Waters

Nicholas Ellor

Brad Barrett

Benefits and Applications of Ultra Performance LC™ for MS Analysis

For Complete Confidence

Waters

Demands of LC/MS
I need …

- Productivity, faster analysis times
- To meet regulatory compliance
- Rapid sample handling and separation
- Better limits of detection
- Quicker method development

Increasing demands for productivity improvements in the laboratory require faster and better results

Waters

LC/MS Today
The state of the science

- LC/MS quality can be limited by both the separation science (LC) and the detection science (MS). Optimization of both technologies can bring speed, sensitivity, and separation benefits to the analytical system
- Today's challenge for all laboratories is to carefully maximize ‘system’ performance
- Selection of instruments from multiple vendors provides minimal cost-savings and offers little competitive business advantages.

History of Innovation
Waters LC/MS at ASMS…

- ASMS 2003
  - Quattro Premier™
  - LCT Premier™
- ASMS 2004
  - ACQUITY UPLC™
  - Q-Tof Premier™
  - nanoACQUITY UPLC™
- ASMS 2005
  - Quattro Premier™ XE

LC/MS Today
The Future of LC…

- ACQUITY UPLC™
  - Fast sample cycle times
  - Ultra-low carryover
  - Reproducible, efficient separations
  - Sharp narrow peaks for increased sensitivity and improved integration
  - Decreased ion suppression effects
  - Low LC system volumes
  - High capacity sample organizer
  - Perfect design for integration to Micromass® Mass Spectrometers

LC/MS Today
The Future of MS…

- Quattro Premier™ XE
  - Robust *Z* Spray™ Ion Source
  - Compact size, < 18.9” wide
  - Intuitive – MassLynx™ software
  - Sensitivity - enhanced ion optics and Whisper™ detection
  - Fast analysis - T-wave™[1]
  - collision cell and high speed electronics
  - Dynamic range - up to 5 orders of magnitude
  - Integrated syringe pump and software controlled gas flow
  - Low LC system volumes
  - Perfect design for integration to ACQUITY UPLC™

1 The traveling wave device described here is similar to that described by Kirchner in US Patent 5,206,506 (1993).

Waters

LC/MS Today

The Future of LC/MS is now

- Resolution 2X better than HPLC
- Analyses times 9X faster
- 3X better sensitivity
- Productivity increases over 100%
- Lower cost of ownership with solvent savings of up to 80%
- Integration by design

Waters

LC/MS Today

The Future of LC/MS is now

Major Challenges in Today's Lab

- Throughput (Speed)
- Detection limits (Sensitivity)
- Robustness (Longevity)
- Carry over
- Chromatographic resolution

Waters

Making UPLC™ a Reality Today
Essential technology achievements

- Small, pressure-tolerant particles
- High pressure fluidic modules (up to15,000 psi)
- Minimized system volumes and optimized flow paths
- Reduced cycle times
- Negligible carryover sample management
- High speed detectors (optical and mass)
- Software designed for system integration
  - Novel communication protocols
  - Advanced diagnostics

Waters

ACQUITY UPLC™

Ultra performance by design

For Complete Confidence

Waters

ACQUITY UPLC™ Technology

Ultra performance by design

Holistic Design

Detectors:
Optical and/or Mass Spec
Tunable UV or Photodiode Array
Optimized flow cell for UPLC™
High speed data sampling

System Considerations:
Small Footprint
Redesigned tubing and fittings
Consolidated waste management
Integrated system diagnostics
Connections Insight™ remote diagnostics

Sample Organizer: (option)
Expands capacity (22/15/8)
Shuttles plate feed
Heated/chilled

Column Manager:
Innovative pivot design
Up to 65°C
Positions column to detector
E-Cord™ connection

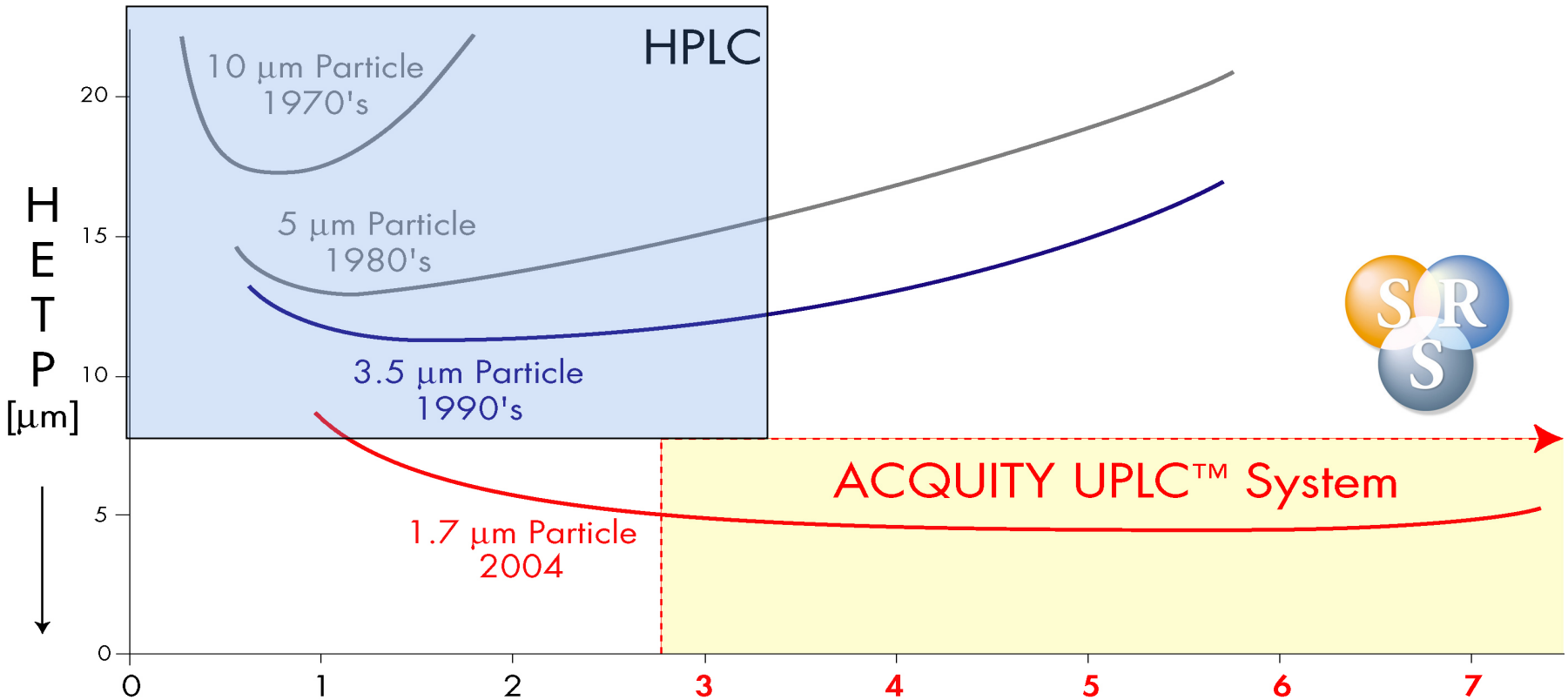
Sample Manager:
Low dispersion XYZZ' Format
Variable vol. fixed loop injector
Low volume injections
Fast cycle times
Dual wash - Low carryover
Plates and/or vials
Thermal control (4-40°C)
Pressure tolerant

Binary Solvent Manager:
High pressure blending
Binary gradients
Four solvent choices
On-line degassing
Low dispersion design
UPLC pressure capabilities

Waters

UPLC™ Separations

A new category of liquid chromatography

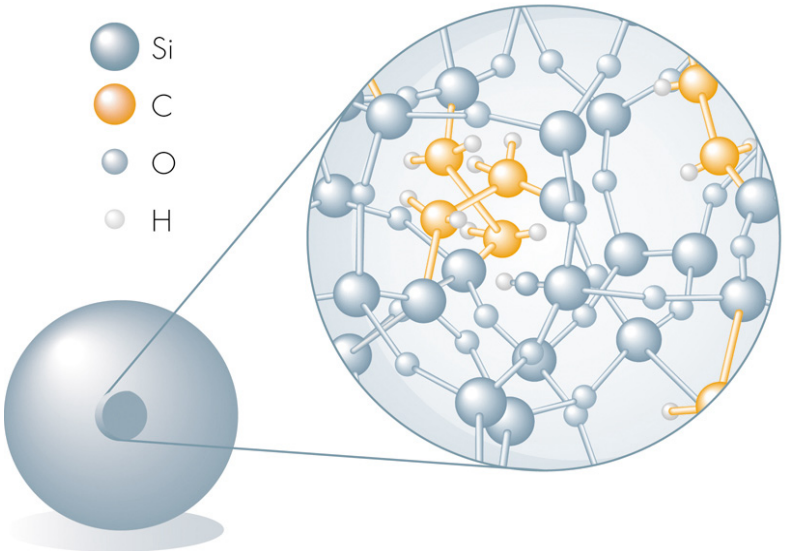


Flow Rate [mL/min]:							
ID = 1.0 mm	0.04	0.07	**0.10**	**0.13**	**0.17**	**0.20**	**0.24**
ID = 2.1 mm	0.15	0.3	**0.45**	**0.6**	**0.75**	**0.9**	**1.05**
ID = 4.6 mm	*0.7*	*1.4*	*2.1*	*2.8*	*3.5*	*4.2*	*4.9*

Advancing the Chemistry of LC

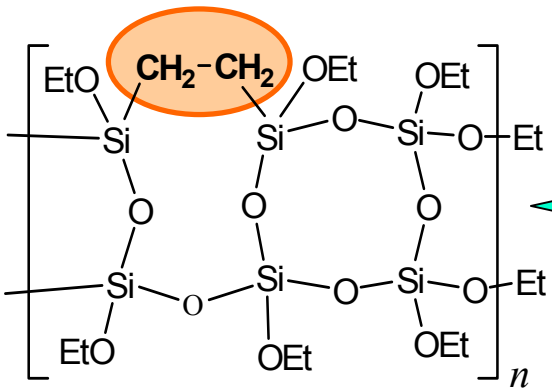
- Sub 2µm particles
  - Porous for optimum mass transfer
  - Bridged hybrid particle required for both high strength and outstanding chromatographic performance
  - Innovative sizing technology for narrow particle size distribution
- Column hardware
  - Innovative frit technology to retain particles
  - Fittings optimized for high pressure operation
- Packing technology
  - Innovative column packing processes to optimize stability
- eCord™
    - New information chip to store column history

New 2nd Generation Hybrid
ACQUITY UPLC™ Chemistry

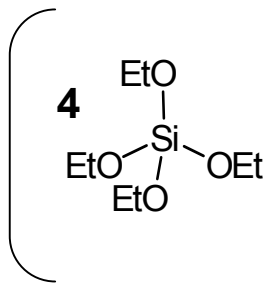


- Improved Strength
- Improved Efficiencies
- Improved Peak Shape
- Wider pH Range

BEH Technology™

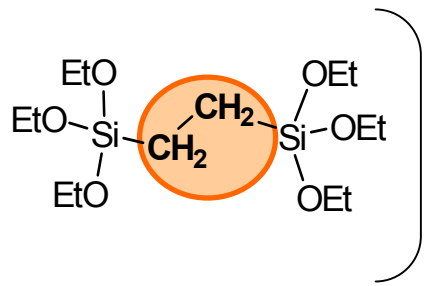


Polyethoxysilane
(BPEOS)



Tetraethoxysilane
(TEOS)

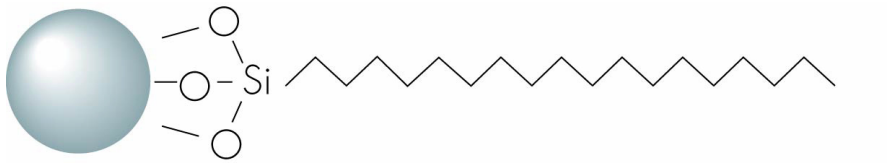
+



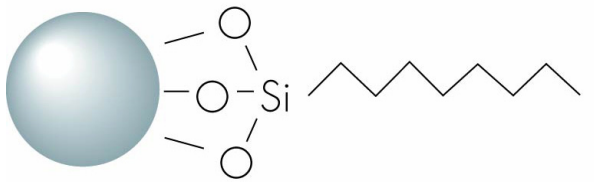
Bis(triethoxysilyl)ethane
(BTEE)

ACQUITY UPLC™ Column *Chemistries*

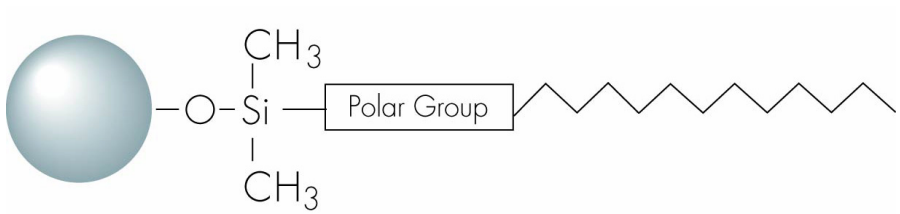
- C18
  - Trifunctionally Bonded C_{18}
  - Proprietary Endcapping
  - Widest pH range



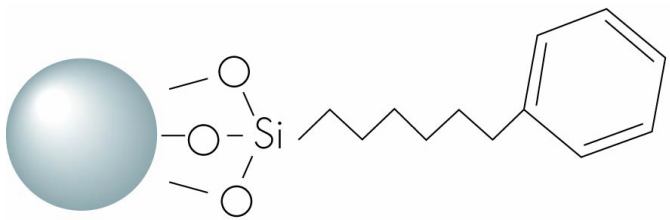
- C8
  - Trifunctionally Bonded C_8
  - Proprietary Endcapping
  - Widest pH range



- Shield RP18
  - Monofunctionally bonded
  - Embedded polar group



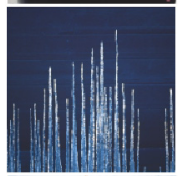
- Phenyl
  - Trifunctionally Bonded C_6 Phenyl
  - Proprietary Endcapping



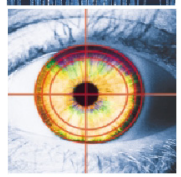
UPLC™ advantages

- UPLC™ demonstrated
  - 9 x increase in throughput
  - 3 x increase in sensitivity
  - 2 x increase in resolution
- Enriched quality data
- More productivity

Speed

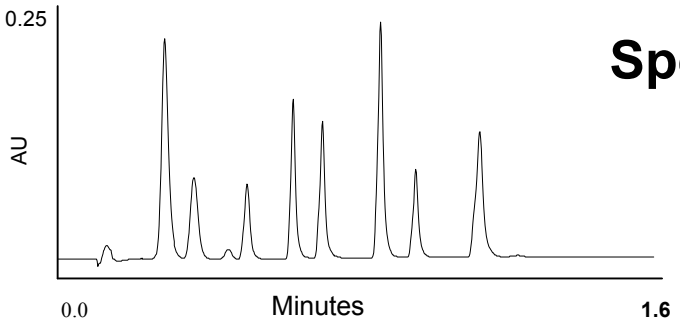


Sensitivity

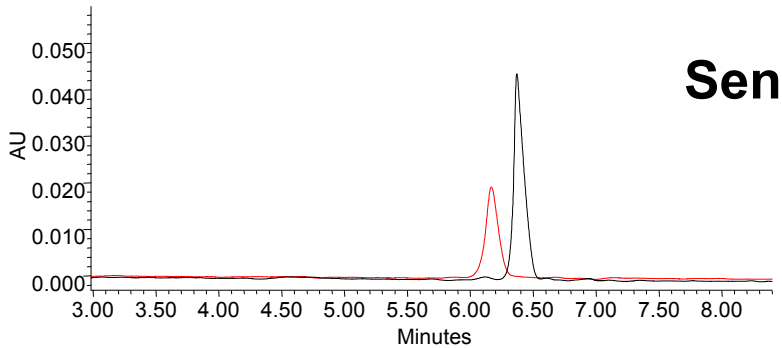


Resolution

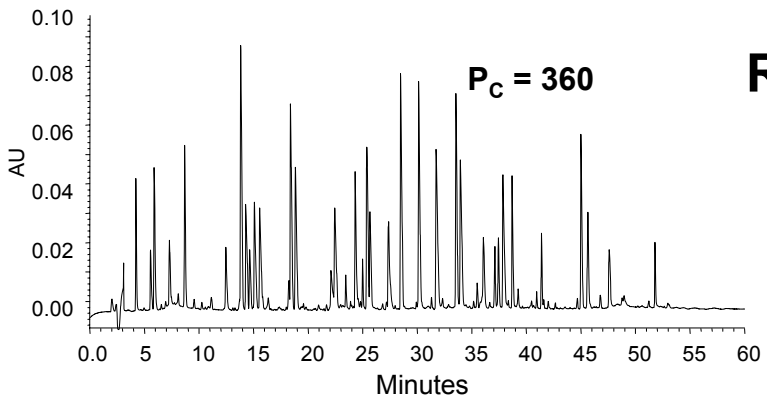
Ultra Performance LC™
Speed, Sensitivity and Resolution



Speed: 9X faster separations

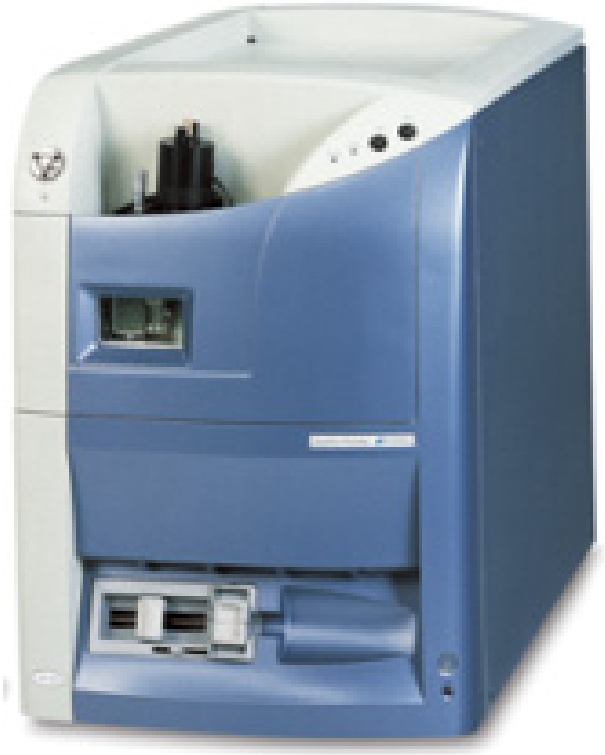


Sensitivity: 3X increase



Resolution: 1.7X increase

Waters



Quattro Premier™

Designed to allow you to acquire more data in less time with T-Wave technology.

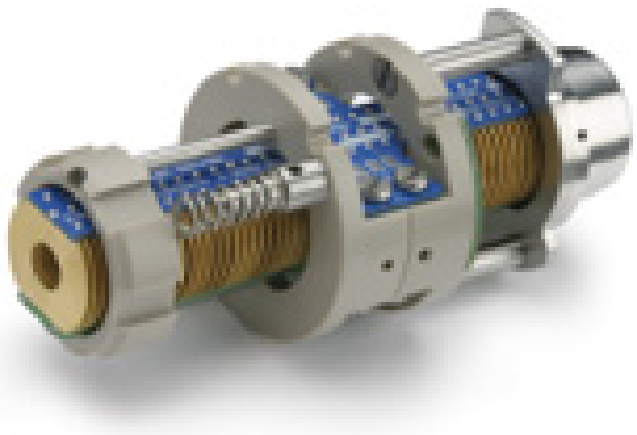
Designed to be compatible with UPLC™

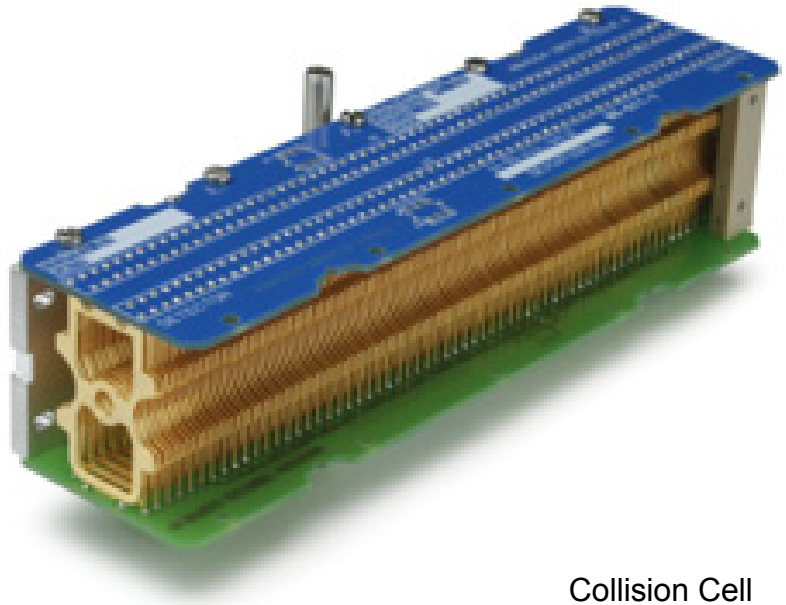
For Complete Confidence

T-Wave Devices

- Designed as a means of propelling ions through rf-only collision cells and transfer optics to enhance the fast acquisition performance of a tandem quadrupole mass spectrometer.

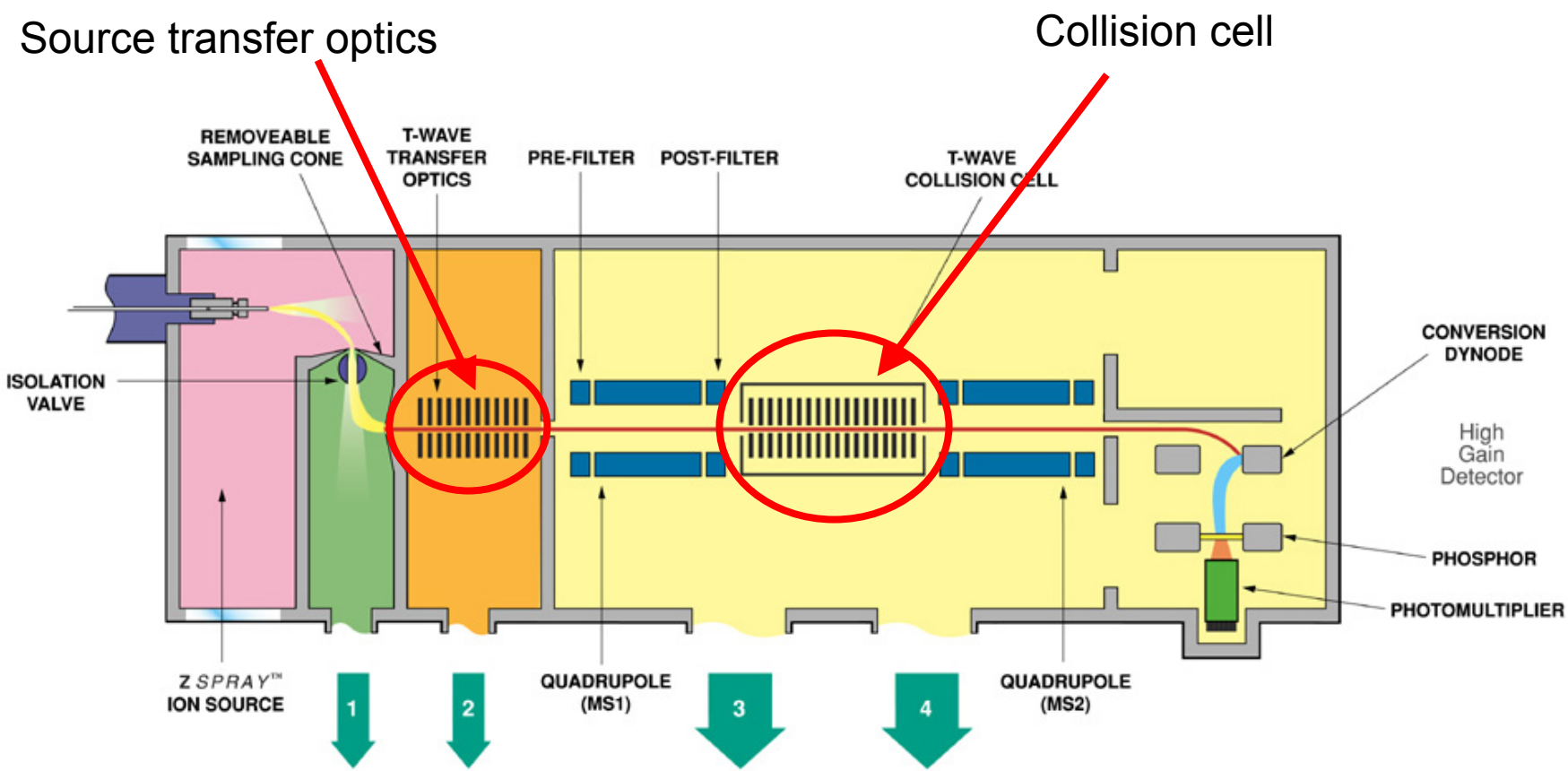
Source Transfer Optics





Collision Cell

T-Wave Devices

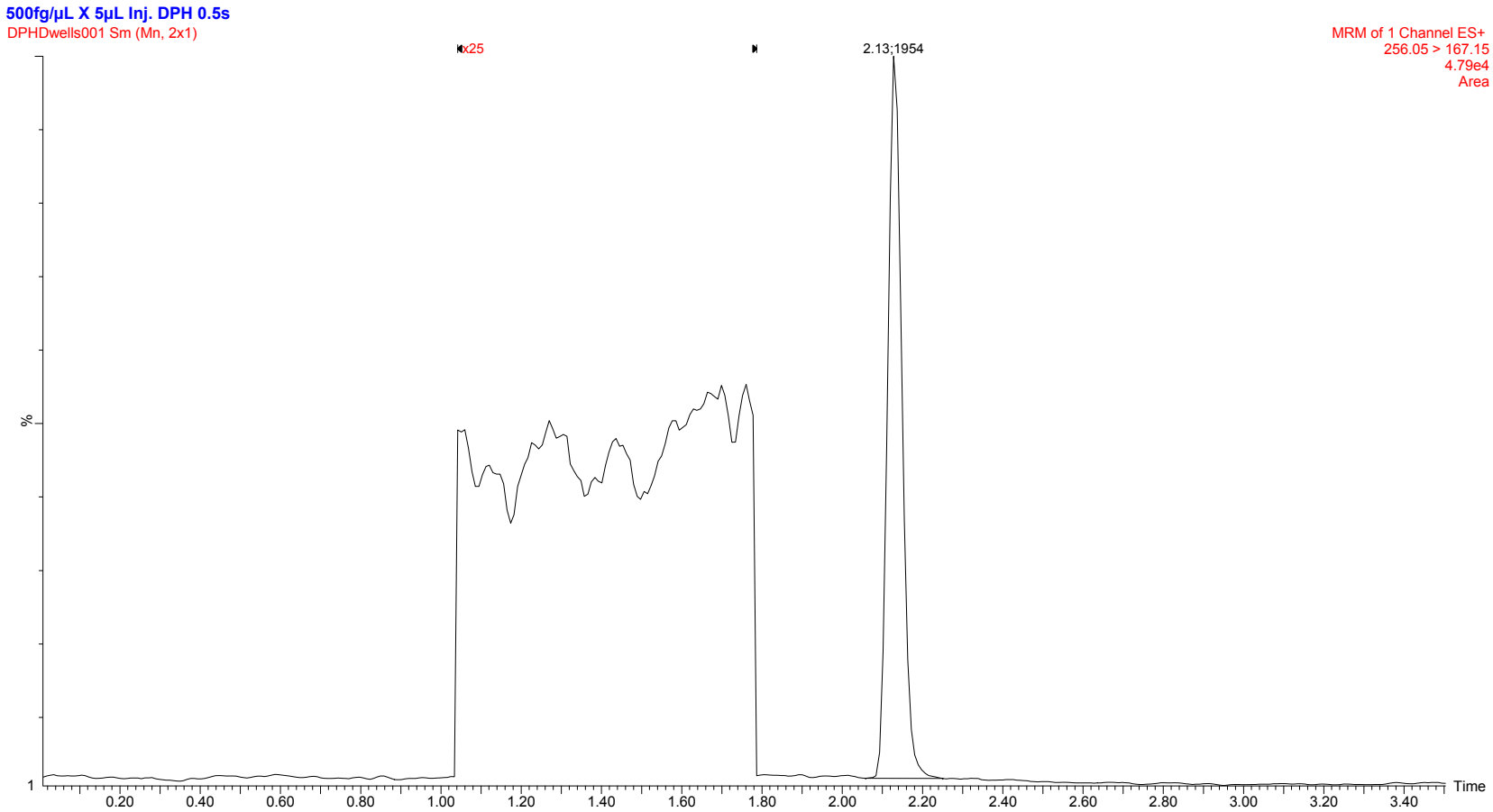


Waters

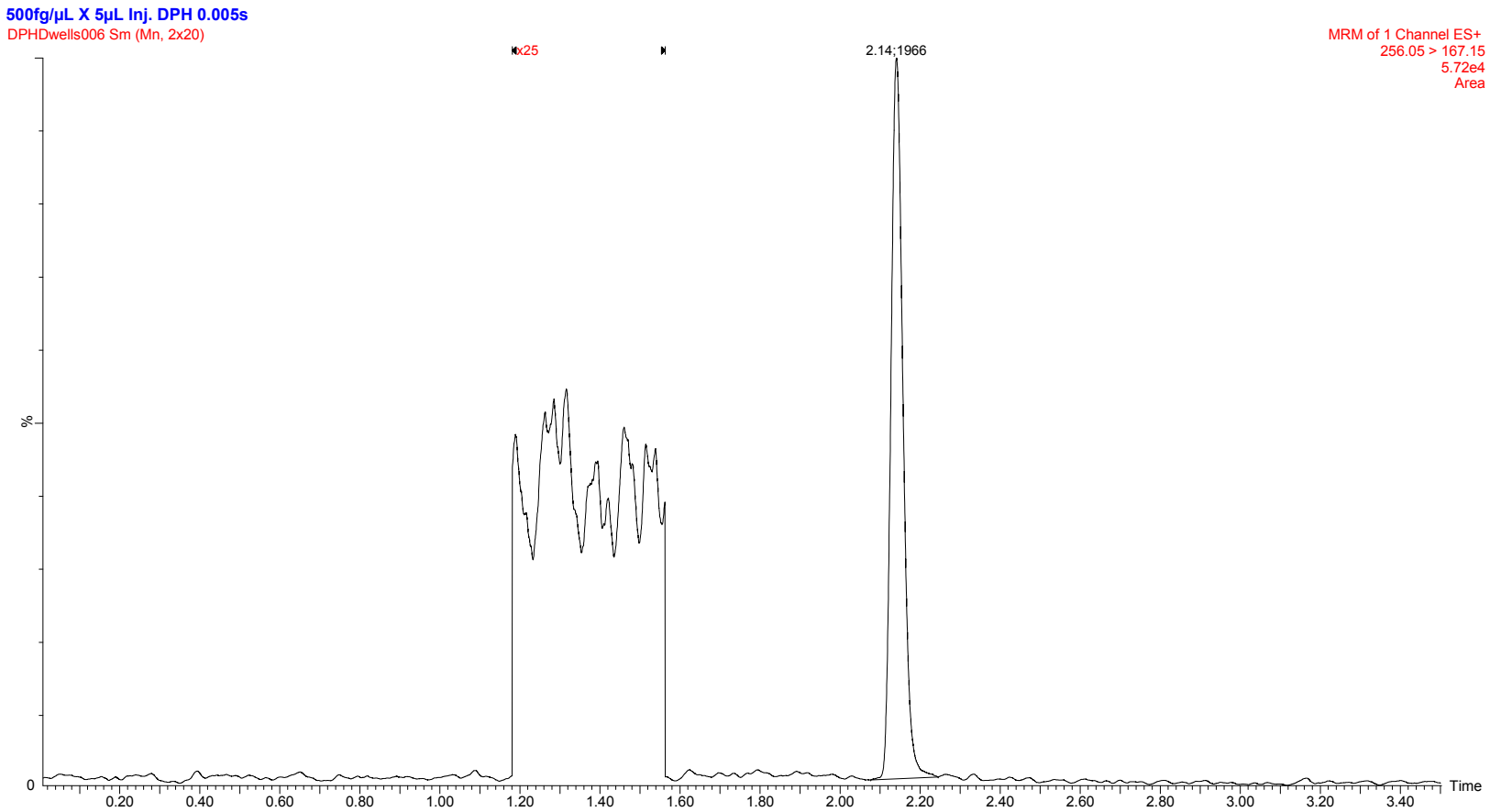
Speed

- More runs per unit time
  - Quicker method development
  - More samples completed

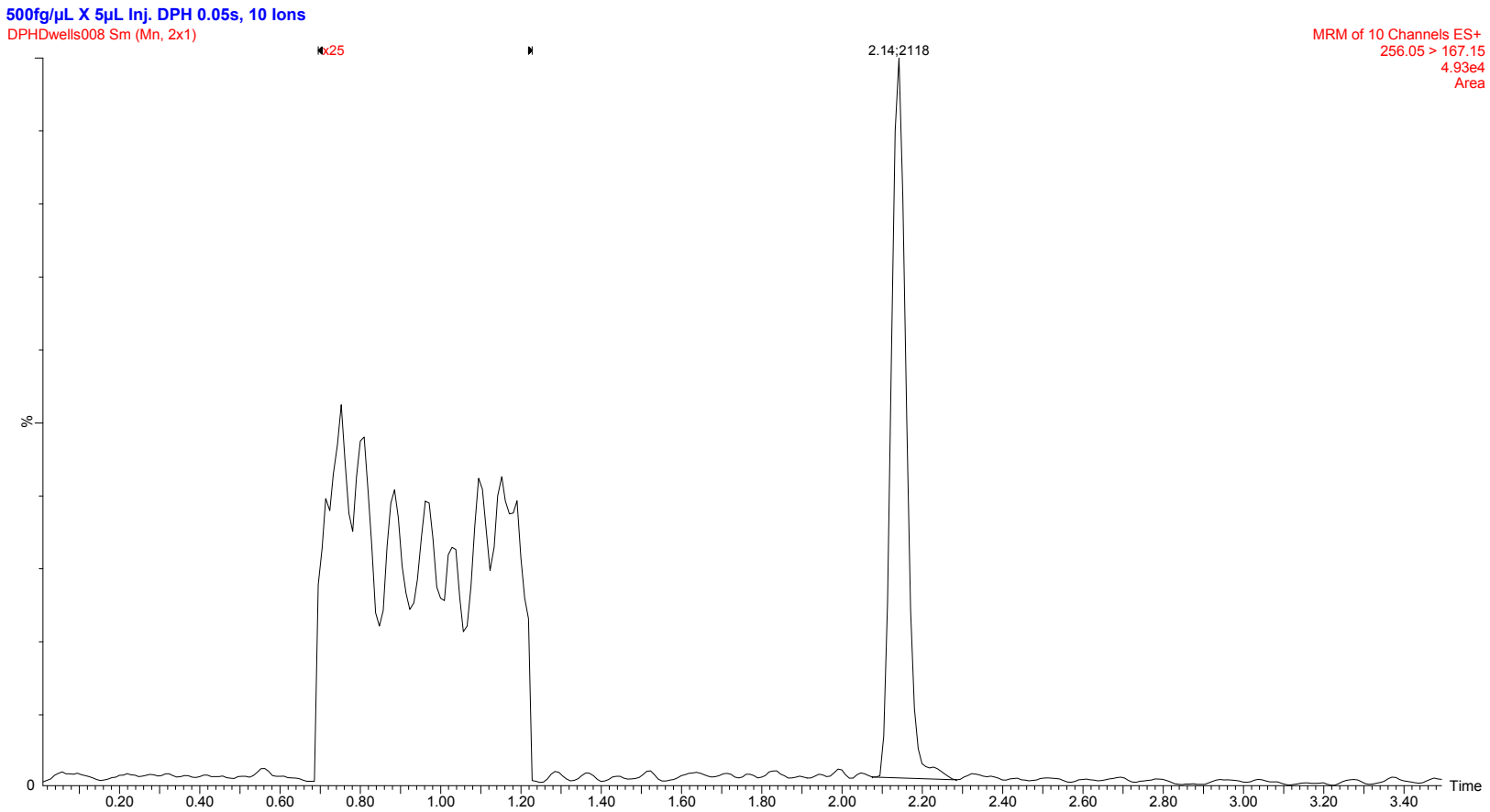
- Fast MRM sample dwell times and inter channel delay
  - Better quantitative results, more data points per peak possible
  - No cross talk during MRM transitions



- 500ms dwell time



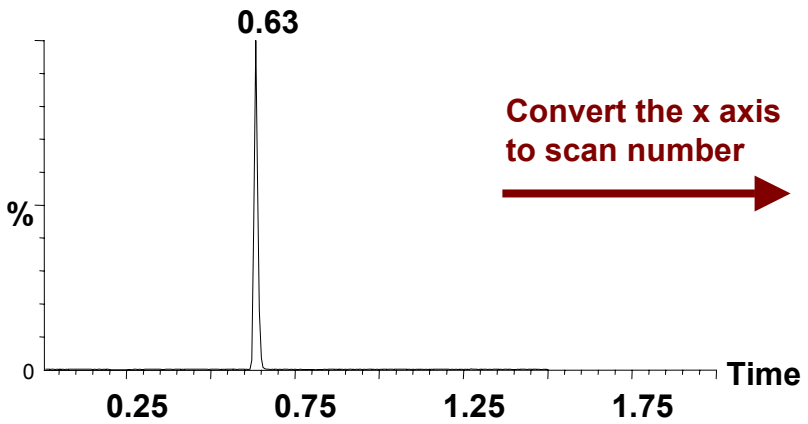
- 5ms dwell time



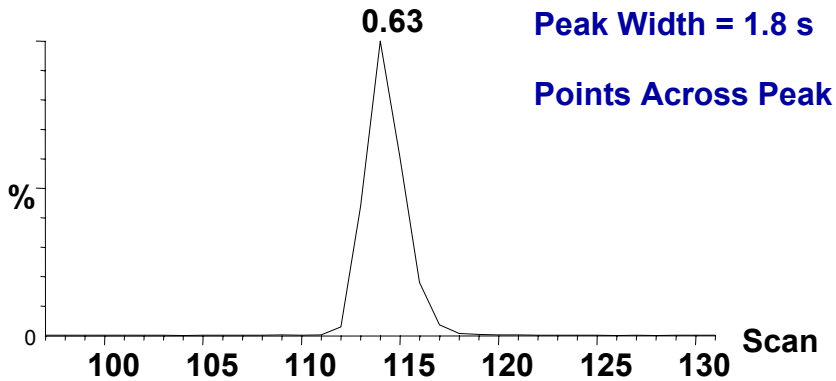
- 50ms dwell time, monitoring 10 different transitions

Quattro Premier™ UPLC™ Compatibility

100 ms Dwell Time, 10 ms Delay



Convert the x axis to scan number

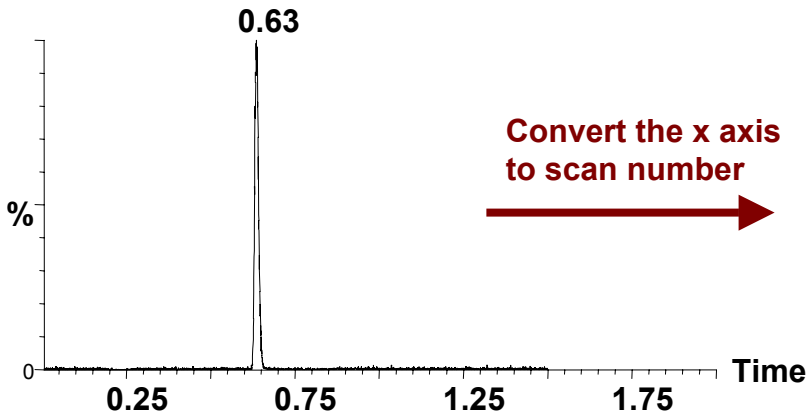


Peas Area = 16791

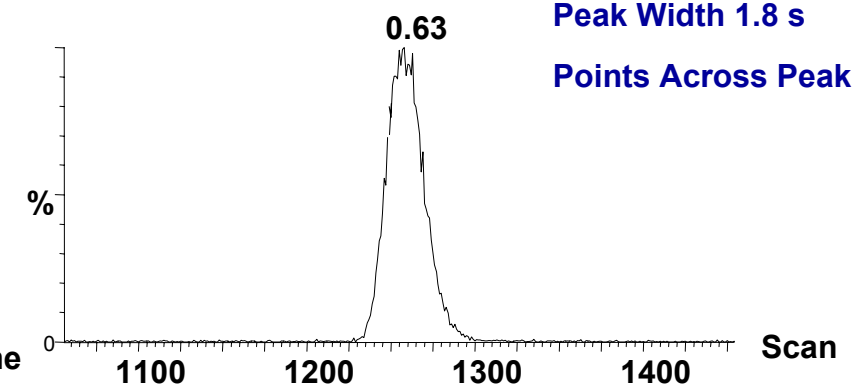
Peak Width = 1.8 s

Points Across Peak = 7

5 ms Dwell Time, 5 ms Delay



Convert the x axis to scan number



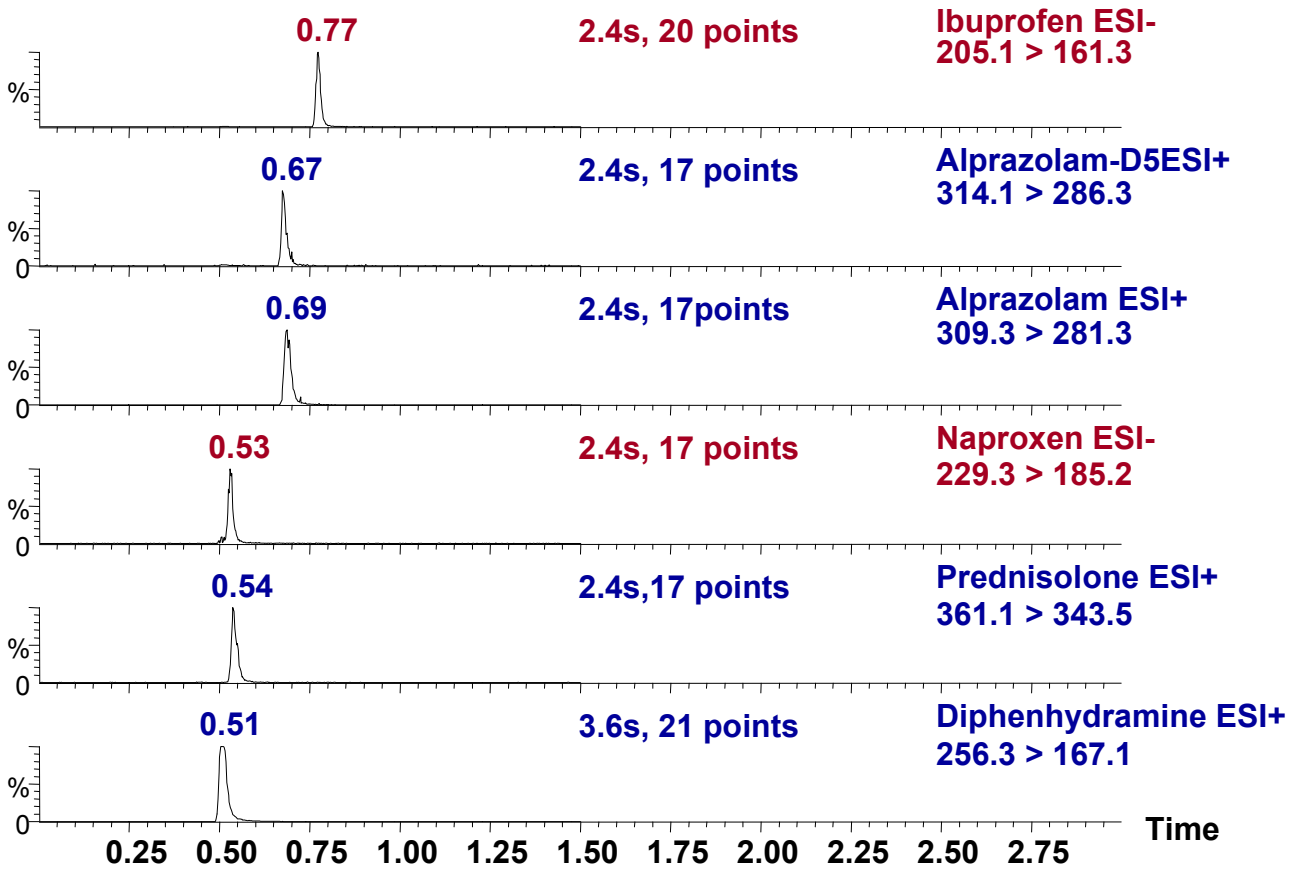
Peak Area = 16262

Peak Width 1.8 s

Points Across Peak = 60

Do You Need to Quantify in Both Positive and Negative Ion Modes From the Same Injection?

Quattro Premier Polarity Switching Time UPLC Compatibility

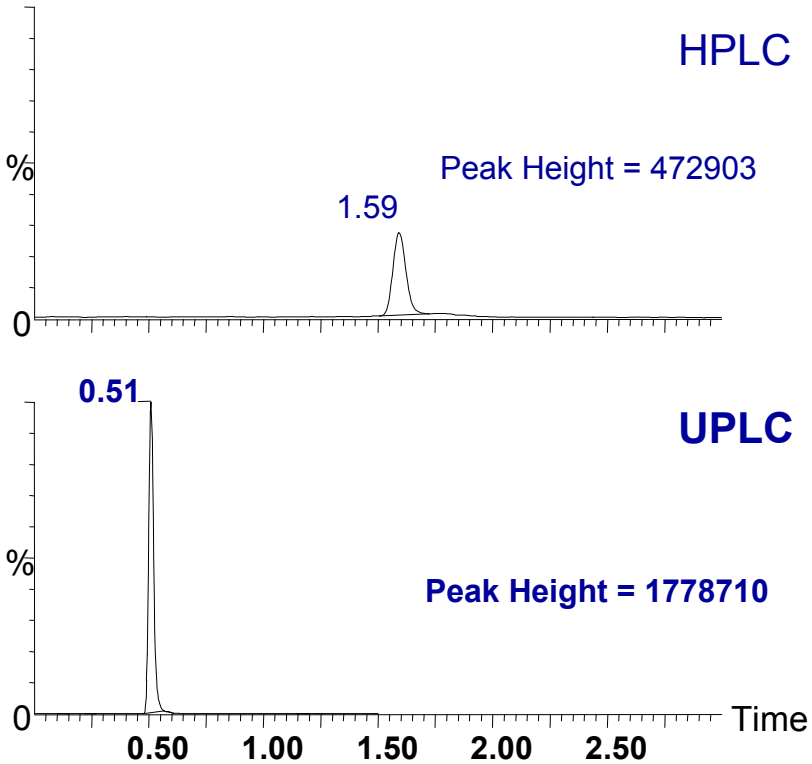


Sensitivity

- Increased detection limits
- Less sample required

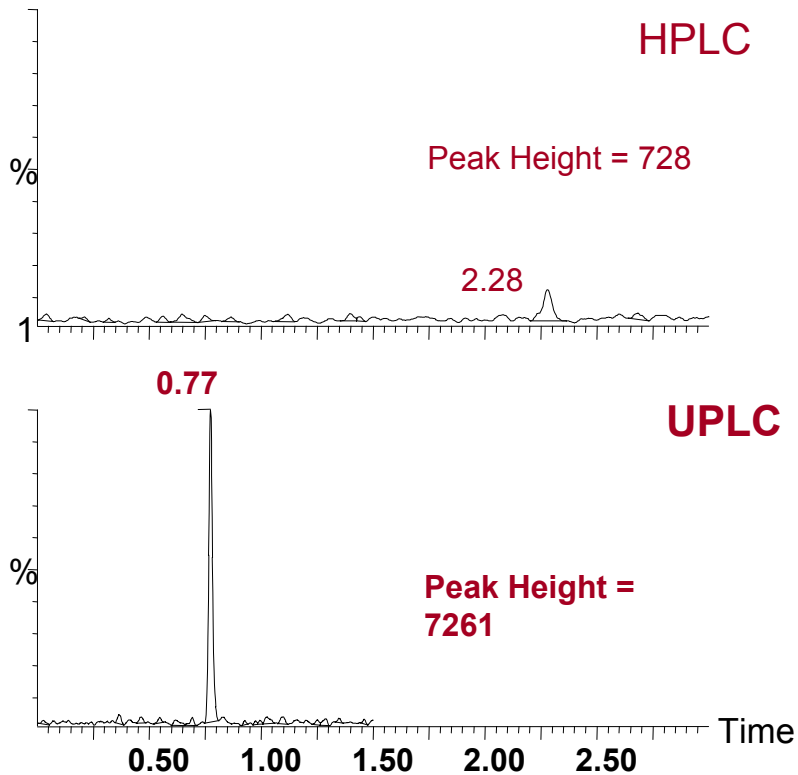
Quattro Premier™ Designed with UPLC™ in Mind

Diphenhydramine, 255.8>167.0, ESI+



Signal Increase 3.76 Times in UPLC™

Ibuprofen, 205.0>160.9, ESI-



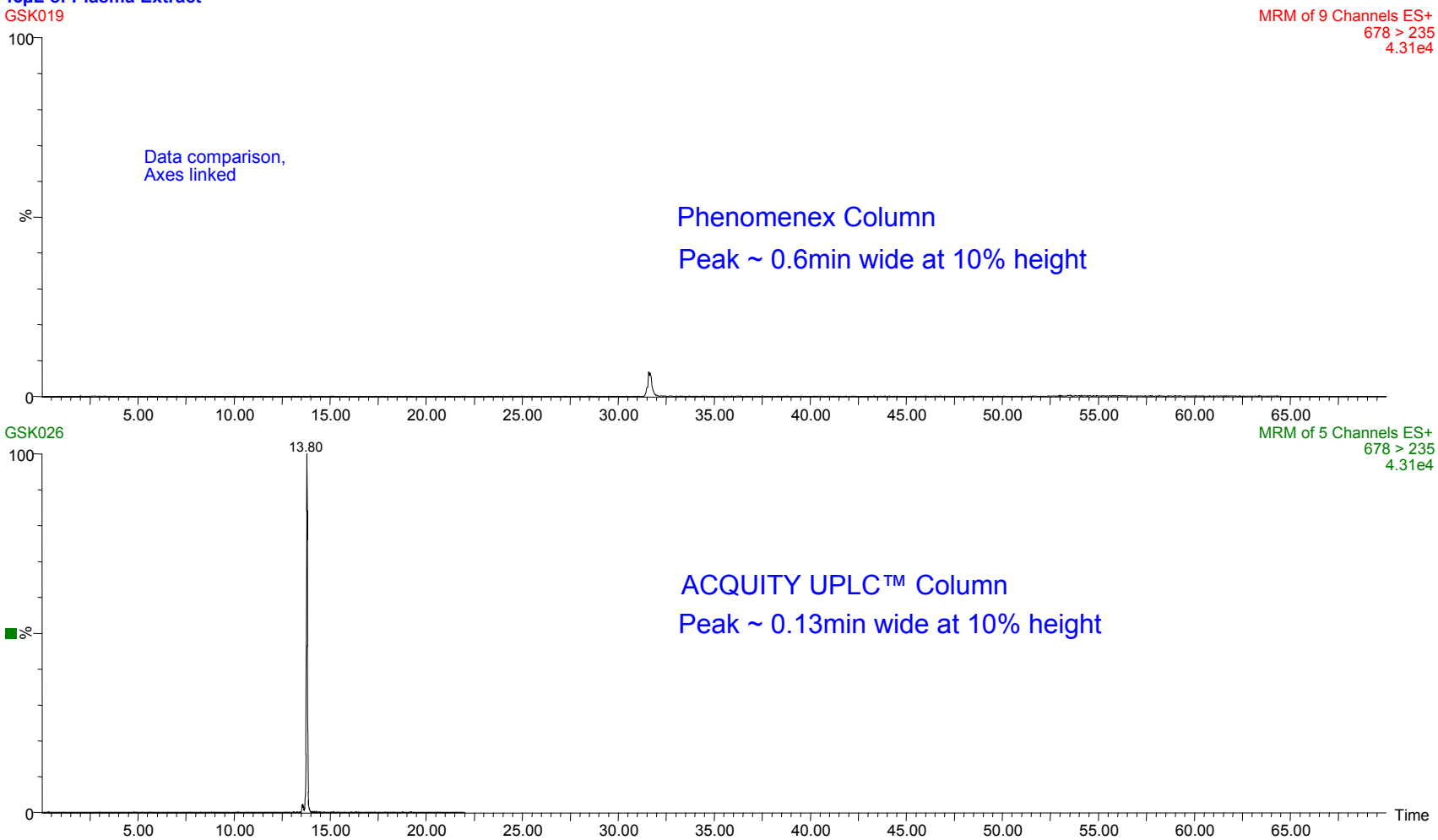
Signal Increase 9.97 Times in UPLC™

GSK Sample, 45µL Injection of Plasma Extract

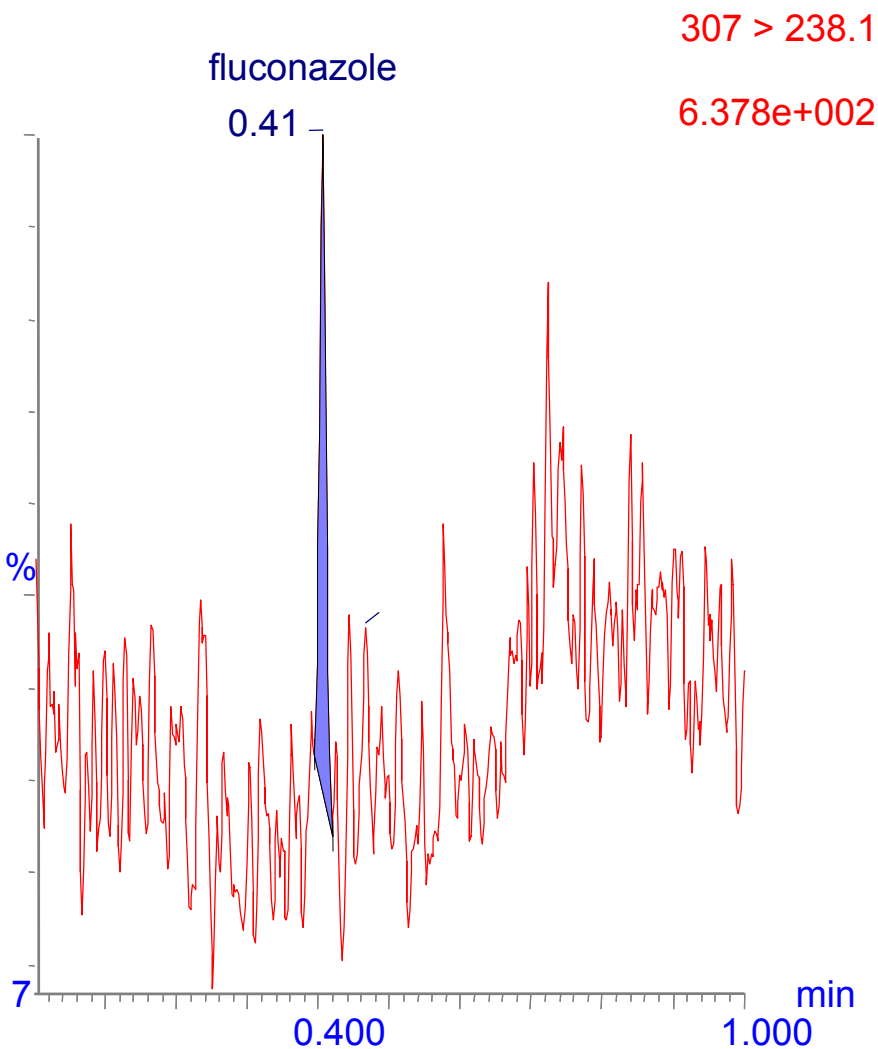
- ACQUITY UPLC™ C18 2.1 X 50mm
- 0.6mL/min
- A=50mM Ammonium Acetate
  B=Acetonitrile
- 0mins A=90%, 18mins A=50%, 19mins A=5%, 20mins A=5%, 20.5mins A=90%, 22mins A=90%
- 22min run time
- 3X Throughput
- 10X Sensitivity increase

- Phenomenex Luna C18 4.6 X 250mm
- 1.0mL/min
- A=50mM Ammonium Acetate
  B=Acetonitrile
- 0mins A=72%, 50mins A=40%, 51mins A=5%, 62mins A=5%, 63mins A=72%, 70mins A=72%
- 70min run time

45µL of Plasma Extract



Quattro Premier™ with ACQUITY UPLC™



Mobile Phase

A= 0.1% HCOOH in H2O

B= 0.1% HCOOH in ACN

Column

BEH 1.7µm C18

2.1 x 50mm

Time	% A	% B	Curve	Flow
0.00	90	10	-	0.6
0.50	5	95	6	
1.00	90	10	11	

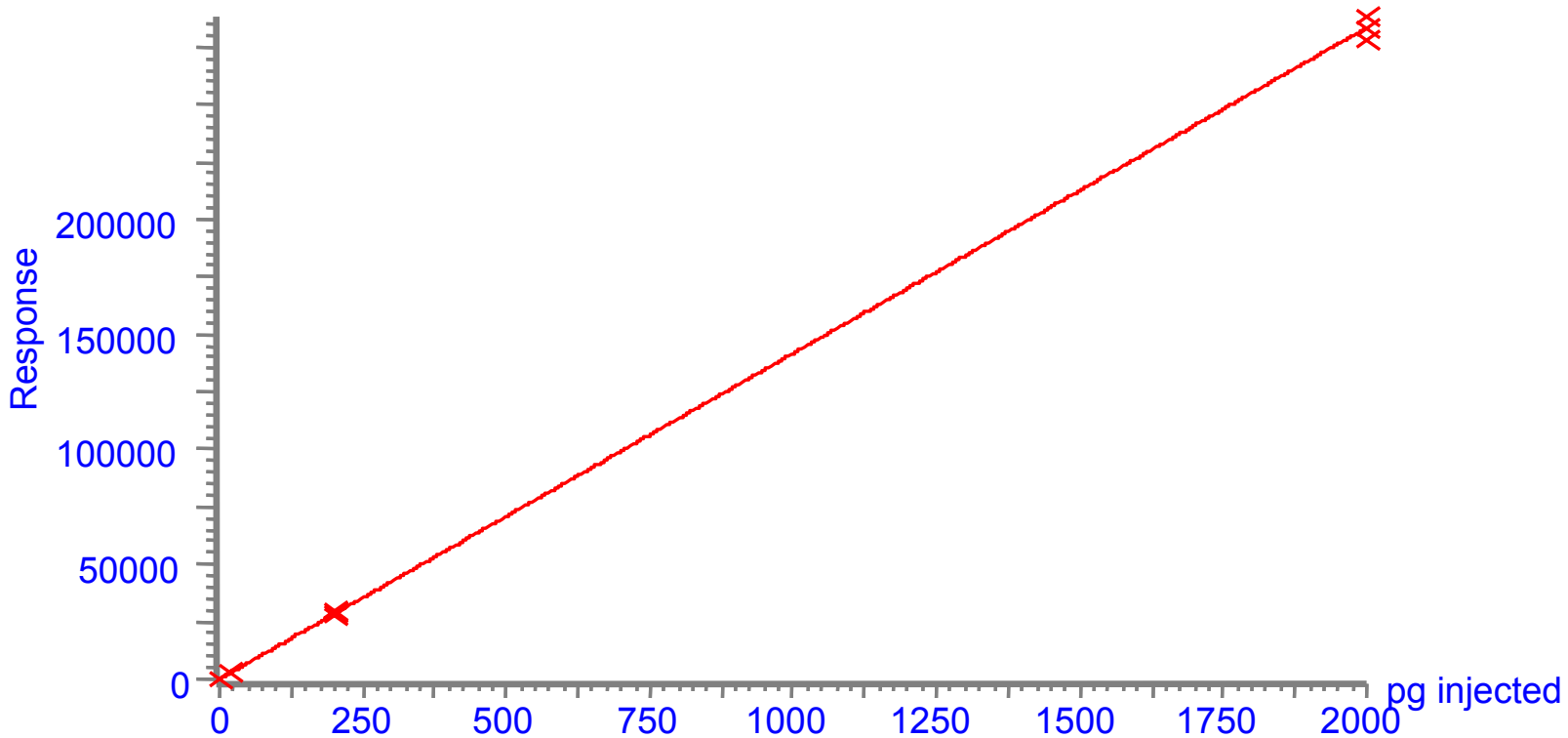
1 pg/mL, 20µL injection

Peak Width = 1.8 sec

Dwell time =0.1 sec

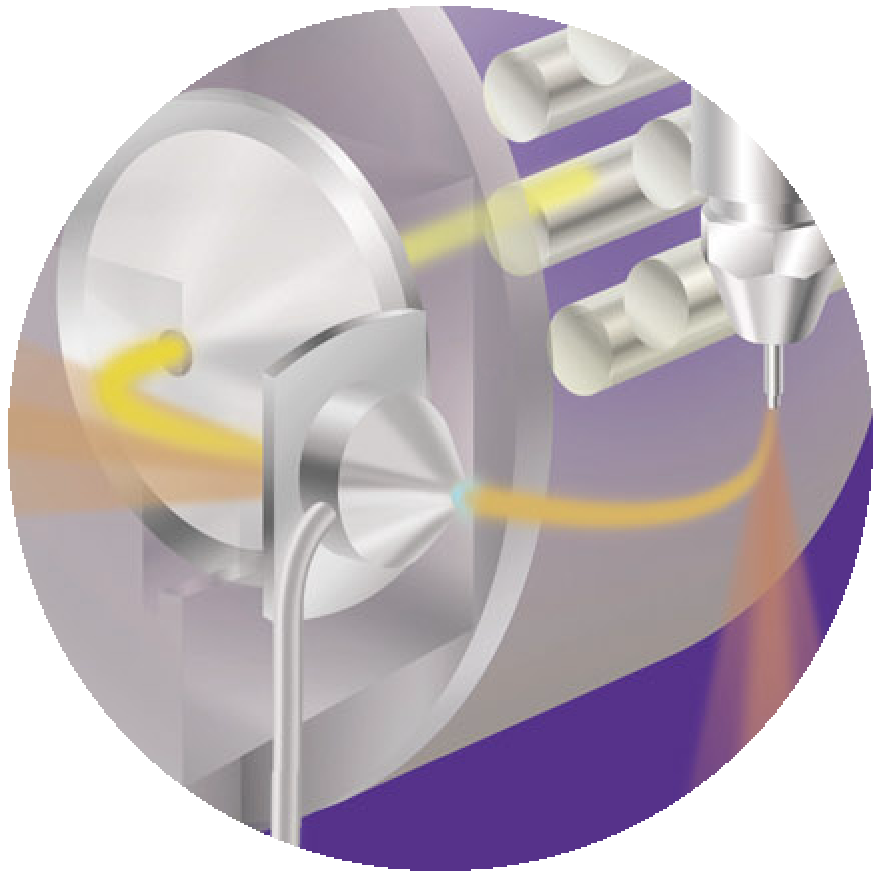
MT

Compound name : Fluconazole
Correlation coefficient : r = 0.999846 , r^2 = 0.999691
Calibration curve : 141 .751 * x + 1.70877
Response type : External Std, Area
Curve type : Linear, Origin : Exclude, Weighting : 1/x, Axis trans : None



Robustness

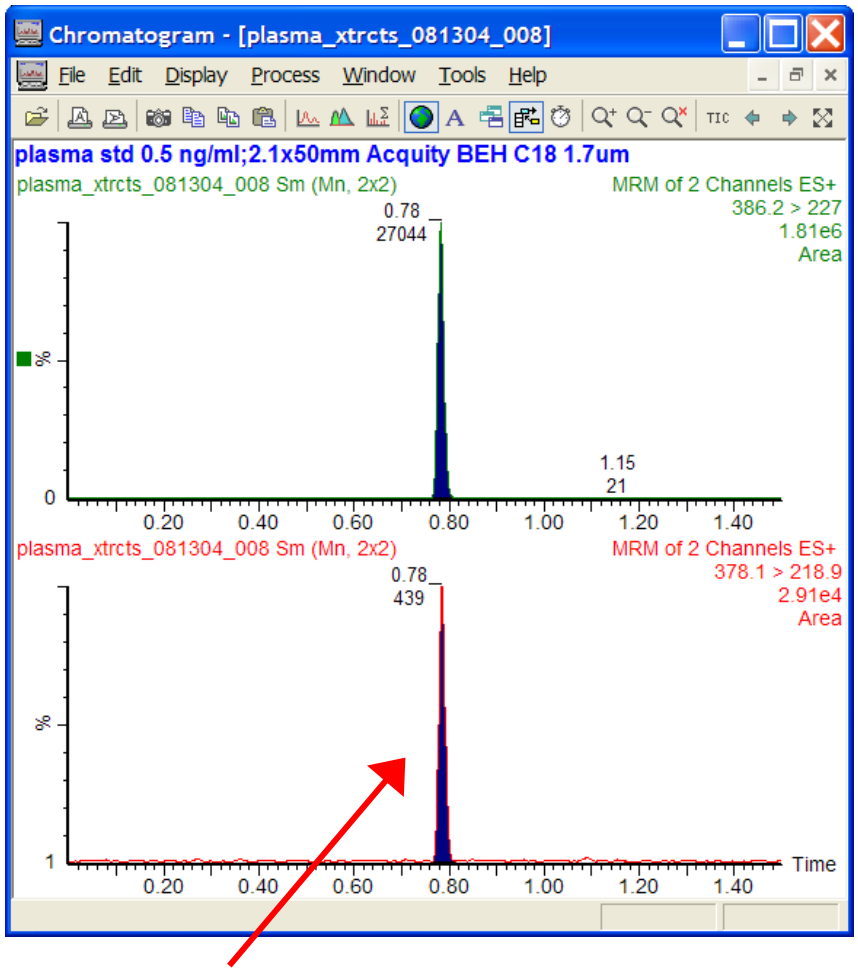
- Most rugged API source ever
  - Z-Spray is an industry standard
  - Increased productivity
  - Simple source cleaning, no need to vent instrument
- Excellent column lifetime
  - Over 4000 injection of “clean” samples
  - Over 1000 injections of precipitated plasma

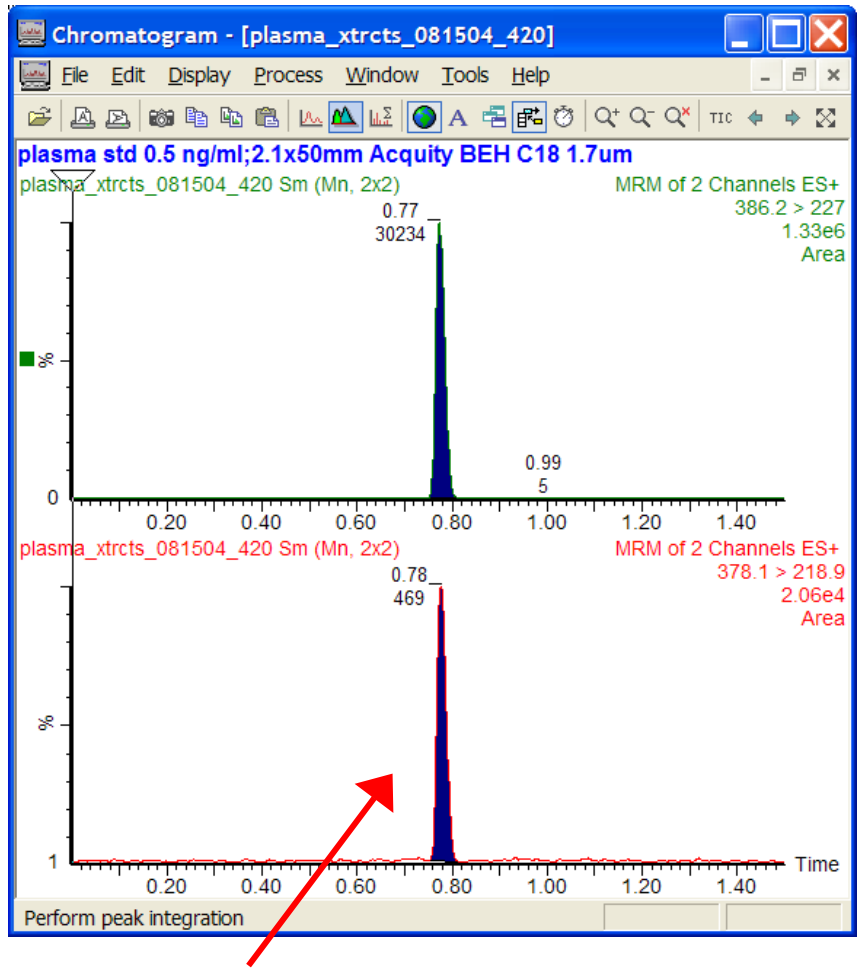


Quattro Premier™ ACQUITY UPLC™ DMPK Application

- Demonstration sample set
- Human plasma samples DMPK study
- Samples prepared by ACN precipitation
- Seventy samples in this DMPK study; Drug and d8 ISTD
- ACQUITY UPLC™ 2.1 x 50 mm C18 BEH column used
- Study set injected twelve (12) times until sample depleted
- 840 plasma samples injected AFTER 950 solvent stds. Injected
- ACQUITY UPLC™ ruggedness challenged
- Quattro Premier reproducibility and ruggedness challenged

Quattro Premier™ – DMPK Application





First low std injection

Last low std injection

Waters

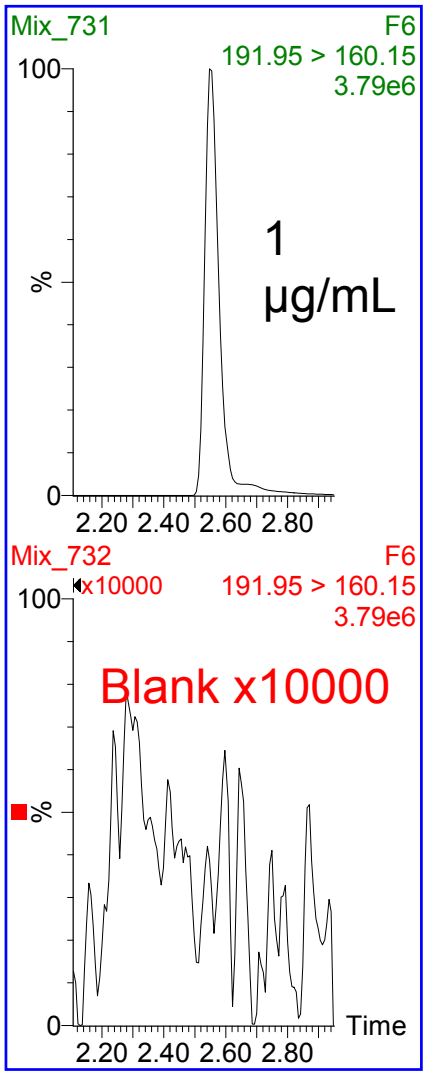
- Quattro Premier™ with ACQUITY UPLC™ demonstrated excellent ruggedness and reproducibility
- ACQUITY UPLC™ peak shape was consistent after nearly 1800 injections

No Carry Over

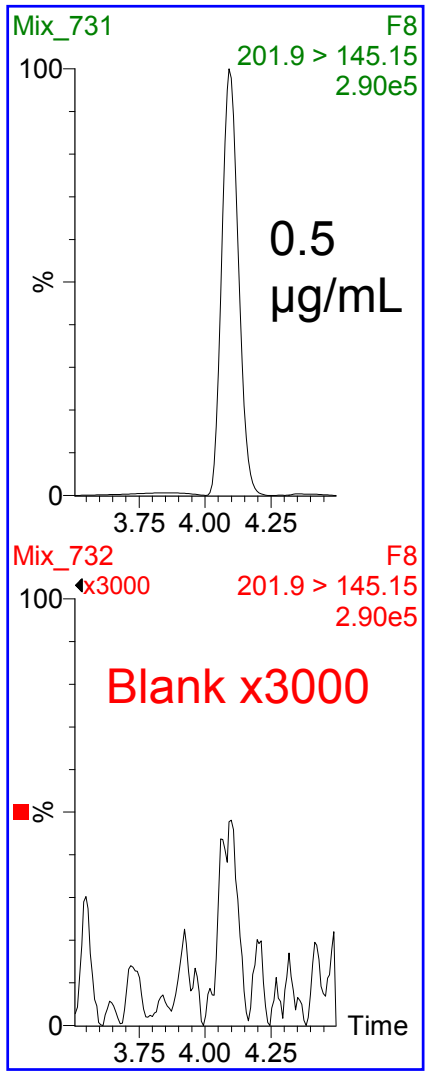
- Sample manager with negligible carryover
  - Greater confidence in results
  - Fewer reanalysis due to poor results

Very Low Carryover – Pesticide Sample Followed by a Blank

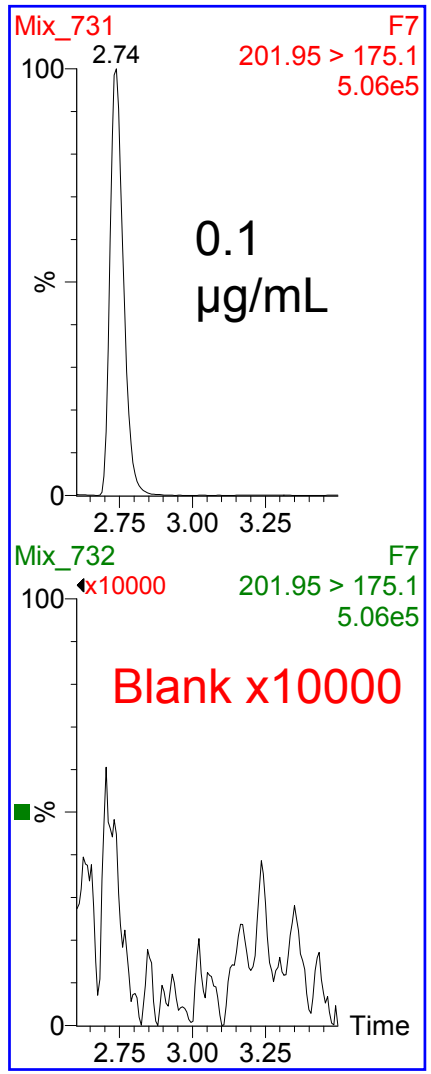
Carbendazim



Carbaryl



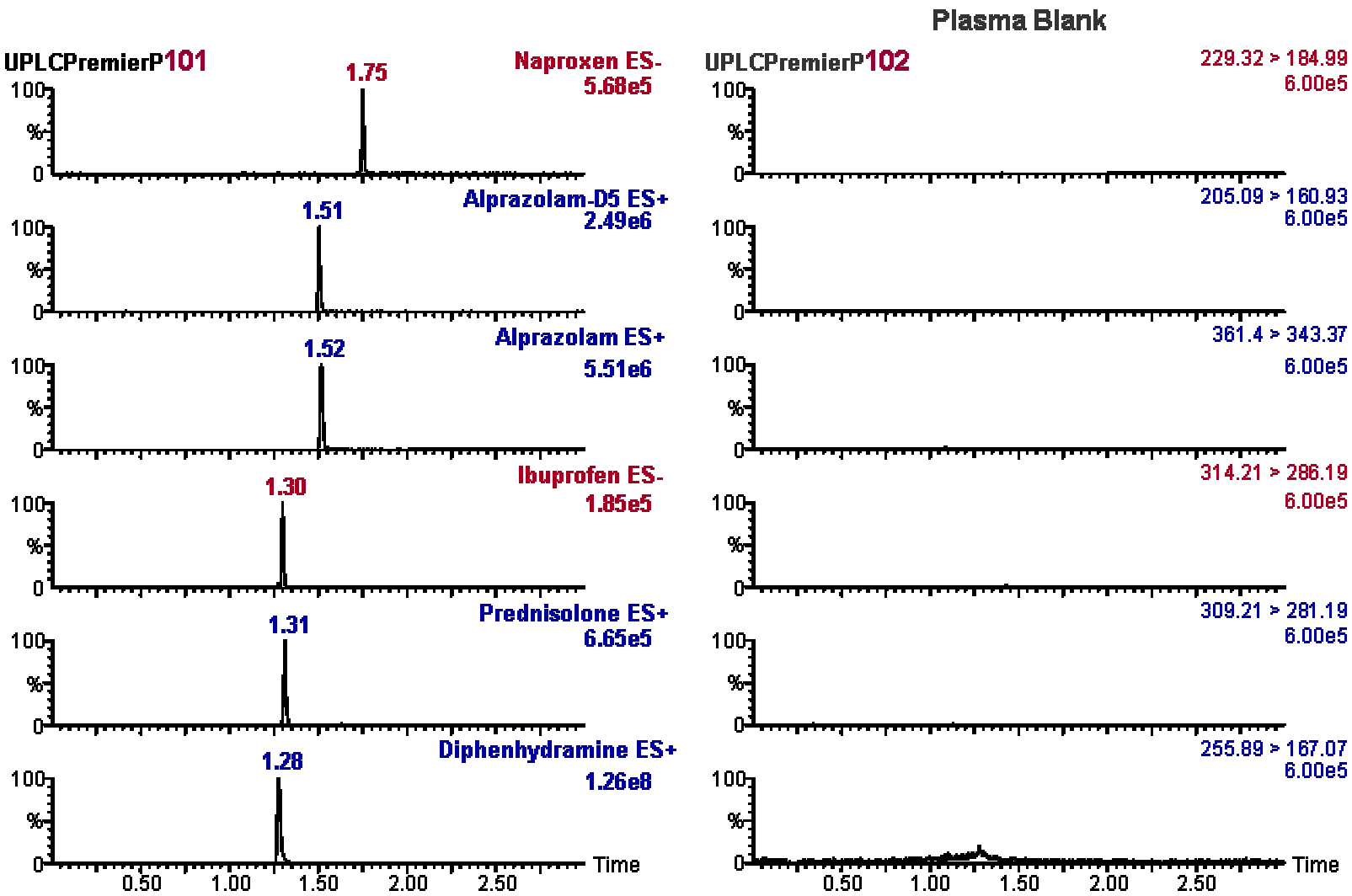
Thiabendazole



10 µL Injections

Waters

ACQUITY UPLC™
Carry Over



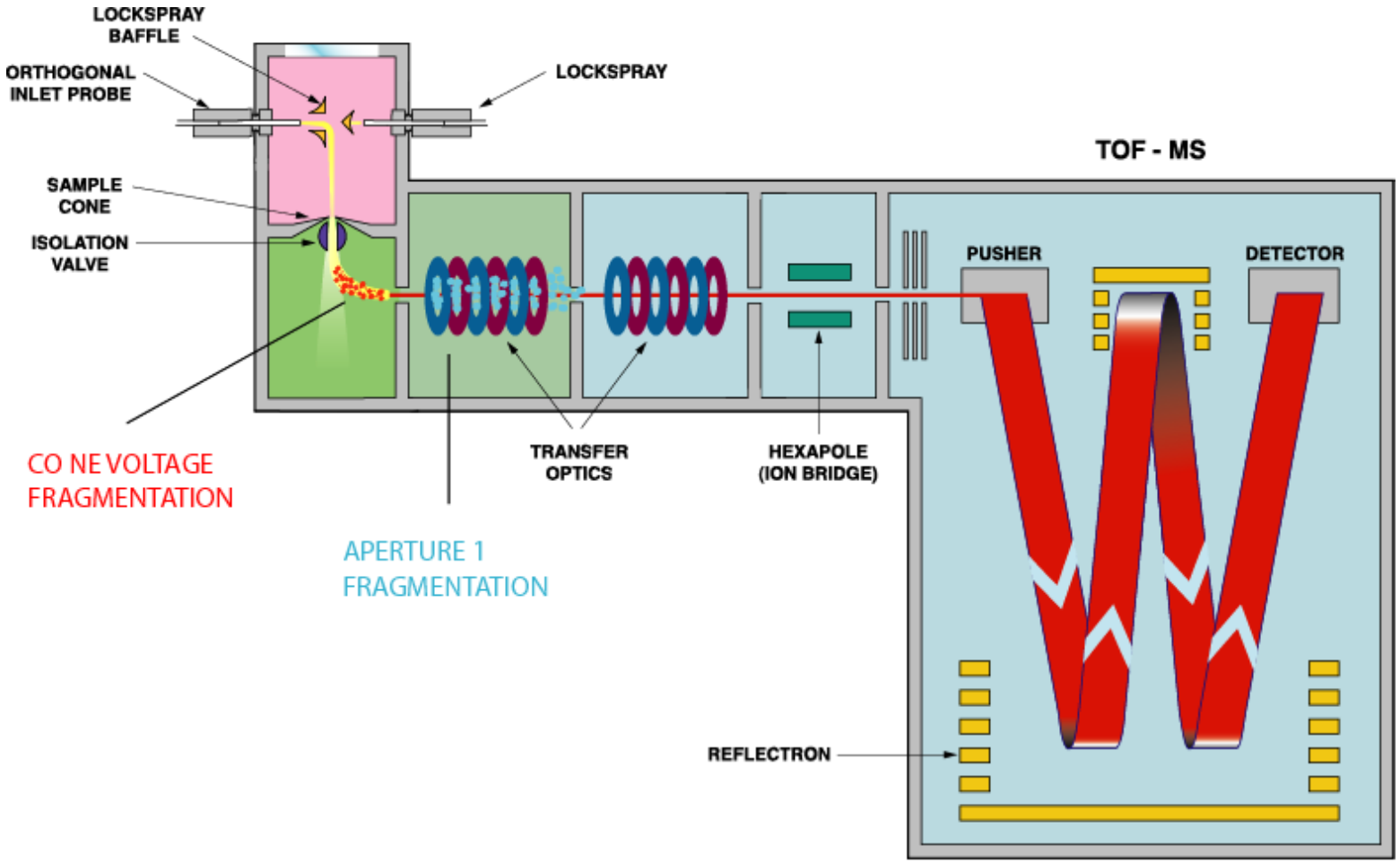
Waters

ACQUITY UPLC™
LCT Premier™

Designed to be compatible with UPLC™

For Complete Confidence

LCT Premier™ W-mode



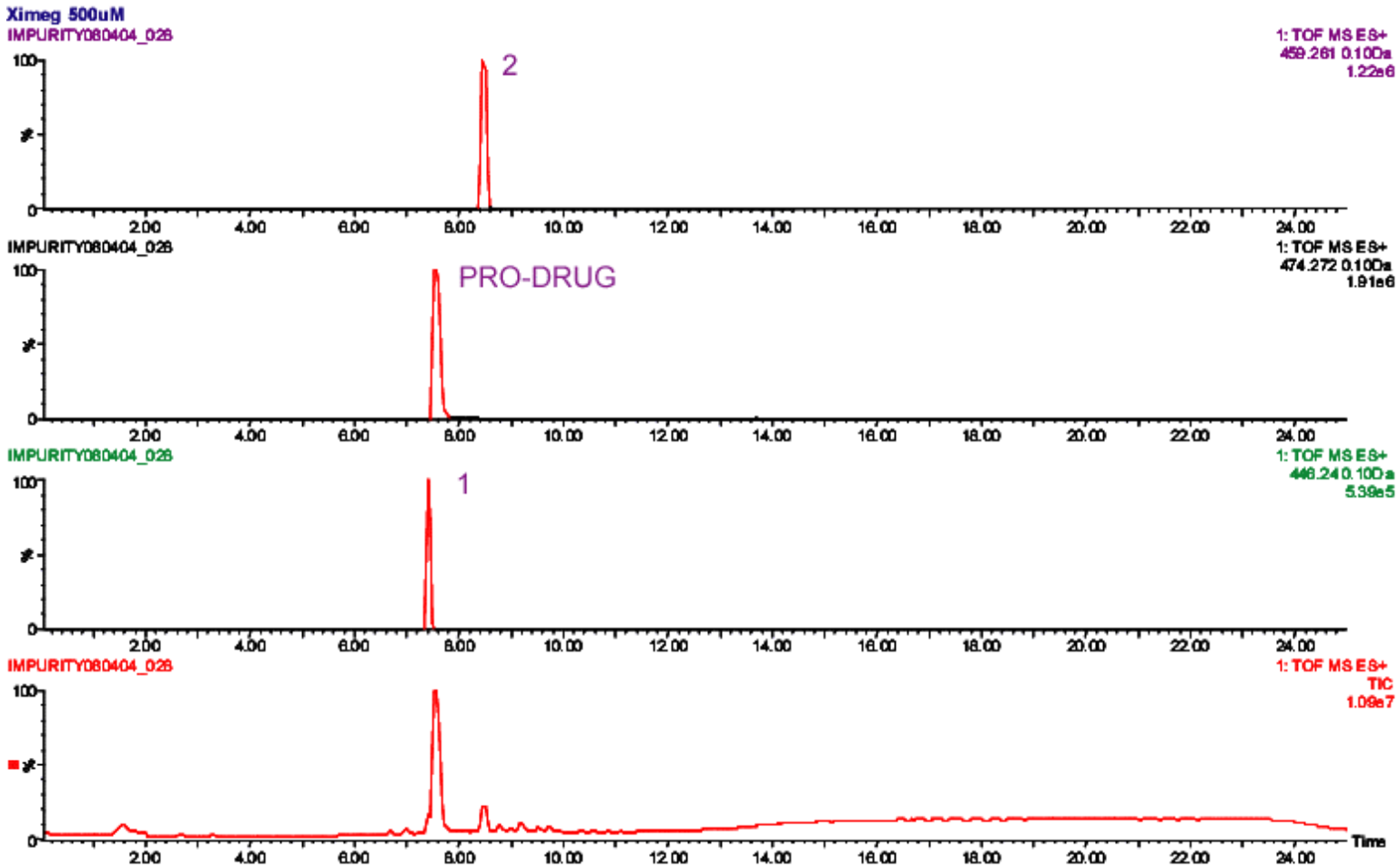
UPLC™ Resolution

- More information per sample
  - Impurity analysis
  - Degradation products
  - Metabolite ID
  - Metabonomic markers
  - Natural products

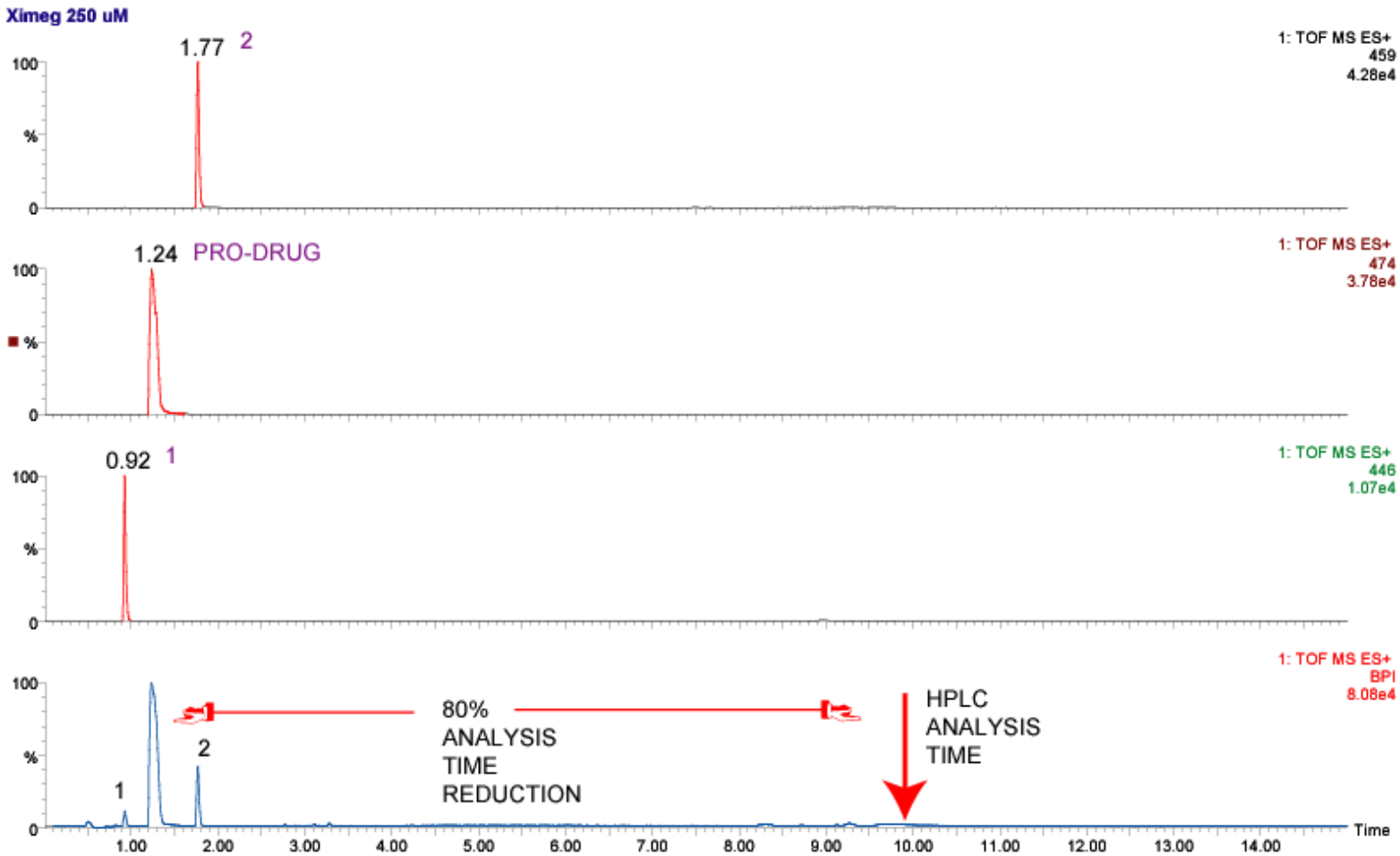
Waters

- Impurity Profiling Utilizing UPLC™ and HPLC combined with Oa-TOF
- Sources of impurities
  - Solvents
  - Synthesis reagents
  - Catalysts

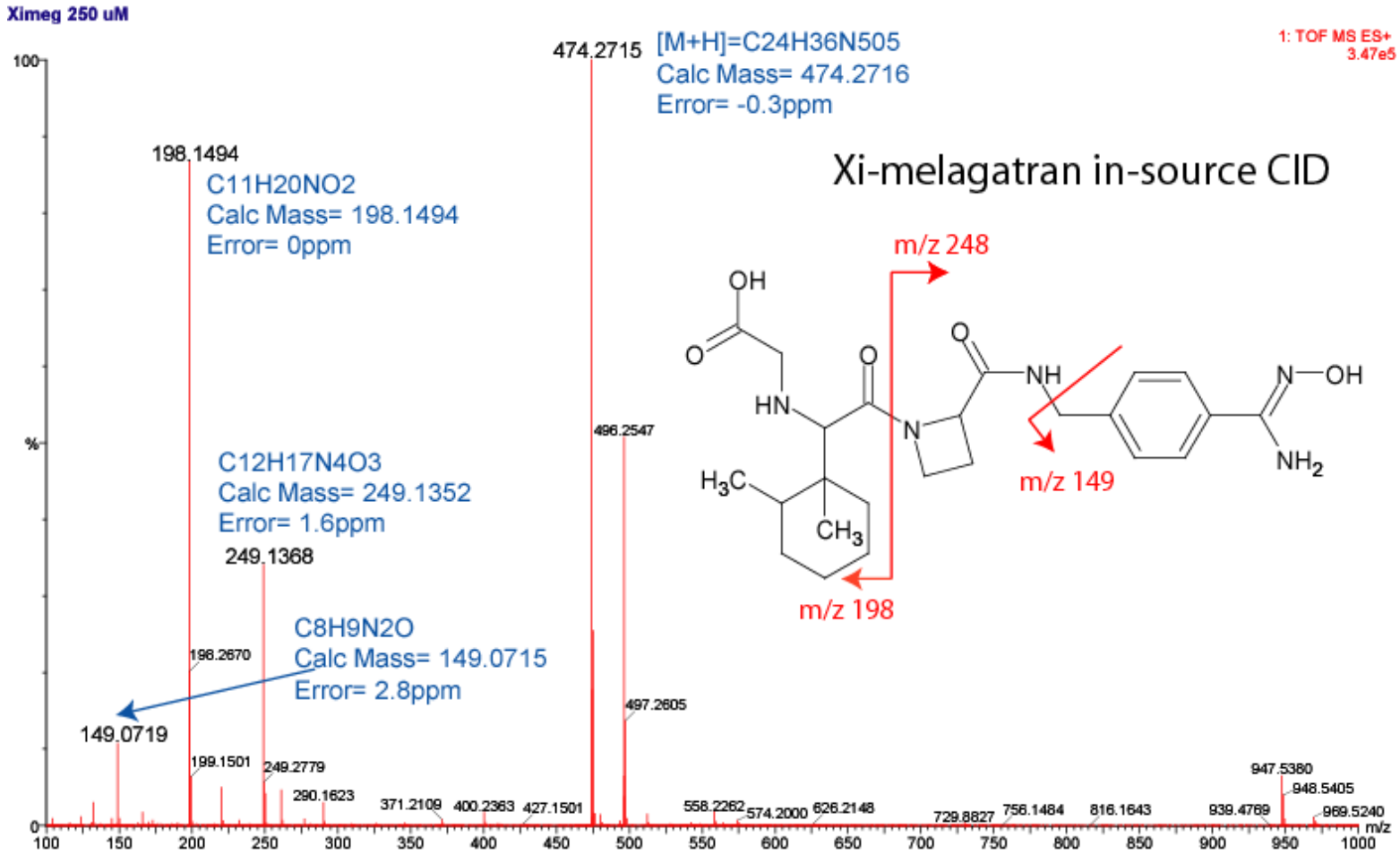
Impurity Profile of Xi-Melagatran DRE HPLC/MS LCT Premier™



Impurity Profile of Xi-Melagatran DRE
UPLC™/MS LCT Premier™

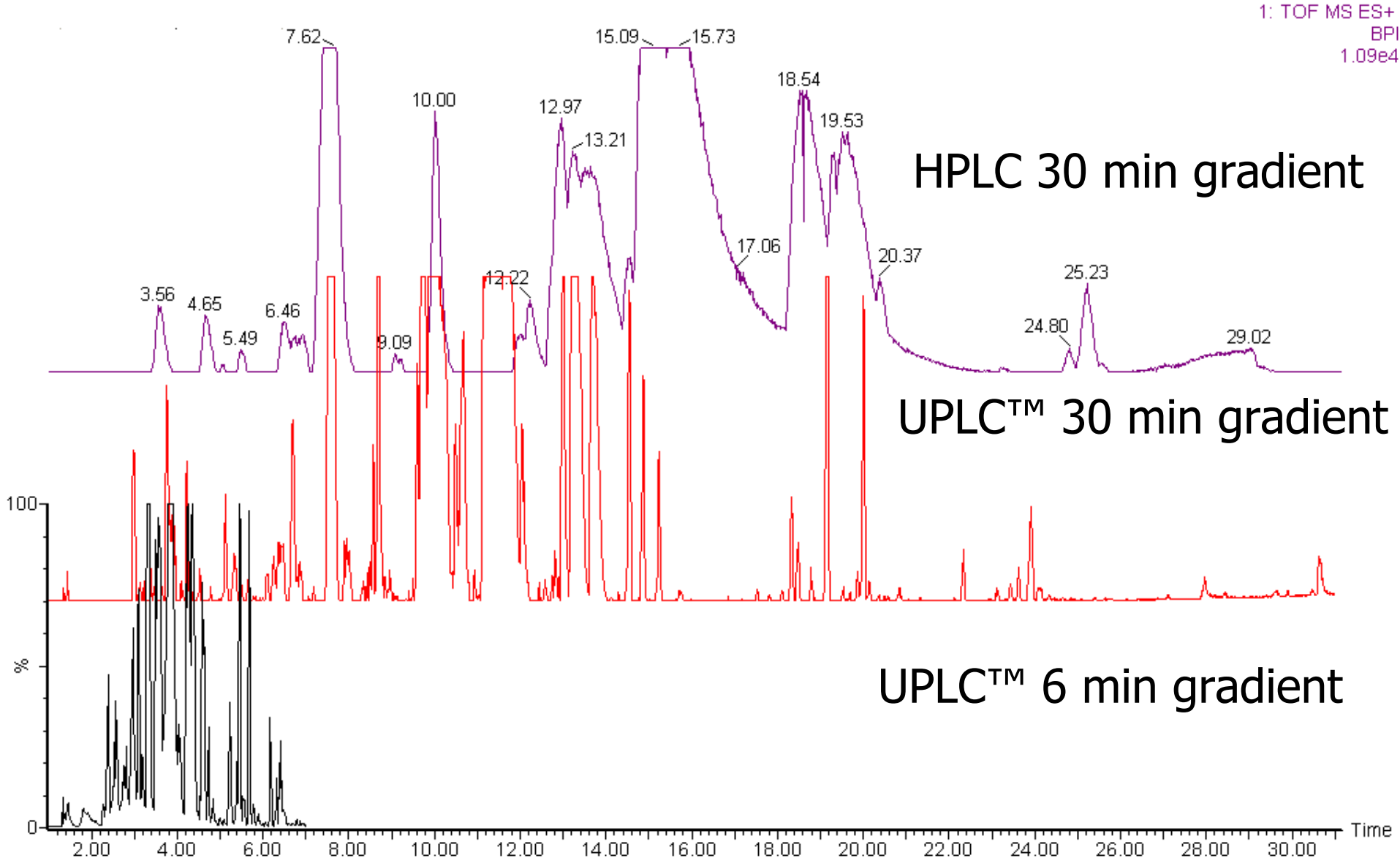


Xi-Melagatran DRE UPLC™/MS In-source CID LCT Premier™



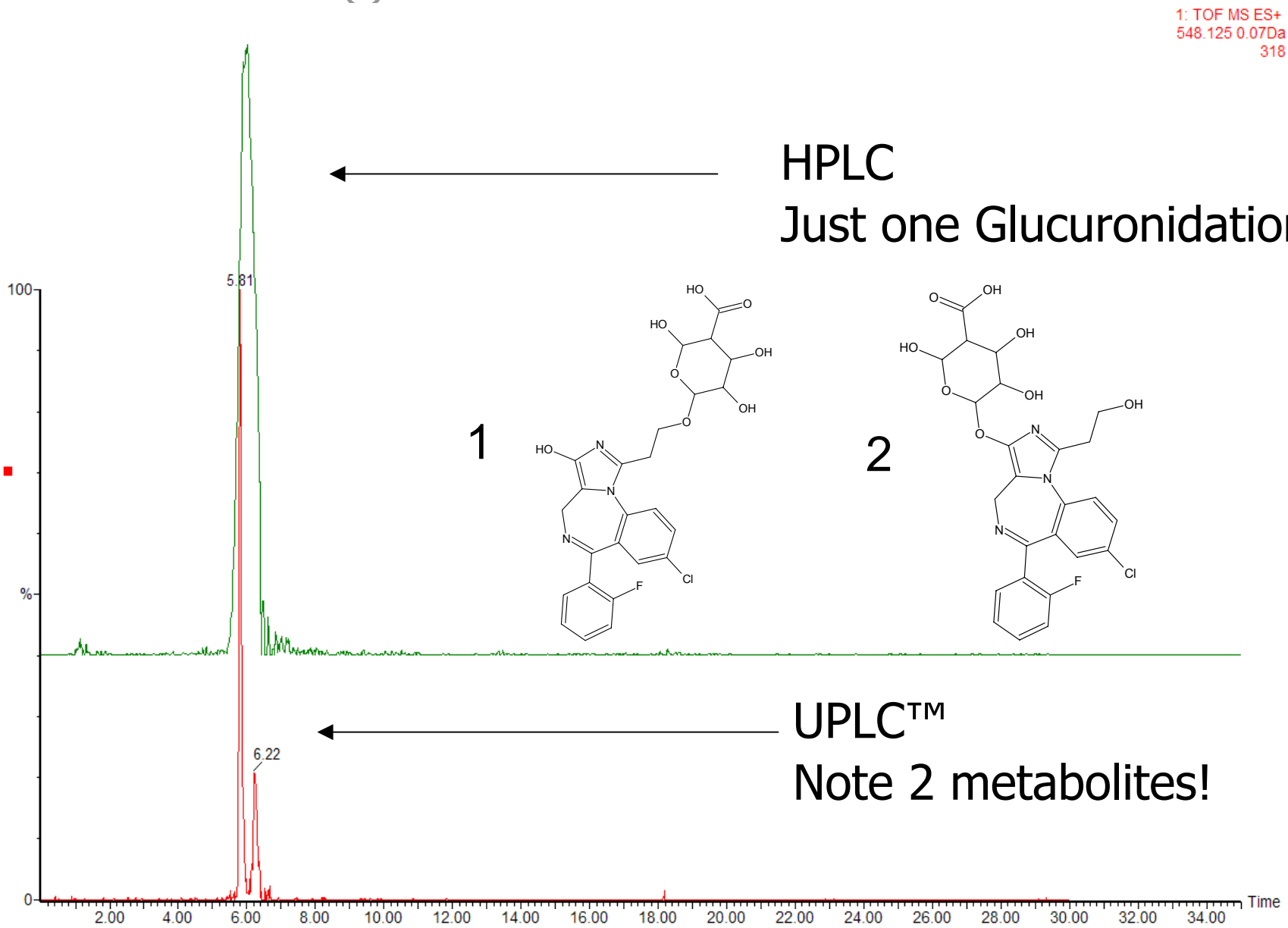
Waters

Faster UPLC™ Gradient Conditions



Waters

Extracted Ion Chromatogram Glucuronide Metabolite



Conclusion
The Future of LC/MS is now

- UPLC™/MS/MS allowed shorter analysis times (3x faster)
- Chromatographic resolution enhanced using 1.7μm particles
- Higher resolution improved peak height and LOD
  - Theory predicts 3x Some compounds showed 10x improvement
- Identification of drug metabolites simplified

Waters

Acknowledgements

- With thanks to Frances Gorycki and colleagues at Worldwide Bioanalysis, DMPK, GSK for providing samples
- Lena M von Sydow, Astra Zeneca, Mölndal, Sweden
- Rob Plumb, Kate Yu, Waters, Milford, MA
- Lisa Calton, Michael McCullagh, Waters, Manchester, UK
- Mike Wakefield, Waters, Dublin, CA
- Gordon Fujimoto, Marian Twohig, Waters, Beverly, MA