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LC-MS/MS Technology and Applications

" Transform your routine science "

Jonathan Beck, Ph.D

Marketing Scientist

Environmental & Food Safety

October 20th, 2015

The world leader in serving science

Outline

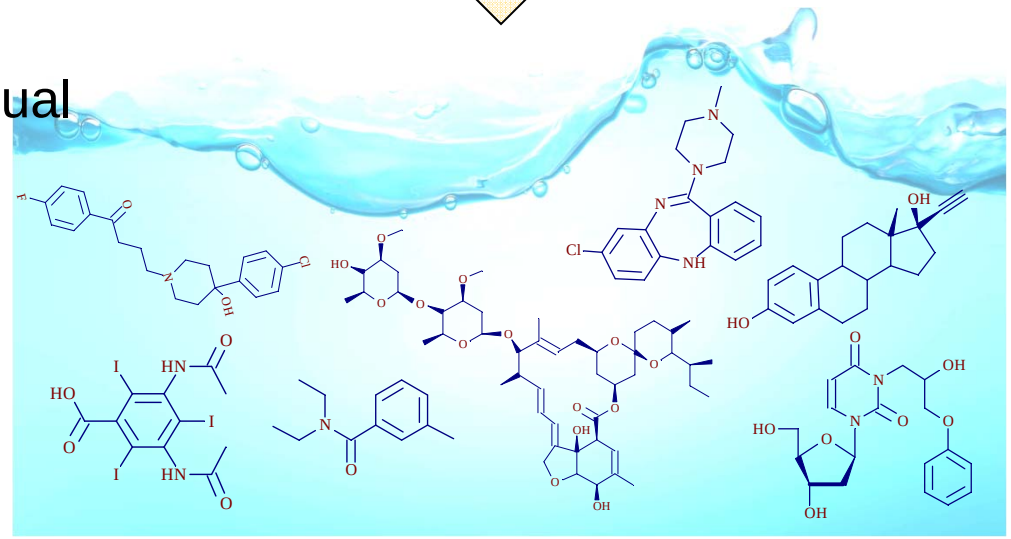
- High Resolution Accurate Mass for Environmental Analysis
- EQuan MAX Plus online sample preconcentration and cleanup
- New Triple Quadrupole developments from Thermo Scientific
- Project with Duke University for Emerging contaminants

What is Environmental Analysis Challenge?

- Analysis of thousands of pollutants
- High diversity of chemical characteristics
- Different matrices
- Screening Workflows with Quan/Qual capabilities needed
- New issues: Hydraulic fracturing
- Regulatory

- ***EU Water Framework Directive***
- ***Emerging Contaminants in The Environment – USGS study***

Pharmaceuticals, Pesticides, Endocrine Disruptors, Personal Care Products



Contamination of ground and drinking water is a risk for human health

EPA United States Environmental Protection Agency

USGS science for a changing world

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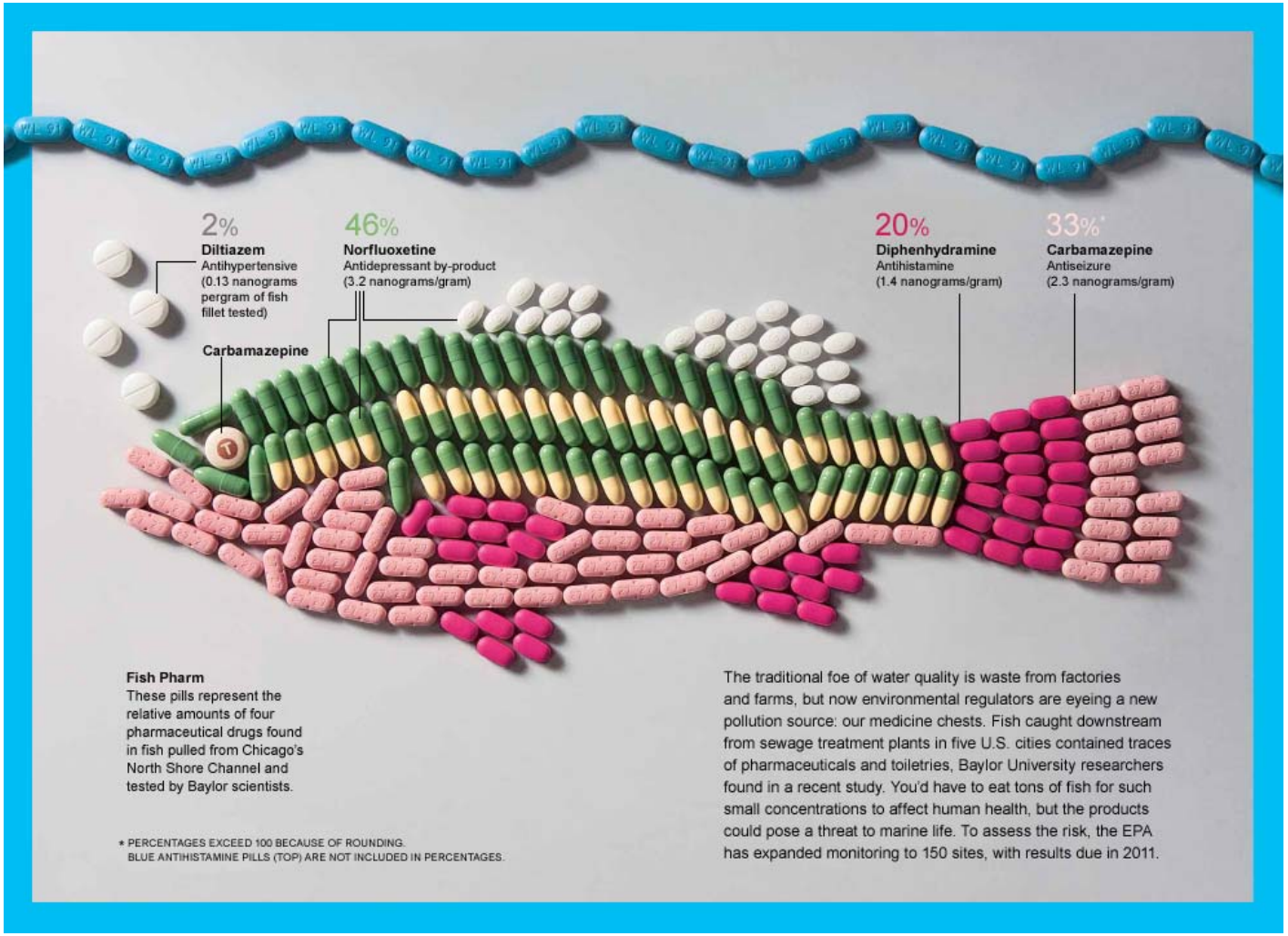
What's in our water supplies?



Pharmaceuticals, Personal Care Products, Pesticides

*Image from *National Geographic, April 2010*

Sample preparation/analyte detection strategy

Clean-up efficiency

Simple extraction

LLE

Dispersive SPE

GPC

SPE

Detection selectivity

HRAM-MS/MS

MS/MS

LC-MS

HPLC-FLD

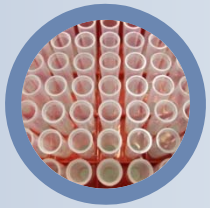
GC-MS, ECD

HPLC-UV

Major Challenges in EFS Analysis

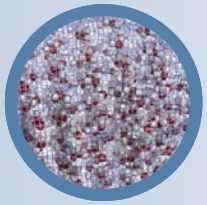
Low Concentration

Being able to detect and identify trace level analytes in simple or complex samples.



Large Sample Numbers

High throughput for routine labs.



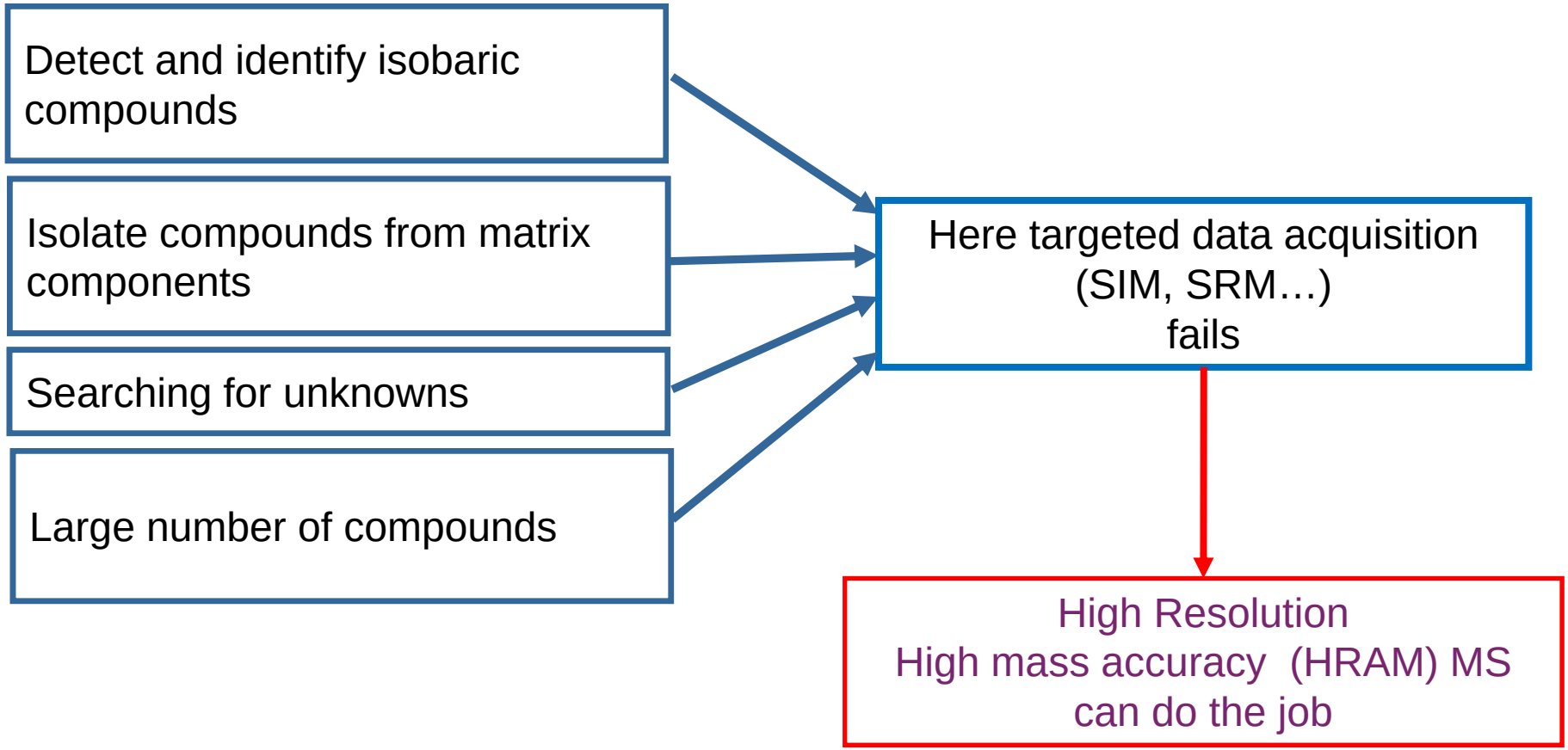
Matrix Complexity

Identifying low abundance analytes obscured by high abundance compounds.

Small Sample Volumes

Getting the most accurate information from precious samples

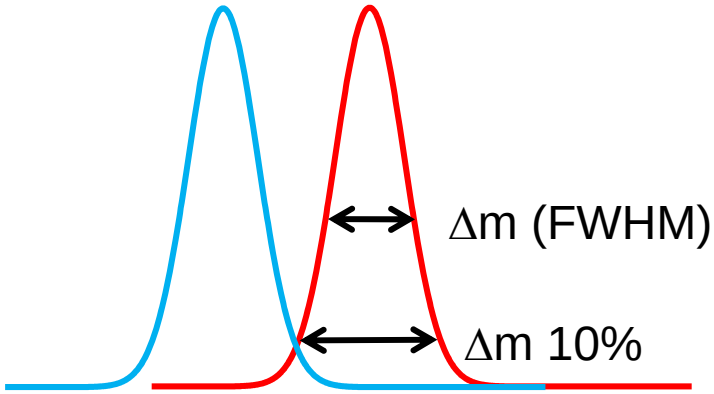
Analytical Challenges for Traditional Mass Spectrometry



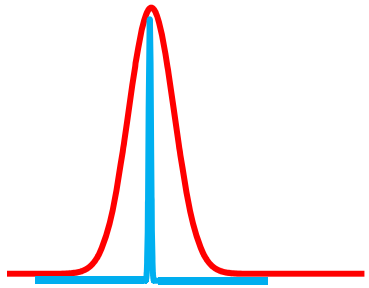
Resolution (resolving power)

- Resolution

$$R = \frac{m}{\Delta m}$$



- Quadrupole MS $R = \frac{m}{\Delta m} = \frac{500}{0.6} = 833$

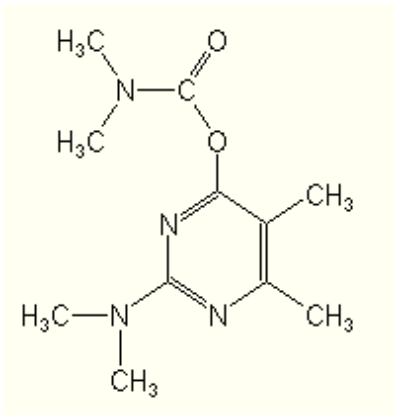


- Orbitrap MS $R = \frac{m}{\Delta m} = \frac{500}{0.005} = 100000$

Detection of analytes in heavy matrix

Pesticide in Matrix

Pirimicarb



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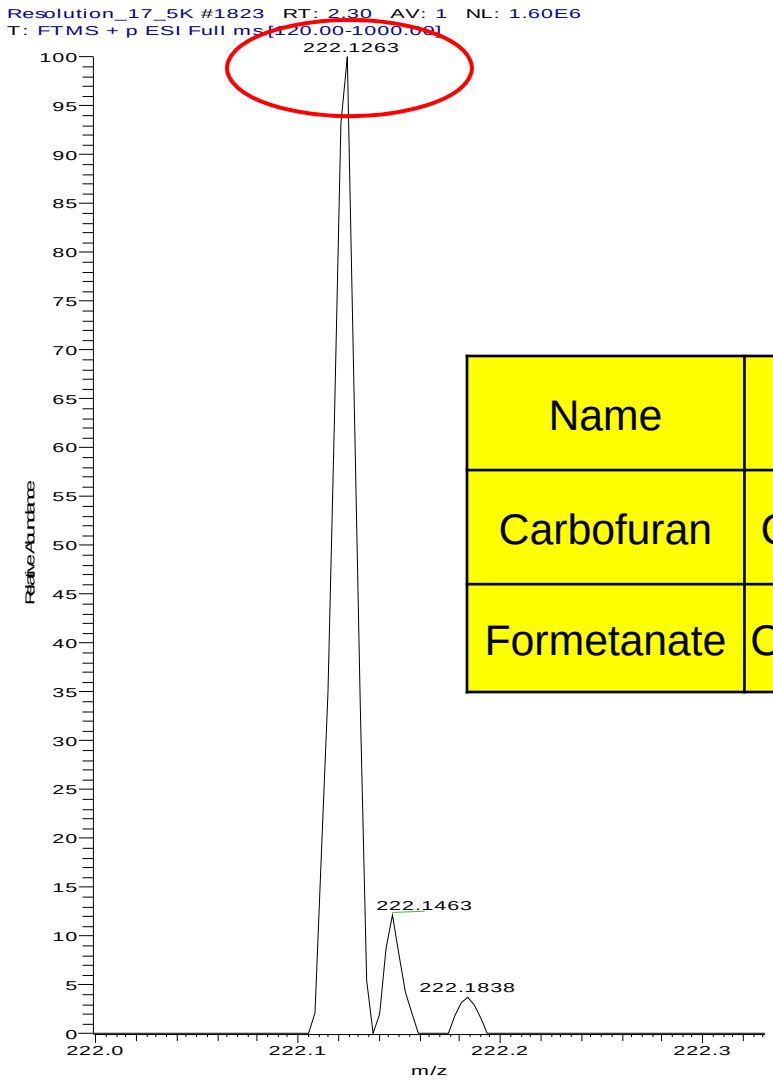
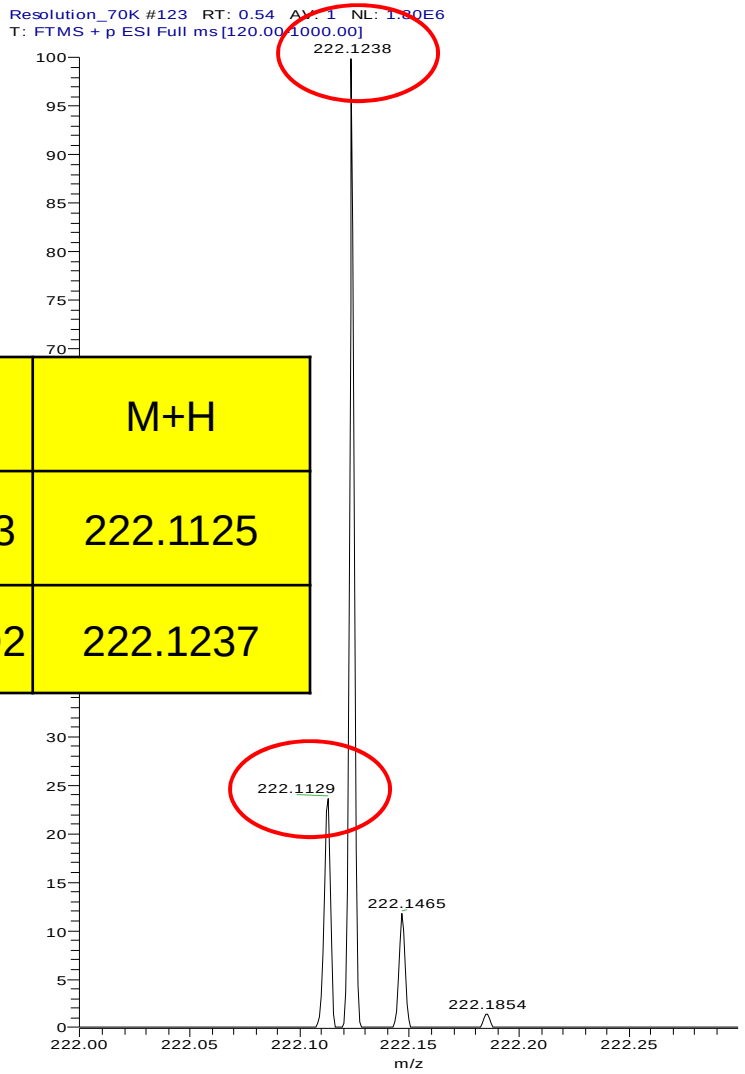
Resolution influence (17.500 vs. 70.000)



Name	Molecular Formula	M+H
Carbofuran	C12H15NO3	222.1125
Formetanate	C11H15N3O2	222.1237



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Mass accuracy

- Mass accuracy

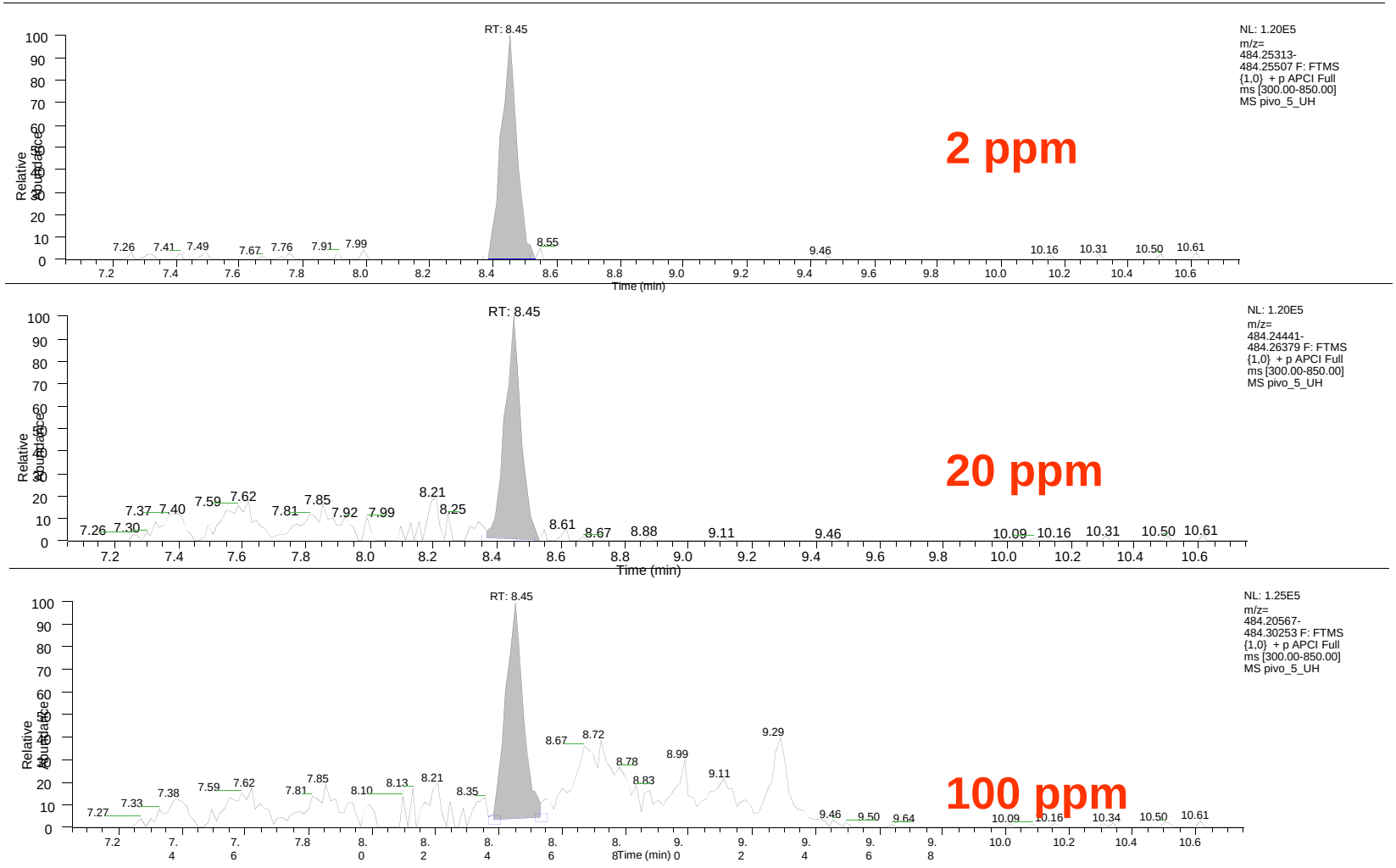
$$\Delta m / z = \frac{m_{meas} - m_{true}}{m_{true}} \cdot 10^6$$

- Quadrupole MS $\Delta m / z = \frac{500.1 - 500.0}{500} \cdot 10^6 = 200 ppm$

- Orbitrap MS
  TOF MS $\Delta m / z = \frac{500.10314 - 500.10214}{500.10314} \cdot 10^6 = 2 ppm$

Selectivity increases with higher mass accuracy

Beer extract, T-2 toxin 5 µg/L



**Zachariasova M et al, Anal. Chim. Acta*

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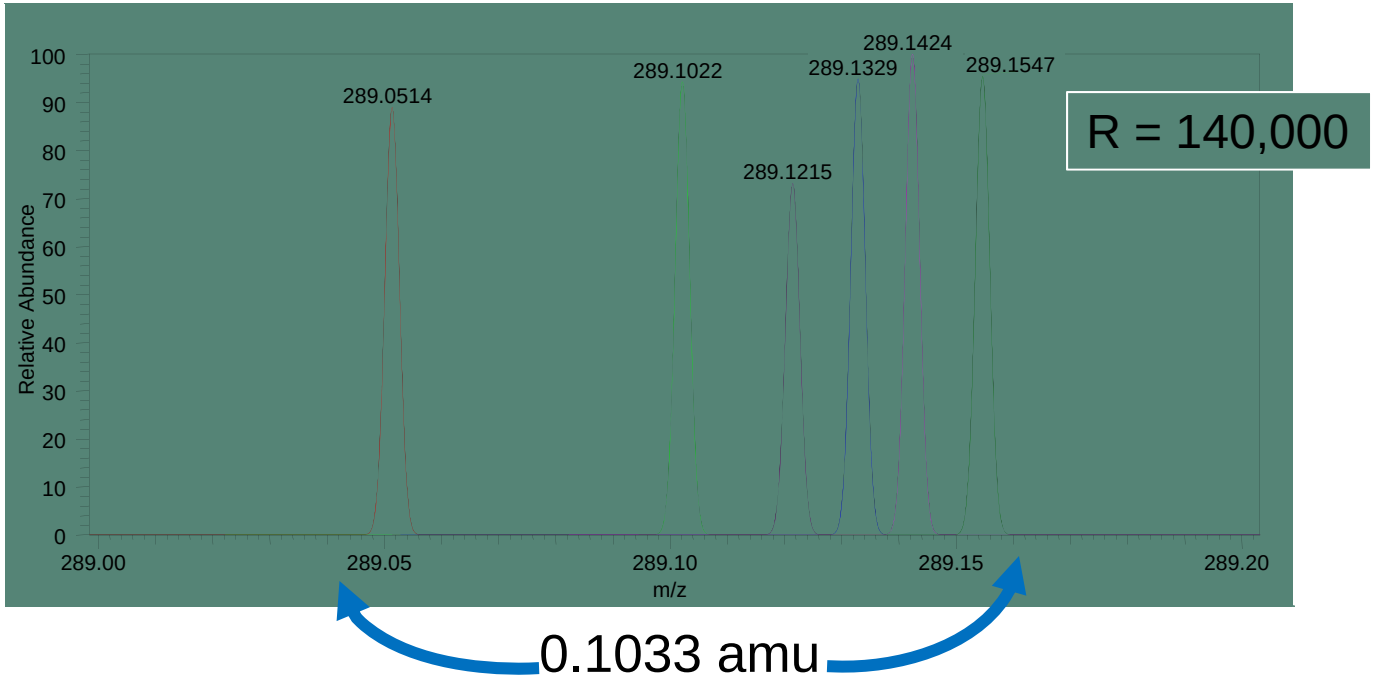
Detection of Isobaric Compounds in mixtures

Element	Exact Mass
H	1.007825
C	12.000000
N	14.003074
O	15.994915

Is a simultaneous measurement possible?



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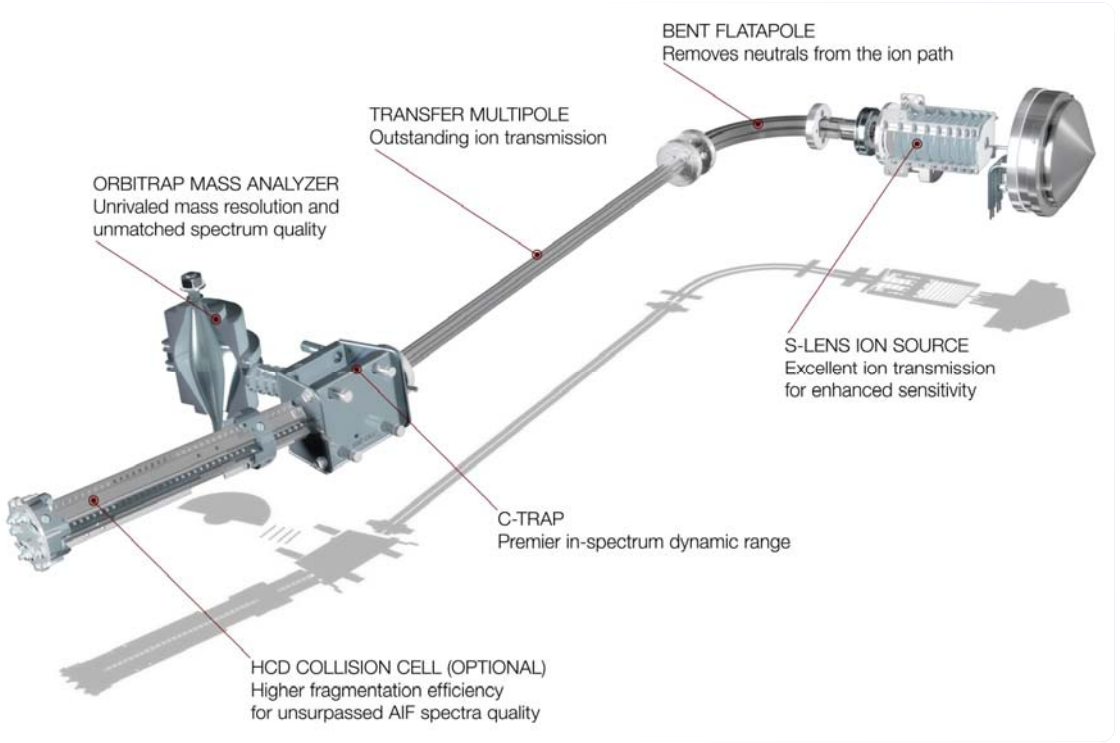
The Exactive Plus Orbitrap LCMS system



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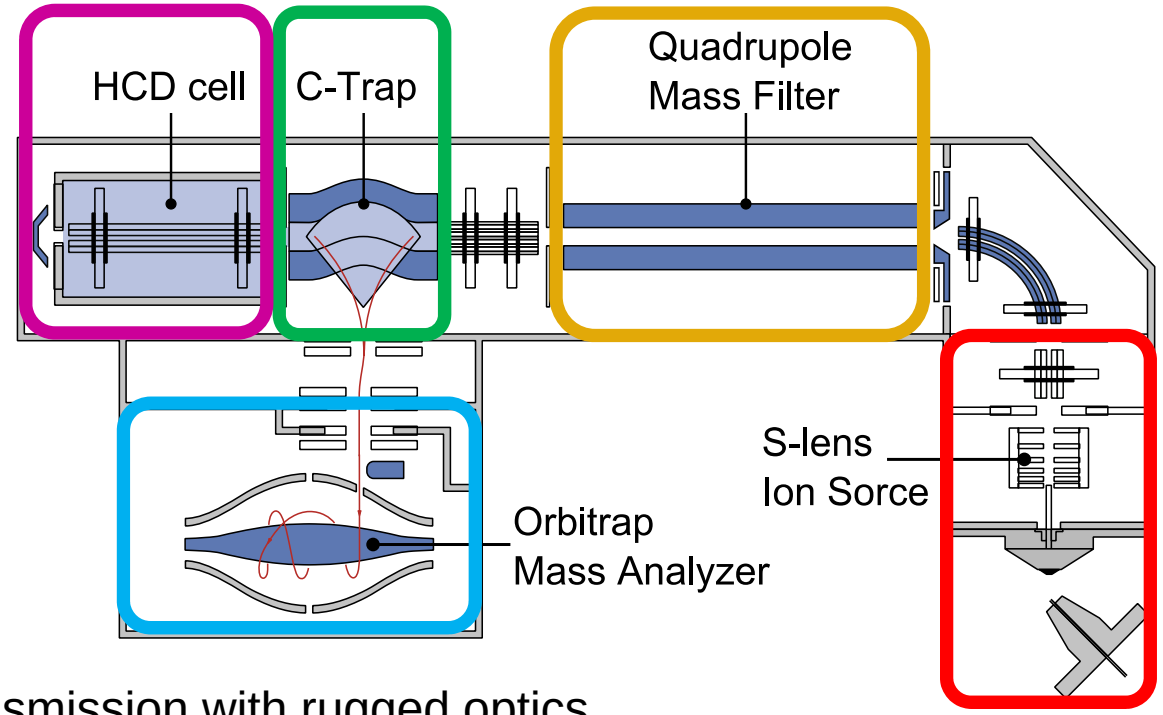
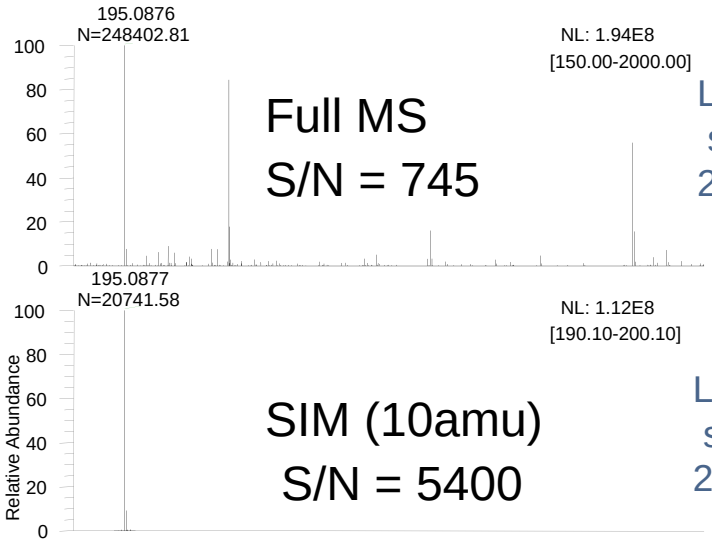
Q Exactive: Orbitrap MS/MS system



- Higher ion transmission with rugged optics
- Increased sensitivity, selectivity, true MS/MS
- Directly interfaced to HCD increases spectrum quality
- Multiple fills for spectrum multiplexing increases duty cycle
- Predictive automatic gain control for parallel filling & detection brings more speed
- Advanced signal processing brings more resolution at same speed

What do we gain by using the quadrupole ?

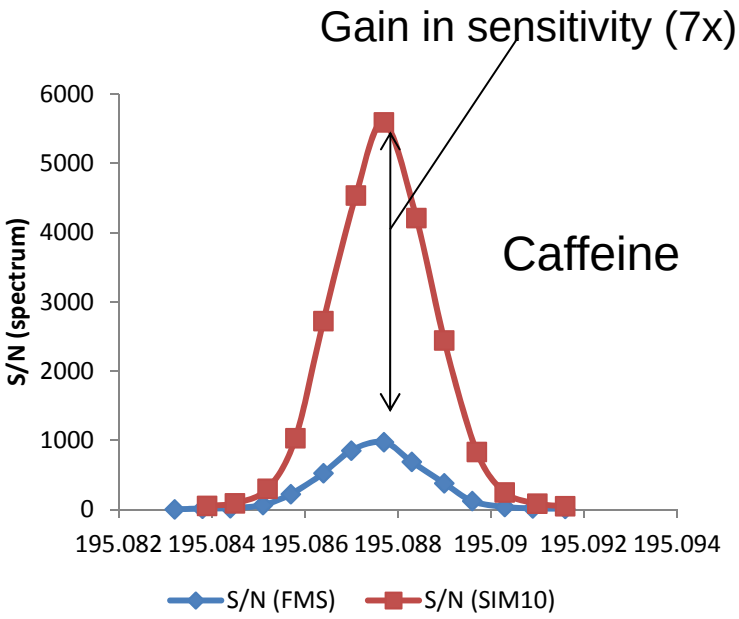


Full MS
S/N = 745

Lowest detected signal/scan 250330

SIM (10amu)
S/N = 5400

Lowest detected signal/scan 28240



Sensitivity gain 5 – 10 x with SIM mode

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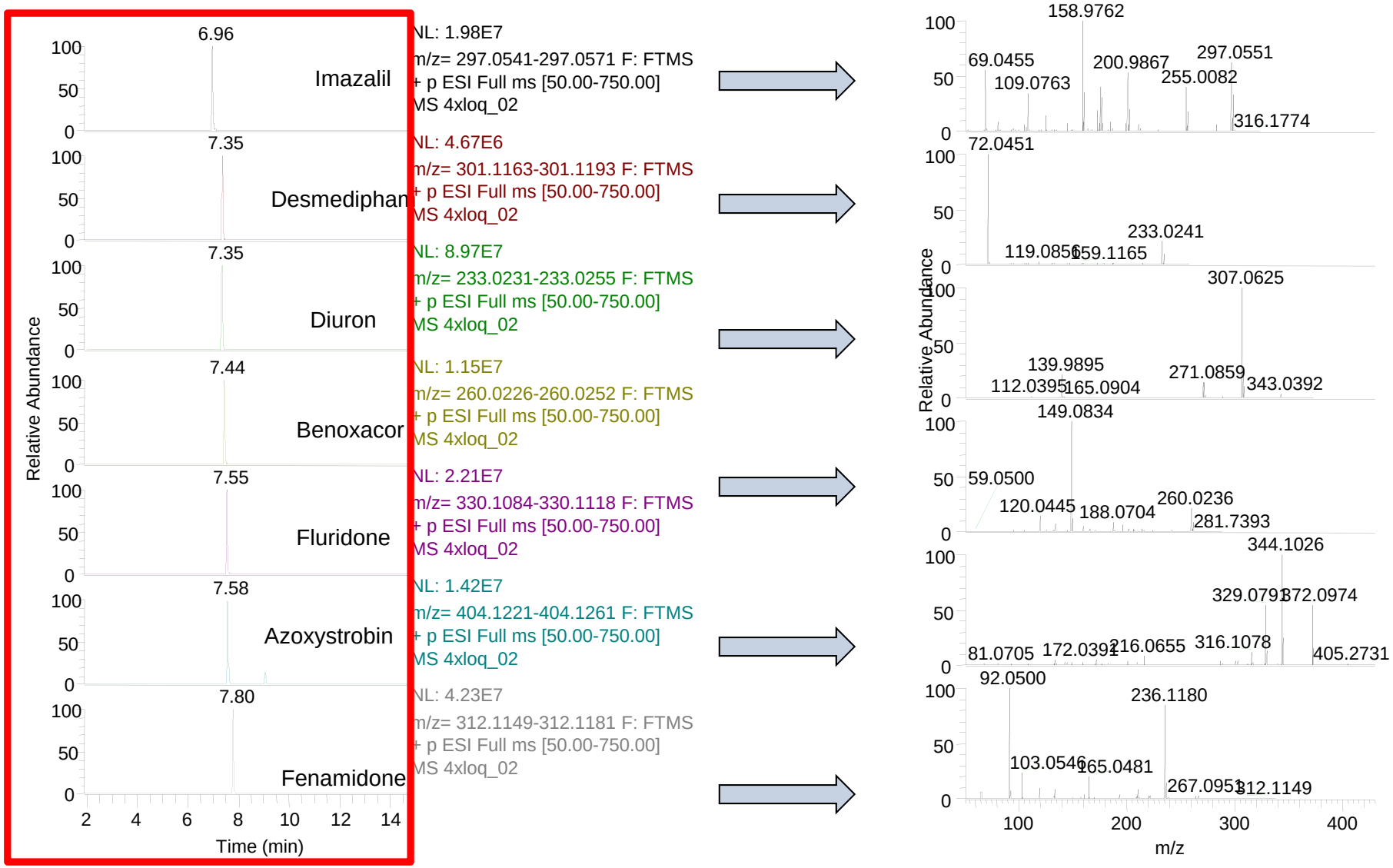
Data dependent MS/MS confirmation



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Q Exactive – “Quanfirmation” what is it?

- High performance HRAM *Quan*titation and Con*firmation* bench top LCMS system, capable of :

  - Multi-residue quan performance similar to mid-high end Triples
  - Ideal for targeted and general unknown screening
  - Highest confidence confirmation with R = 140K, and MS/MS
  - UHPLC compatible
  - Let‘s use the QExactive to combine these 2 experiments into one.

Outline

- What is EQuan MAX Plus?
- EQuan MAX Plus
  - How can EQuan MAX Plus help your workflow?
- Customer testimonials
  - Who uses EQuan MAX Plus?

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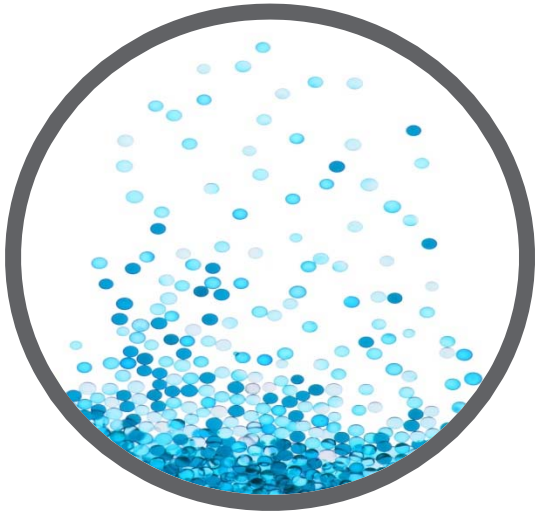
Low Sample Concentration



Challenge

Demanding assays which require the absolute lowest limit of detection

TSQ Quantiva

Powered by AIM technology, the TSQ Quantiva MS is the world's most sensitive triple quadrupole MS, detecting compounds at the ppt level.

EQuan MAX Plus

For targeted quantitation (TSQ)

or

Targeted/non-targeted screening and quantitation using High Resolution Accurate Mass (Orbitrap platform)

EQuan MAX Plus: What is it ?

- Turnkey method 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 uL**

EQuan MAX Plus: Targeted Quantitation

- Couple EQuan MAX Plus with any TSQ Quantum from Thermo Scientific for the most sensitive and selective experiments.
- **Fast Positive Negative Switching**
- **TraceFinder Software**
  - **Built in SRM parameters**
  - **Built in EQuan Methods**
- **Quantum Access MAX**
- **Quantum Endura**
- **Quantum Quantiva**

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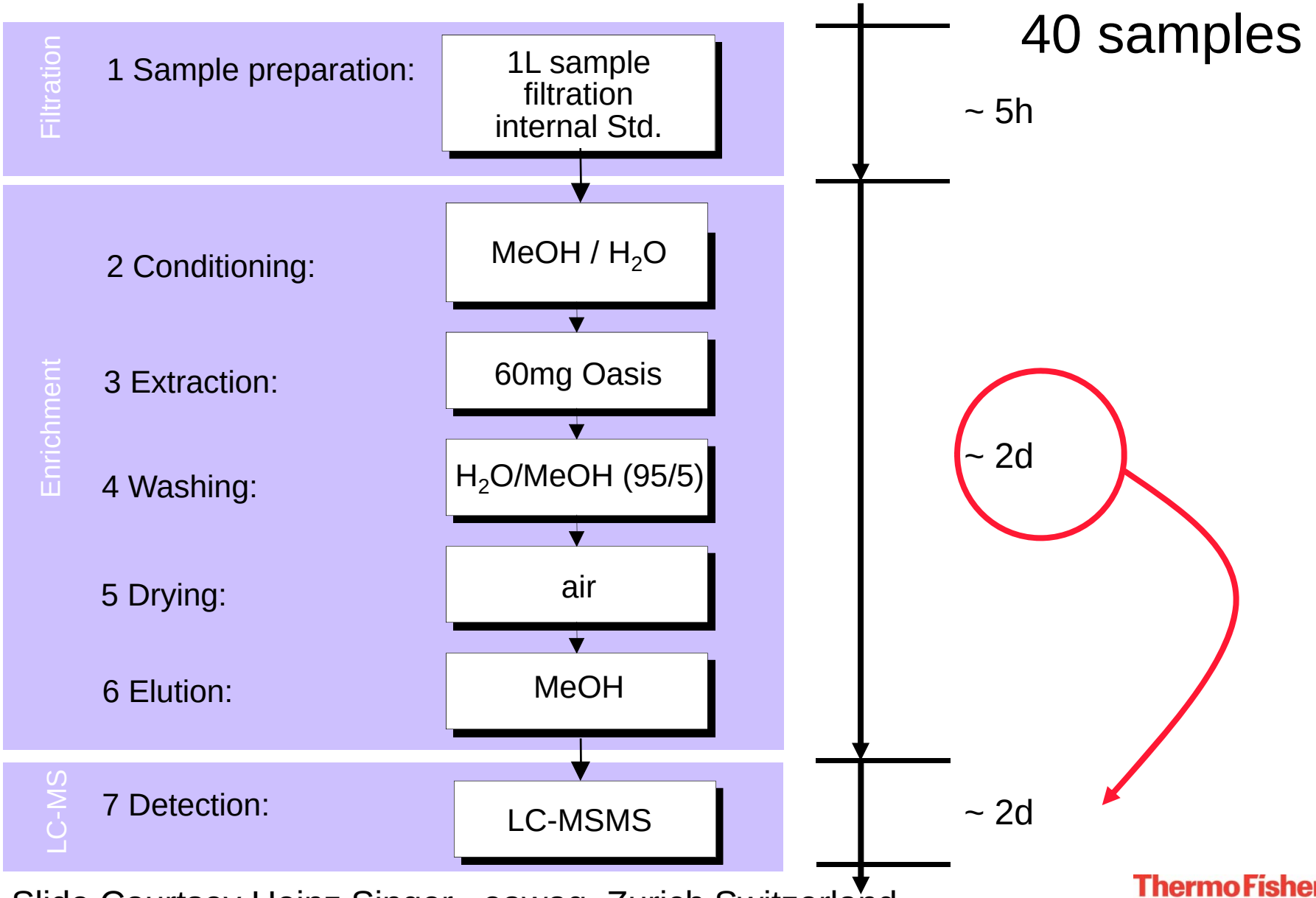
EQuan MAX Plus: Non-targeted screening and Quantitation

- Couple EQuan MAX Plus with the Exactive Orbitrap instruments (Exactive Plus or Q-Exactive).
- **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.**

SPE - standard enrichment procedure



 Slide Courtsey Heinz Singer –eawag, Zurich Switzerland

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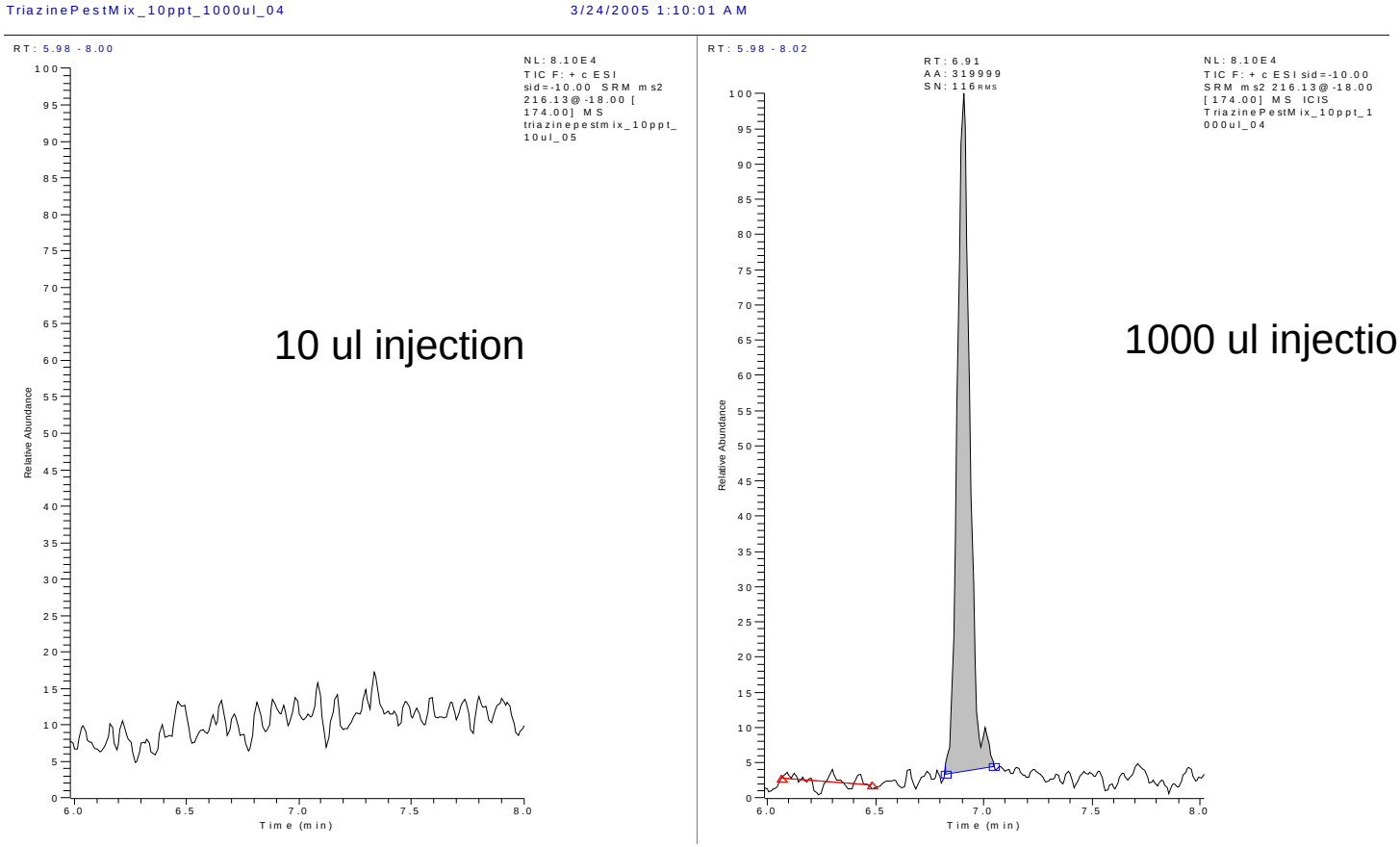
EQuan MAX Plus Solution – Gain Vs. Conventional Injections



10 ppt Atrazine in ground water

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Sensitivity, Speed & Robustness for EFS high throughput laboratories

	TSQ Quantiva	TSQ Endura
Mass Range	10-1850	10-3400
Max SRM Number	30,000 SRMs	30,000 SRMs
SRM/Sec	500 SRMs/sec	500 SRMs/sec
Ion Optics	**Active Ion Management (AIM)** • Ion Max NG source • Electrodynamic ion funnel • ion beam guide with neutral blocker • 6 mm HyperQuad quadrupoles with asymmetric RF drive	S- LENS with Beam Blocker Technology
Quadrupole Design		4mm Quadrupoles with Asymetric RF
Reserpine Specification	100,000 :1 S/N for 1 pg Reserpine	10,000 :1 S/N for 1 pg Reserpine

TSQ Quantiva

Extreme quantitative performance

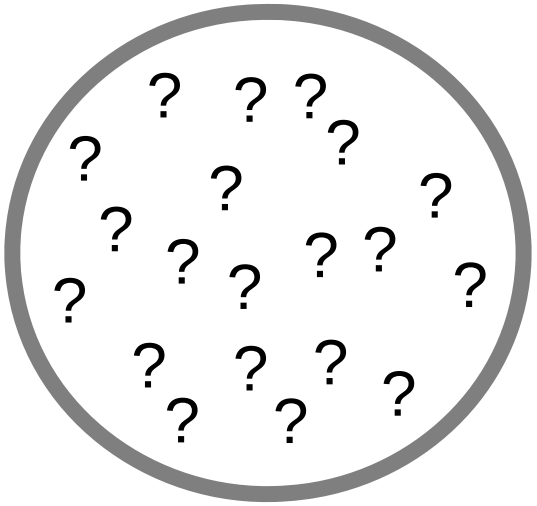
- Designed for the most challenging assays.
- For scientists needing to stay at the forefront of analytical technology

TSQ Endura

Extreme quantitative value

- Designed for non-stop operation.
- For scientist who need to run routine samples day in and day out.

From Identification.....



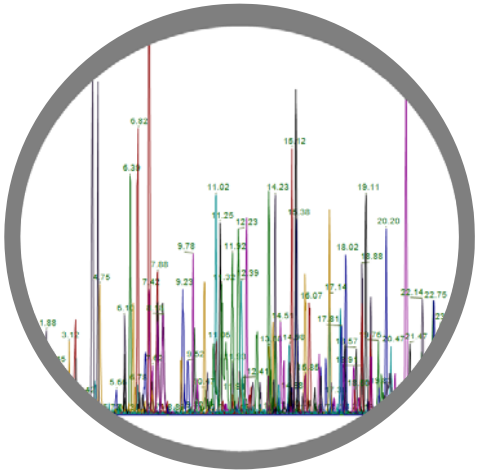
Challenge

Confidently identify unknown emerging contaminants……

Orbitrap Velos™ MS

High-Resolution Accurate Mass for identification of unknown emerging contaminants.

To Routine Quantitation



Challenge

Followed by routine quantitation.

EQuan MAX Plus and TSQ Quantiva™ MS

Online sample prep LC/MS for quantitation of emerging contaminants identified by the Orbitrap Velos

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Analysis of Targeted and Non-targeted Contaminants in Storm Water Retention Ponds

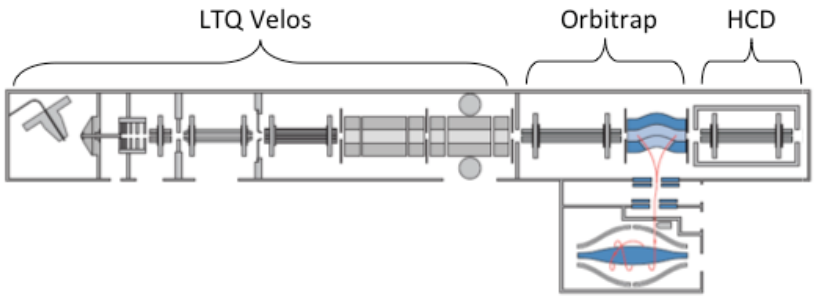
Emerging Contaminants, from ID to Quantitation.
The Total Thermo Scientific Solution

Gordon Getzinger – Duke University

Jonathan Beck, PhD –
Thermo Fisher Scientific

UHPLC-HRMS: An Emerging Technique for “Helping Contaminants Emerge”

- Target screening:
  - Monitor known contaminants using reference standards.
- Suspect screening
  - Screen high-resolution accurate-mass data against molecular databases of suspected contaminants.
- Non-target screening
  - Assign molecular and structural formula to chromatographic features without previous knowledge of contaminant presence or identity.



Krauss *et al.* LC-high resolution MS in environmental analysis: from target screening to the identification of unknowns. (2010) *Anal Bioanal Chem.* **397** [3].

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Emerging Contaminant ID to Quantitation Workflow

- Reclaimed waste water used for irrigation on a golf course for emerging contaminants (PPCPs)
- Lee Ferguson and Gordon Getzinger, Environmental Chemistry, Duke University, USA

Acquire Fast HRAM Data

Identify Contaminants

ExactFinder Software

Etofenprox

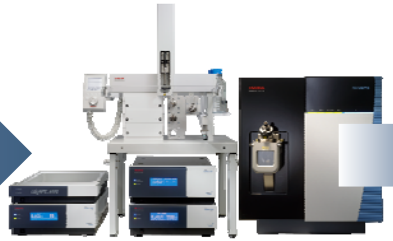
Benzotriazole

Propanamide

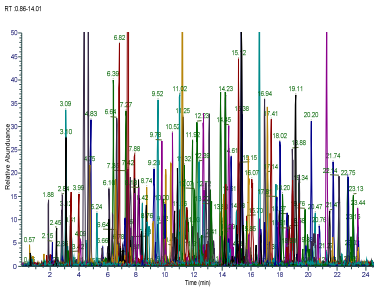
Acephate

Allethrin

Develop Quantitative Assay



Routine Quantitation



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Kiawah Island, SC, USA

Question: Which microcontaminants a entering the aquatic environment as a result of wastewater reuse practices?

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Typical Sampling Site

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Variety of Sampling Sites

Sample Site	Inputs
Pond 5	Golf course runoff
Pond 25	Golf course runoff
Pond 43	Residential stormwater
Wastewater lagoon	Treated municpal wastewater
Wastewater Composite	24hr composite effluent
Well 1	Infiltration from pond 25
Well 7	Infiltration from pond 5

Quantitation of Samples

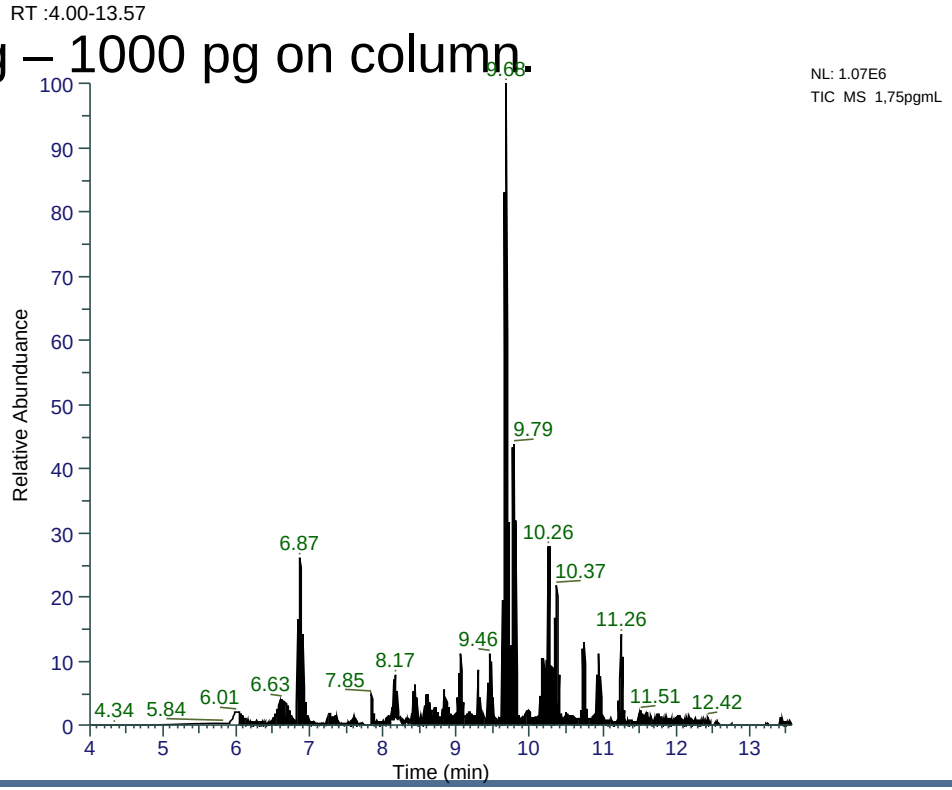
- Use a triple quad for quantitation.
- Analyze samples directly, no offline SPE to save time and money.
- Remove variables and sources of experimental error by automated extraction and preconcentration.
- An ideal solution for this challenge is…

EQuan MAX Plus & TSQ Quantiva MS!

What Did We Want to See in the Quantitation Experiment?

- Quantitative results at low concentrations… low and sub PPT levels
- Calibration range:
  - 0.06 pg/mL – 1000 pg/mL
  - With a 1mL injection, that's 60 fg – 1000 pg on column.

TIC of 1.75 pg/mL injected standard
1.75 pg on column



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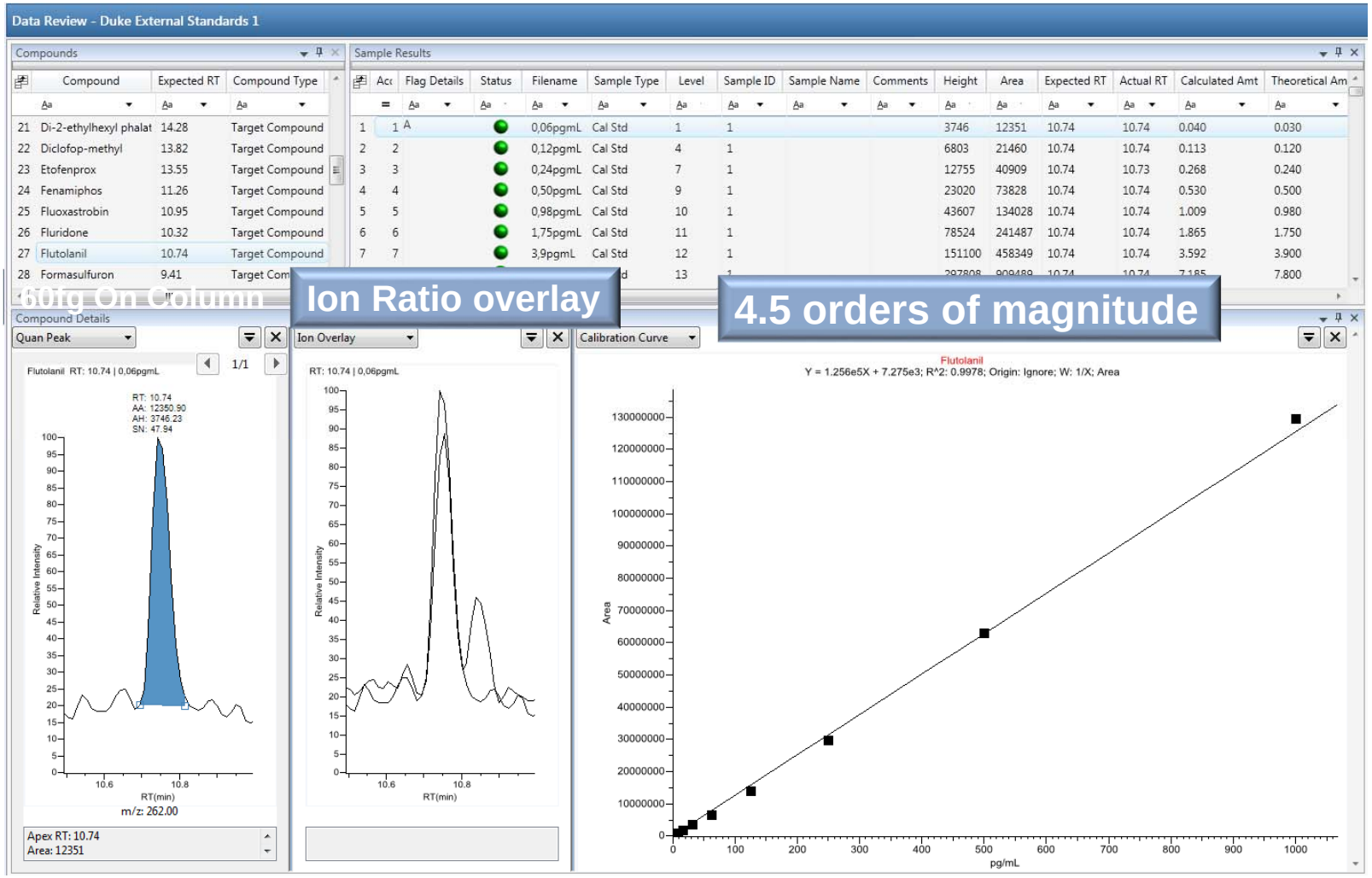
Calibration range, 0.06-1000 pg/mL - Flutolanil



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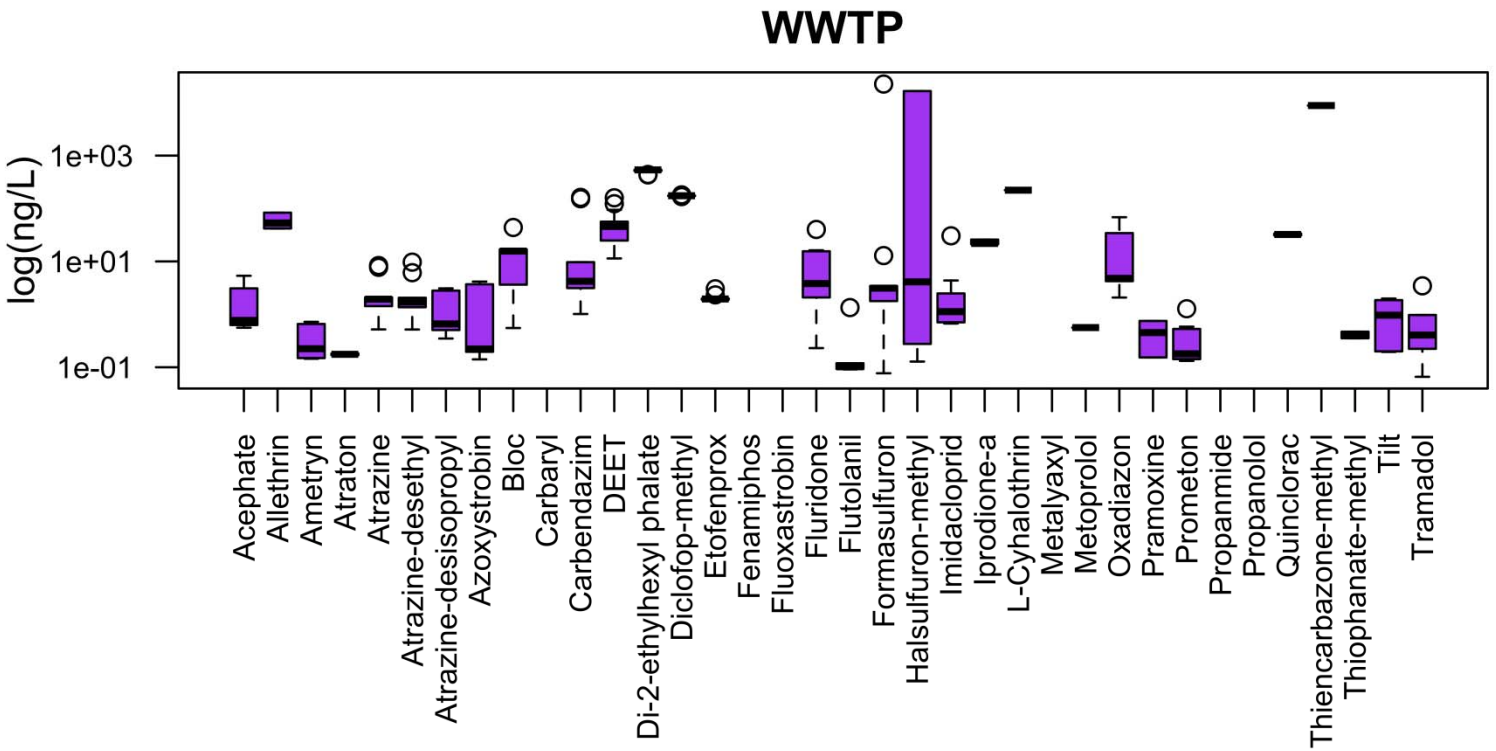
Results of Compounds Detected in a WWTP Sample



Identification of Emerging Contaminants

Data Analysis Workflow

Target screening

Are compounds *x, y, & z* present in this sample?

Suspect screening

Which compounds of a defined list are present in this sample?

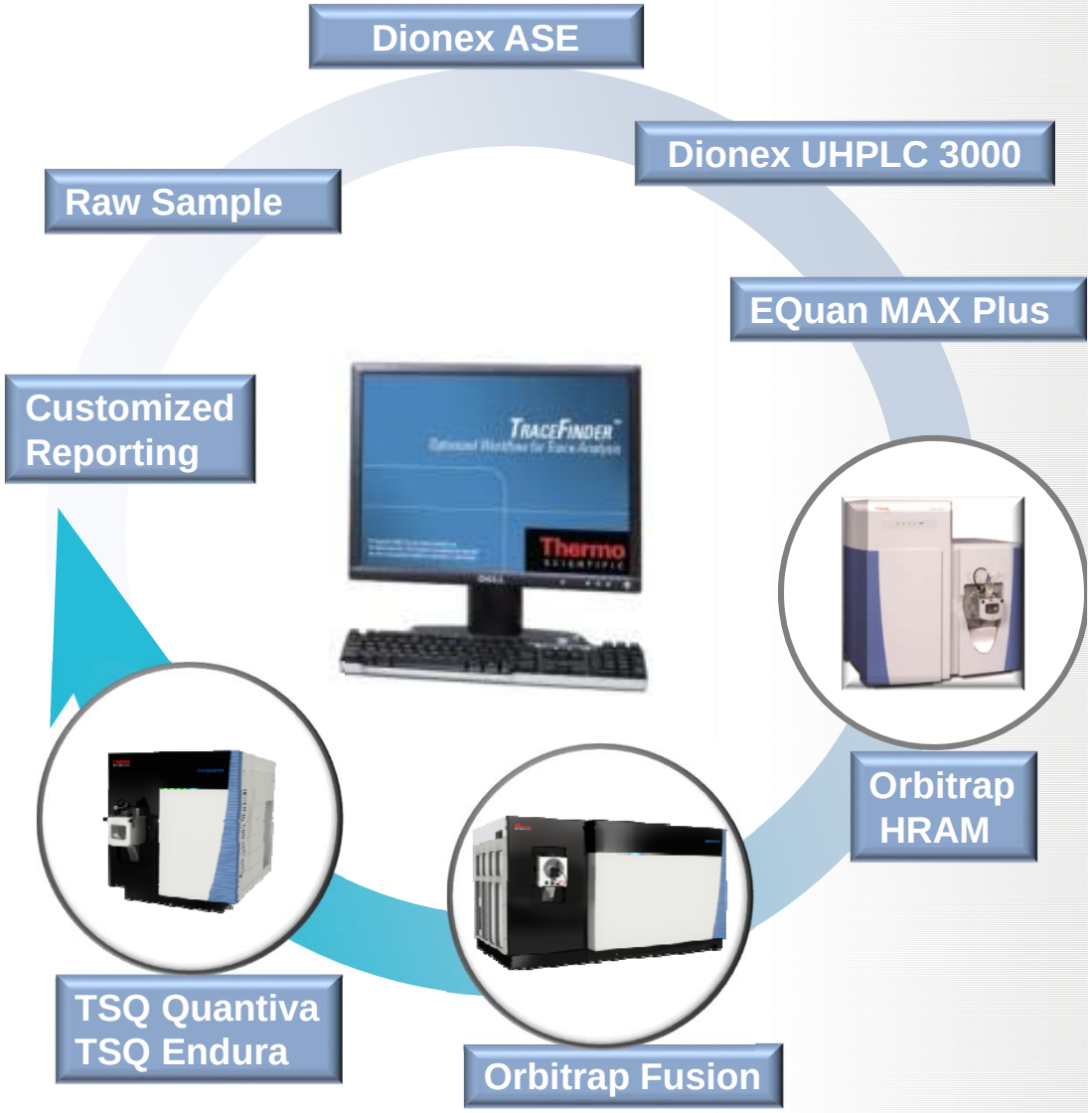
Non-target screening

Which compounds are present in this sample?

LC/MS Workflows for Environmental and Food Safety

Tools to Develop methods for Known and Emerging contaminants

- Accelerated Solvent Extraction (ASE)
- Equan MAX Plus online sample prep LCMS
- Exactive Plus and Q Exactive Mass Spectrometers: High Resolution Accurate Mass to identify, quantify and confirm
- TSQ Series Mass Spectrometers: triple stage quadrupole LC-MS/MS and GC MS/MS for high sensitivity quantitation
- Custom software to meet the needs of your workflow
    - TraceFinder, SIEVE, mzCloud



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