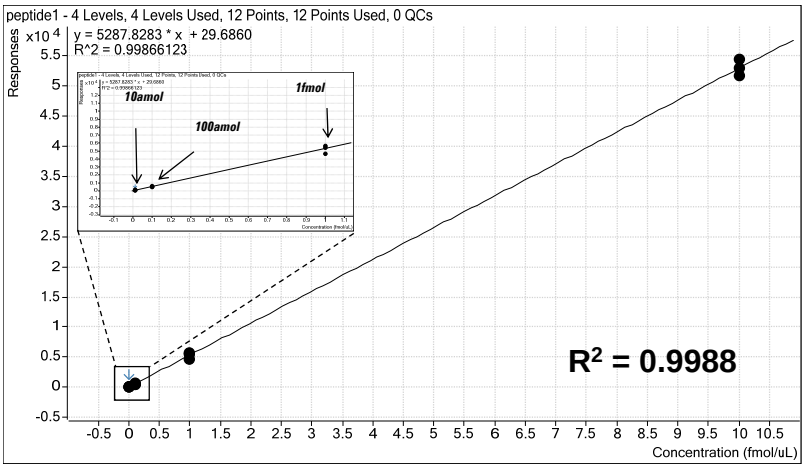
Compound Name	Method	Precursor Ion	Fragmentor	Product Ion	Collision Energy	Abundance
HSA peptide KVP	D:\MassHunter\m	547.32	90	575.88	8	10928
				589.34	18	18576
				740.43	12	4931
				900.5	16	3453
HSA peptide AAF	D:\MassHunter\m	686.29	90	852.35	22	963
				1082.42	24	1253
				1246.12	30	85
				1339.48	6	18
HSA peptide AVM	D:\MassHunter\m	671.82	90	1041.49	20	6031
				1172.17	18	3581
				1202.6	32	10
				1234.01	32	17
HSA peptide YLY	D:\MassHunter\m	464.25	90	488.31	14	4159
				651.33	12	31063
				651.98	12	31063
				764.49	14	2815
HSA peptide LVN	D:\MassHunter\m	575.31	90	595.31	18	7911
				694.37	16	6176
				937.41	16	22612
				1120.1	24	110

Limit of Quantitation in the Low Amol Range

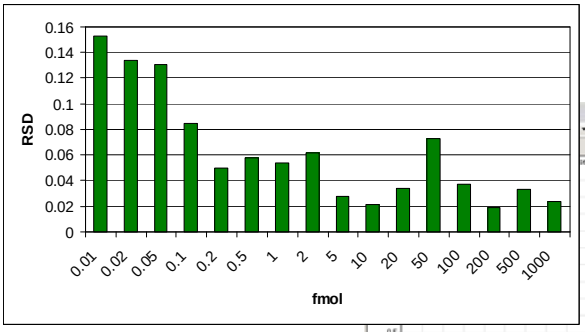
Peroxidase 10 amol to 10 fmol spiked into 1 µg human serum

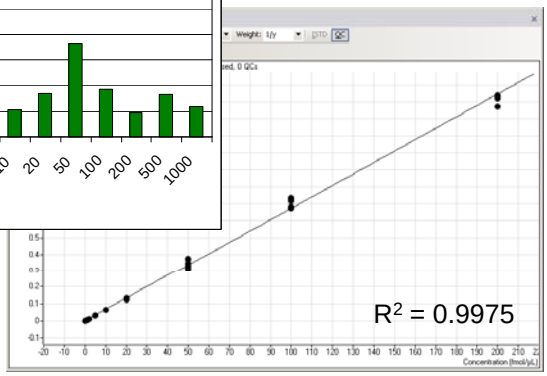


External Quantitation Curve of Peroxidase Peptide DTIVNELR From 10 amol to 10 fmol Spiked into Human Serum



Excellent Reproducibility of MS Response
HSA Peptide LVNEVTEFAK from 10 amol to 1 pmol (n=6)





All RSDs are within 15%

New 6460 Triple Quad LC/MS

- Breaks the femtogram detection barrier for many compounds
- New Ionization Technology – Agilent Jet Stream
- Higher Signal Strength, great RSDs even in low fg region
- New Acquisition Software - Scheduled MRMs
- New Method Development Tool – MH Optimizer
- **Agilent is committed to continuous innovation in:**
  - **Mass analyzer technology**
  - **Brighter sources**
  - **Innovative software**