Choosing the Optimum Pesticide GC Workflow

Massimo Santoro

GC and MS Marketing Manager

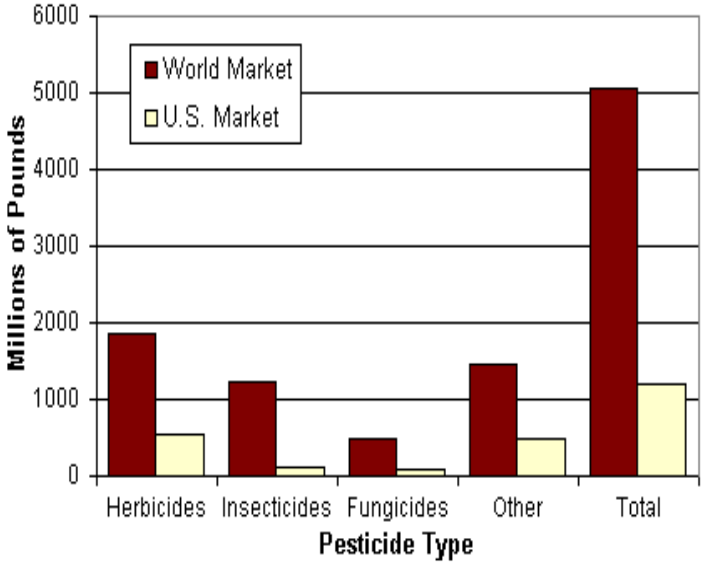
Agenda

- Why pesticide analysis?
- Why still use GC for pesticide analysis?
- GC analytical choices and examples
  - Sample preparation
  - GC parameters
  - GC detection
- Conclusions

Importance of Pesticide Analysis

- Extensively used in agriculture to control pests & improve yields
- More than 1,000 different active substances used
- Thoroughly tested and evaluated before approval

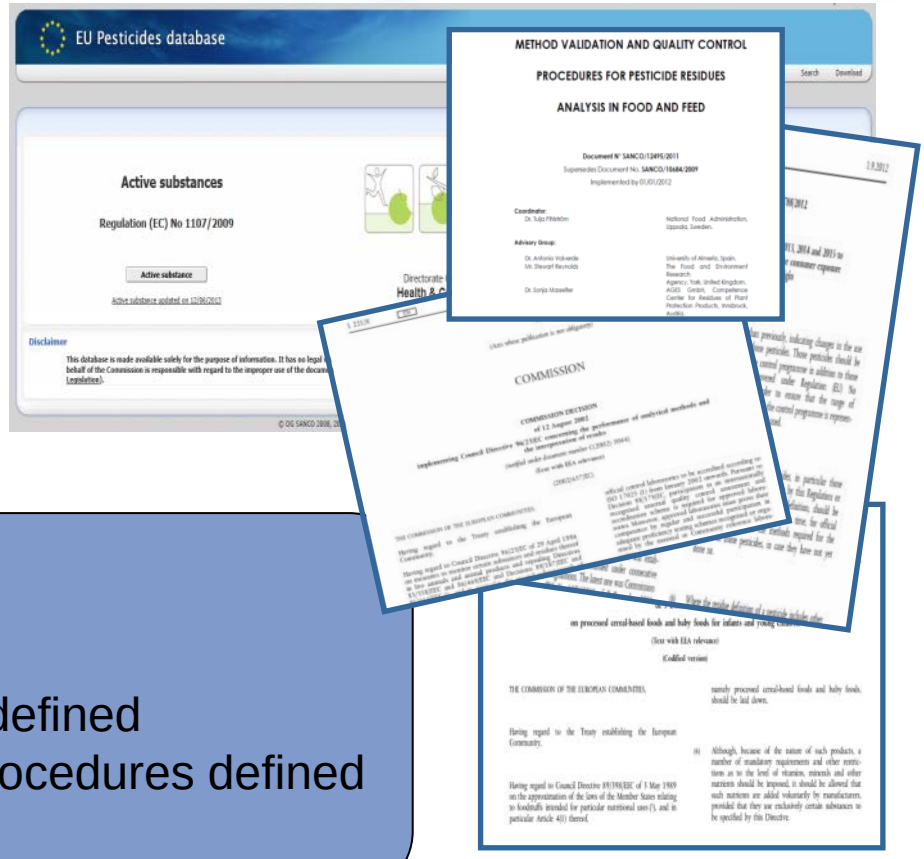
- Sometimes not applied in accordance with intended purpose
- Banned pesticides still used in some countries
- Significant public concerns about pesticide use
- Regulatory requirements: Regulation 396/2005 500 pesticides in variety of crops



Regulatory Requirements

Most stringent regulation adopted in the European Commission

- 657/2002/EC
- 125/2006/EC
- 788/2012/EC
- SANCO/12495/2011
- 395/2005/EC



Regulatory requirements:

- > 145.000 MRLs defined
- MRL ranges mostly ~ 10-100 µg/kg
- Compound identification requirements defined
- Method validation and quality control procedures defined

U.S. EPA Methods for Pesticide Testing

- EPA Method 505: Organohalide Pesticides and Commercial Polychlorinated Biphenyl (PCB) Products in Water
- EPA Method 507: Nitrogen- and Phosphorus-Containing Pesticides in Water
- EPA Method 508: Chlorinated Pesticides in Drinking Water
- EPA Method 527: Selected Pesticides and Flame Retardants in Drinking Water
- EPA Method 553: Benzidines and Nitrogen-Containing Pesticides in water
- EPA Method 608: Organochlorine Pesticides and PCBs in wastewater
- EPA Method 614: Organophosphorus Pesticides in Municipal and Industrial Wastewater
- EPA Method 619: Triazine Pesticides in Municipal and Industrial Wastewater
- EPA Method 622: Organophosphorus Pesticides in Municipal and Industrial Wastewater
- EPA Method 1618: Organo-Halide, Phosphorus Pesticides, and Phenoxy-acid Herbicides
- EPA Method 1699: Pesticides in Water, Soil, Sediment, Biosolids, and Tissue
- EPA Method 8081A: Organochlorine Pesticides in Water
- EPA Method 8141B: Organophosphorus (OPs) Pesticides in Water

Pesticide Chemistry

- Organophosphate
- Carbamate
- Organochlorine
- Pyrethroid
- Sulfonylurea
- Biopesticides

- Herbicides
- Bactericides
- Fungicides
- Insecticides
- etc...

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Screening and Quantitative Methods Needed

Multi-analyte methods to identify non-compliant samples

Positive sample

Most screening methods focus on single analytes – ONLY instrumental screening give multi-residue capability

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Why Still Use GC for Pesticide Analysis?

- Many compounds not amenable to LC separation
- Low polarity - poor atmospheric pressure ionization

- GC offers good separation efficiency
- Choice of detectors
- Easy coupling with MS

Thermo Scientific™
TRACE™ 1300 Series GC

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Analytical Choices

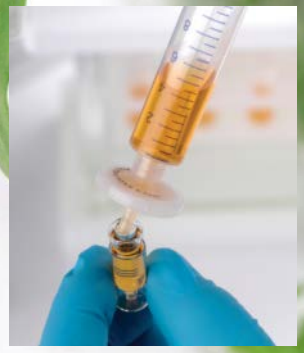
- **Sample preparation**
- **GC parameters**
- **GC detection**

Potential Sample Extraction Issues

- **Sample handling is the primary source of errors**
- **Sample extraction causes the biggest bottleneck for most analysis methods**
- **Loss of pesticides during sample processing at ambient temperatures may occur**
- **Costs are increasing for solvent purchase and disposal**
- **The data is only as good as the sample preparation**
  - **High-price chromatography and data systems do not improve the quality of data from poorly-prepared samples**

Fully Integrated Workflow - Sample Preparation

- **QuEChERs - Fast, effective and easy to implement sample extraction from complex matrices**
- **SPE - Targeted, clean and reproducible sample extraction**
- **Filtration - Quick and effective sample preparation**
- **Accelerated Solvent Extraction - Fast extractions, low solvent usage, less labor, better reproducibility**



QuEChERS Acetonitrile Extraction Features

- **Easy to use**
- **Covers broad range of analytes**
- **Suitable for high moisture samples (fruits, vegetables)**
- **Low costs and fast response**
- **Compatible with both LC and GC**

But

- **Limited clean-up (only dSPE)**
- **Not suitable for fatty food**
- **Acetonitrile is not optimal for GC analysis**

Anastassiades et al. (2003) J.AOAC Int, 86:412

Benefits of Using Automated SPE

- Compatible with EPA-approved clean water and groundwater methods
- Replaces tedious manual liquid-liquid extraction (LLE)
- Automates all four steps of SPE
- Runs unattended
- Removes sample interferences
- Isolates and concentrates analytes from liquid matrix
- Reduces solvent consumption
- Reduces exposure to solvents
- Increases productivity
  - Users can load six samples using only 15 minutes of their time
  - Six samples completed and ready for injection onto GC or LC in just 2-3 hours

Accelerated Solvent Extraction Overview

- **Using Thermo Scientific™ Dionex™ ASE™ Accelerated Solvent Extractor Systems**
- **Automates sample preparation for solid samples using solvent extraction**
- **Operates above the boiling point of most extraction solvents**
- **Pressure is used to keep solvents liquid during extraction**
- **Requires small quantities of solvent and short periods of time**
- **Equivalent or superior to Soxhlet extraction**

Dionex ASE 350
Accelerated Solvent Extractor

Meets the requirements of U.S. EPA Method 3545A

(OCP, OPP, BNA, TPH, PCDD, herbicides and semi-volatiles)

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Accelerated Solvent Extraction Standard Methods

- US EPA Method 3545A (OCP, OPP, BNA, TPH, PCDD, herbicides and semi-volatiles)
- NIST (National Institute of Standards and Technology) uses two extraction techniques to certify all standard reference materials (SRMs) for organics: Soxhlet and accelerated solvent extraction
- Accepted under CLP SOW OLM04.2
- Method 6860/6850: perchlorate from solid waste; Accelerated solvent extraction for extraction and clean-up
- NOAA Method NWFS-NWFSC-59 for hydrocarbon and chlorinated hydrocarbon contamination in marine animal tissues, soils and sediments
- Chinese Method GB/T 19649-2005 for 405 pesticides in grains and grain products
- German Method DIN-EN-12393 (extended and revised version of DFG Method S19) for pesticides in foodstuffs
- ASTM Standard Practice D-7210 and D-7567 (additives in polymers and gel content of polyolefins)
- Mexican National Standard NMX-AA-146-SCFI-2008 for PAHs in soils and sediments

Recovery of Organochlorine Pesticides

Standards Spiked onto a Raw Banana at 100 ppm level

Compound	Av. Recovery (%)	SD (µg/kg)	RSD (%)
α-BHC	100.3	2.3	2.3
β-BHC	102.2	2.3	2.3
γ-BHC	98.9	3.2	3.2
Heptachlor	89.2	7.6	8.5
Aldrin	89.4	2.2	2.5
Dieldrin	93.7	1.6	1.7
4-4‘ –DDE	92.1	1.8	1.9
2,4‘ – DDD	95.4	2.5	2.6
Endrin	94.4	2.7	3.0
4,4‘ – DDD	88.8	2.7	3.0
4,4‘ – DDT	89.6	5.8	6.4

* n = 3

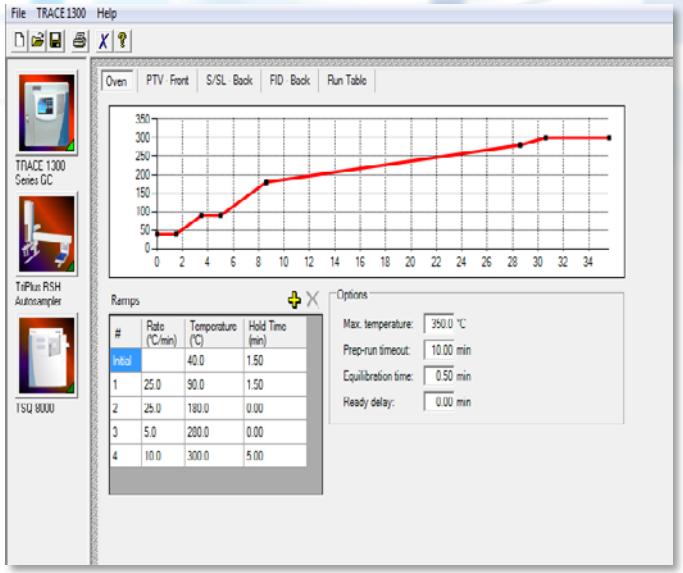
Analytical Choices

- **Sample preparation**
- **GC parameters**
- **GC detection**

Analytical Choices

GC Parameters

- Column
- GC temperature program
- Injector type
- Injection volume
- Backflush
- Detection



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TRACE 1300 Series GC: Tailor Instrument Configuration

- **Instant Connect modules are user-installable in less than two minutes**
- **GC injector and detector modularity enables you to:**
  - **Tailor GC configuration to the application**
  - **Adopt a future-proof GC platform**
  - **Ease and scale up investments**
  - **Maximize instrument uptime and Resume operations quickly**
  - **Ensure constant response time**
  - **Reduce capital investments**
  - **Make troubleshooting easy**

TRACE 1300 Series GC

- **Instant Connect Helium Saver Module injector offers innovative and unique features and benefits**
  - **Up to 14 years for one helium cylinder life time**
  - **Always saves – works during analytical run and idle time**
  - **Always ready – no switch-back equilibration time**
  - **Can maintain existing methods with the use of helium for the separation**

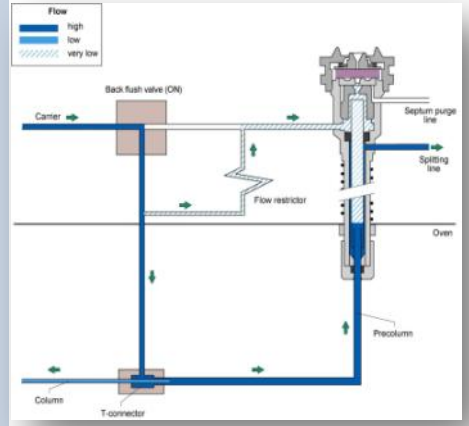
- **Instant Connect PTV & SSL with Backflush modules maintain column performance in heavy matrix**

✓**Efficient removal of non-volatiles**

✓**Longer column lifetime**

✓**Less frequent inlet maintenance**

✓**Lower running costs and higher system uptime**



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Analytical Choices

- **Sample preparation**
- **GC parameters**
- **GC detection**

Analytical Choices

- **GC Detection**
  - **Conventional, Selective GC Detection (ECD, NPD, FPD,)**
  - **Single Quadrupole MS**
  - **Triple Quadrupole MS**
- **Data Reporting**

Thermo Scientific™ TRACE™1300 Series GC

Thermo Scientific™ ISQ™ Series Single Quadrupole GC-MS

Thermo Scientific™ TSQ™ 8000 Triple Quadrupole GC-MS/MS

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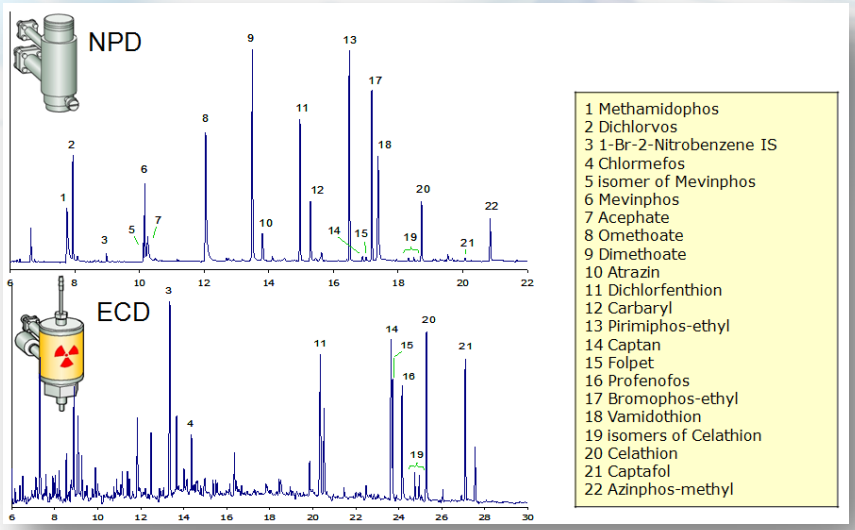
Large Volume Splitless Technique for Increased Sensitivity

- 30X increased sensitivity in the analysis of pesticides in food by GC - Large Volume Splitless Technique
- Large Volume Splitless (LV-SL) allows injections of up to 50 µL in a conventional split/splitless injector
- Robust versus sample byproducts or contaminants and extremely suitable for food matrices
- LV-SL uses the patented Concurrent Solvent Recondensation technique (CSR)
- CSR allows injection of large volumes by combining a restricted evaporation rate with an accelerated sample transfer granted by the pressure surge generated by solvent evaporation and by the quick solvent recondensation in a precolumn. It requires a split/splitless injector with low dead volume and an uncoated precolumn with a capacity for retaining liquid at least corresponding to the volume of sample to be injected (e.g. 5 m x 0.32 mm i.d. or 3 m x 0.53 mm i.d. for 30 µL volumes)
- Steps of the process
  - Fast, automated injection, minimizing contact between syringe and injector, and exploiting liquid band formation
  - Auto pressure surge strongly accelerating transfer of vapors in the precolumn
  - Recondensation of the solvent vapors in the precolumn
  - Transfer of solutes into the precolumn
  - Solvent evaporation in the precolumn

Evaluation of Pesticides Recoveries and Linearity

- **Results injecting 30 µL of the low concentrated standard solution compared with the injection of 1 µL of the 30 times more concentrated solution in COC and SL**
  - **NPD response is the same with SL and LV-SL**
- **A high level of linearity obtained with NPD by injecting 30 µL of standard solution at different concentrations**
  - **Similar results were obtained with ECD**
- **Linearity is respected even at the sub ppb level and also with compounds known as labile (i.e. carbamates) from literature**



COMPOUND	R^2
Metamidophos	0.9998
Dichlorvos	0.9999
Acephate	0.9989
Mevinphos (trans)	0.9991
Omethoate	0.9999
Dimethoate	0.9998
Atrazin	0.9999
Carbaryl	0.9998
Pirimiphos-ethyl	0.9998
Captan	1.0000
Folpet	0.9998
Bromophos-ethyl	0.9998
Vamidothion	0.9997
Captafol	0.9999
Azinphos-methyl	1.0000

Table 2: LV-SL Linearity between 0.5 and 100 ppb (NPD) with examples.

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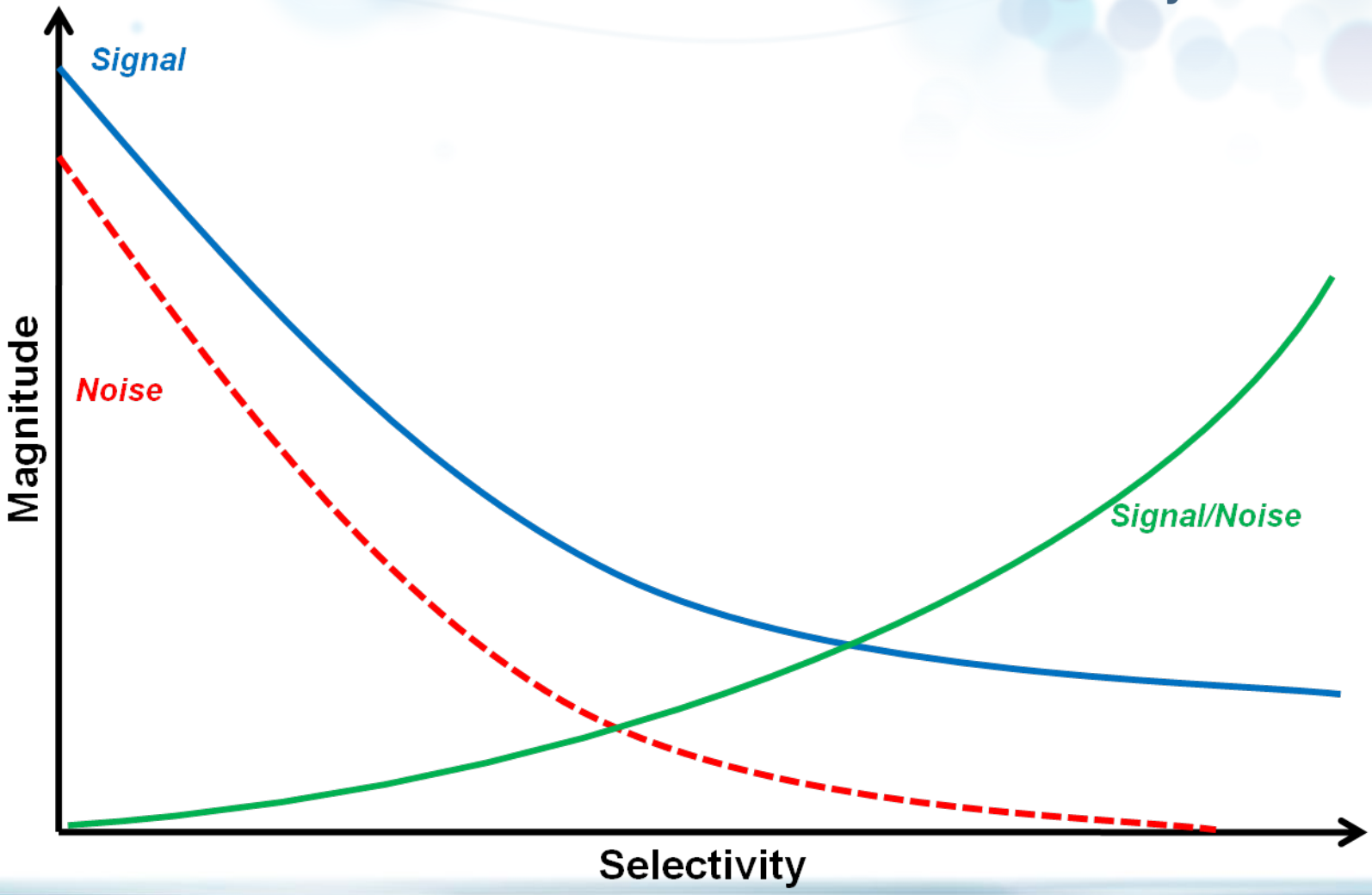
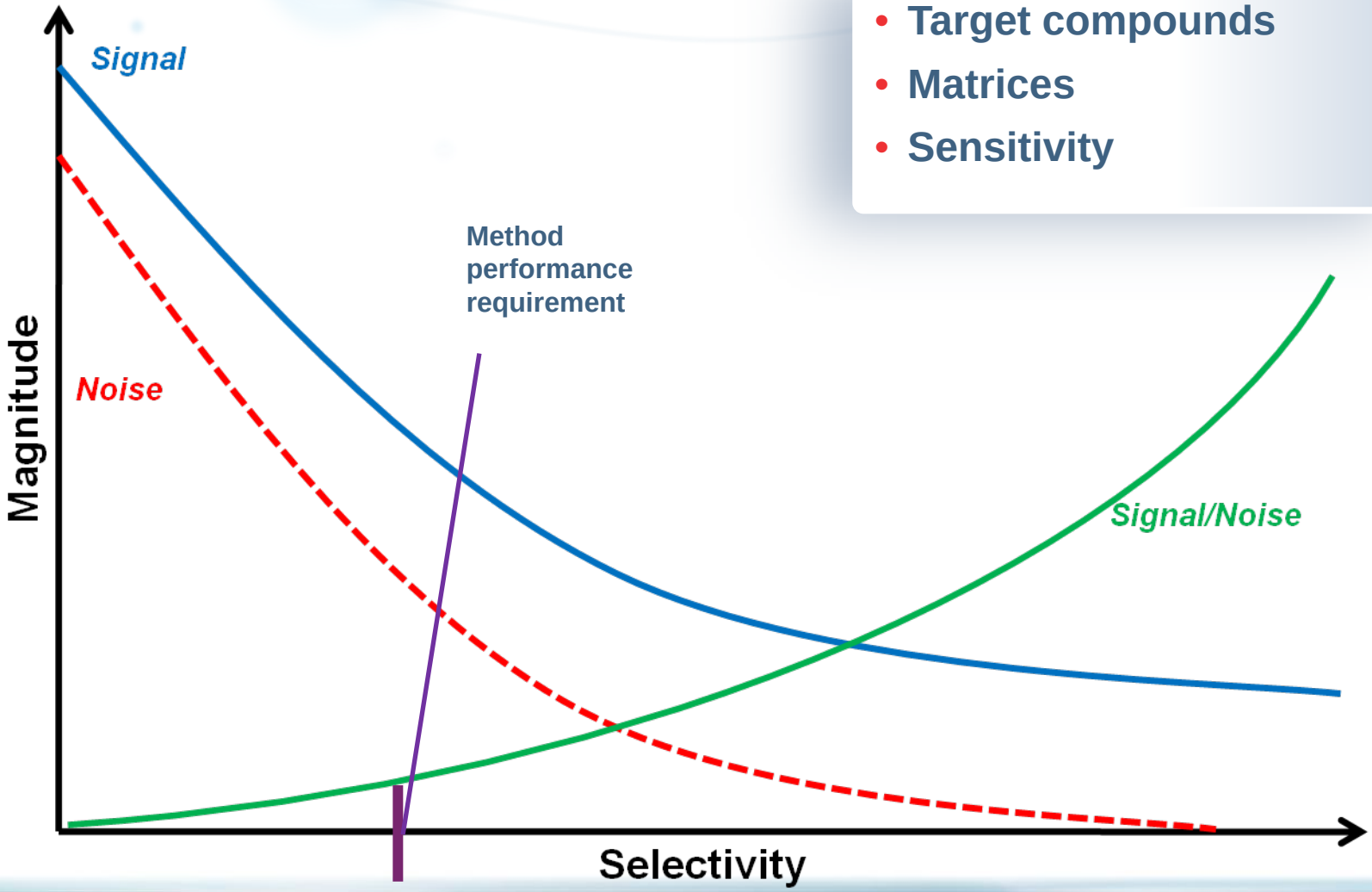
Selectivity in a Method

McLafferty circa. 1980

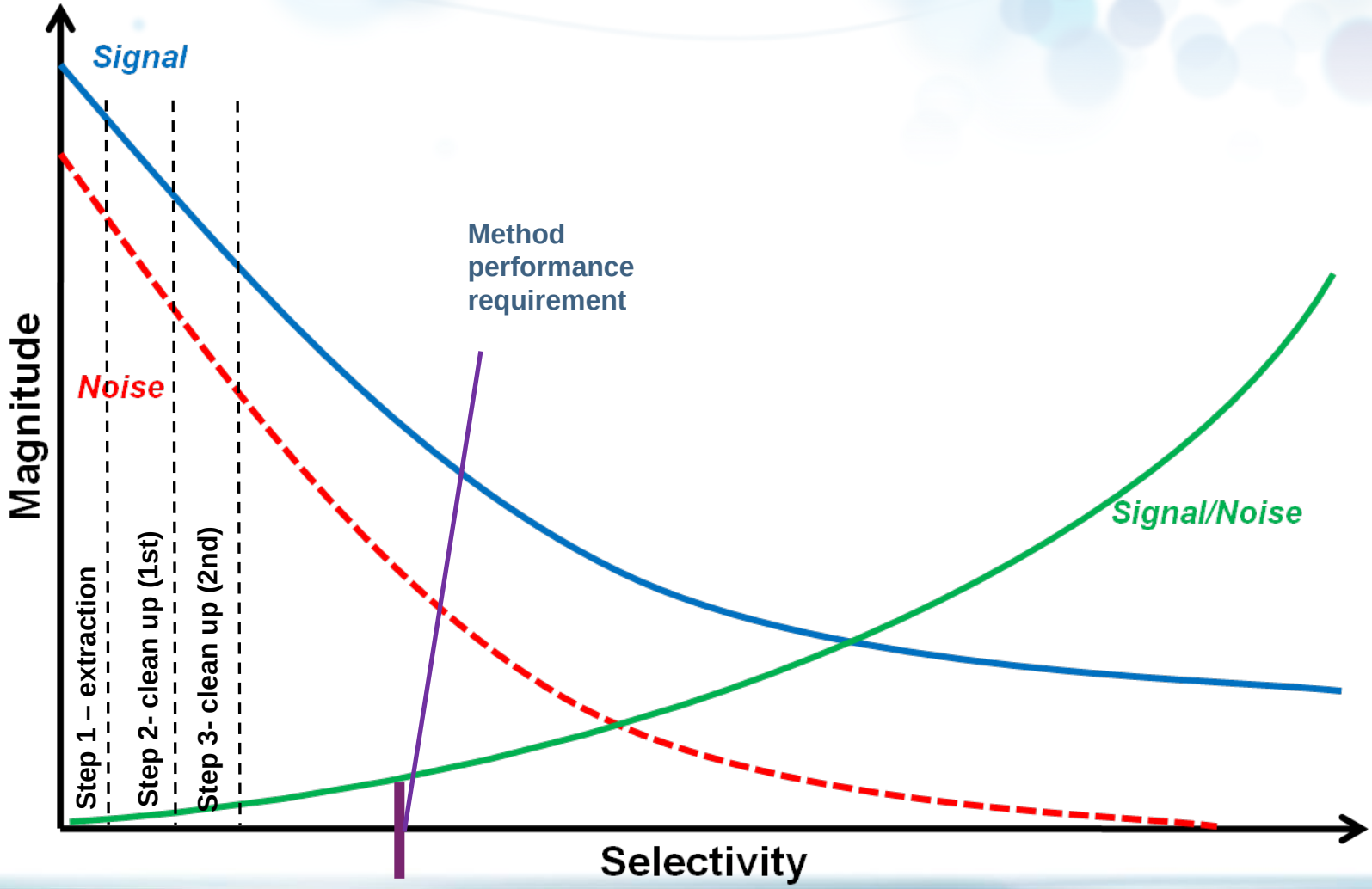


Method Performance Requirement

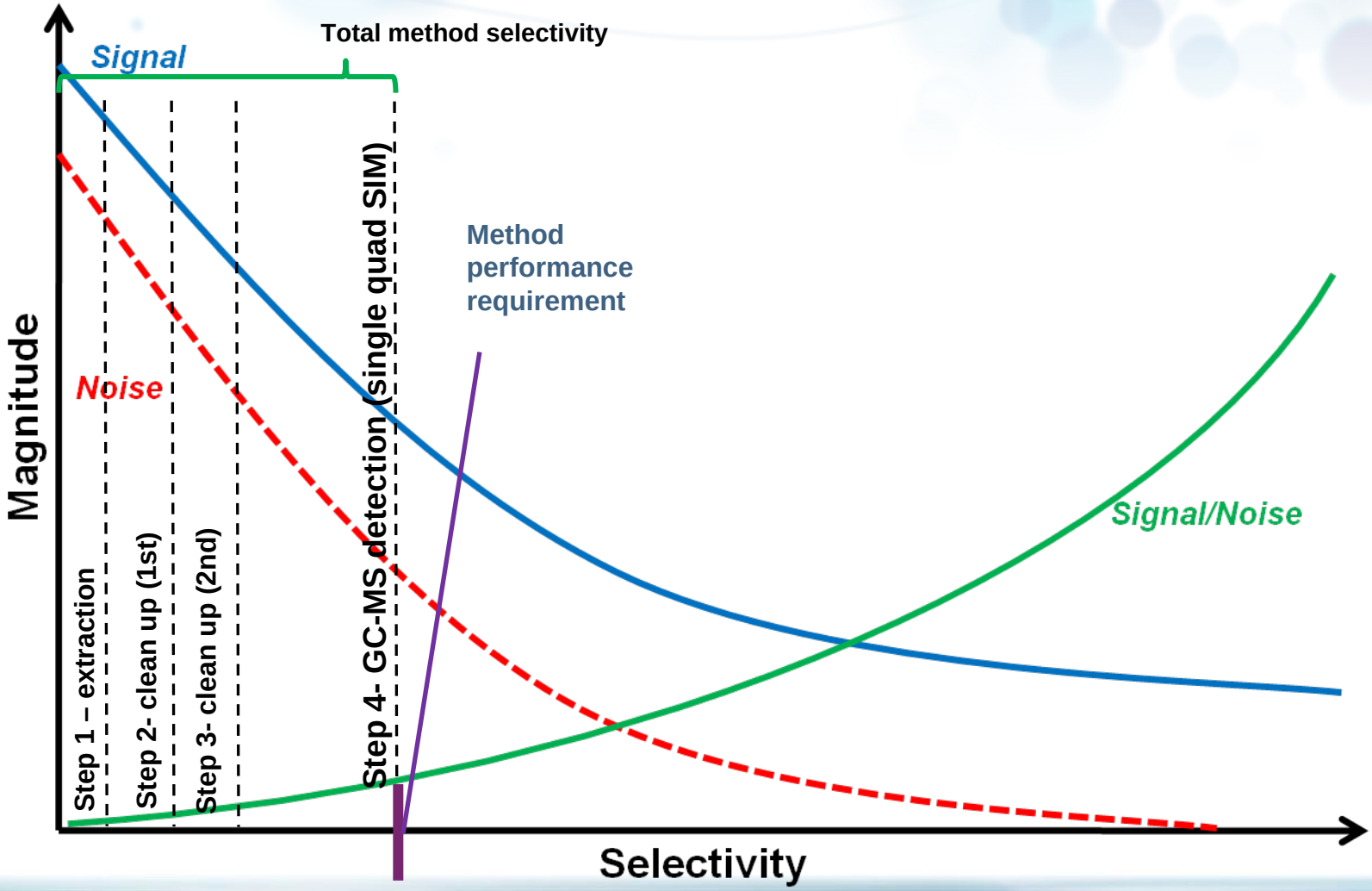
- Target compounds
- Matrices
- Sensitivity



Sample Preparation



Instrument Detection



Analytical Benefits for Single Quadrupole GC-MS

- **Robust and easy to use and maintain**
  - **Run more samples between cleaning**
- **Sensitive precision**
  - **Accurate and reproducible results at the lowest levels**
- **Unknown analysis**
  - **Full scan for unknown library searches**
  - **Alternating full scan/SIM for unknowns and low level analysis**
- **Flexibility**
  - **Switch quickly between dedicated EI and CI sources**

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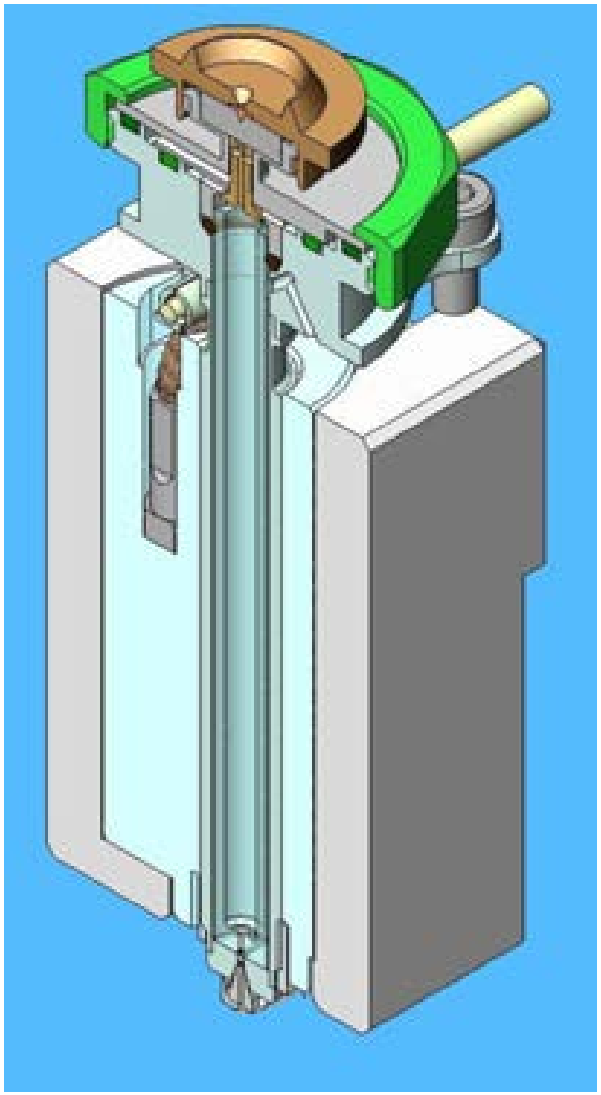
GC Systems Designed with MS in Mind



TRACE 1300 GC and TRACE 1310 GC

Minimal Septum Bleed,
By Design

Immeasurable Air Diffusion*,
By Design

No Gas Lines to Plumb,
You Guess

* by Thermo Scientific™ Delta V Ion Ration Mass Spectrometer (irMS)

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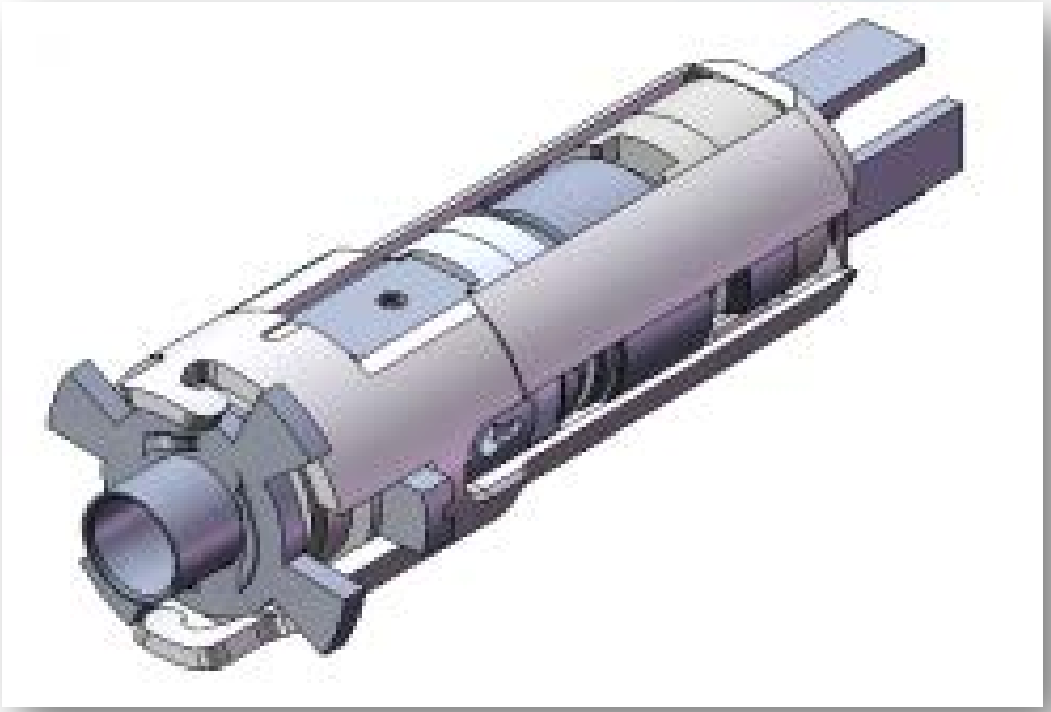
ISQ Series GC-MS Ion Source

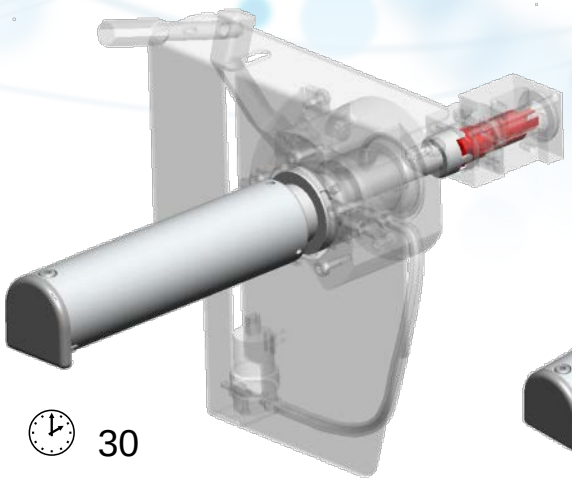
Eliminates the need for scheduled down time

Thermo Scientific™
ExtractaBrite™
Ion Source



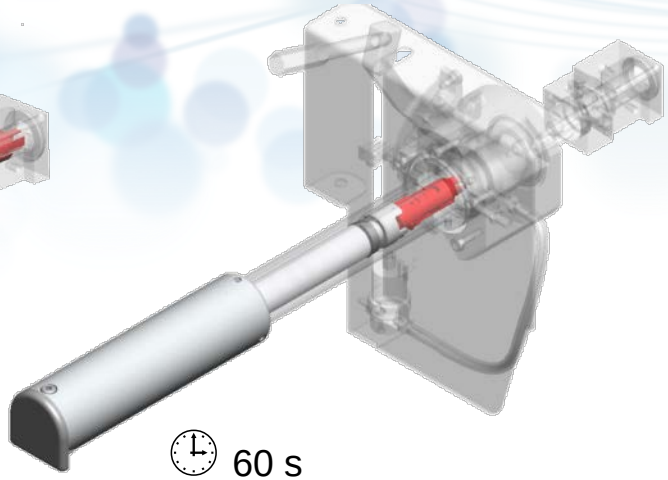
When the unplanned happens, it still requires no down time

Single Quadrupole MS: Forget Maintenance Downtime



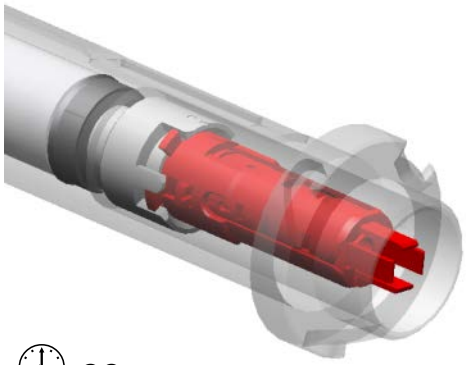
30 s

Step 1. Insert removal tool



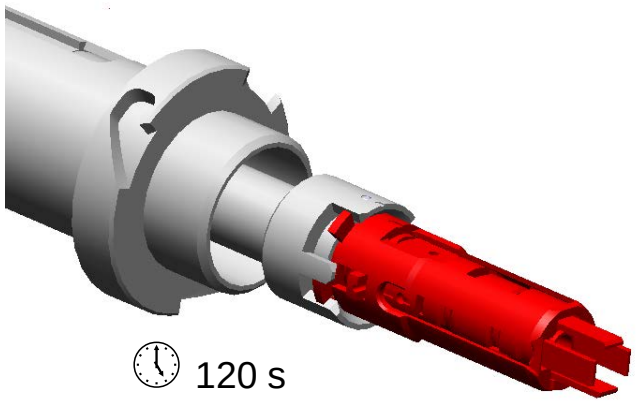
60 s

Step 2. Remove source



90 s

Step 3. Hot source is held in tool



120 s

Step 4. Push source out of tool

[VIEW: Source Removal Video]

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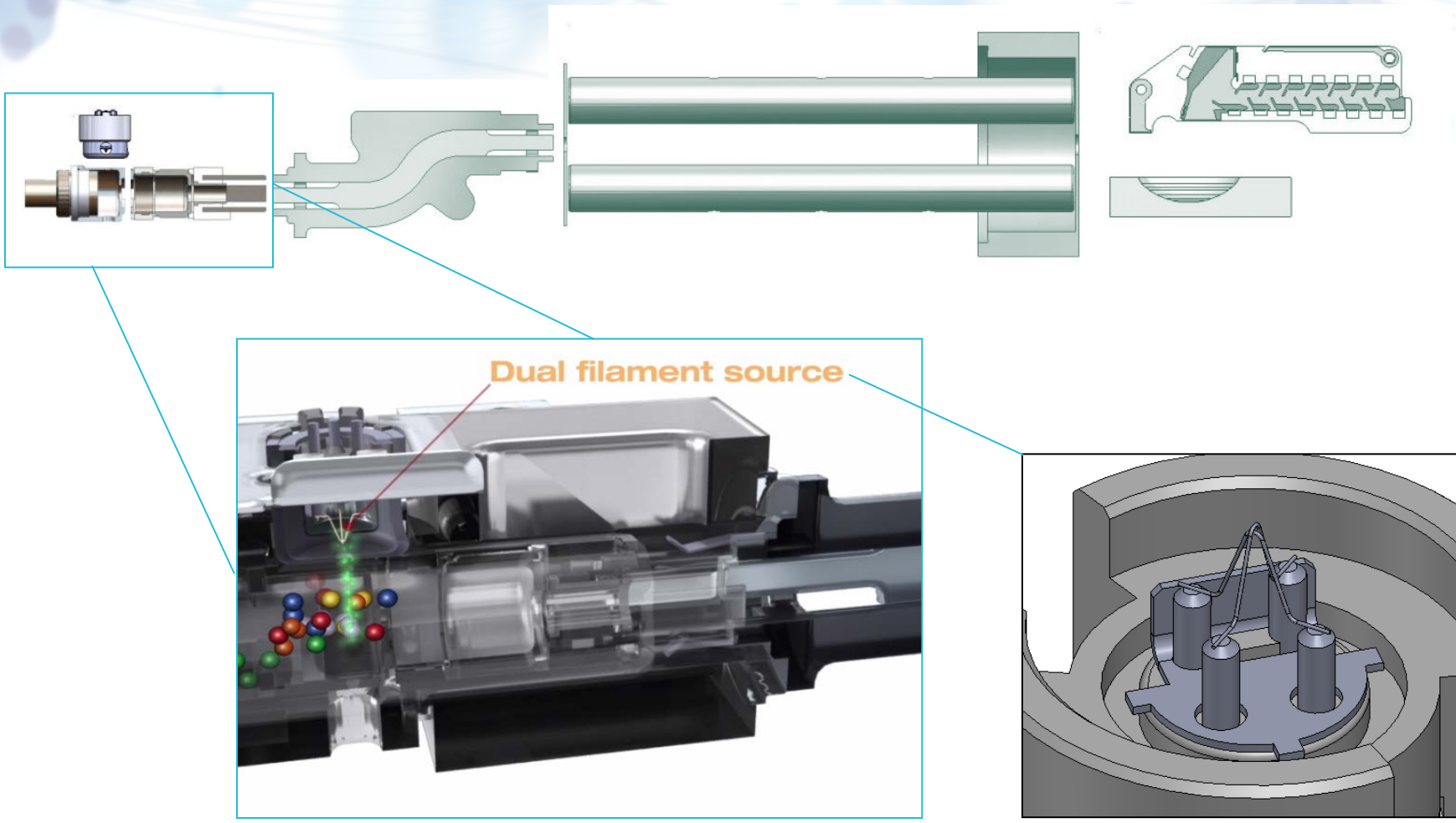
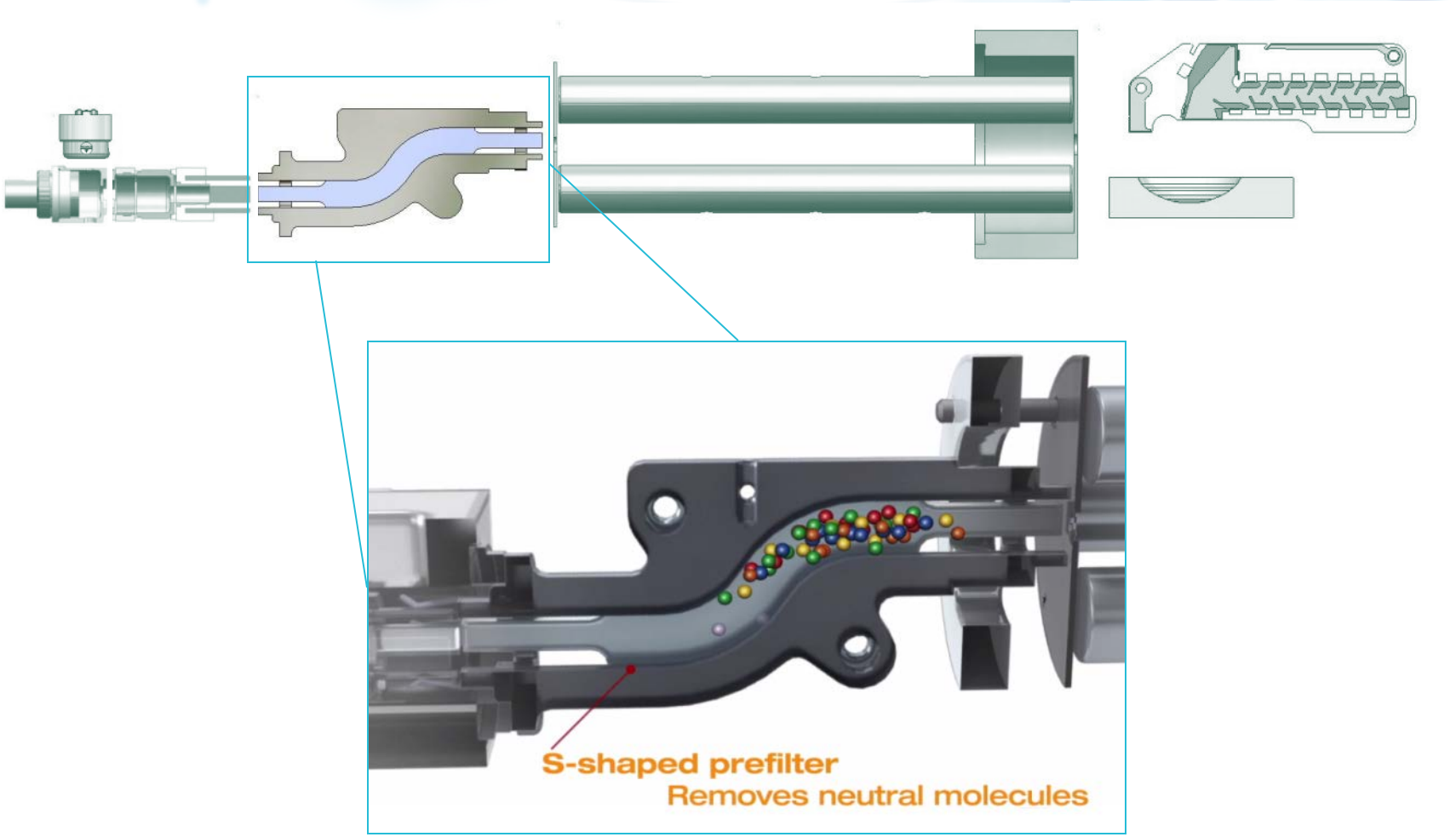
Ion Source: Dual Orthogonal Filaments



Removing Neutrals Before the Quad and Detector



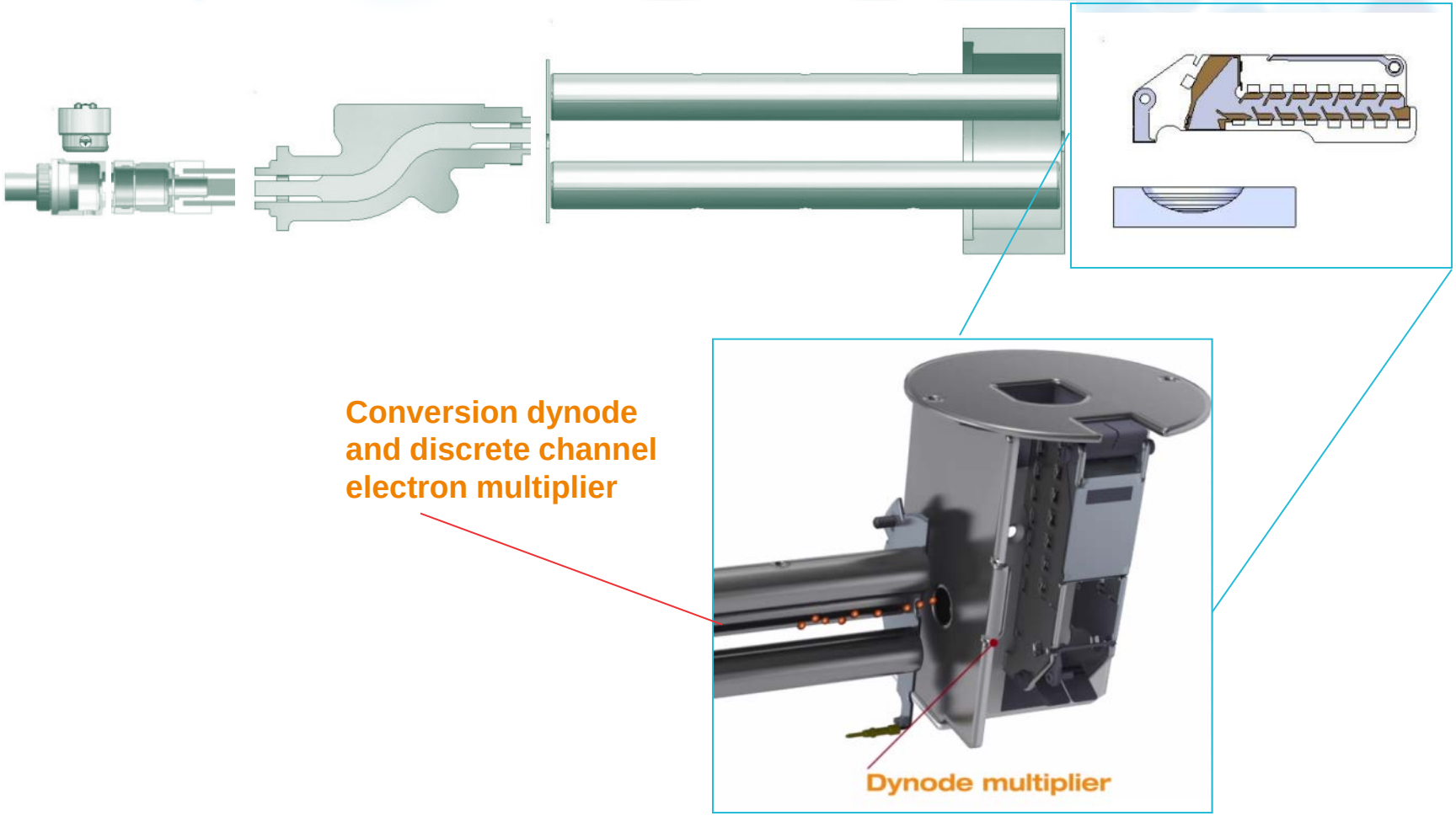
Keeping the Quad Clean for Longer

Quad Rods stay cleaner longer — with the Pre-Filter

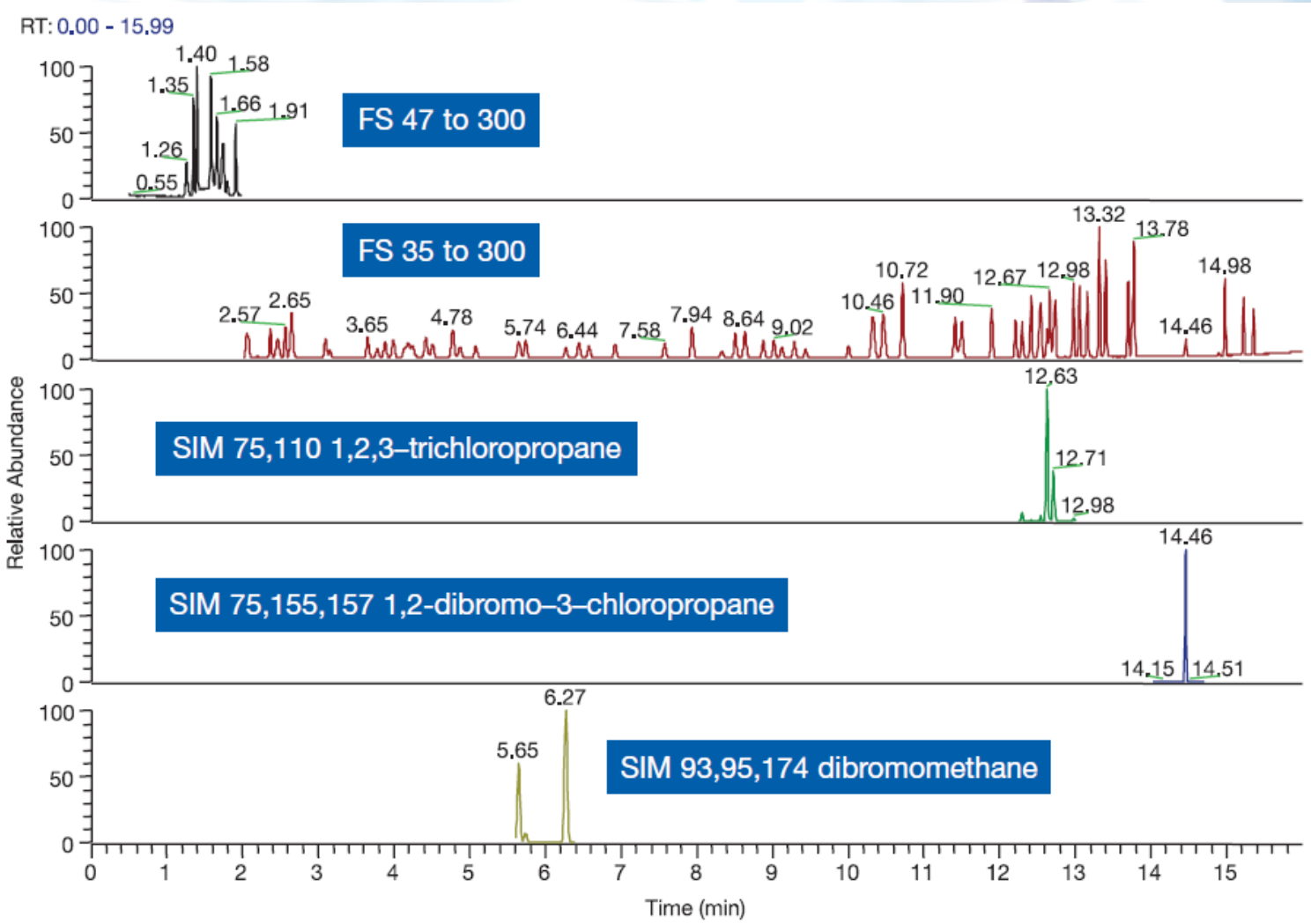
…and can still be cleaned (if needed)

Detect Your Ions, Not Excited Neutrals

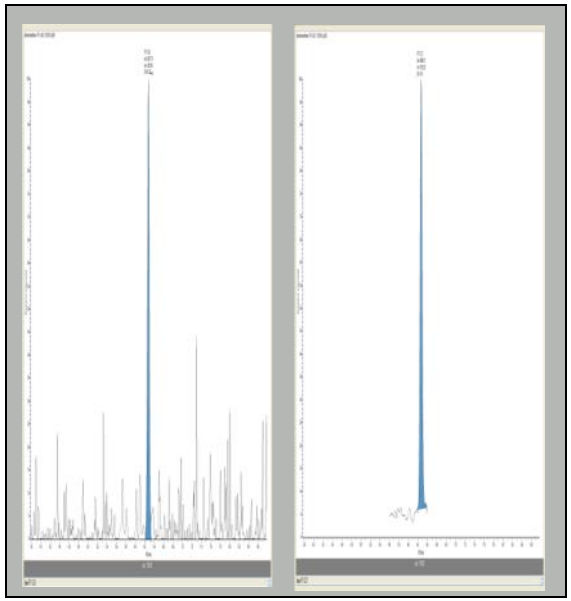
with Linear-Log Detector for Wider Dynamic Range



Full Scan/SIM Methodology for Drinking Water



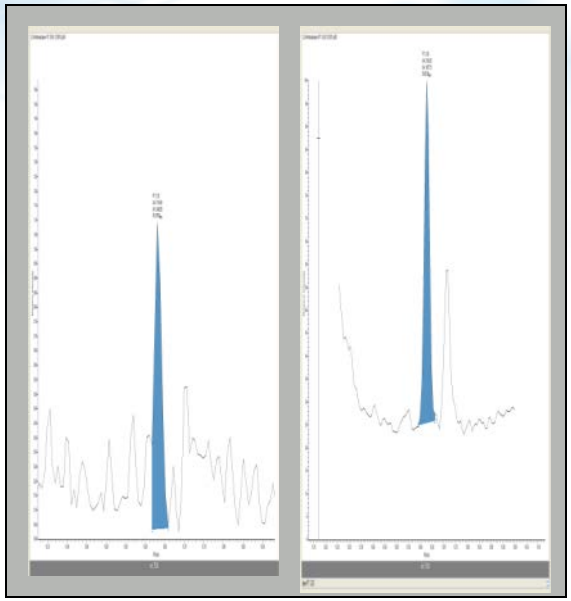
Low Level Drinking Water Analysis FS/SIM



0.020 ppb Full Scan

0.020 ppb SIM

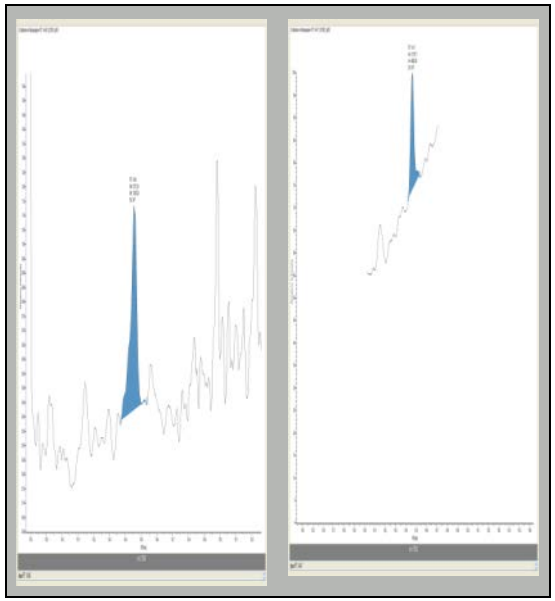
Dibromomethane



0.020 ppb Full Scan

0.020 ppb SIM

1,2,3-trichloropropane

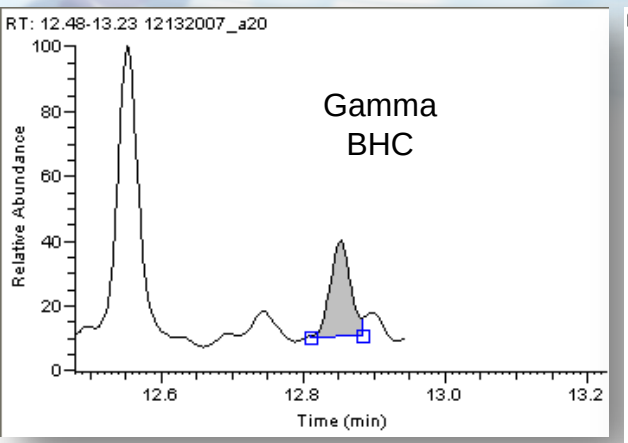


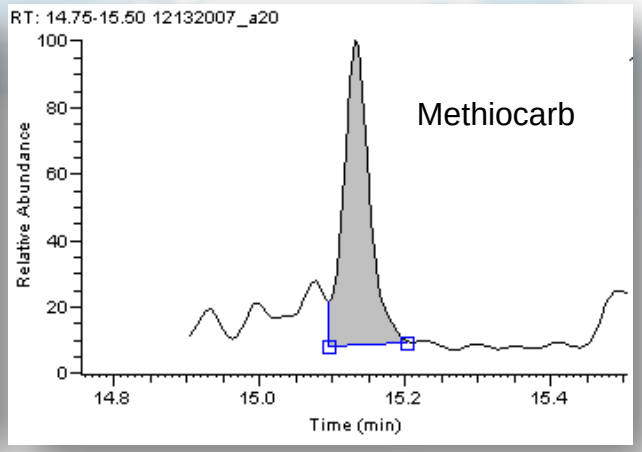
0.100 ppb Full Scan

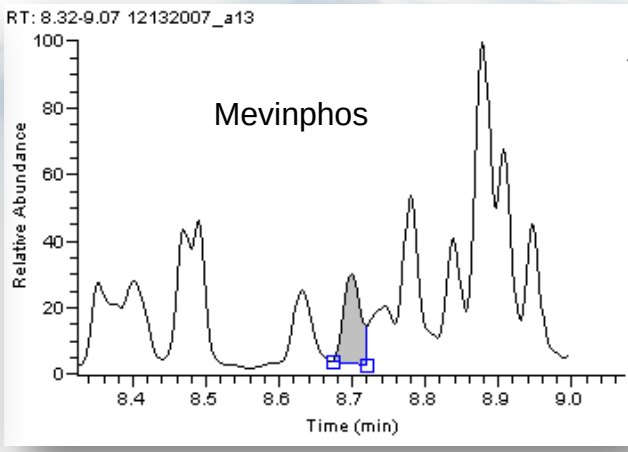
0.020 ppb SIM

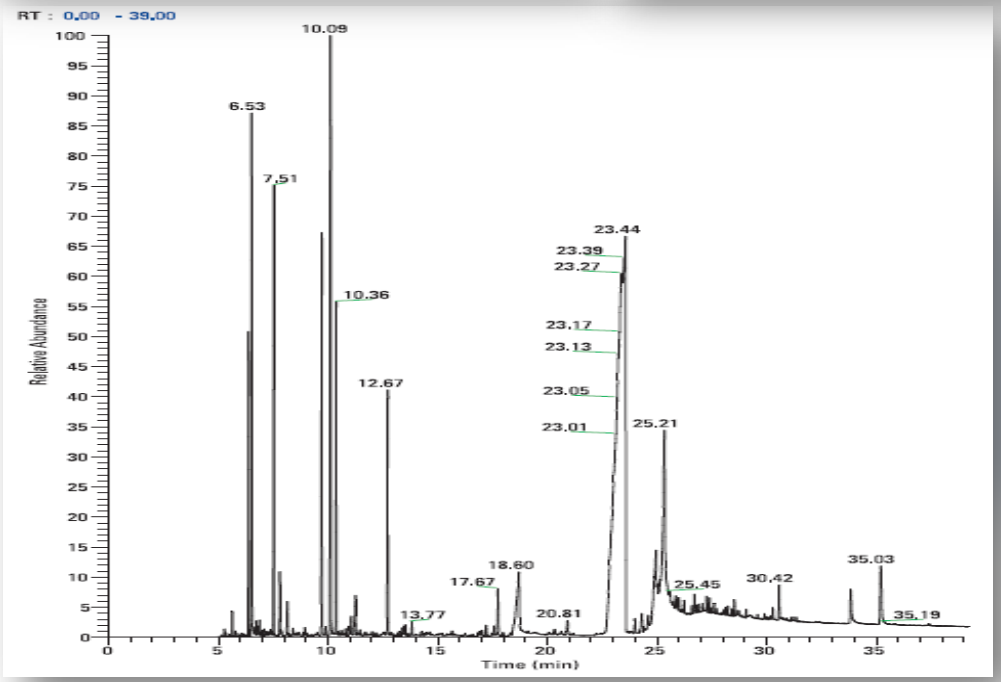
1,2-dibromo-3-chloropropane

Challenging Samples for Single Quadrupole GC-MS









- **Matrix challenges**
- **Concentration challenges**
- **Difficult to prove contamination**

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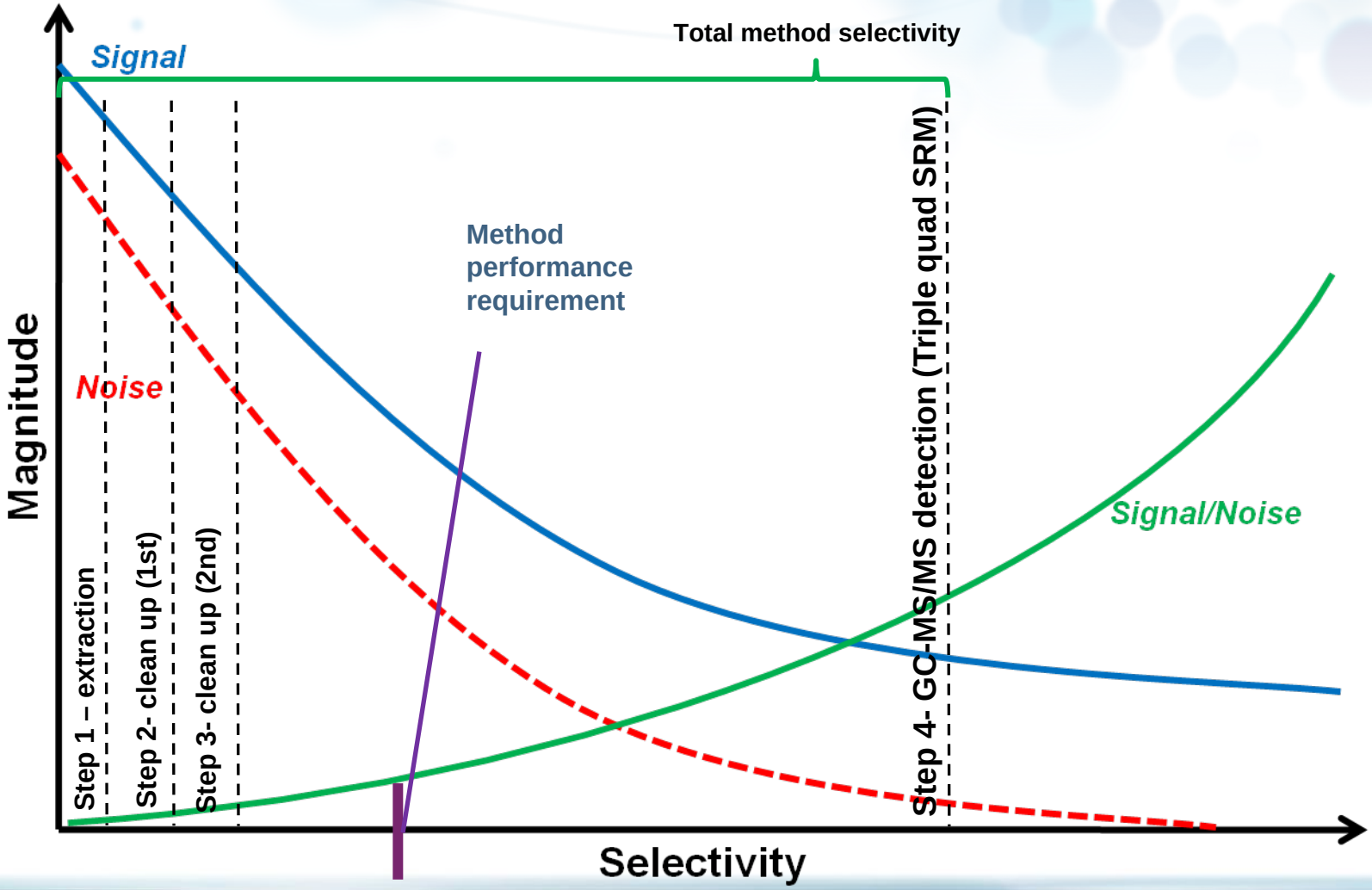
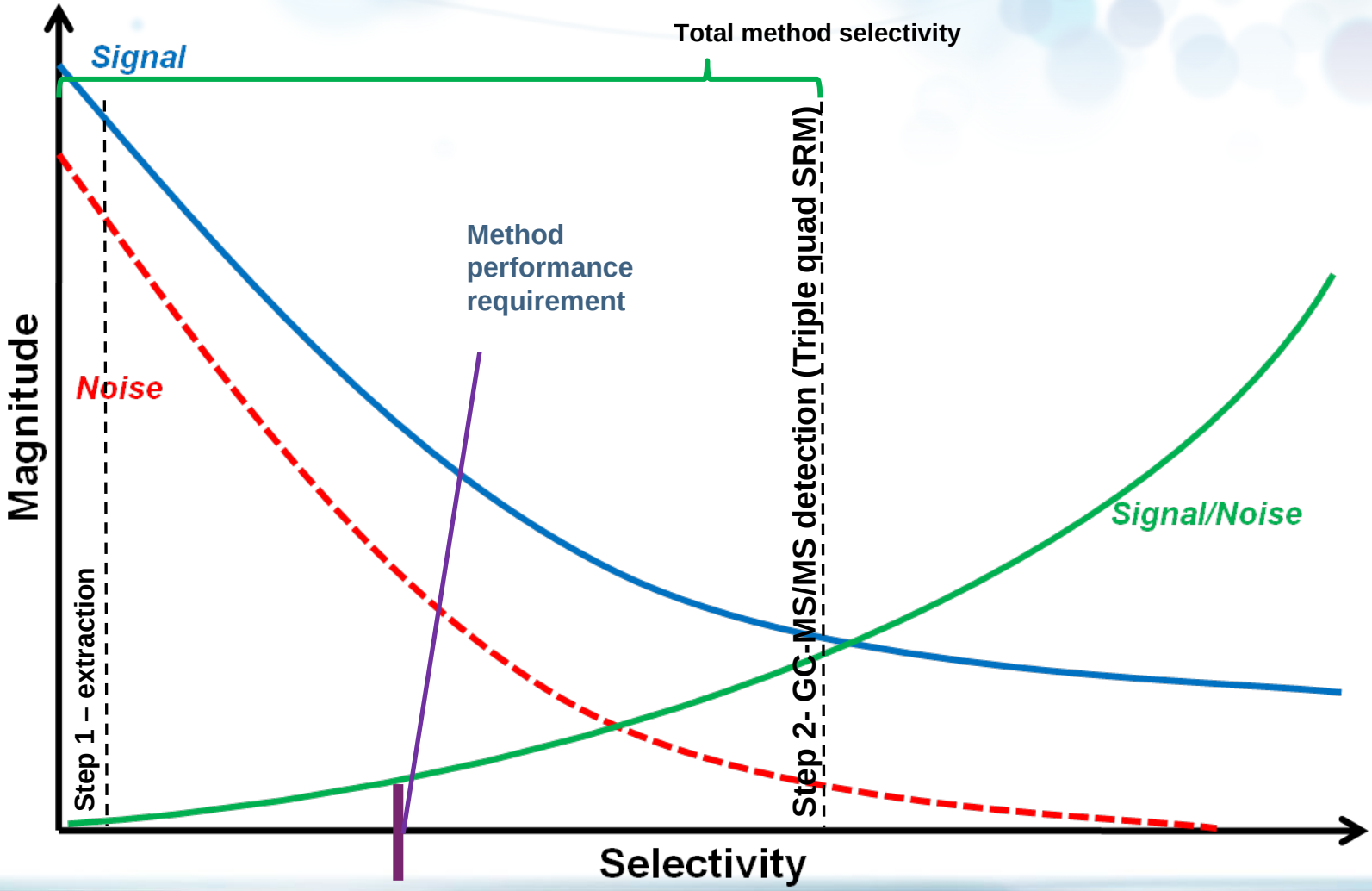
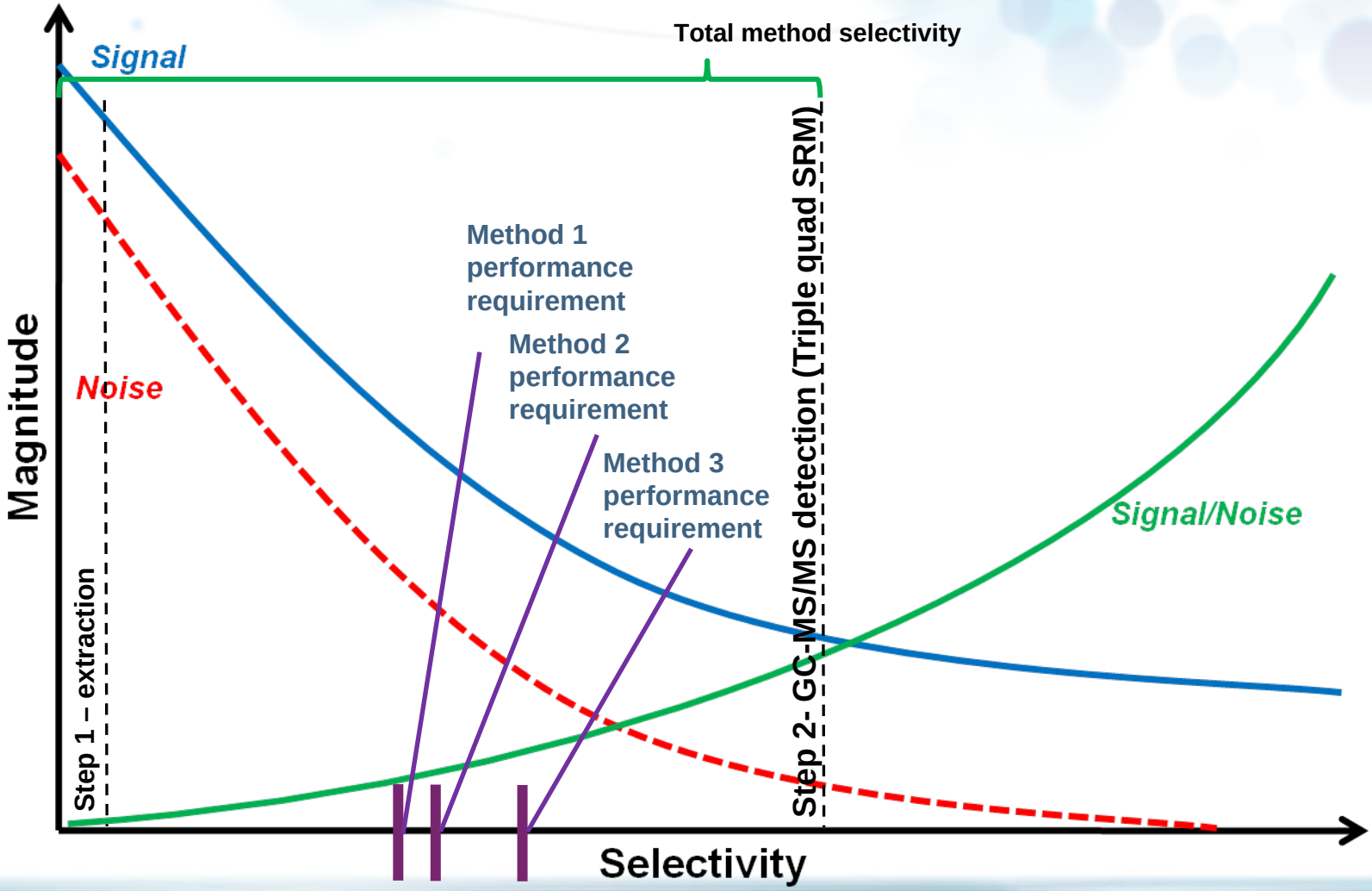
What About Triple Quadrupole GC-MS/MS?



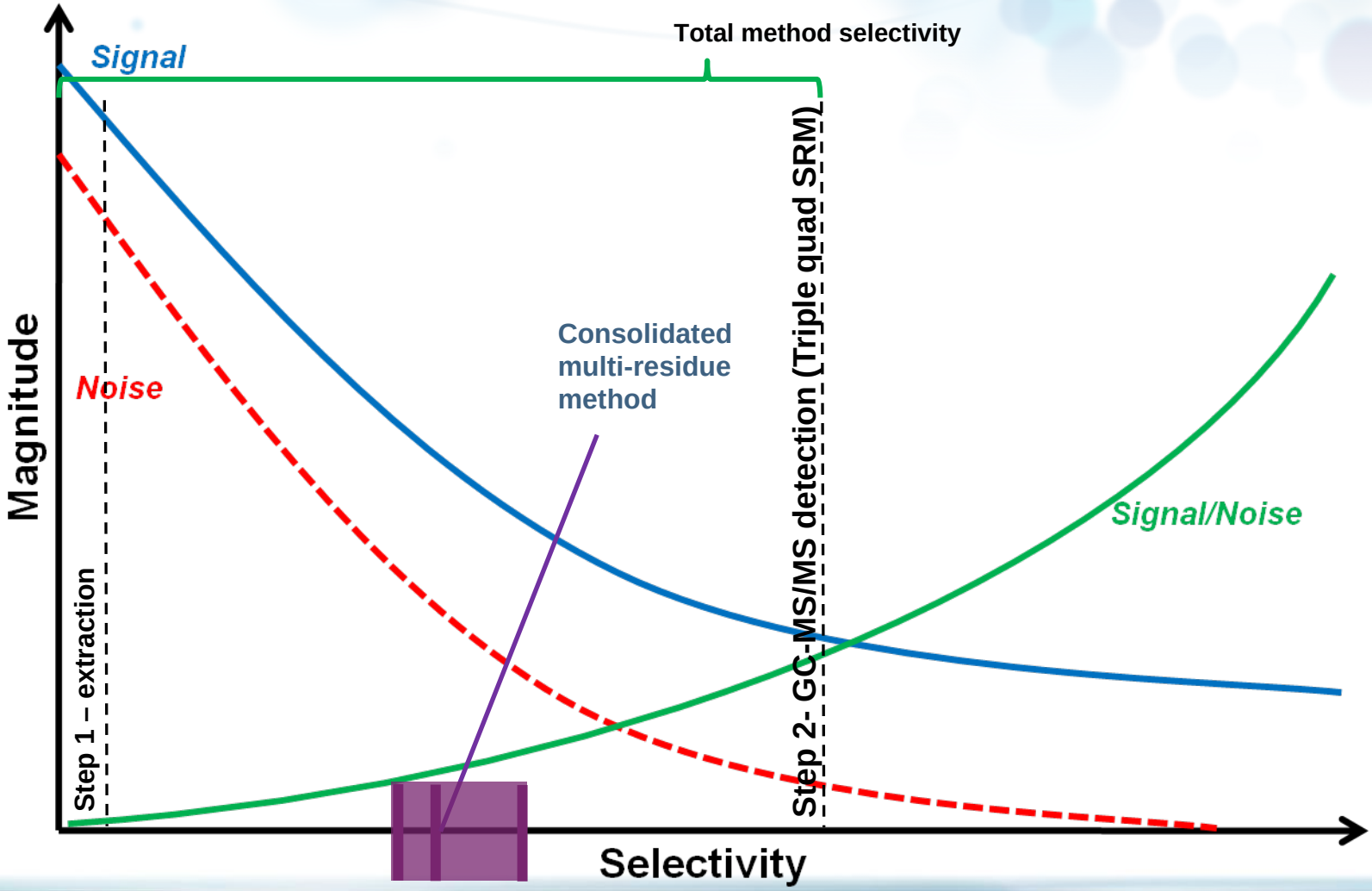
Use GC-MS/MS to Reduce Clean-up



Use GC-MS/MS to Consolidate Methods



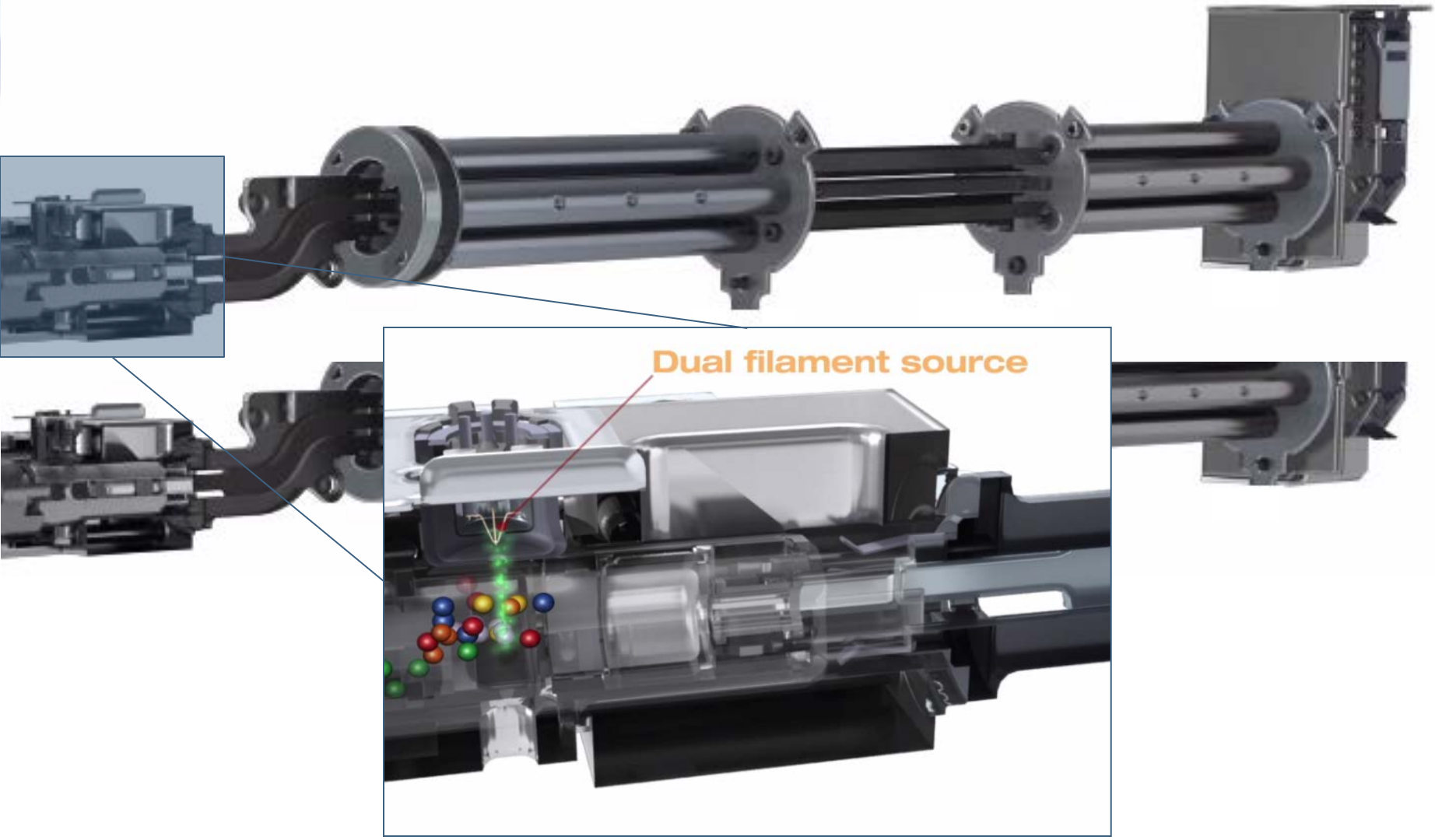
Use GC-MS/MS to Consolidate Methods



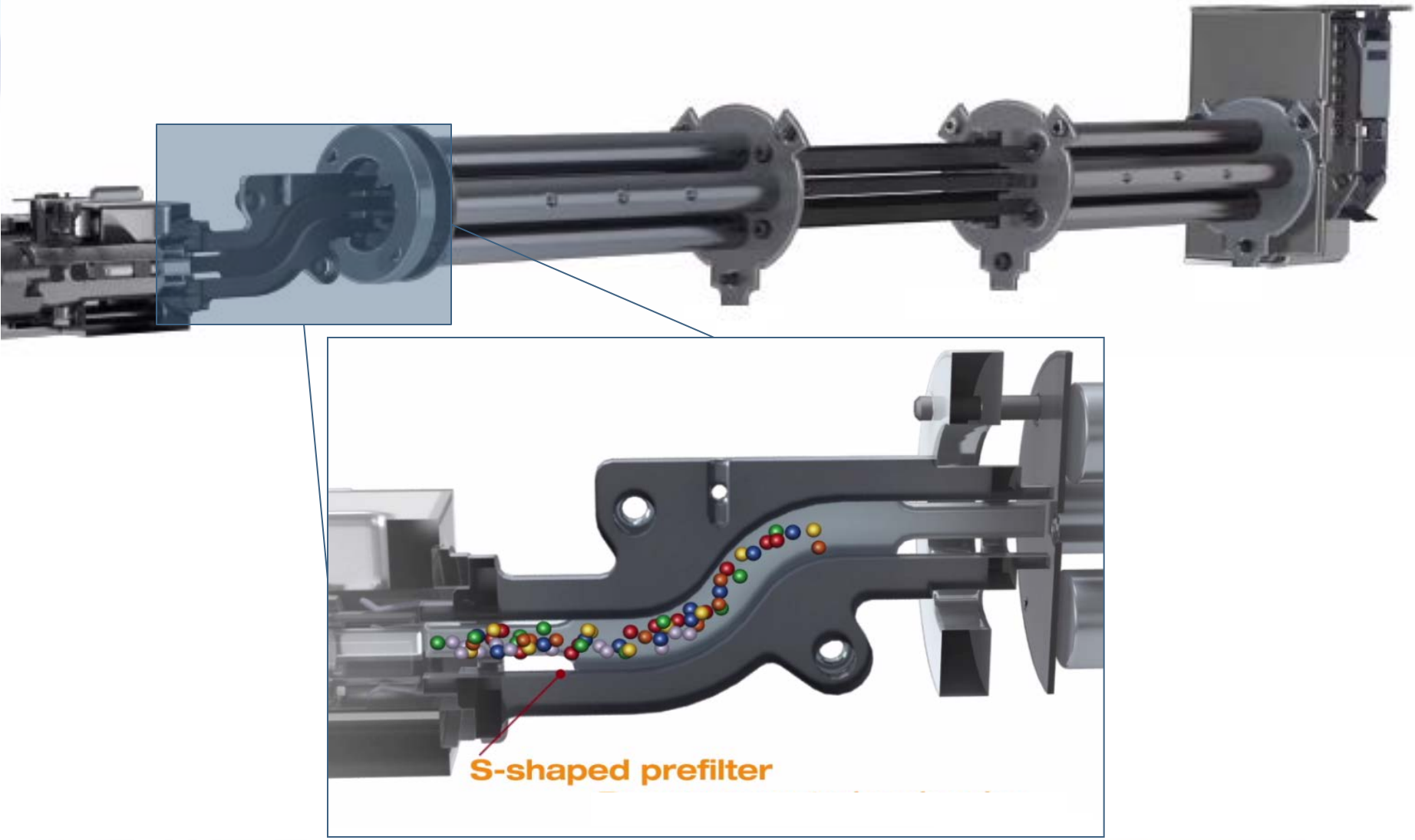
Benefits of GC-MS/MS

- High selectivity
- Possibility to reduce selectivity in sample preparation
- Reduced sample prep steps creates a more generic sample prep method – more compounds & matrices
- Consolidated GC-MS methods due to high performance – buffer against requirements
- Compressed chromatography possible
- Easy peak evaluation – auto-integrators

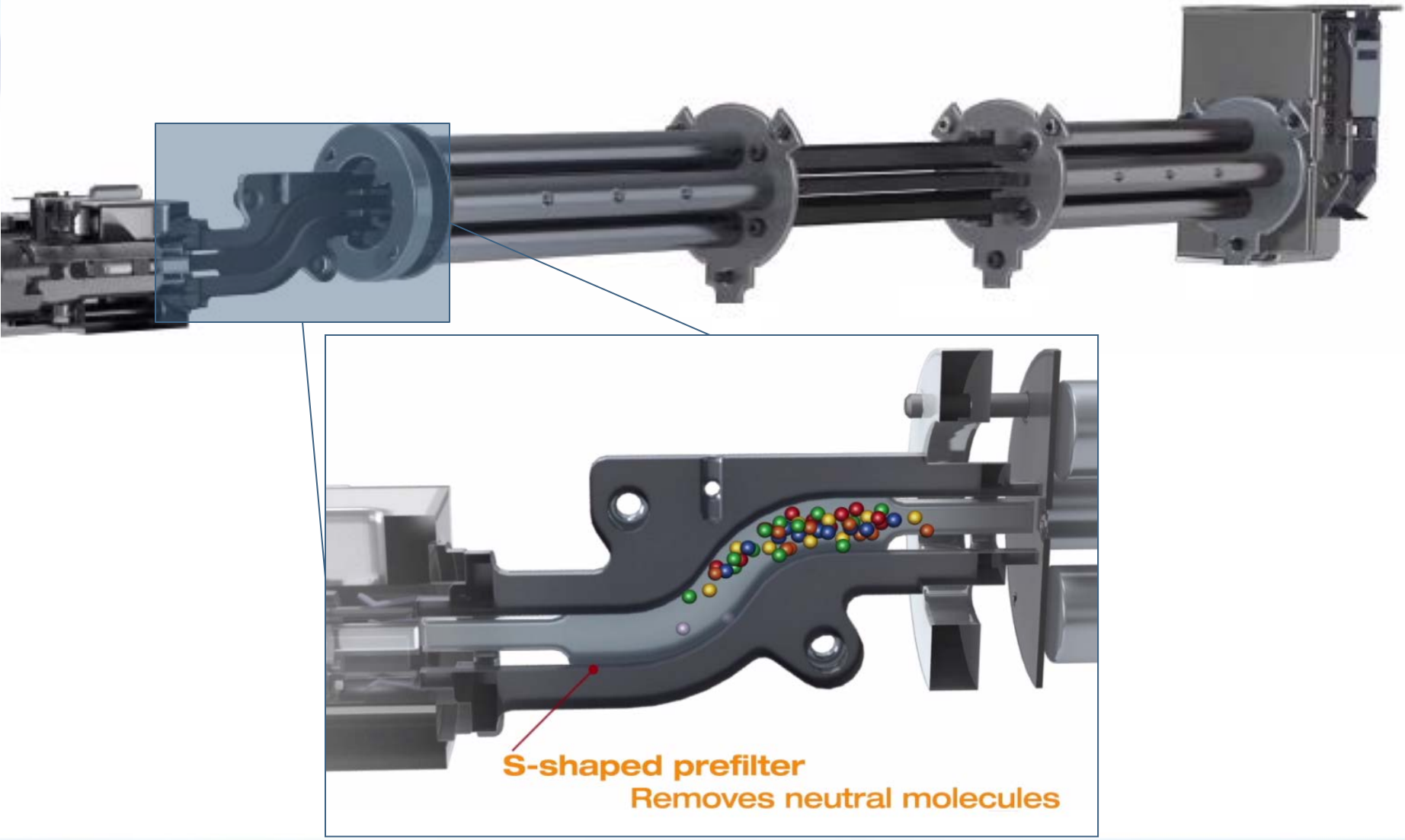
Step 1: Ions are Produced in the Source



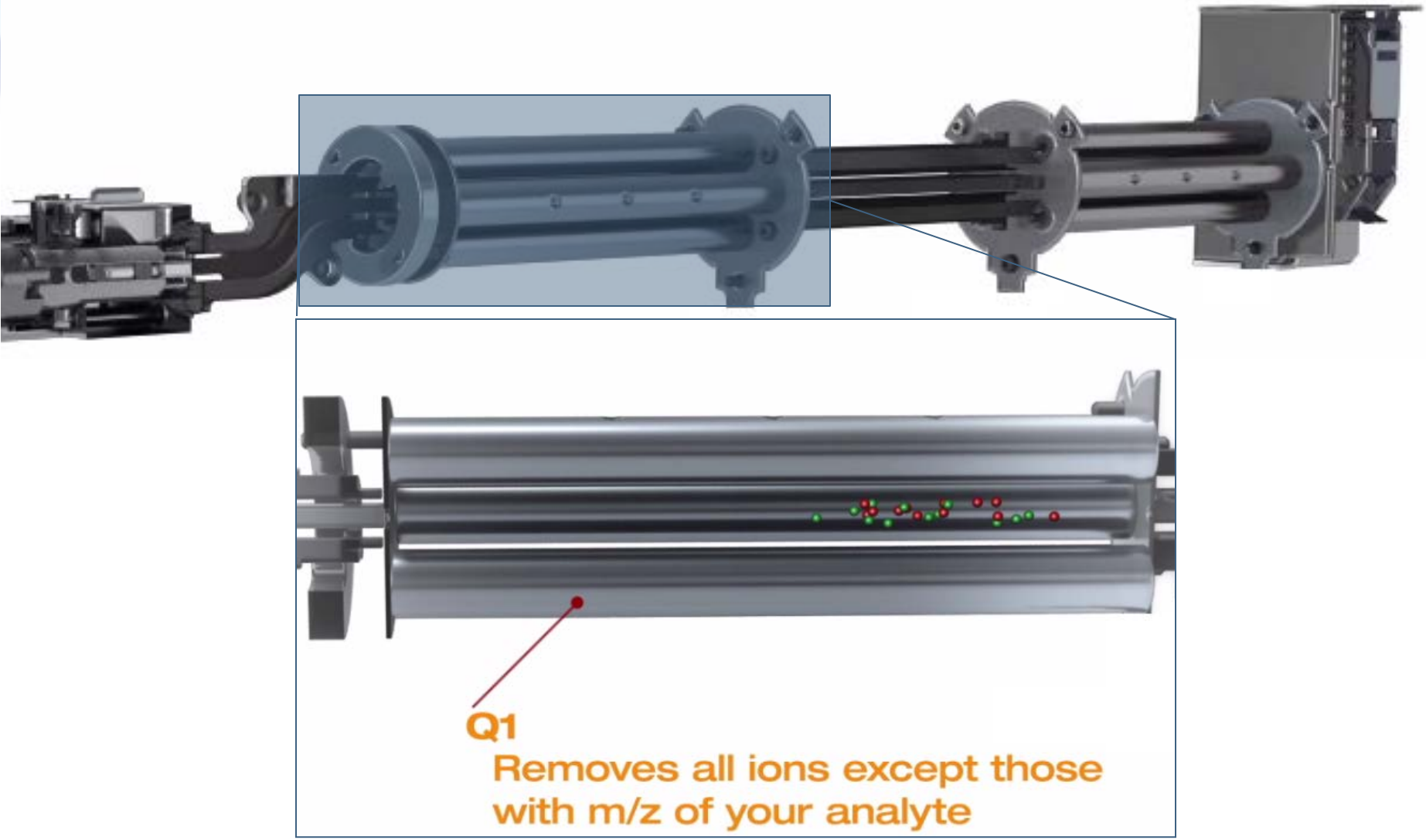
Step 2: Ions are Focused in the S-shaped Prefilter



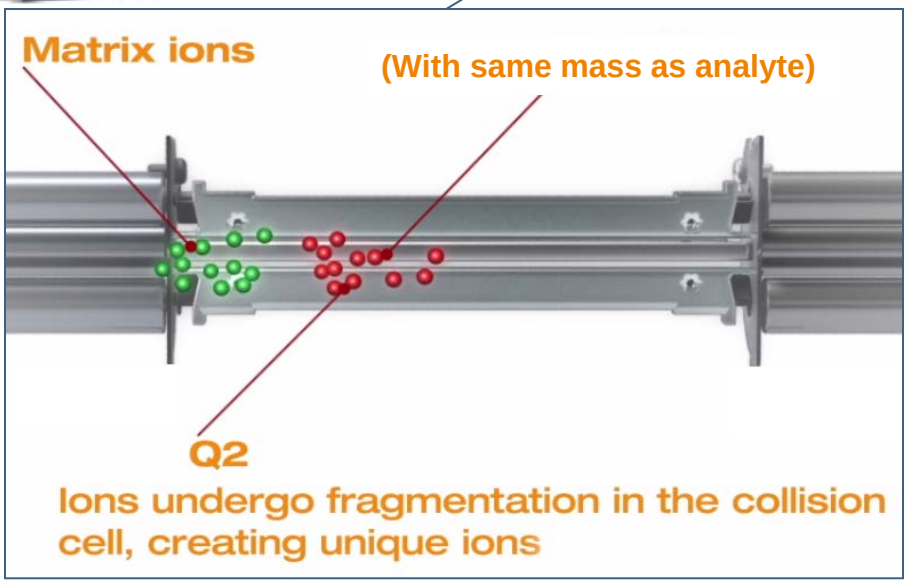
Step 2: Remove Neutral Molecules

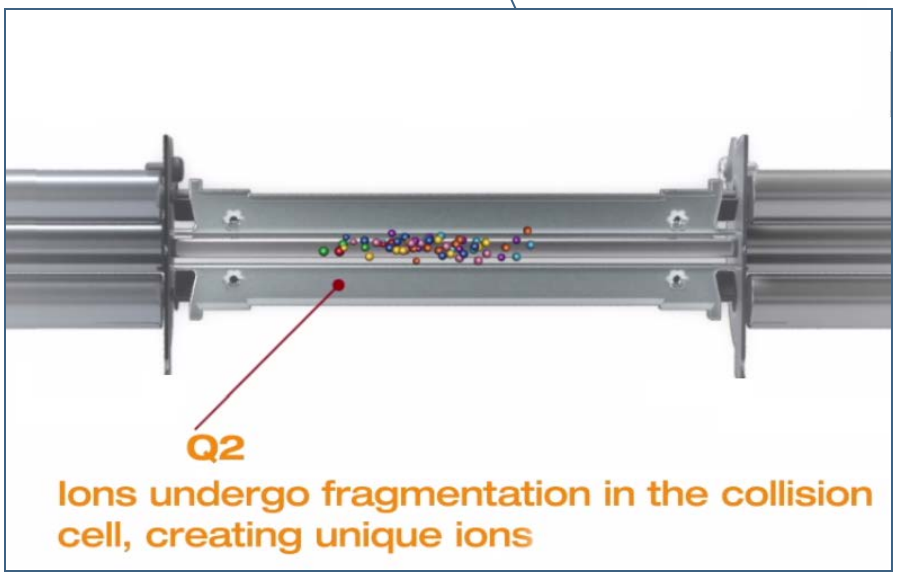


Step 3: Ions are Isolated by Molecular Weight in Q1



Step 4: Ions Further Fragmented in Collision Cell





Step 5: Unique Ions to Analytes Chosen in Q3

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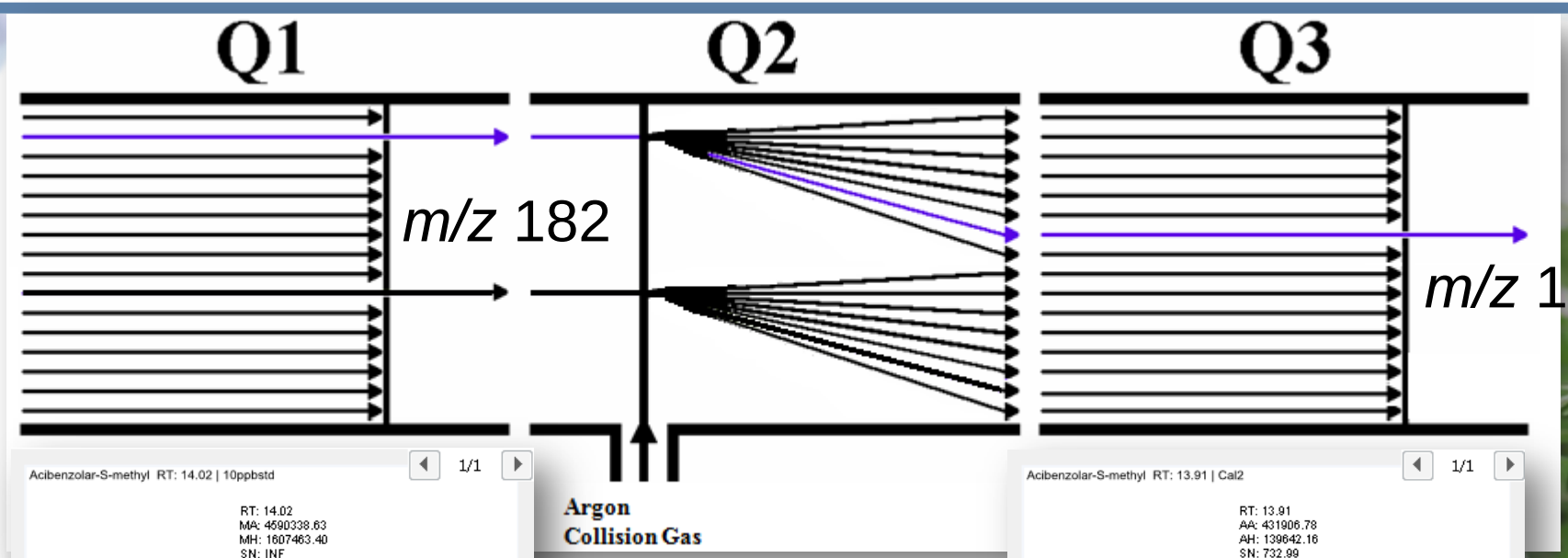
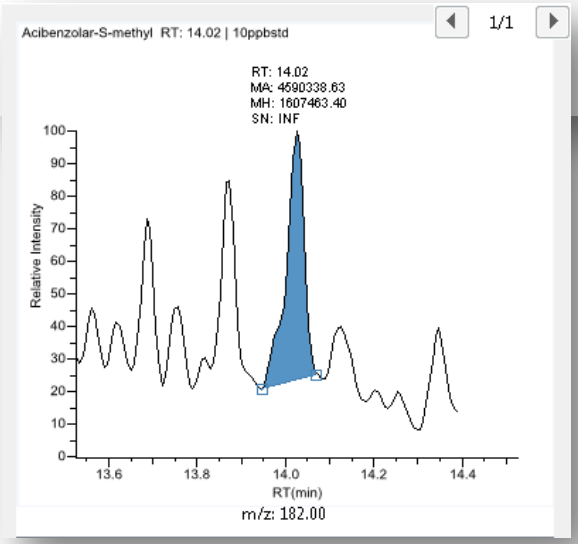
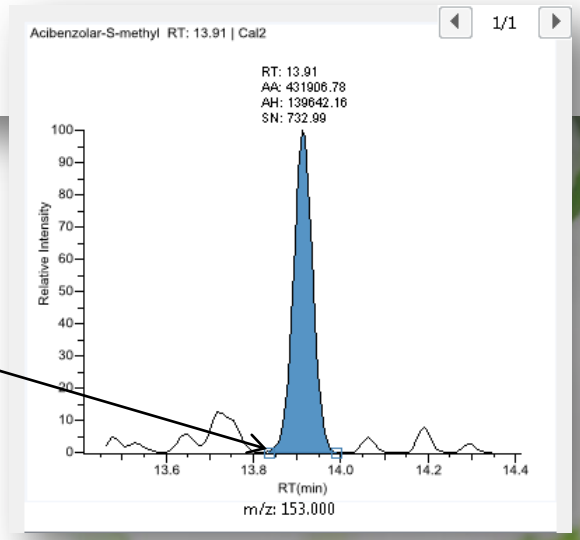
Product Ion Selection





SIM on *m/z* 182

SRM removes the shoulder from matrix interference & reduces background



SRM on *m/z* 182 > 153

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Comprehensive Pesticide Analyzer

A complete pesticide method implementation, management and maintenance solution to drive unstoppable result productivity

Thermo Scientific™

TSQ™ 8000 Pesticide Analyzer is designed to create powerful pesticide methods that are:

1. **Self-customized**
2. **Auto-optimized**

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Powering the TSQ 8000 Pesticide Analyzer

- Preconfigured performance leading TSQ 8000 GC-MS/MS system featuring the award winning TRACE 1310 GC
- Pre-loaded acquisition methods
- Thermo Scientific™ TraceGOLD™ GC Column and consumable technology
- Thermo Scientific™ TraceFinder™ 3.1 EFS Data Processing software
- 600+ Pesticide compound database (CDB) with 1500 + SRM transitions
- AutoSRM & timed SRM (t-SRM)
- Pesticide Analyzer Installation Guide

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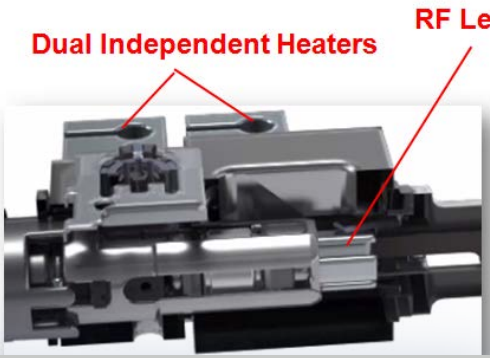
Integrated Workflow with GC Columns/Consumables

- **Liners – PTV Baffle (Siltek™), deactivated, 2 mm x 2.75 mm x 120 mm**
- **Columns - TraceGOLD TG-5 SilMS, 30 m x 0.25 mm x 0.25 mm**
- **Consumables – septa, ion volumes, etc…**
- **Instrumental method**
- **Compound database**

TSQ 8000 Triple Quadrupole GC-MS/MS Features

- No venting needed to clean the optics: the ExtractaBrite Ion Source is exchanged via vacuum interlock
- Dual orthogonal filaments for both EI and CI
- Source temperature up to 350 ° C for improved robustness
- S-Shaped ion guide for excited neutrals noise reduction
- Full Scan data match Single Quadrupole data
- Solid probe capability via vacuum interlock
- User-customizable tuning and tune reporting
- Automated preventative maintenance alarms

- Simple method transfer from ISQ Single Quadrupole GC-MS to TSQ 8000 Triple Quadrupole GC-MS/MS
- Integrated software tools like AutoSRM ease the route to productive analysis regardless of your starting point, whether it be from the very start, a single quad method or an existing MRM method



- Never clean or replace quads
- Heated RF Lens contains ion burn

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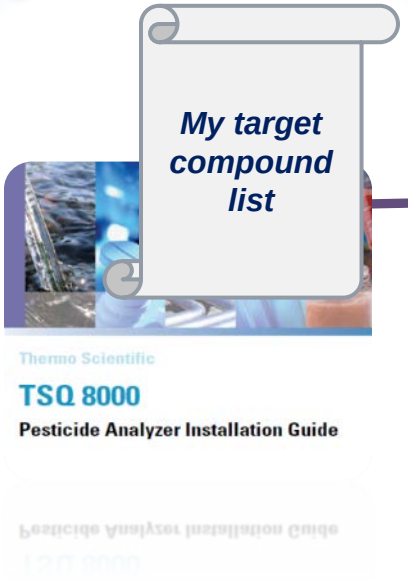
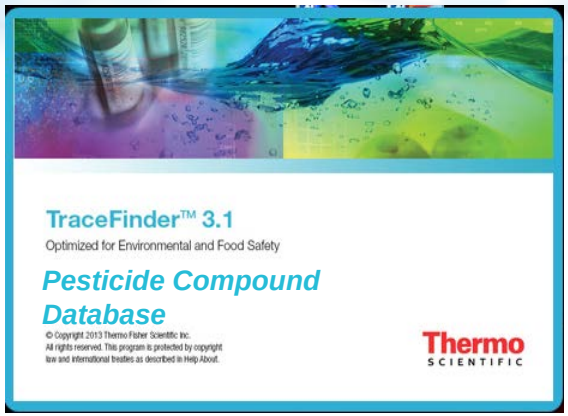
TSQ 8000 Pesticide Analyzer Workflow





Develop missing compound/ SRM

Add to database

Automatic MRM method creation

RT Update

My completed pesticide method

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AutoSRM: Fast, Simple Route to Optimized SRM

1) Precursor ion selection

2) Product ion

- **AutoSRM automates the development of SRM methodology**
  - Creation of full scan, product ion scan and SRM methods
  - Creation of sample sequences
  - Creation of data layouts for analyzing results
  - Selection of precursor, product and collision energies

End result showing optimized transition

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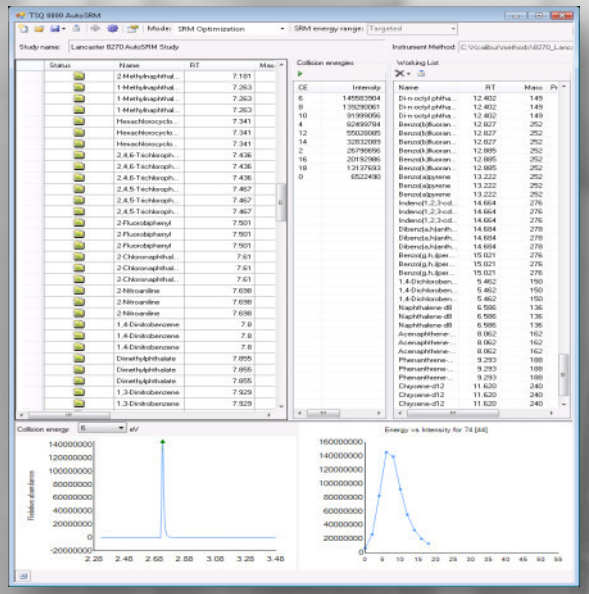
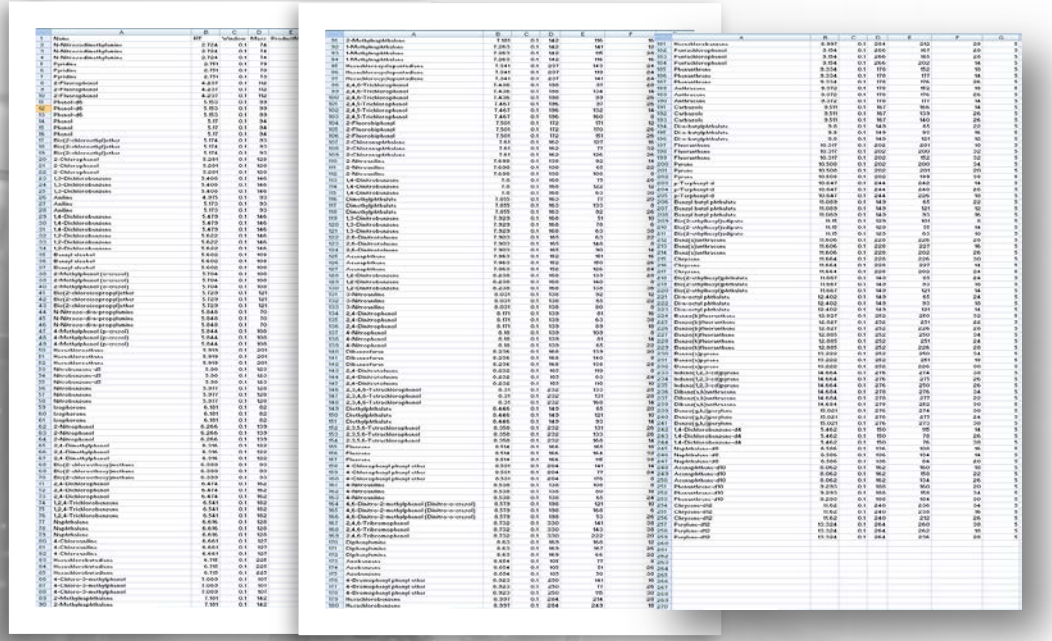
AutoSRM User Case

- Created and optimized > 250 transitions for > 80 compounds
- Minimal user interaction (less than an hour of “face time”) over 24 hours period.





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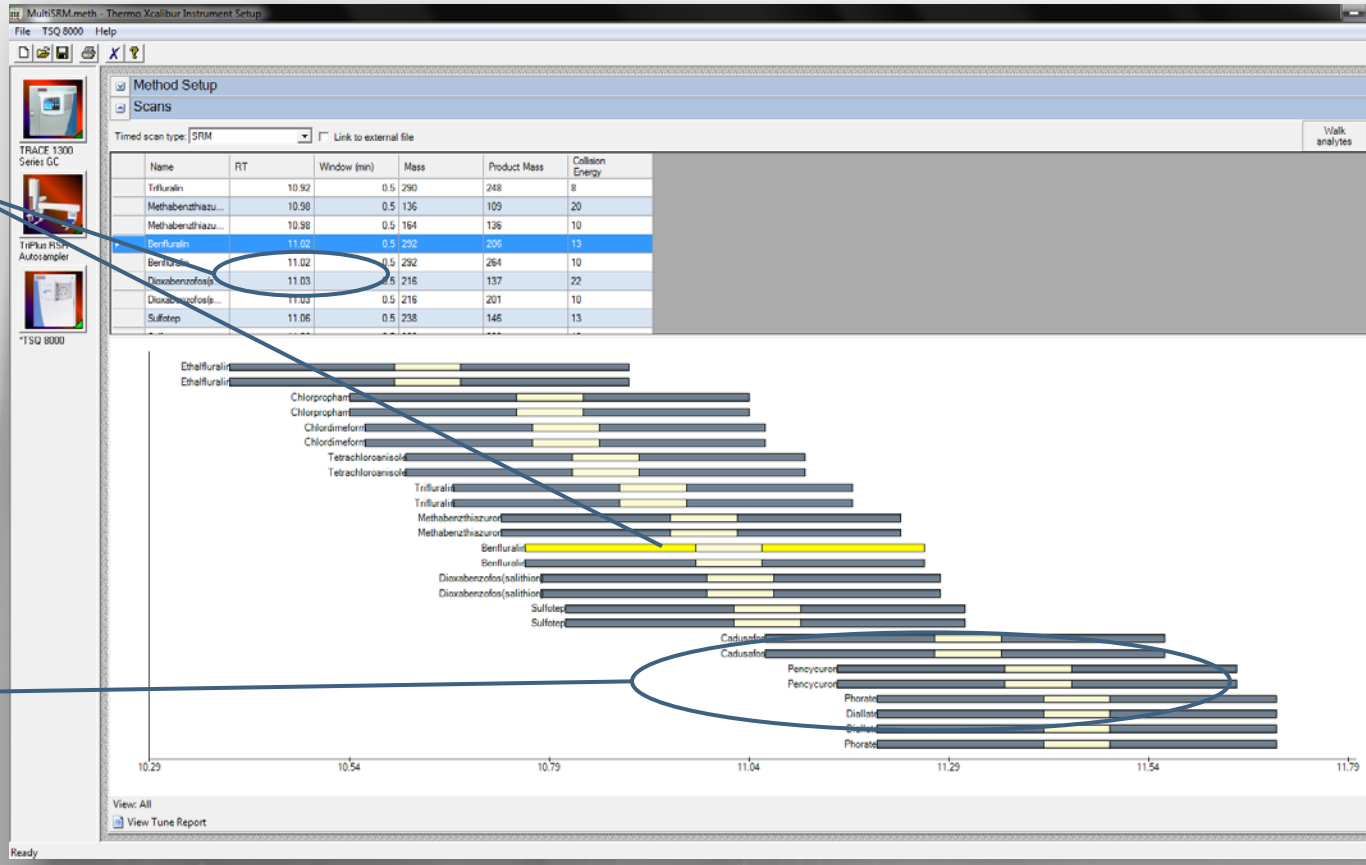
Timed-SRM Method Overview

- We created Timed-SRM
- Break the segmented methodology paradigm with individual SRM's centered around peak retention time

Acquisition windows centered around retention time

Acquisition windows allowed to overlap



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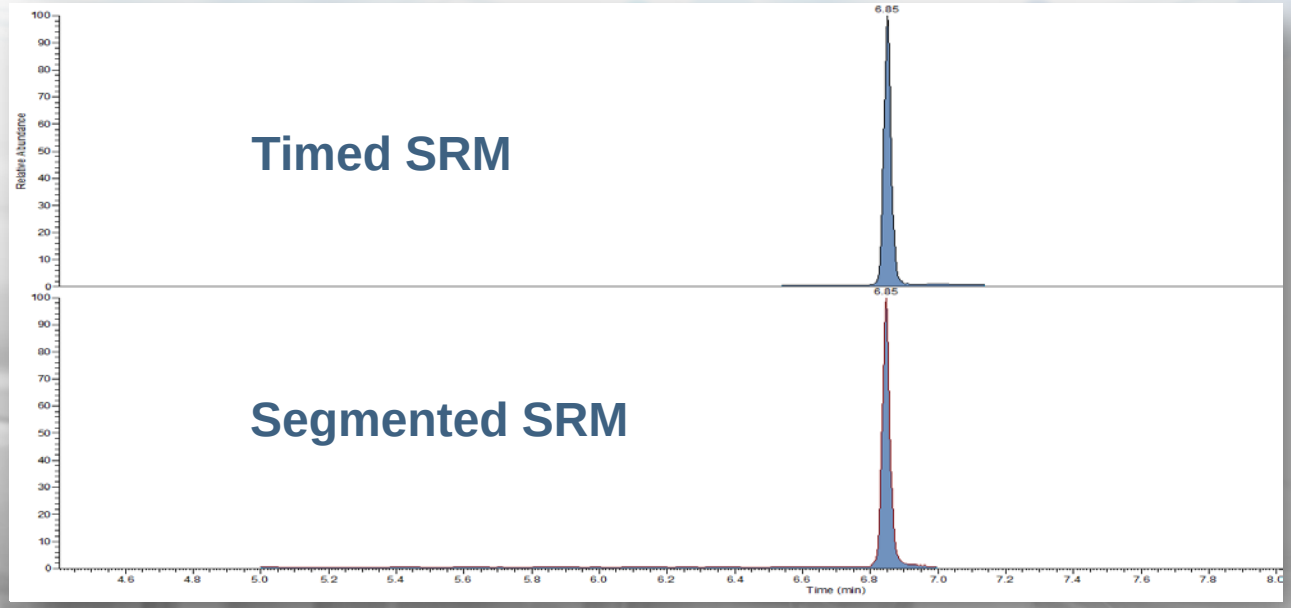
Timed-SRM Advantages



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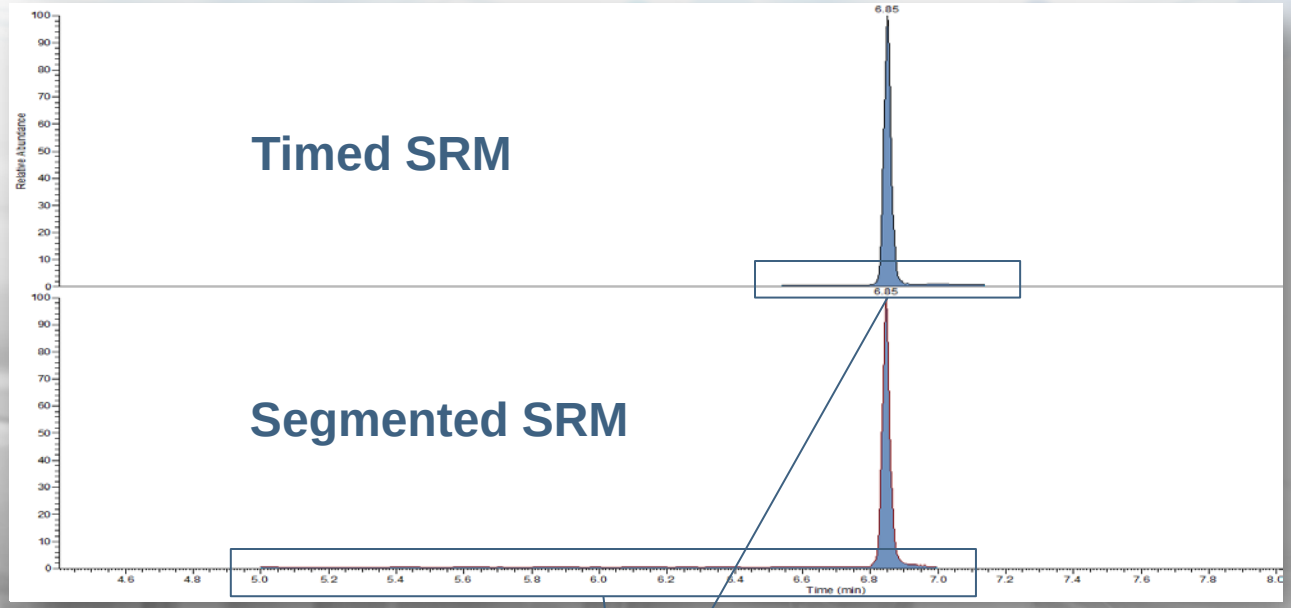
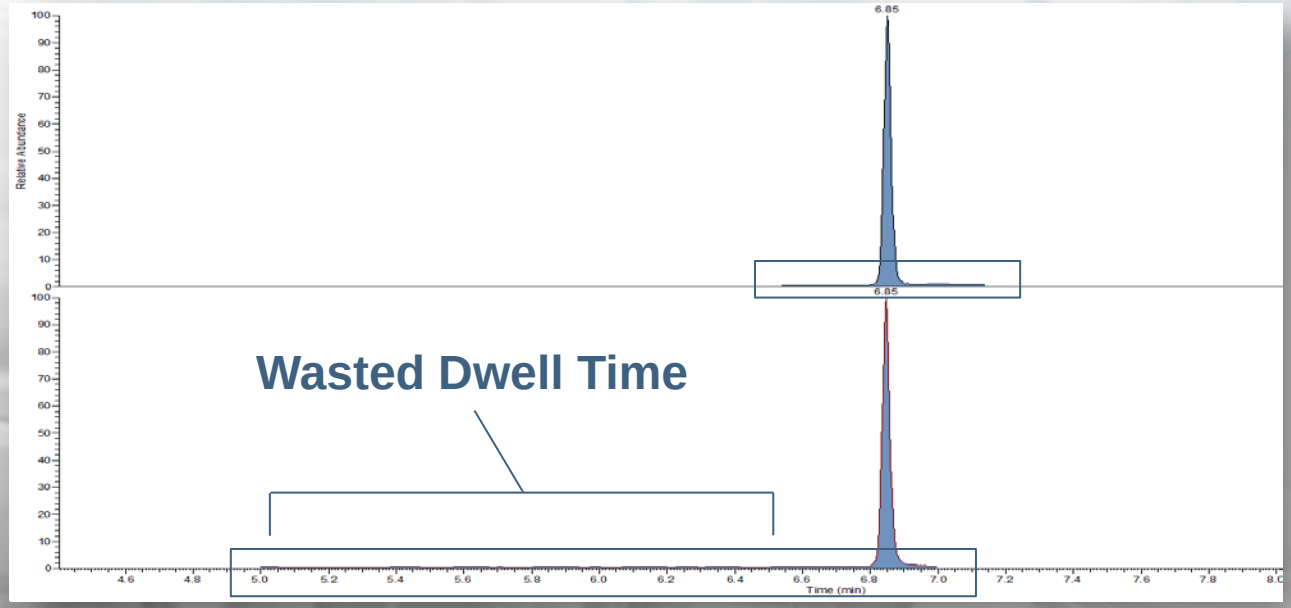
Timed-SRM Advantages



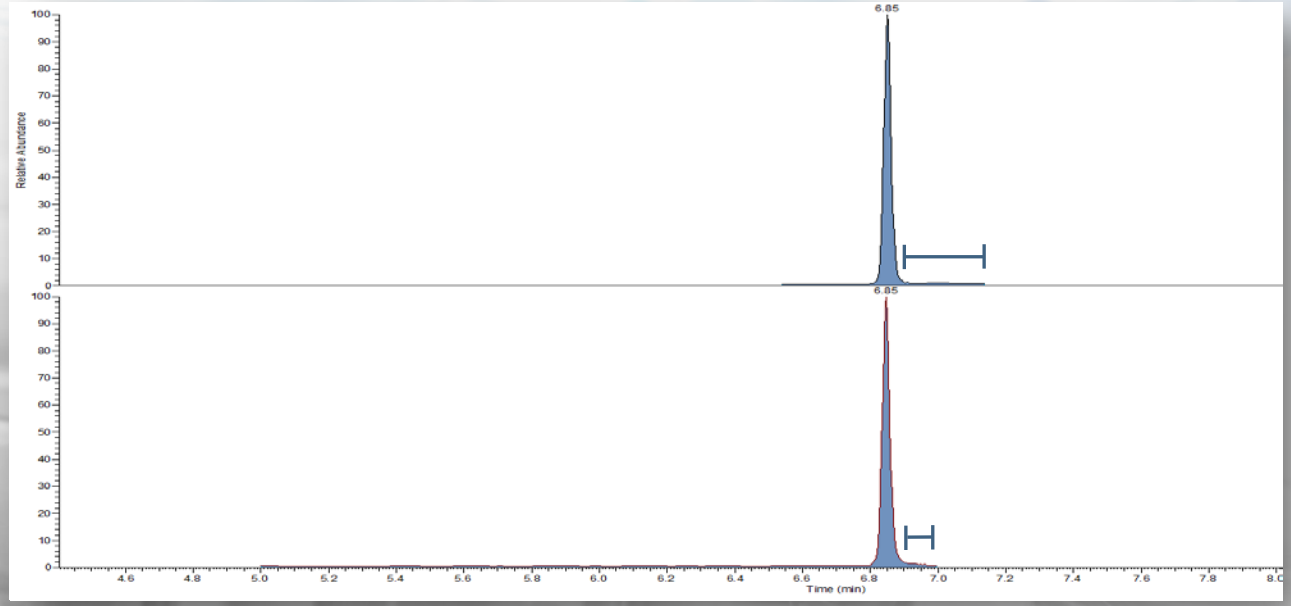
Acquisition Windows

Timed-SRM Advantages



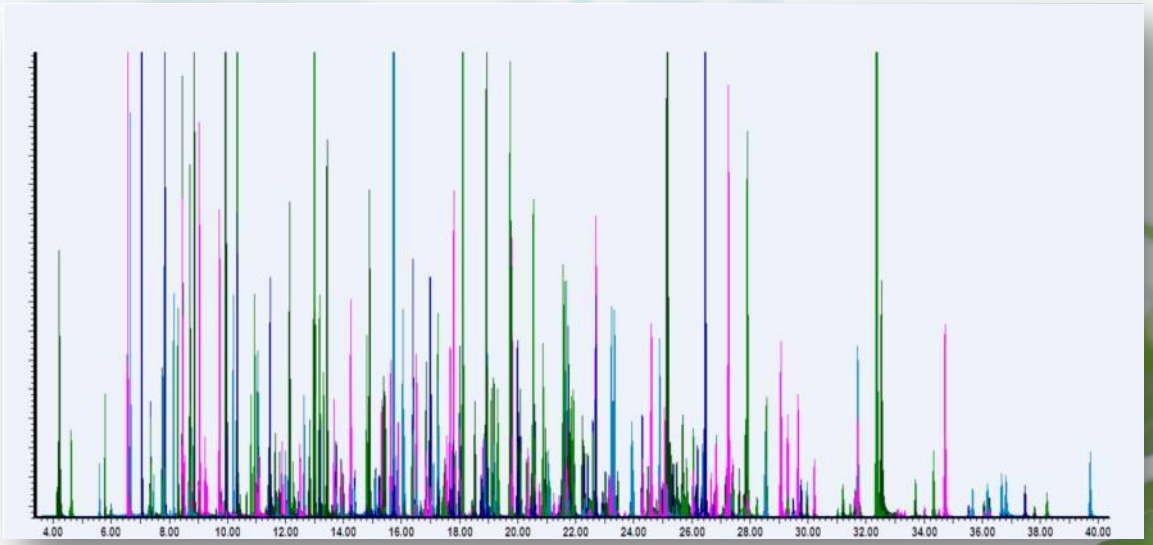
- **Removes wasted dwell time**
  - Allow higher overall dwell times
  - Leads to higher sensitivity

Timed-SRM Advantages



- **Peaks centered in acquisition window**
  - No peak elutes near acquisition break
  - Allows for retention time shift (e.g. due to heavy matrix)

Analysis of 300 Pesticides – Segmented vs. Timed



Segmented SRM	Timed SRM
Closest compound to segment break: **5 seconds**	Closest compound to segment break: **15 seconds** reduced risk of false negatives due to peaks shifting (example for a heavy matrix load)
Average number of simultaneous transitions: **55**	Average number of simultaneous transitions: **15** **(4X higher dwell times- better sensitivity)**

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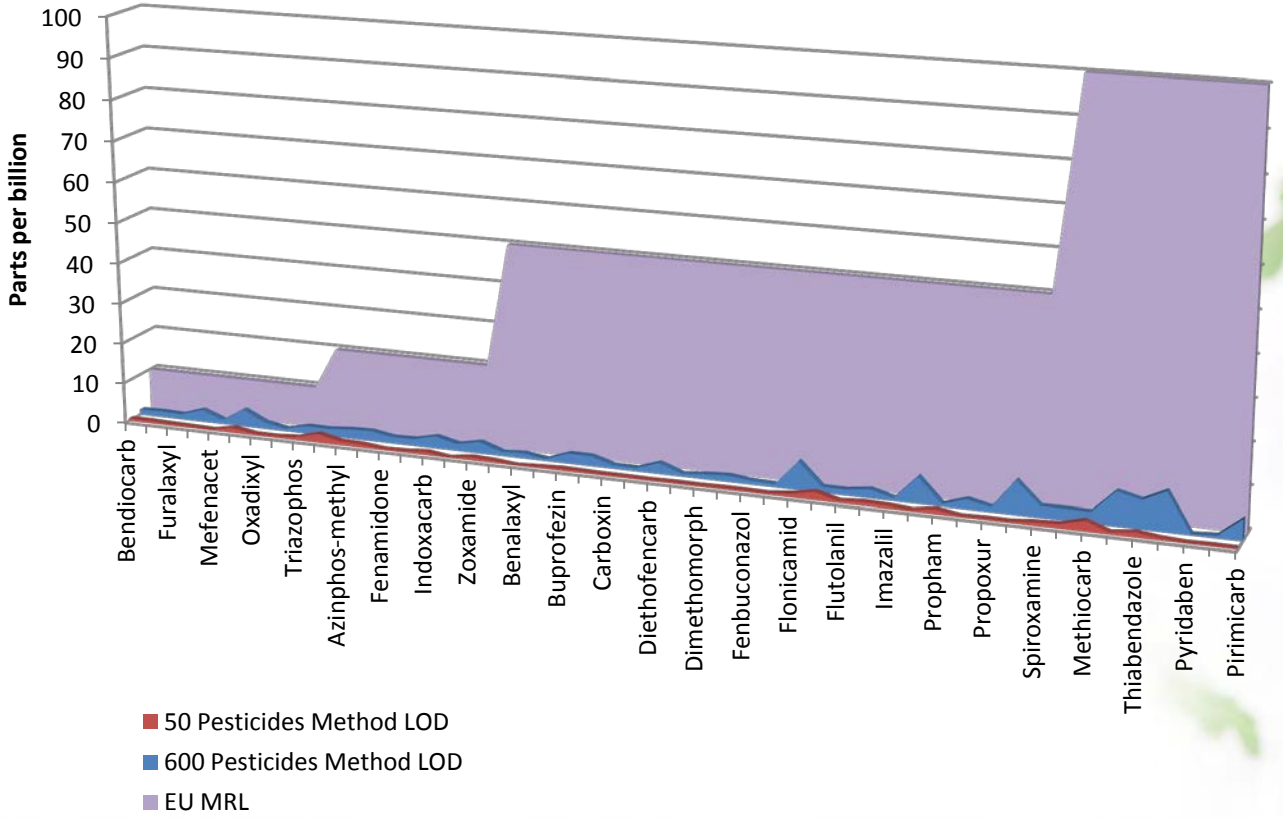
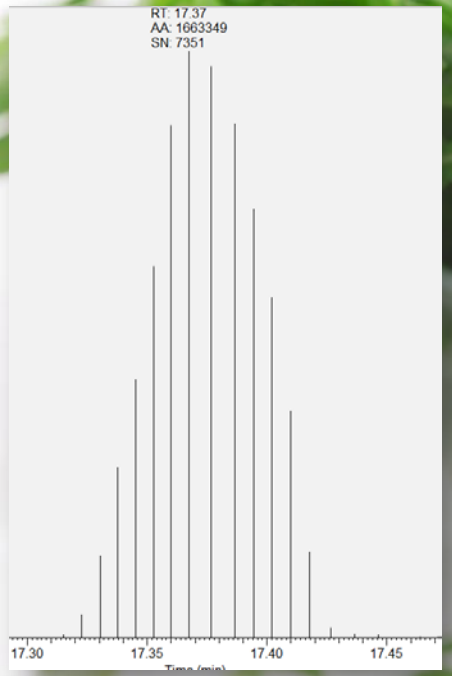
Incredible Pesticide Capacity in a Single Run

- **Example Statistical IDLs calculated for pesticides monitored when acquiring for 50 or 600 residues with a least 2 SRMs transitions each**
- **Instrument detection limits are always well within European Union Maximum Residue Limits requirements**





Peak sample frequency flutolanil in 600 method

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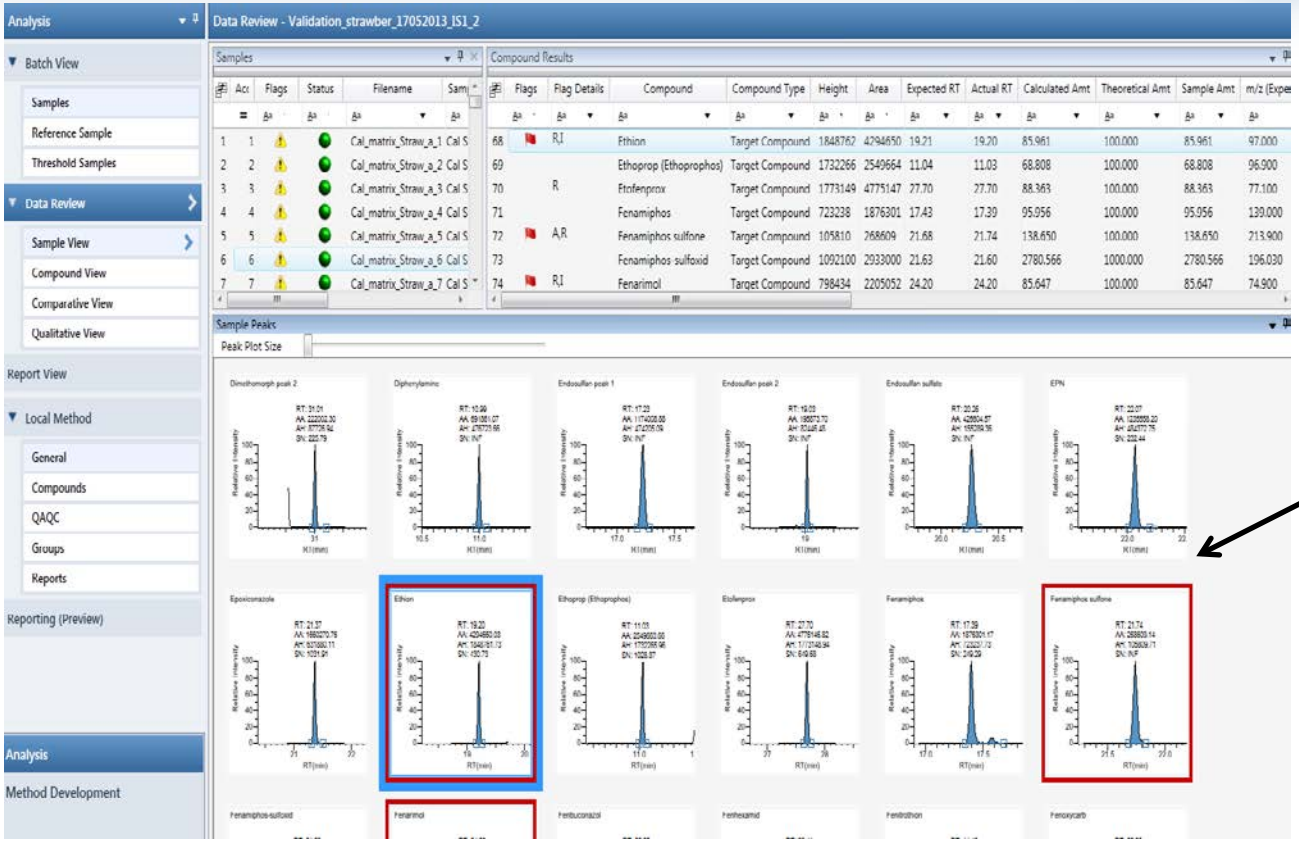
TraceFinder 3.1 Software Data Evaluation

Easy and quick overview of high number of sample results



Overview of all compounds in a sample at a glance with indication of any exceed of pre-set values

Summary

- **Pesticide analyses in food and environmental matrices is one of the most common yet more complex analyses run by gas chromatography**
- **Different approaches can be taken using selective conventional detectors, the “golden standard” single quadrupole GC/MS, or the powerful triple quadrupole GCMSMS technique**
- **Complete workflow solutions exist from sample preparation to data evaluation – including ready to use methods, databases**
- **There are benefits and limitations of each of these techniques for pesticide screening and confirmation**

More technical details and support: http://www.thermoscientific.com/pesticideanalysis

Thank You for Your Attention!

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