

High Resolution LC-MS for Screening and Quantitative Analysis of Antibiotics in Drinking Water using an Orbitrap and Online Sample Preparation

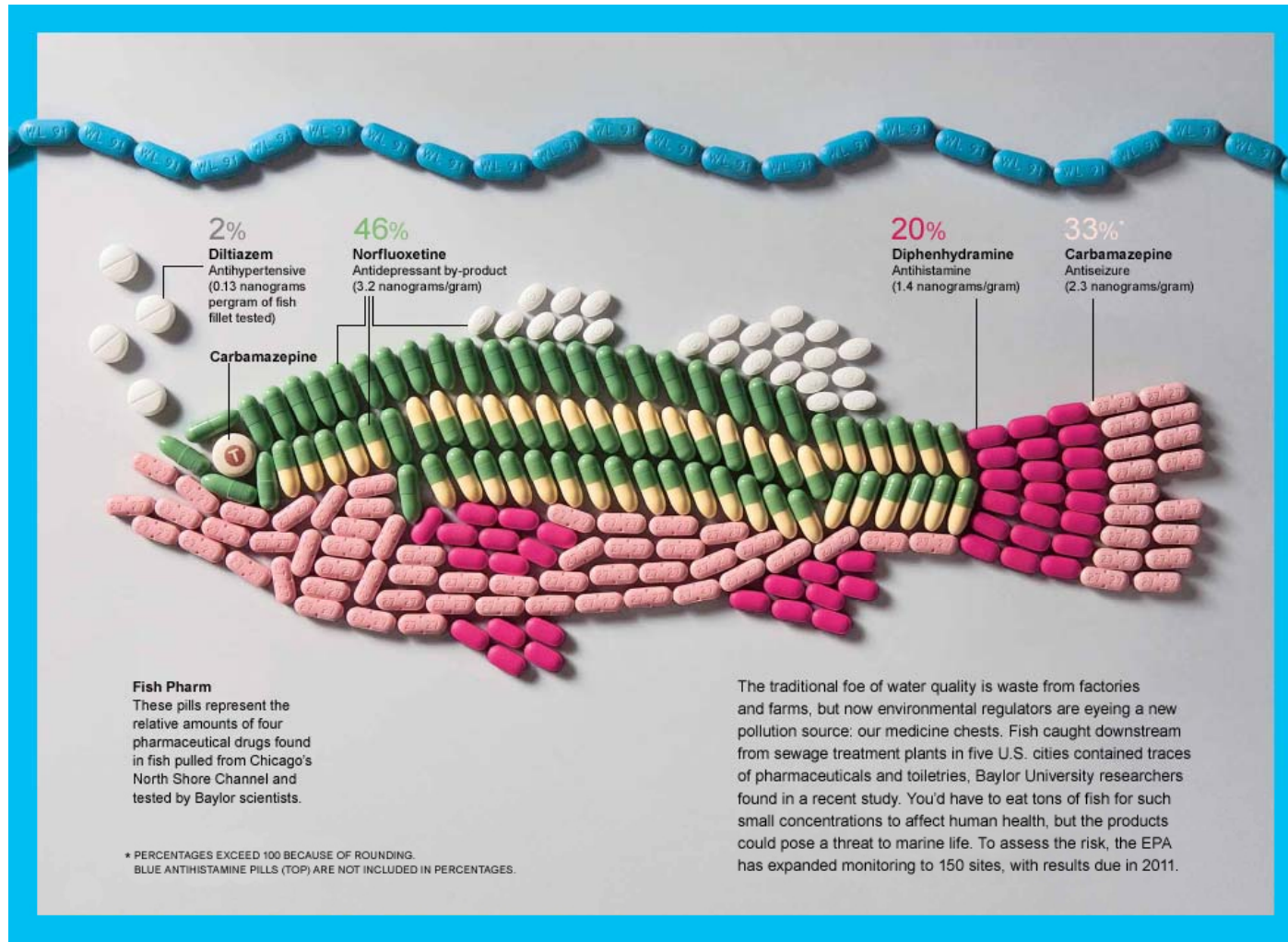


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Thermo Scientific



What's in our water supplies?



Pharmaceuticals, Personal Care Products, Pesticides

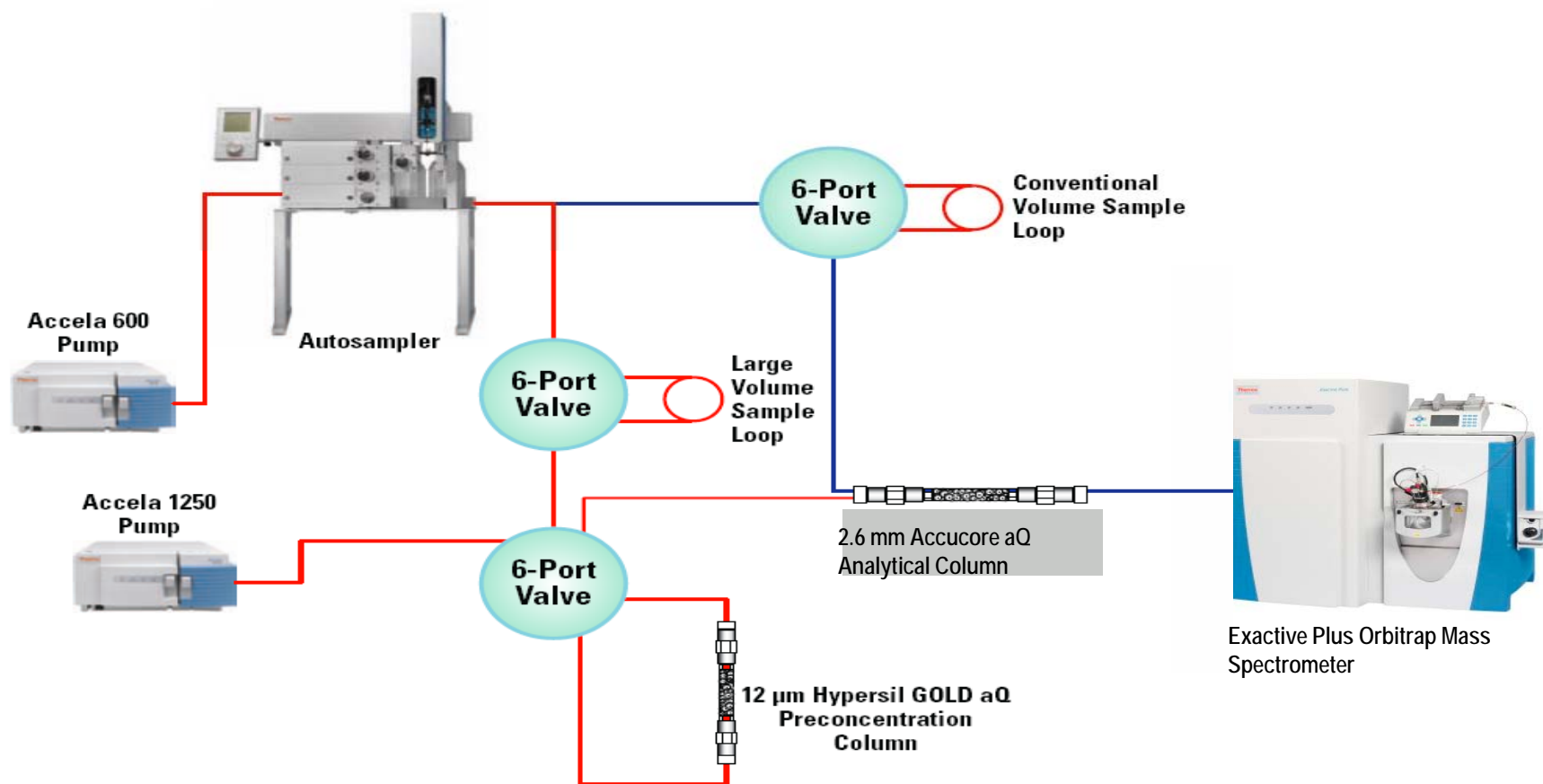
EQuan MAX: What is it ?

- Turnkey system for assaying environmental water samples (pesticides, antibiotics, etc.) at low ppt levels
 - **On-line sample clean-up and preconcentration**
 - **2 Columns : Loading and Analytical**
 - **2 pumps**
 - **High injection volumes**
 - **1mL-20mL**
 - **Standard injection volumes**
 - **1-100 μ L**
 - **Can be used with TSQ or HRAM MS instruments**



EQuan Plumbing Schematic

FIGURE 1. EQuan MAX system flow schematic

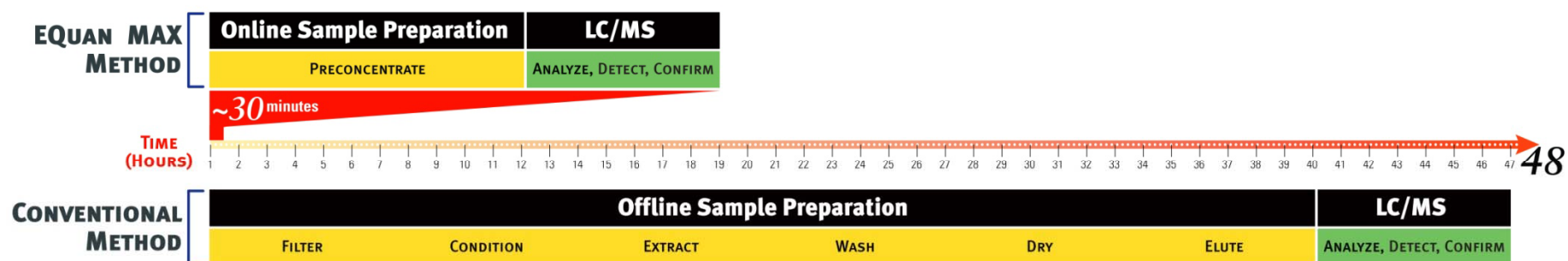


Chromatography Steps in Online SPE (EQuan)

- 3 sections per sample
 1. Loading Sample onto load column
 2. Transfer from load column to analytical column for separation
 3. Re equilibrate columns
- Large sample volumes (1-20mL) coupled with high flow rate (1-5 mL/min) for the load column necessitates large particle size
- During step 2, both columns are in-line, trapped compounds are back flushed from the load column and are separated on the analytical column
- In step 3, the columns can be washed with high organic to clean the columns before being re-equilibrated for the next sample

EQuan MAX: what's the Benefit ?

- Significant reduction in sample preparation times
 - On-line sample enrichment
 - Lowers cost of analysis by ca. 1-2 days
 - Reduces manual SPE errors and improves reproducibility over



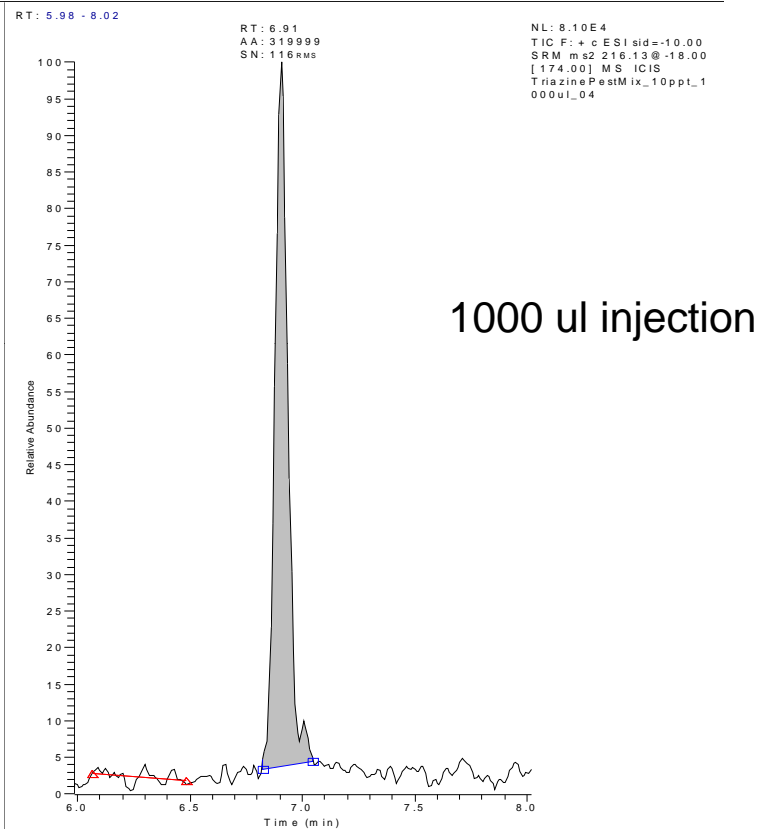
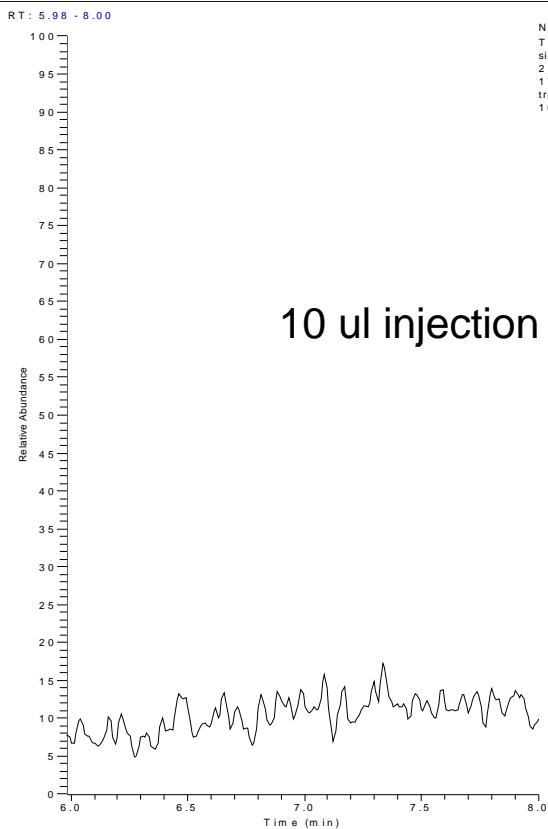
- Launched IUPAC, Japan Aug 2006

Now used in nearly all major European Reference Labs.

EQuan MAX Solution – Gain Vs. Conventional Injections

TriazinePestMix_10ppt_1000ul_04

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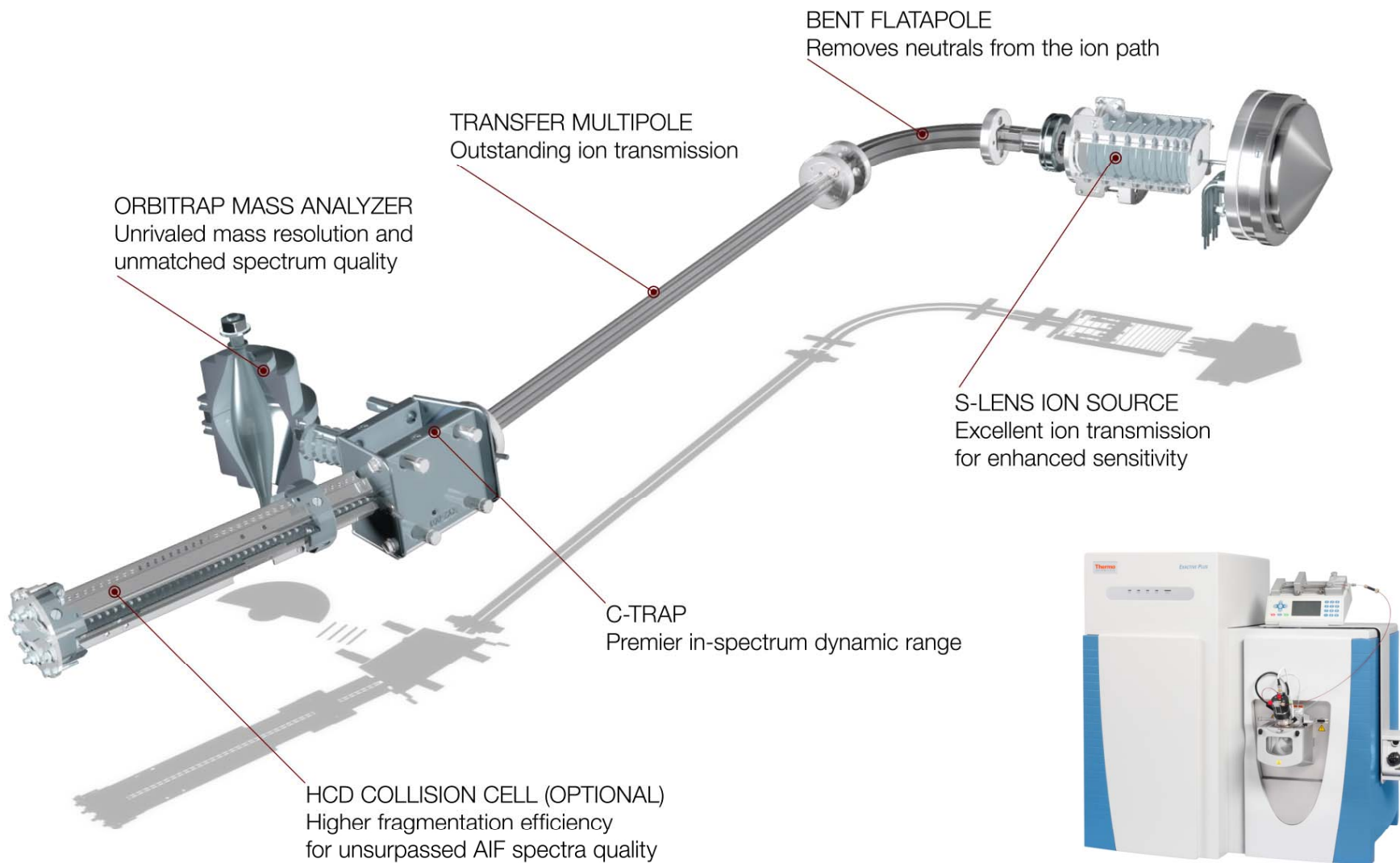
10 ppt Atrazine in ground water

EQuan MAX: Non-targeted screening and Quantitation

- Couple EQuan MAX with the Exactive Plus Orbitrap instrument.
- High Resolution Accurate Mass (HRAM)
- All ions are collected in every experiment.
 - Re-interrogate your data at a later time
- Quantitation and screening methods are easy to set up since there are no compound dependant parameters to optimize.

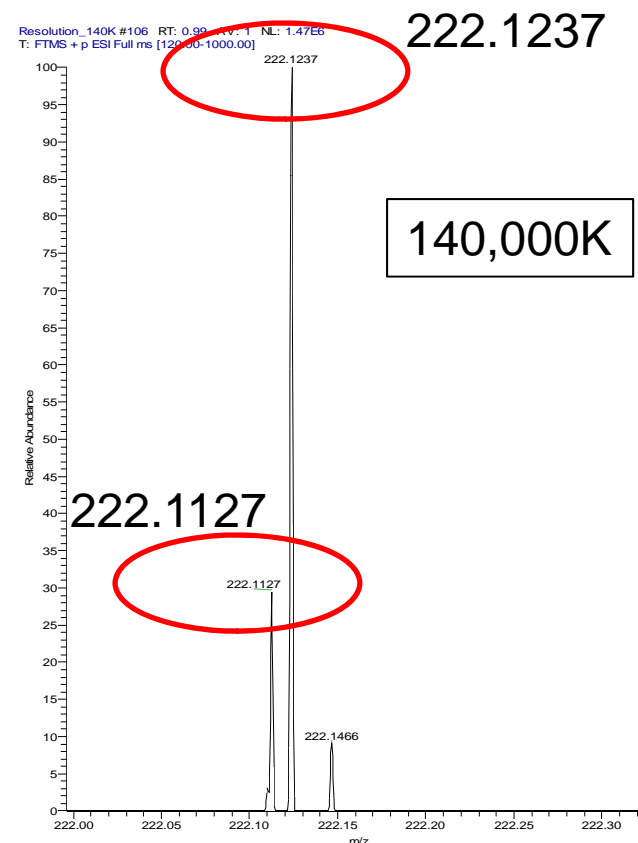
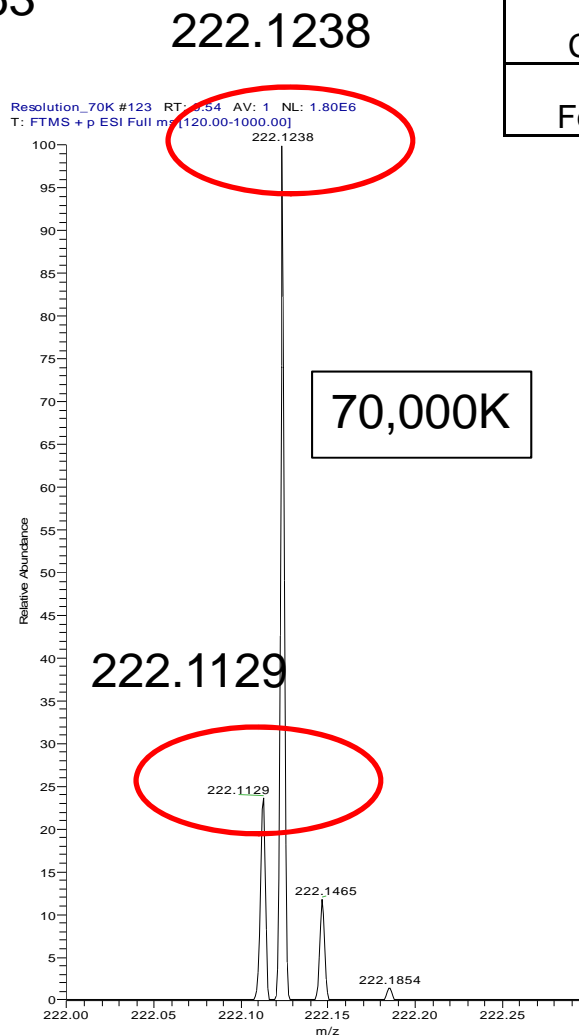
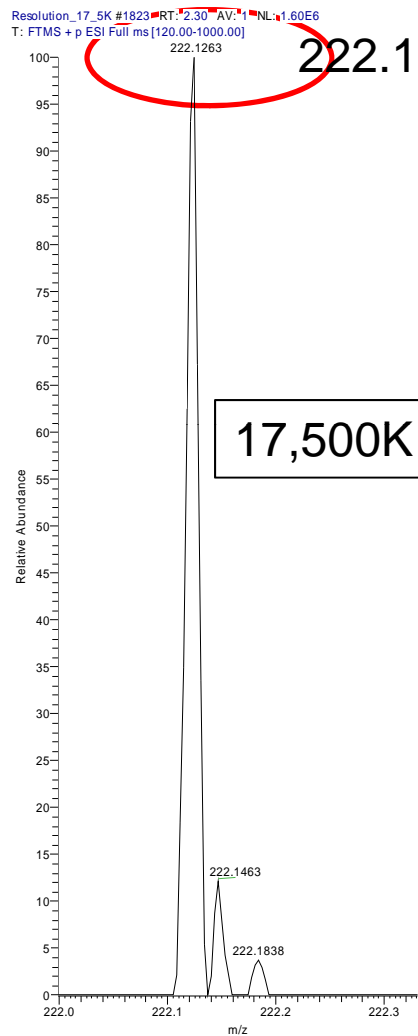


Exactive Plus



Importance of High Resolution

Name	Molecular Formula	M+H
Carbofuran	C ₁₂ H ₁₅ NO ₃	222.1125
Formetanate	C ₁₁ H ₁₅ N ₃ O ₂	222.1237



Importance of High Resolution: Terbutylazine in Water

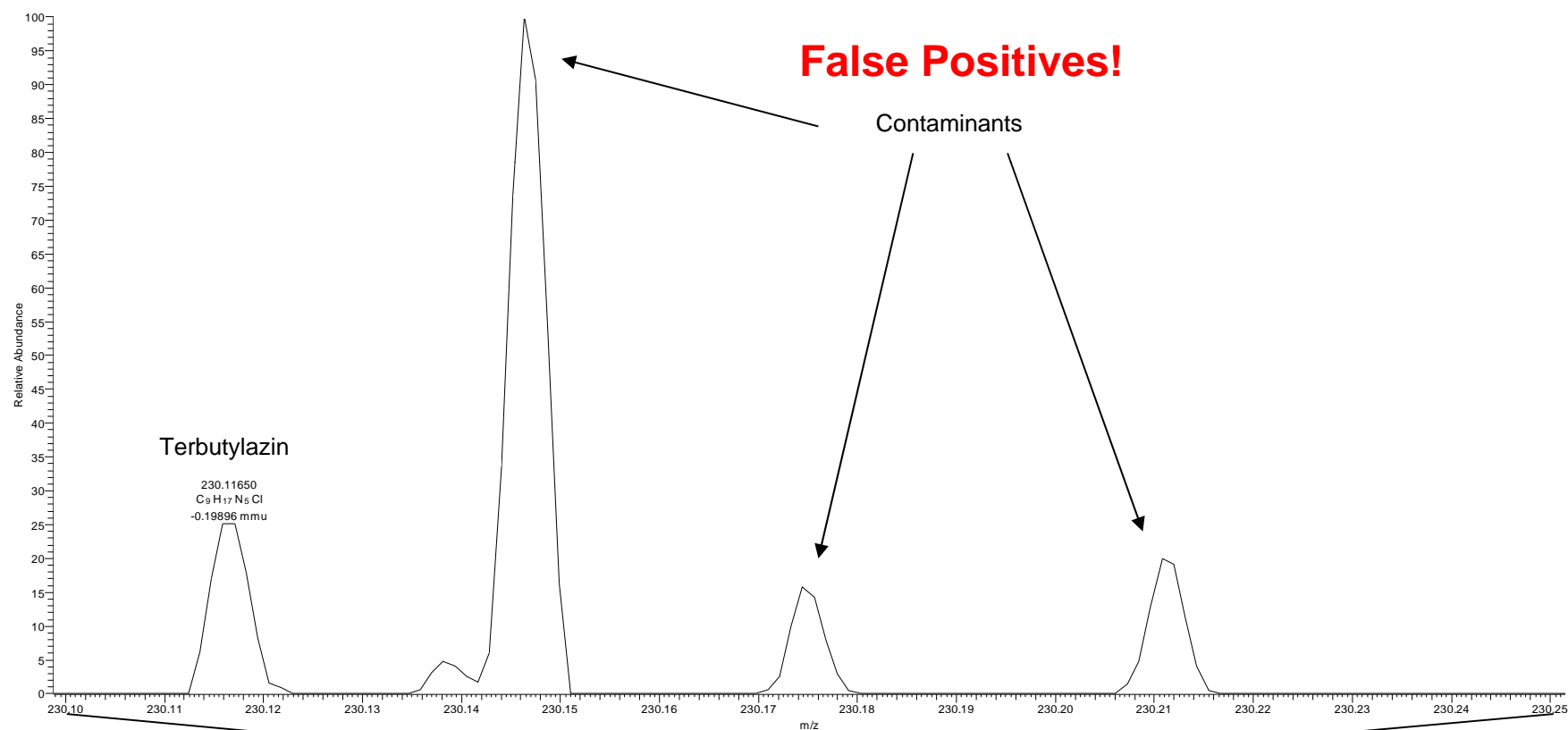
Resolution: 50000 (EQuan + Exactive)

F:\Thermodaten\...\Rawfiles\PSM018

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PSM 0.5 ug/mL

PSM018 #1405 RT: 10.76 AV: 1 NL: 1.29E5
T: FTMS (1,1) + p ESI Full lock ms [100.00-1000.00]



Zoom 0.15 Da!

Exactive Plus – “Quanfirmation” what is it?

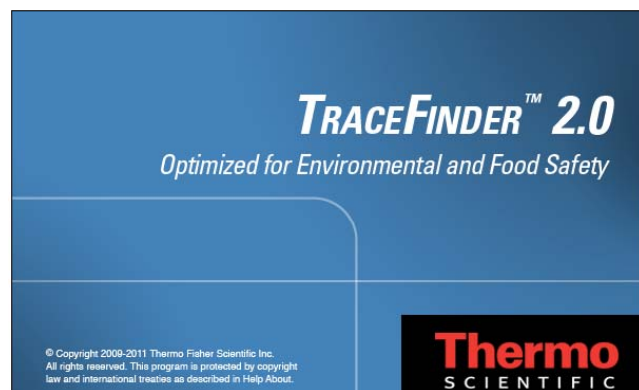
- High performance HRAM Quantitation and Confirmation bench top LCMS system, capable of :
 - Multi-residue quan performance similar to mid-high end Triples
 - “All Ion Fragmentation” where all ions created in the source are fragmented in the HCD cell of the Exactive Plus.
 - In addition to quantitation, the HRAM instruments can also provide us with MS/MS spectra to confirm production ions and compare to a spectral library.
 - Ideal for targeted and general unknown screening
 - Highest confidence confirmation with R = 140K, and MS/MS
 - Let’s use the Exactive Plus to combine these 2 experiments one.



Software Solutions for Screening and Quantitation



- Software tailored for HRAM screening and confirmation workflows
- Built in confirmation library (>1000 compounds)
- Isotopic pattern matching
- Easy for data review



- Software tailored for quantitative work
- Built in methods and compound data store
- >40 report templates
- Streamlined data review
- Ideal for routine work
- HRAM or nominal mass compatible

Sample Preparation – Antibiotics analyzed

- Carbamazepine
- Erythromycin
- Ketoprofen
- Norethindrone
- Roxithromycin
- Sulfachloropyridazine
- Sulfadimethoxine
- Sulfamerazine
- Sulfamethazine
- Sulfamethizole
- Sulfamethoxazole
- Sulfathiazole
- Trimethoprim
- Tylosin

Drinking water samples, spiked with antibiotics

Samples were acidified to 0.1% Formic Acid

Calibration range:

1 ppt (pg/mL) – 10 ppb (ng/mL)

Chromatography

- Loading Pump Accela 600
- Hypersil Gold aQ
20x2.1mm 12 μ
- Analytical Pump Accela 1250
- Accucore aQ column,
100x2.1 mm, 2.6 μ

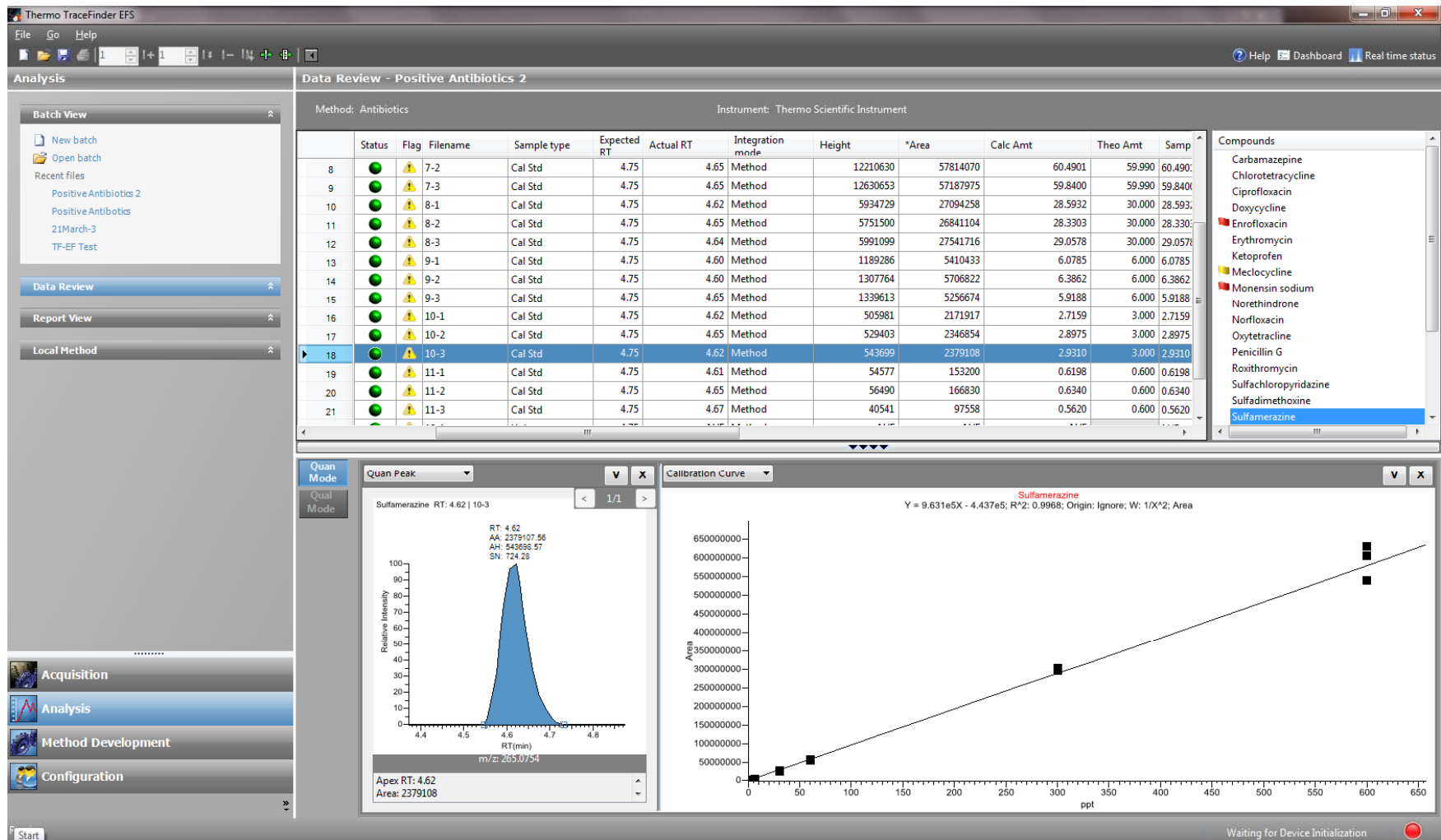
Time (min)	Loading Pump %A	Flow Rate (μ L/min)	Time (min)	Analytical Pump %A	Analytical Pump %B	Flow Rate (μ L/min)
0.0	100	1000	0.0	98	2	350
1.3	100	1000	1.5	98	2	350
1.5	100	100	3.0	70	30	350
12.0	100	100	8.0	2	98	350
12.1	100	1000	9.0	2	98	350
15.0	100	1000	9.1	98	2	350
			15.0	98	2	350

Mobile Phases: A= 0.1% Formic Acid and 4mM Ammonium Formate in Water
B= Methanol with 0.1% Formic Acid and 4mM Ammonium Formate

Results

- All calibrators were run in triplicate
- 1/x weighting
- Linear or quadratic curve fit (due to large concentration range of samples, 1 ppt-10 ppb based on Sulfamethoxazole)
- TraceFinder 2.1 software used for quantitative data analysis, ExactFinder 2.0 used for spectral confirmation.

Sulfamerazine, 3 ppt, 1mL injection volume



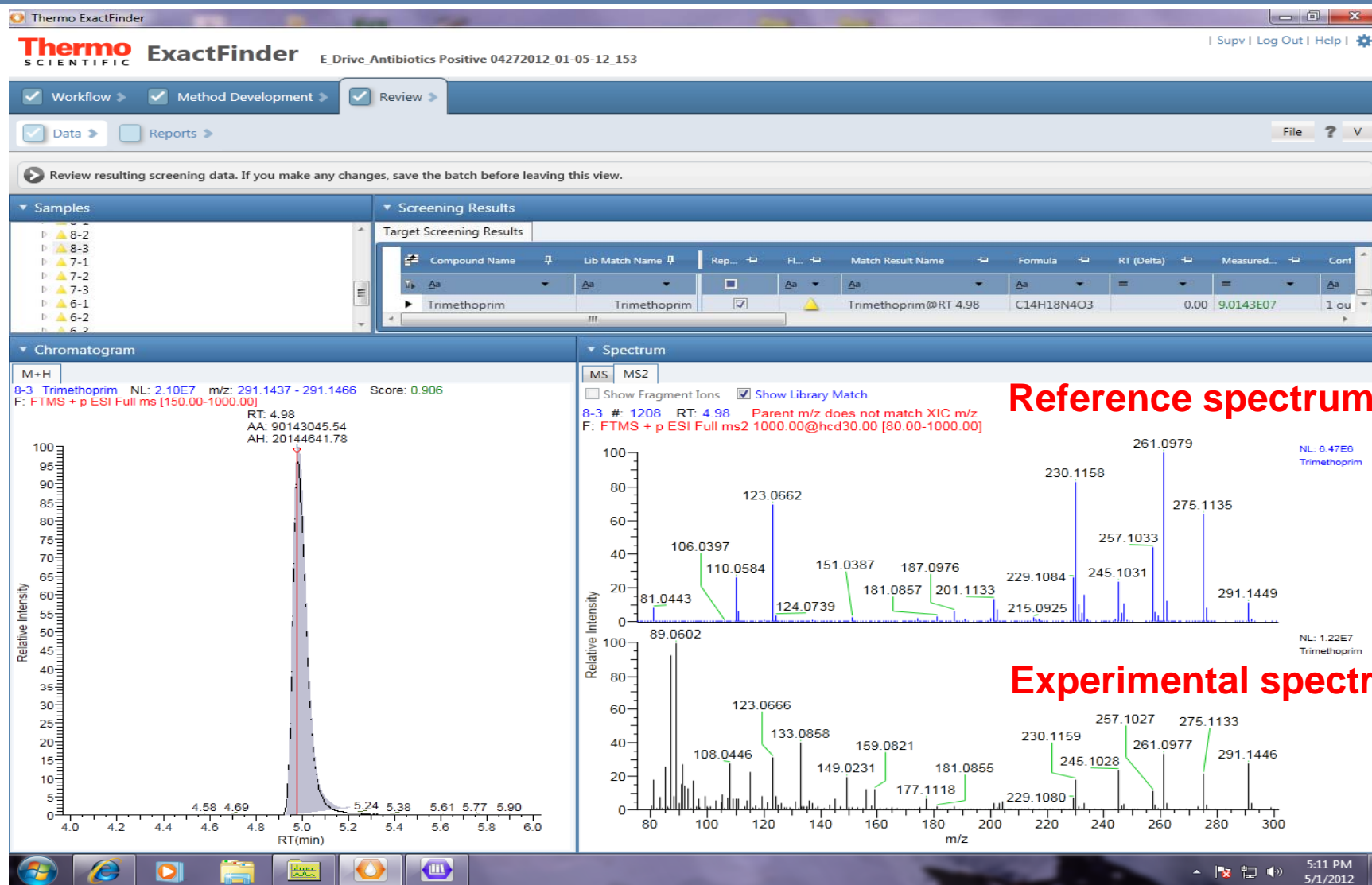
Limits of Quantitation

- Determined by %RSD of the calibration standards, where $LOQ \leq 15\%$
- Some analytes had LOQs lower than the lowest standard
- HRAM instruments have very little noise, so S/N methods for determining LOQs is not applicable

Limits of Quantitation

Compound	Theoretical Mass (<i>m/z</i>)	LOQ (pg/mL)	% RSD at LOQ
Carbamazepine	332.14050	0.2	8.90
Erythromycin	734.46852	40.0	14.30
Ketoprofen	255.10157	1.0	9.90
Norethindrone	299.20056	1.0	13.50
Roxithromycin	837.53185	9.9	4.20
Sulfachloropyridazine	285.02075	1.0	7.30
Sulfadimethoxine	311.08085	0.4	4.80
Sulfamerazine	265.07537	0.6	4.90
Sulfamethazine	279.09102	1.0	3.45
Sulfamethizole	256.02089	1.0	6.30
Sulfamethoxazole	254.05939	1.0	6.60
Sulfathiazole	271.03179	0.6	3.60
Trimethoprim	291.14517	1.6	13.10

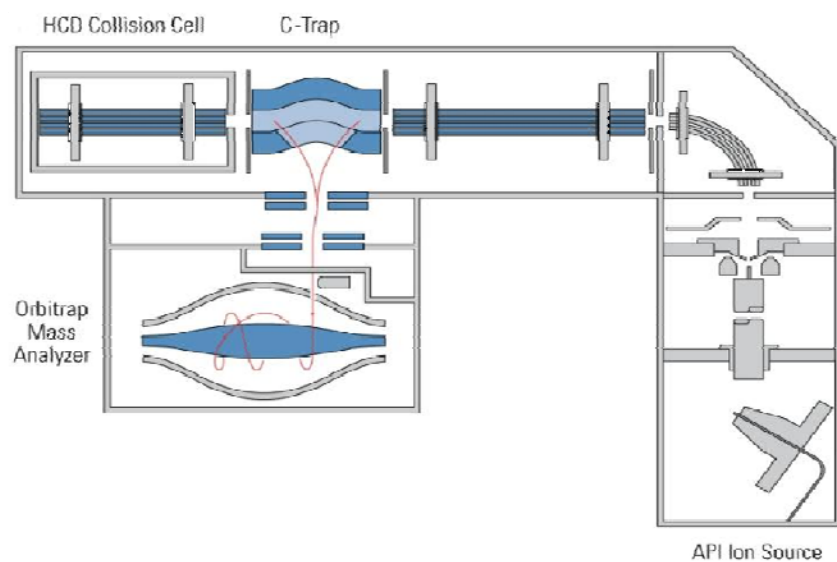
Spectral Comparison – Trimethoprim 80 pg/mL



Conclusions

- Online pre-concentration and extraction for 1mL injections of antibiotics at the ppt level
- The quantitation of HR/AM data using TraceFinder software from the Exactive Plus Orbitrap instrument
- Spectral confirmation of MS² spectrum collected in the same data files as the quantitation data using ExactFinder software
- Ability to quantitate and confirm samples in the same analytical run for antibiotics in water samples

Questions?



Exactive Plus™
Technology

