

Workflow Solution
● **for PCB and Dioxin Analysis**
POPs Analysis from Screening to Confirmation

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Presentation Outline

1. The Integrated POPs Analysis Workflow
2. Screening and Confirmation Analysis – Requirements
3. GC-MS/MS (Triple Quad MS) for *Screening Analysis*
4. GC-HRMS (Magnetic Sector MS) for *Confirmation*
5. How GC-MS/MS and GC-HRMS Work Together
6. Conclusions

What are POPs ?

- POPs are Persistent Organic Pollutants
 - are stable halogenated chemical substances of serious health concern
- POPs are produced as a result of human activity
 - intentionally
 - **Organochlorine pesticides (OCPs, e.g. DDT, aldrine, endrine, HCB, ...)**
 - **Polychlorinated biphenyls (PCBs)**
 - **Toxaphenes**
 - **Perfluorinated compounds (PFOS)**
 - **Brominated flame retardants (BFRs, e.g. PBDEs, PBBs, ...)**
 - **Chlorinated paraffins**
 - but also unintentionally
 - **Polychlorinated dibenzo-p-dioxins and furans (PCDD/Fs)**
- POPs are banned/limited in use by the Stockholm convention (UNEP)
 - signed by more than 160 nations
 - > 170 reporting labs ww
- Analytically challenging – Large number of congeners in difficult matrix

“Dioxin Incidents” – Serious Economic Impacts

- **Dioxin and PCB incidents**

..... **are expensive.**

- Egg production enterprise with \$5M hens would **cost in excess of \$30m.**
- Broiler enterprise producing 3M broilers per week would **exceed \$85m.**
- Times Beach, Missouri, road dust covered by oil spread. The **cleanup cost \$110m.**
- The total costs of the Belgian food crisis are estimated **up to \$1000m.**
- EU farms closed, **damage > \$350m**

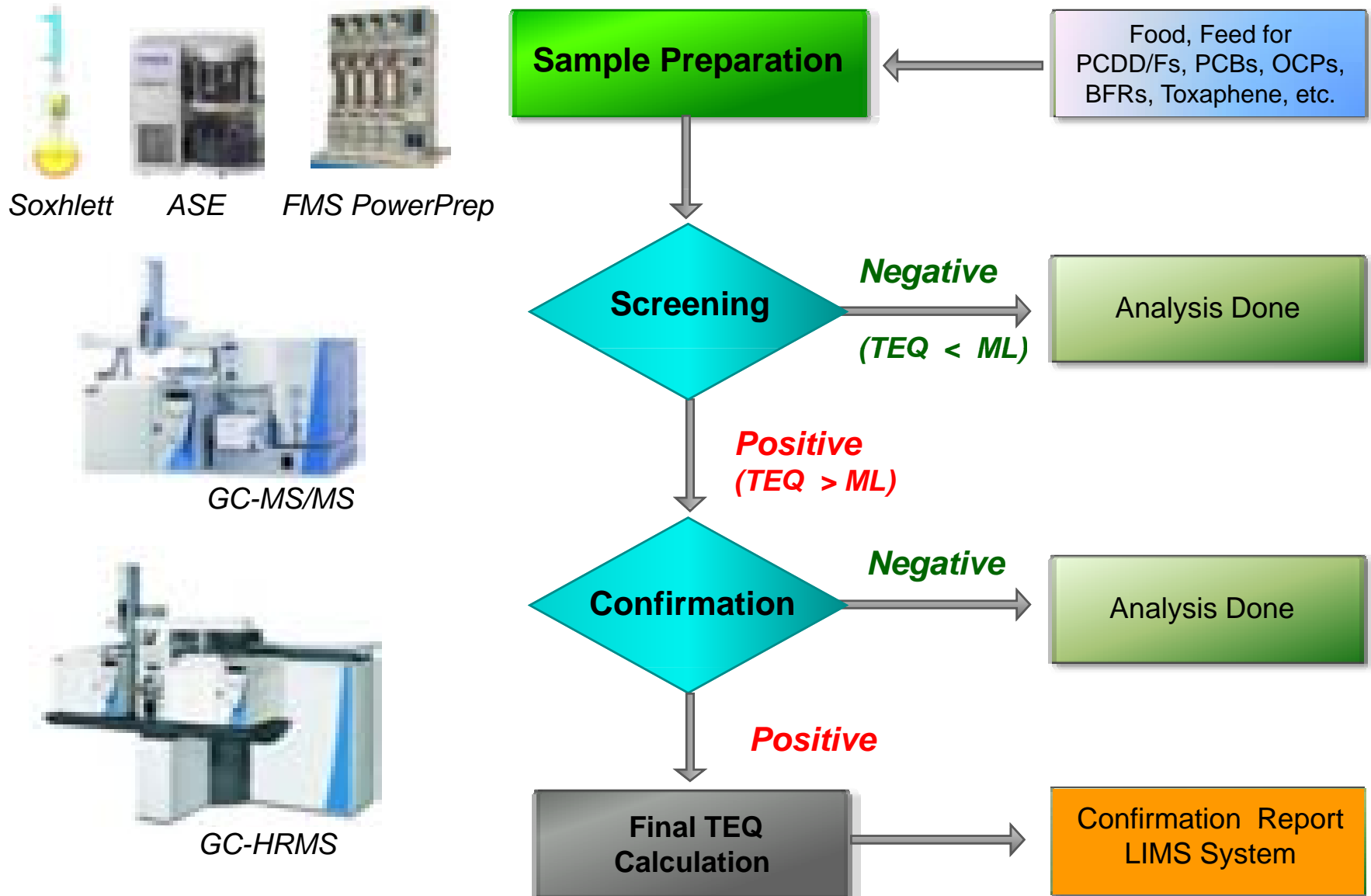
Repeated “Dioxin” cases from only recent years:

- 1998 Milk from citrus pulp pellet feed
- 1999 Belgian PCB/dioxin in eggs, poultry
- 1999 Clay and zeolithes for feed
- 2000 Choline chloride
- 2002 “Carbosan Copper”
- 2004 Potato pulp
- 2005 Hydrochloric acid
- 2007 Indian Guar Gum thickener
- 2008 Irish Pork Meat
- 2008 Italian Mozzarella Cheese ...
- 2011 Contamin’d feed - 4000 farms closed.



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The POPs Analysis Workflow in the Lab



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Why is Screening for POPs done and required?

- **Industrial Analysis - Food Industry**
 - Increasing Food Safety requirements
 - Ensure consistent Quality for raw materials and products
 - High Brand Consciousness, prevent any damages
 - Collaborate with external contract labs for Certification
- **Government Control Labs**
 - Fast in case of “accidents”
 - High number of samples need to be “screened”
- **Requirements for Quality Control and at Incidents**
 - Manage high sample numbers by screening
 - Provide higher efficiency and flexibility, faster results
 - Run indicated samples on HRMS for confirmation analysis



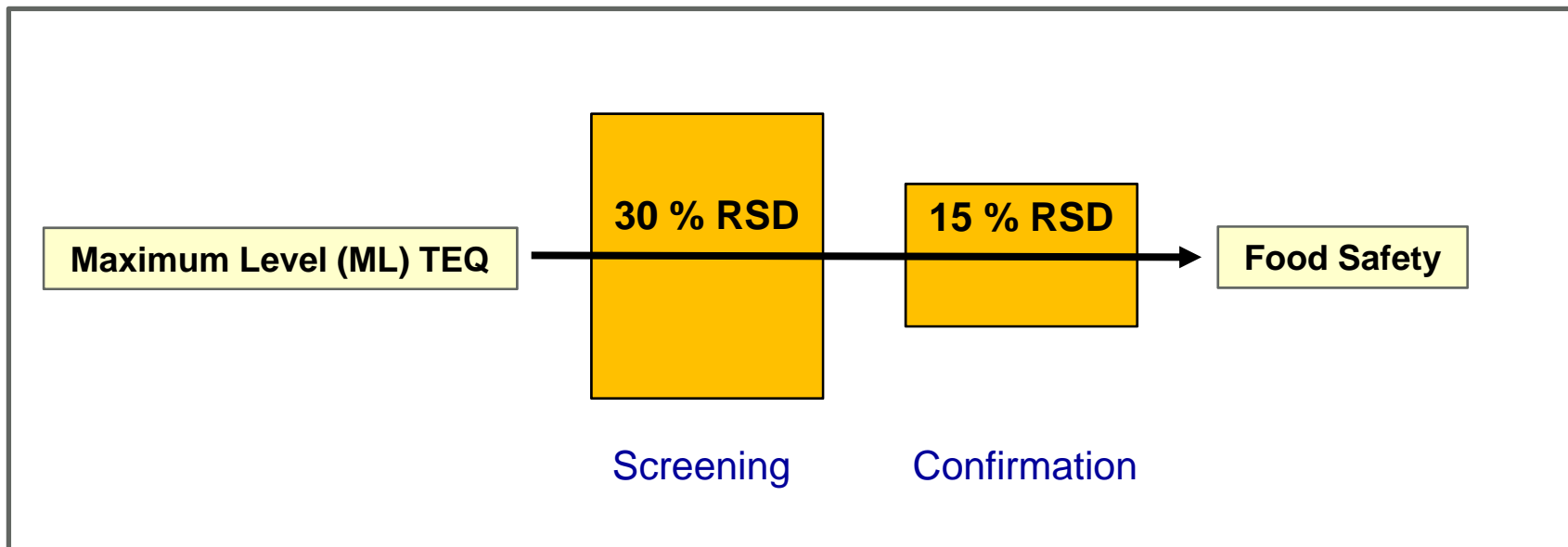
Definitions for Screening and Confirmation Methods

Screening:

- High throughput analysis
- Low false negative rate (< 1%)
- Limited precision (<30 % RSD)
- Sensitivity 25% below of ML


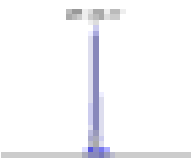
Confirmation:

- HRMS methods required
- High precision (<15 % RSD)
- Higher sensitivity to monitor lower background and action levels.



Impact of analytical performance on screening

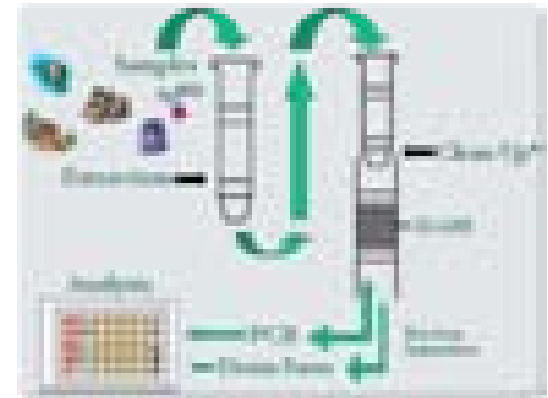
- Compliance with screening regulations is not sufficient
- Resolving power plays a key role in the overall productivity game

		Selectivity	Sensitivity	Sample clean-up	Productivity
Resolving Power	 Low	<ul style="list-style-type: none"> • Poor ability to extract signal from matrix interference 	<ul style="list-style-type: none"> • Low sensitivity • Demand for higher sample weight 	<ul style="list-style-type: none"> • High because of matrix interference impact 	<ul style="list-style-type: none"> • Long sample prep stage limits overall productivity
	 High	<ul style="list-style-type: none"> • Allows clean signal free from matrix intereferents 	<ul style="list-style-type: none"> • High sensitivity • Need for lower sample amount • Better precision 	<ul style="list-style-type: none"> • Minimized due to low impact of matrix 	<ul style="list-style-type: none"> • 20 min sample prep allows very high workload

Why Dioxins Screening with GC-MS/MS?

Bioassays - are in discussion because of

- *Speed and cost*
 - No practical cost advantage – high labor and license fees
 - Similar time of sample prep as for GC-MS/HRMS
- *Analytical Performance*
 - Higher number of false positives and negatives reported in proficiency tests
 - Overestimation > 2x reported in guar gum proficiency test [1]
 - Underestimation > 2x reported for fish oil and pork sausage [1]
- *No TEQs, but REPs*
 - relative potency values - WHO-TEFs > 3x [1]
- *Not congener specific*
 - Results depend on PCDD/F and PCB congener profile [2]



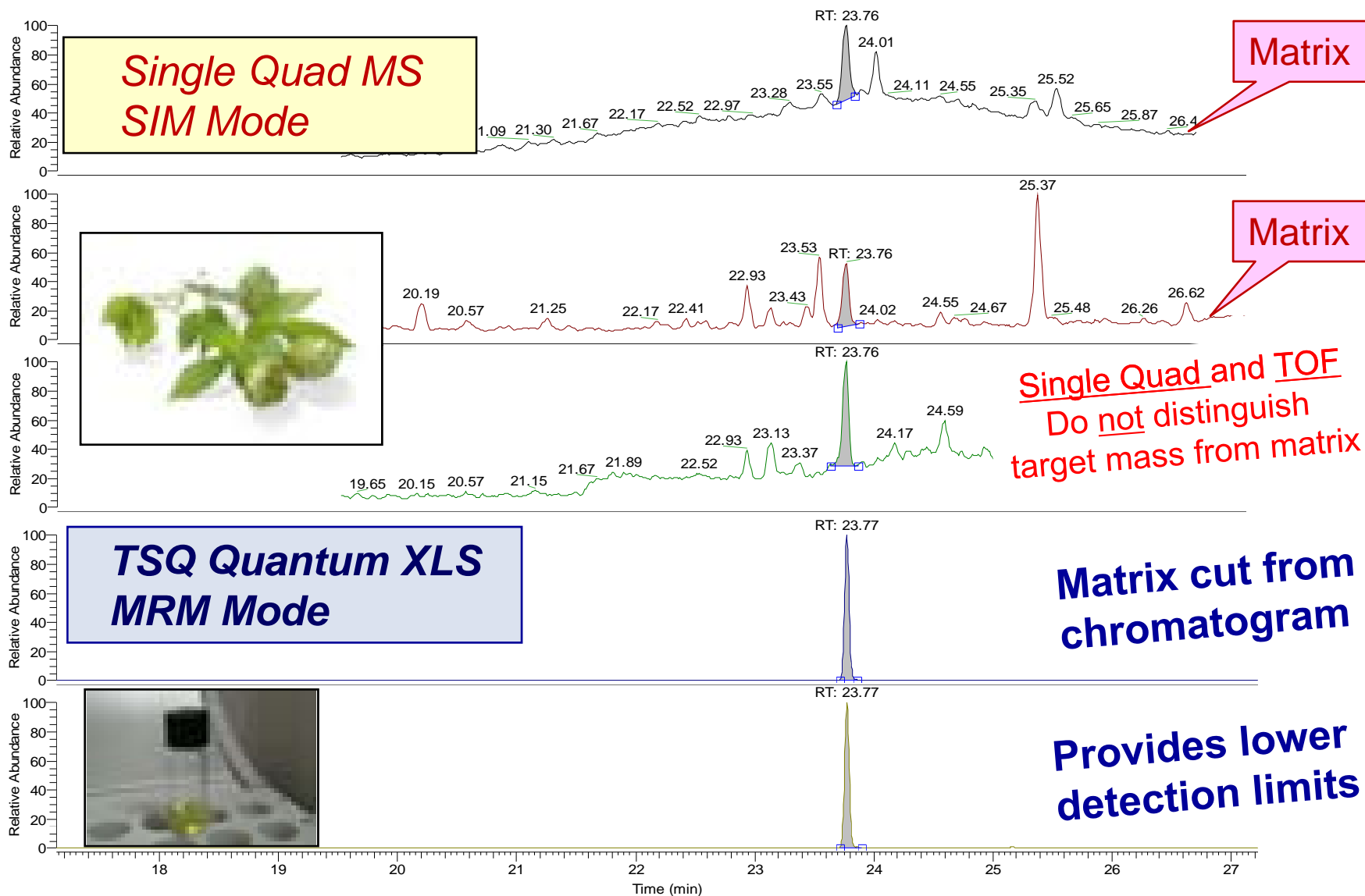
www.dioxins.com

=> Requirement to calibrate bioassays for each matrix by GC-HRMS !

- References:
 - [1] Kotz, Malisch 2010, Dioxin Conference
 - [2] Fochi, Brambilla 2008

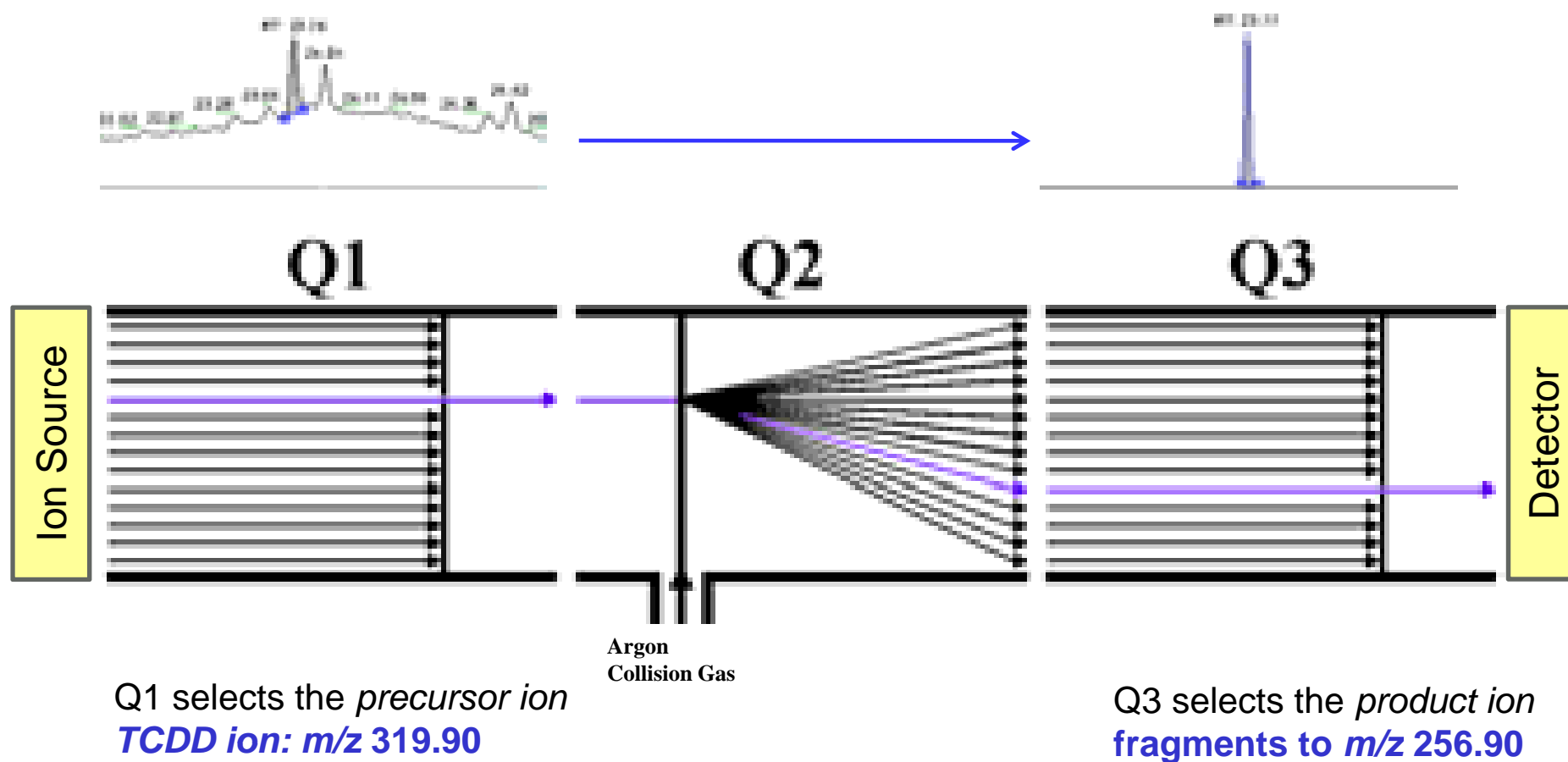
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GC-MS/MS Selectivity in Matrix Extracts



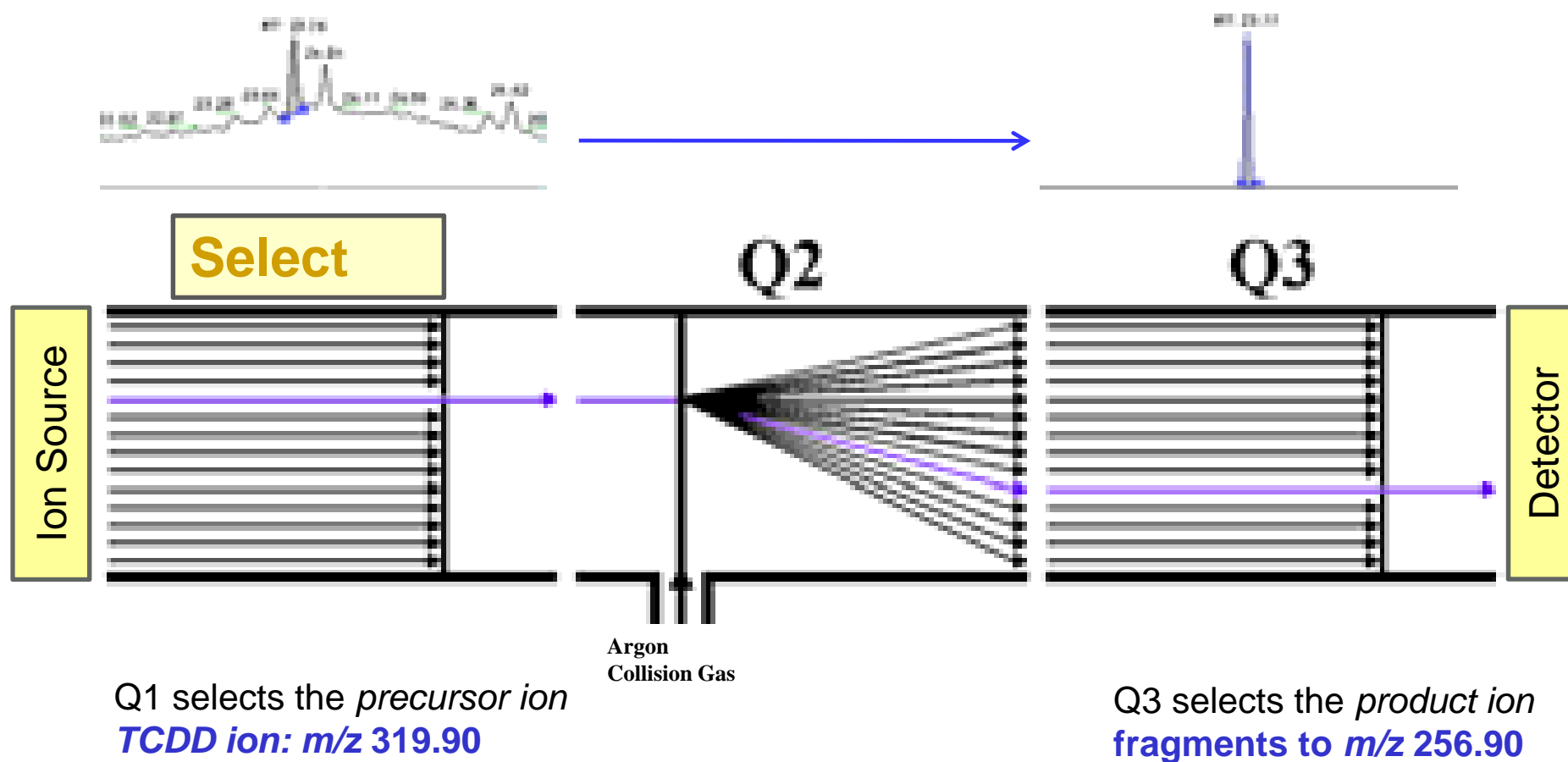
Triple Quadrupole MS – Principle of Operation

Quantitation of target compounds in matrix samples



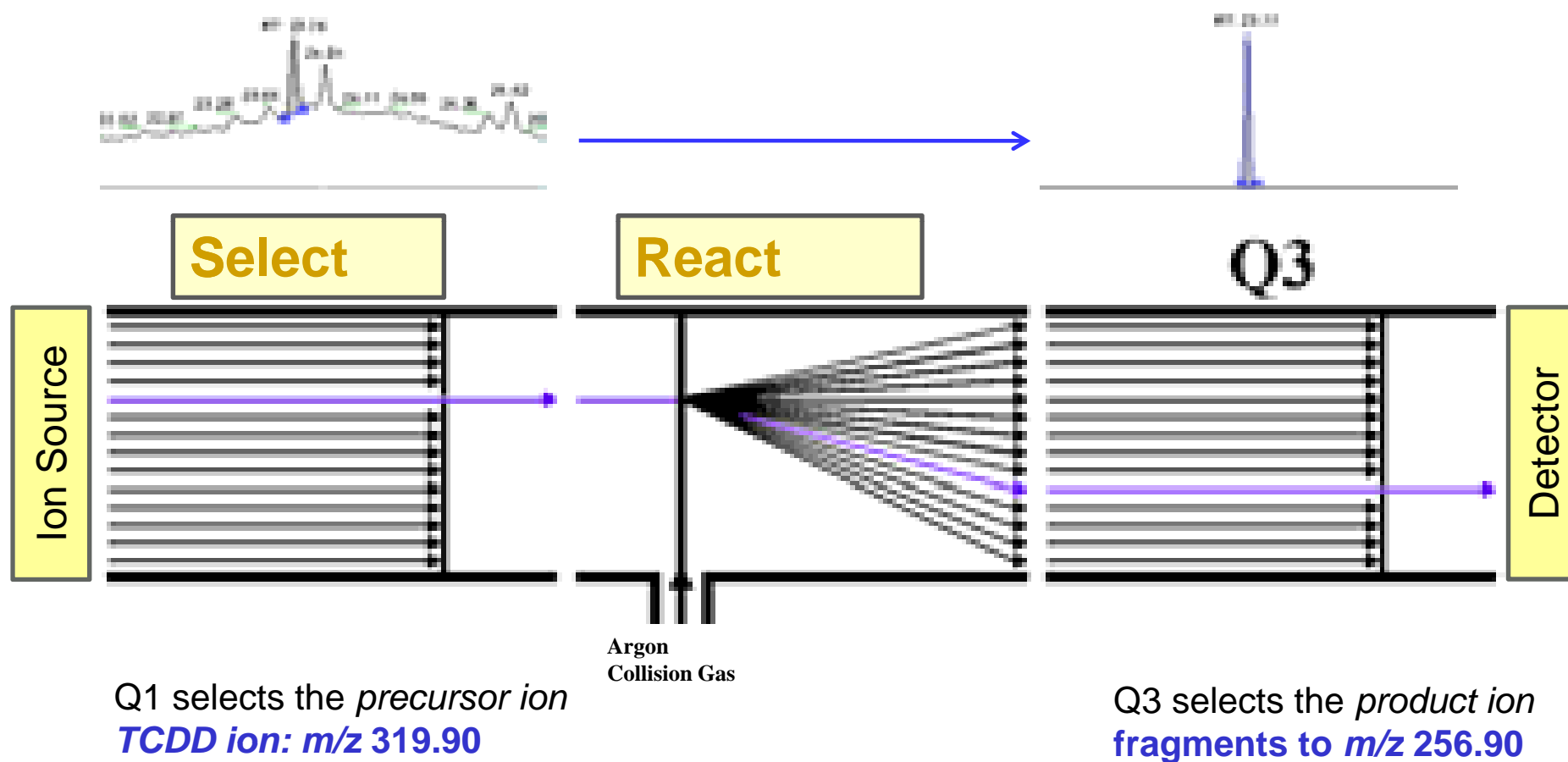
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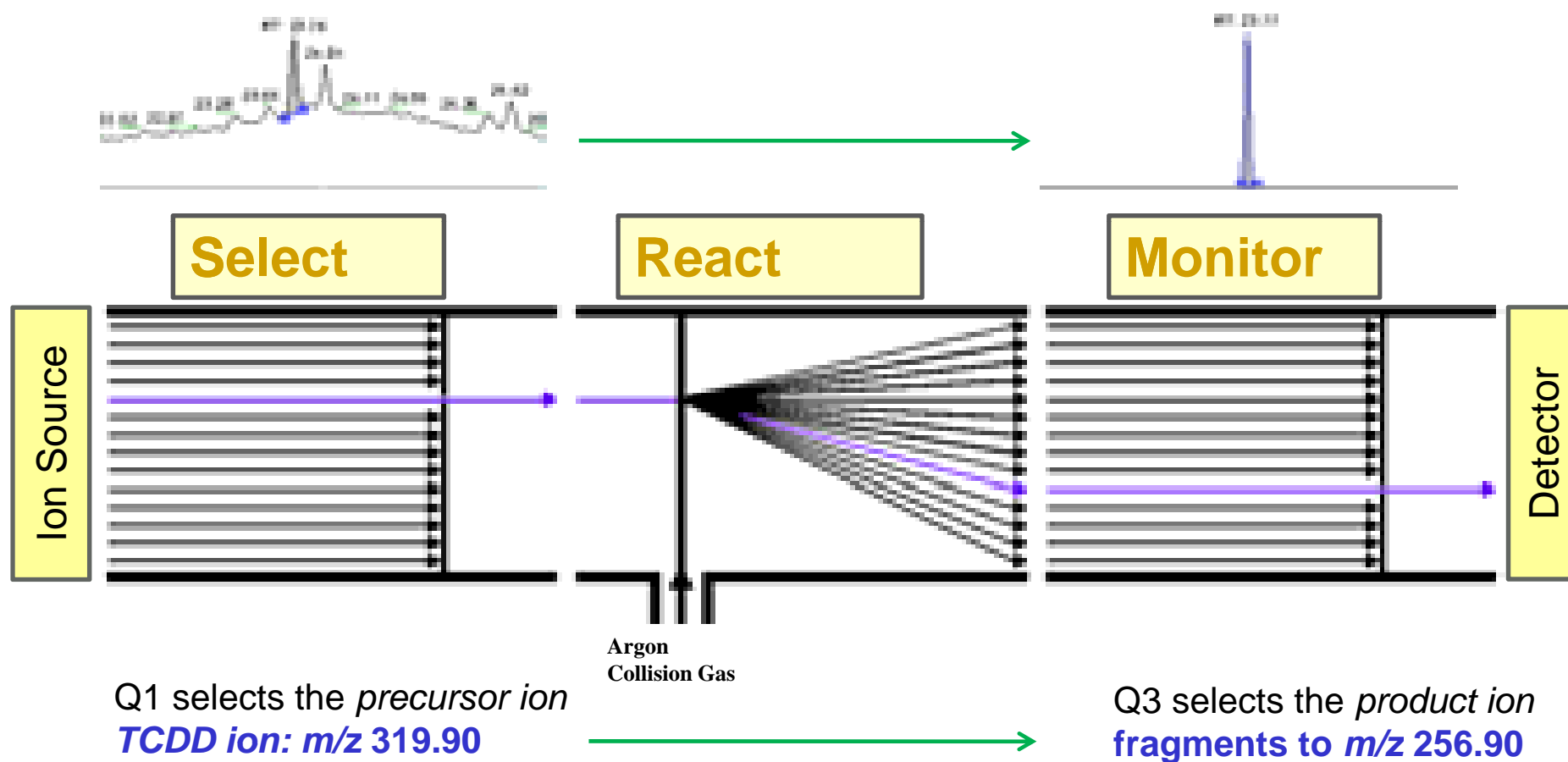
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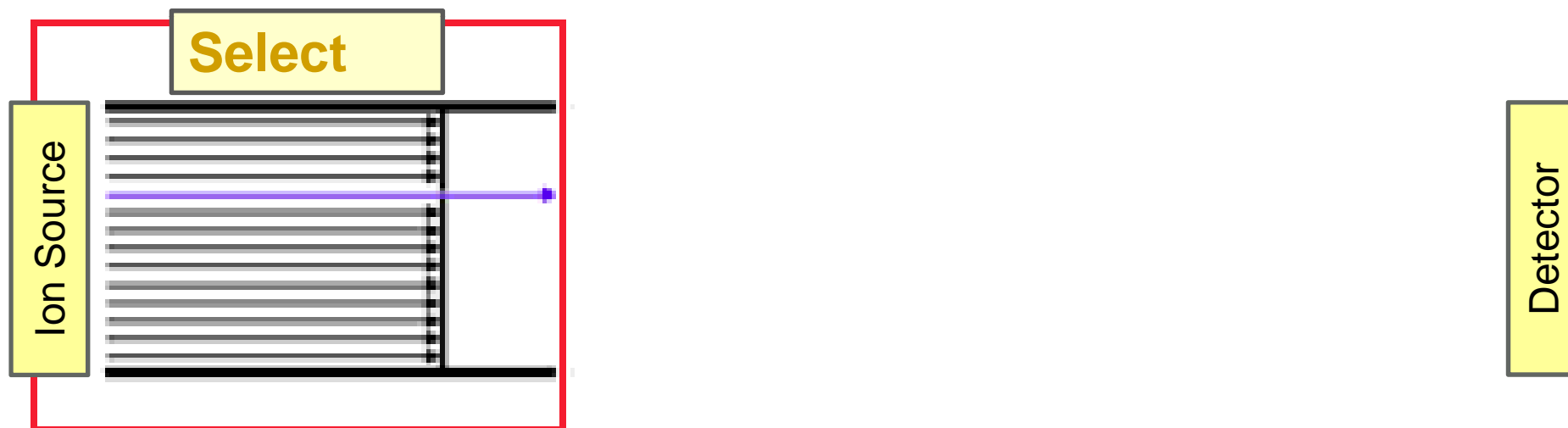
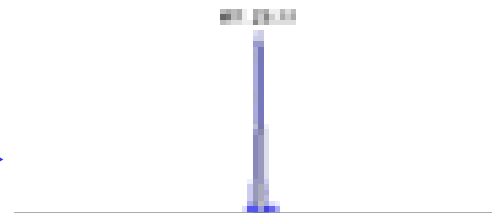
Triple Quadrupole MS – Principle of Operation

Quantitation of target compounds in matrix samples



TSQ Quantum XLS - Selected Reaction Monitoring

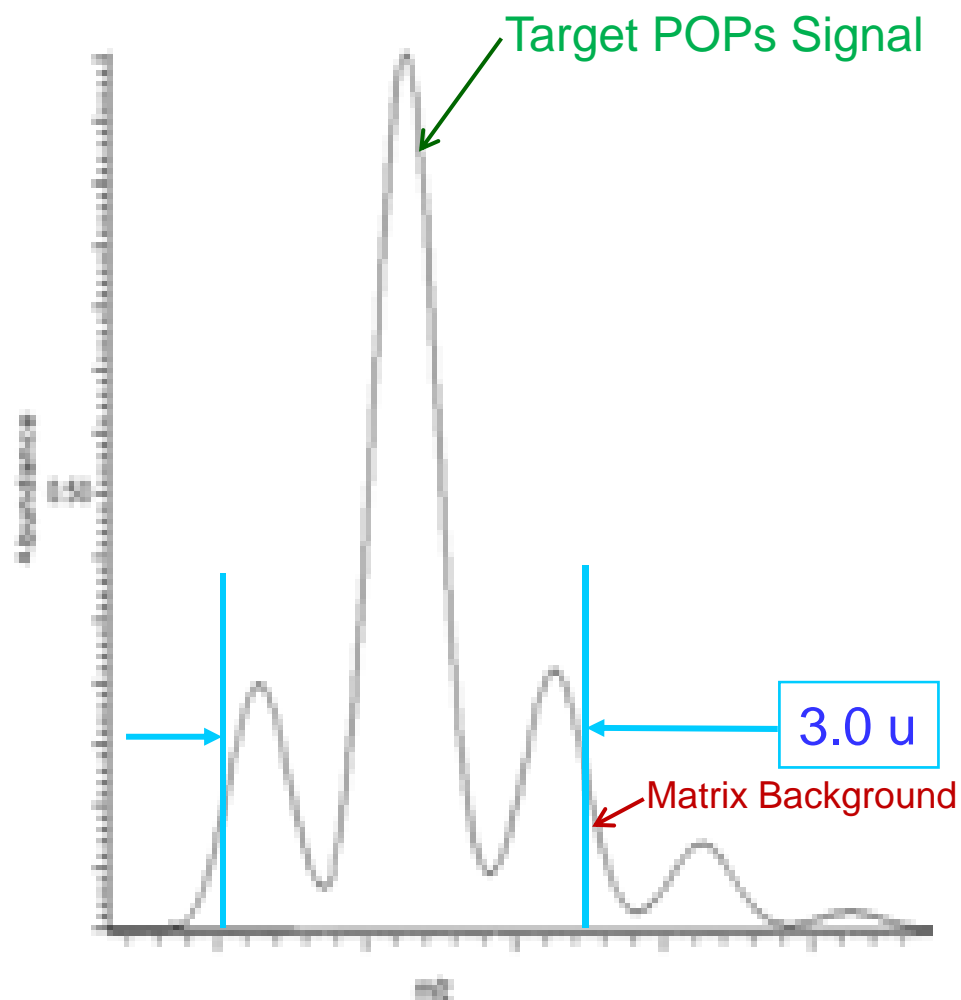
Quantitation of target compounds in matrix samples



Q1 selects the *precursor ion*
TCDD ion: m/z 320

Q3 selects the *product ion*
fragments to m/z 257

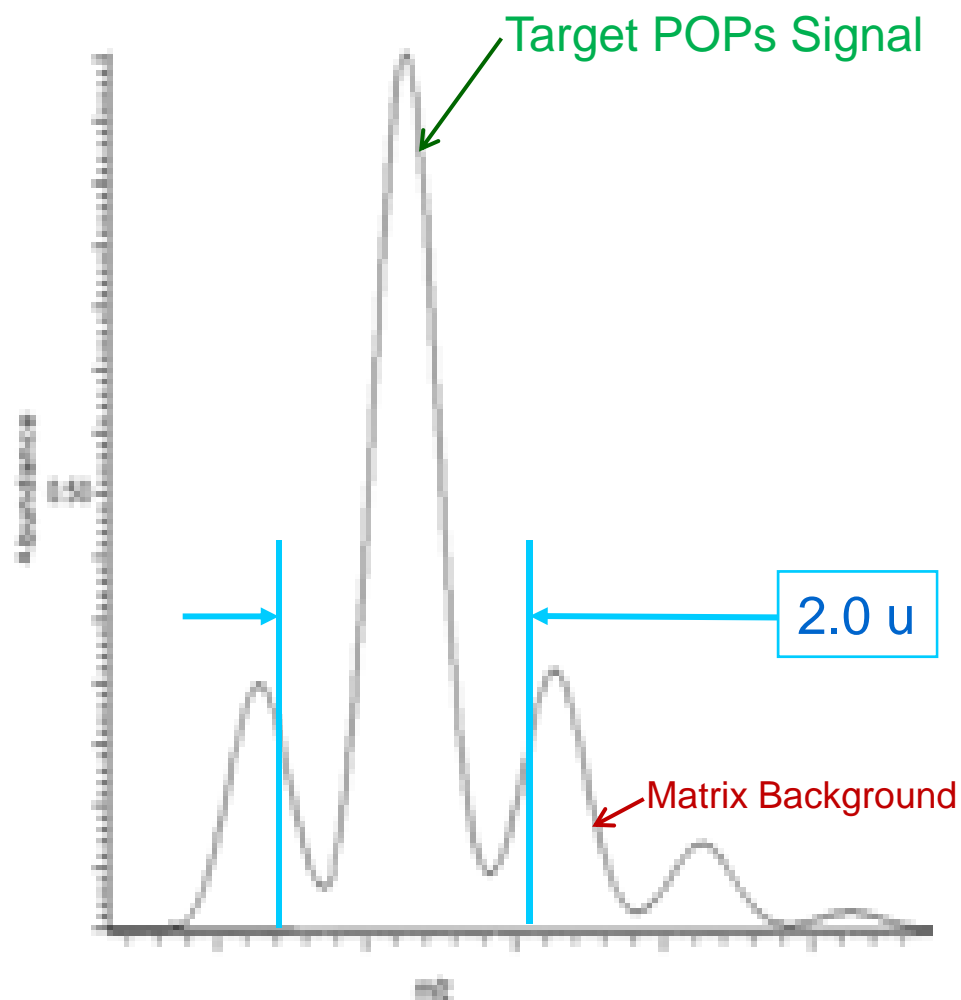
More Selectivity for More Sensitivity



Mass Resolution in Q1 determines selectivity

Other technologies use a wide Q1 mass window
More matrix gets into the collision cell,
and sacrifices selectivity.

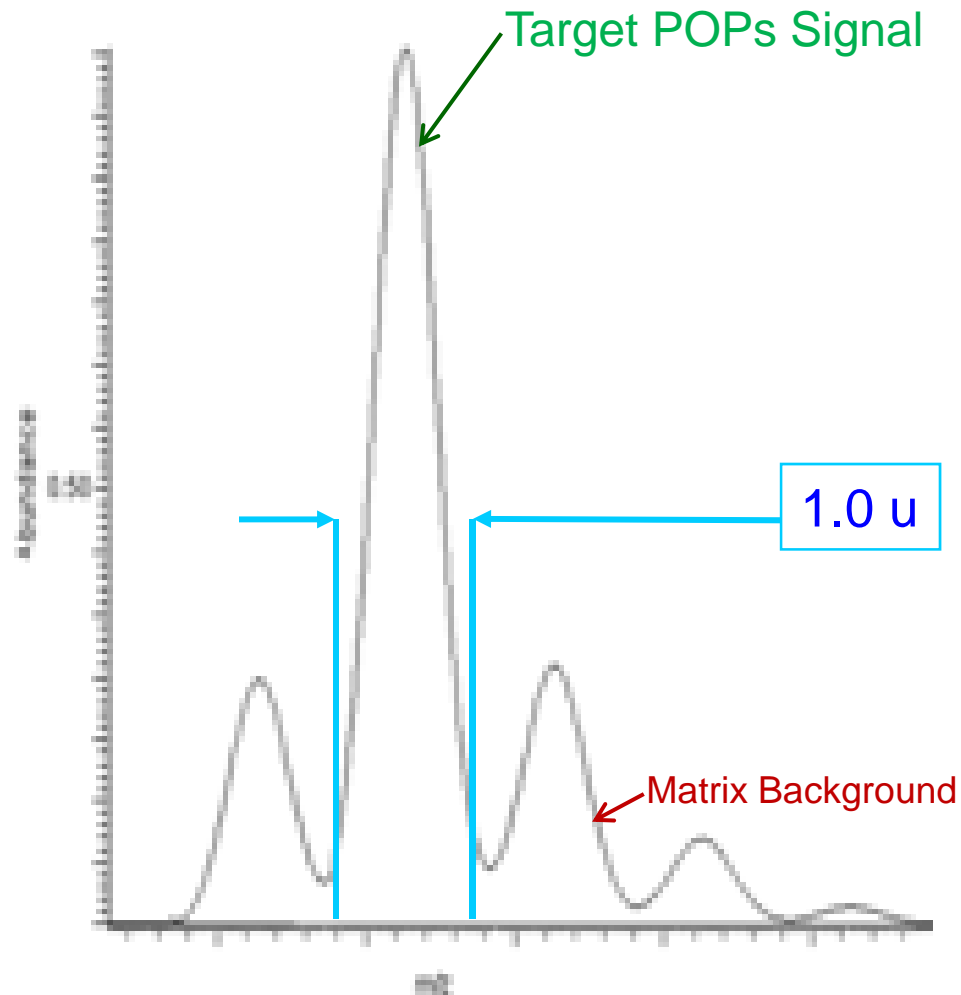
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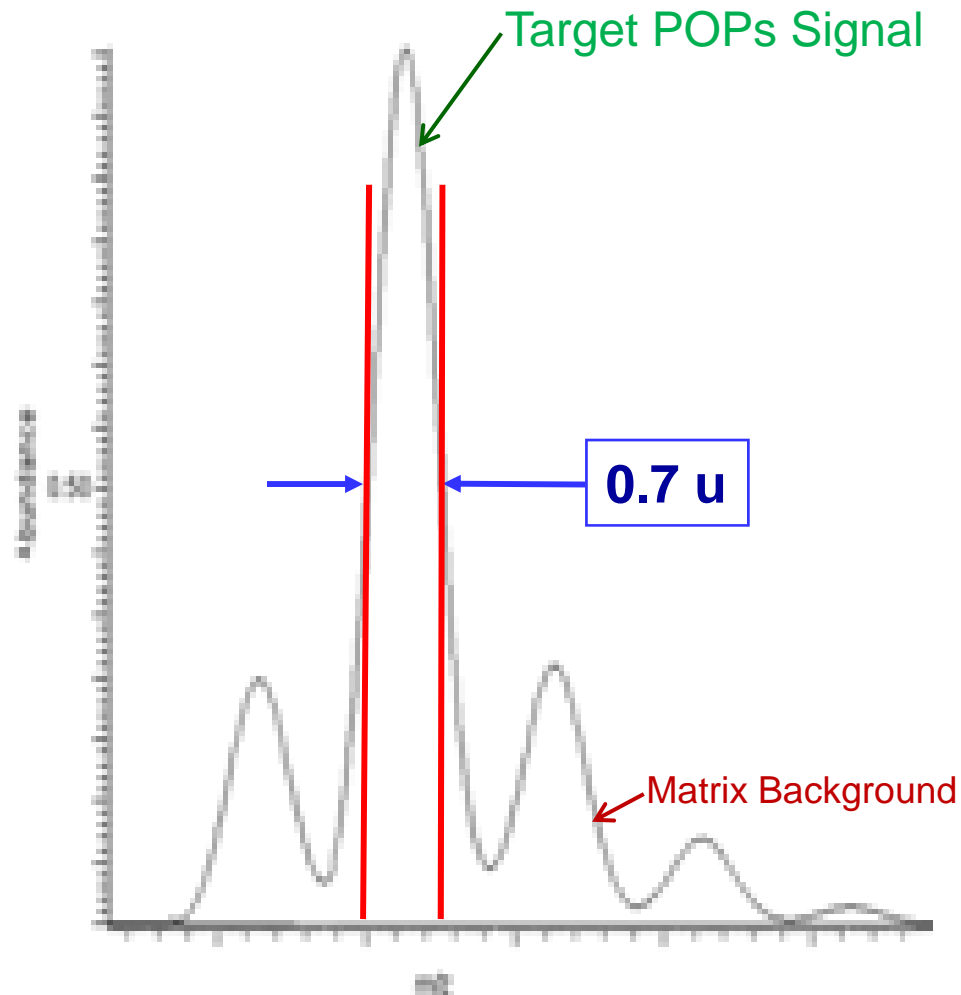
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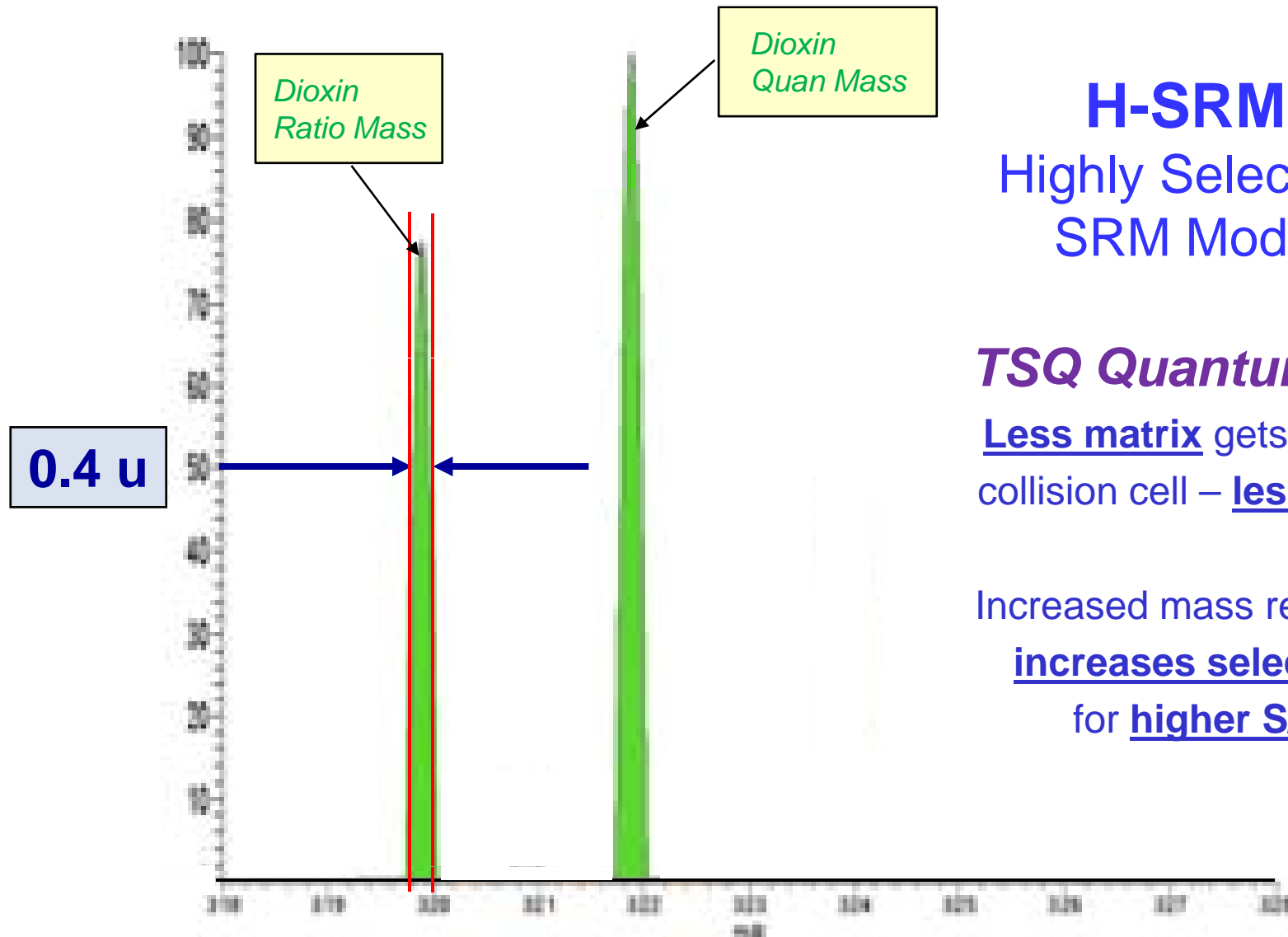
Mass Resolution in Q1 determines selectivity

TSQ Quantum XLS:

Higher Mass Resolution selects the target signal efficiently from the matrix

Higher Selectivity for matrix samples

Higher Mass Resolution for More Selectivity

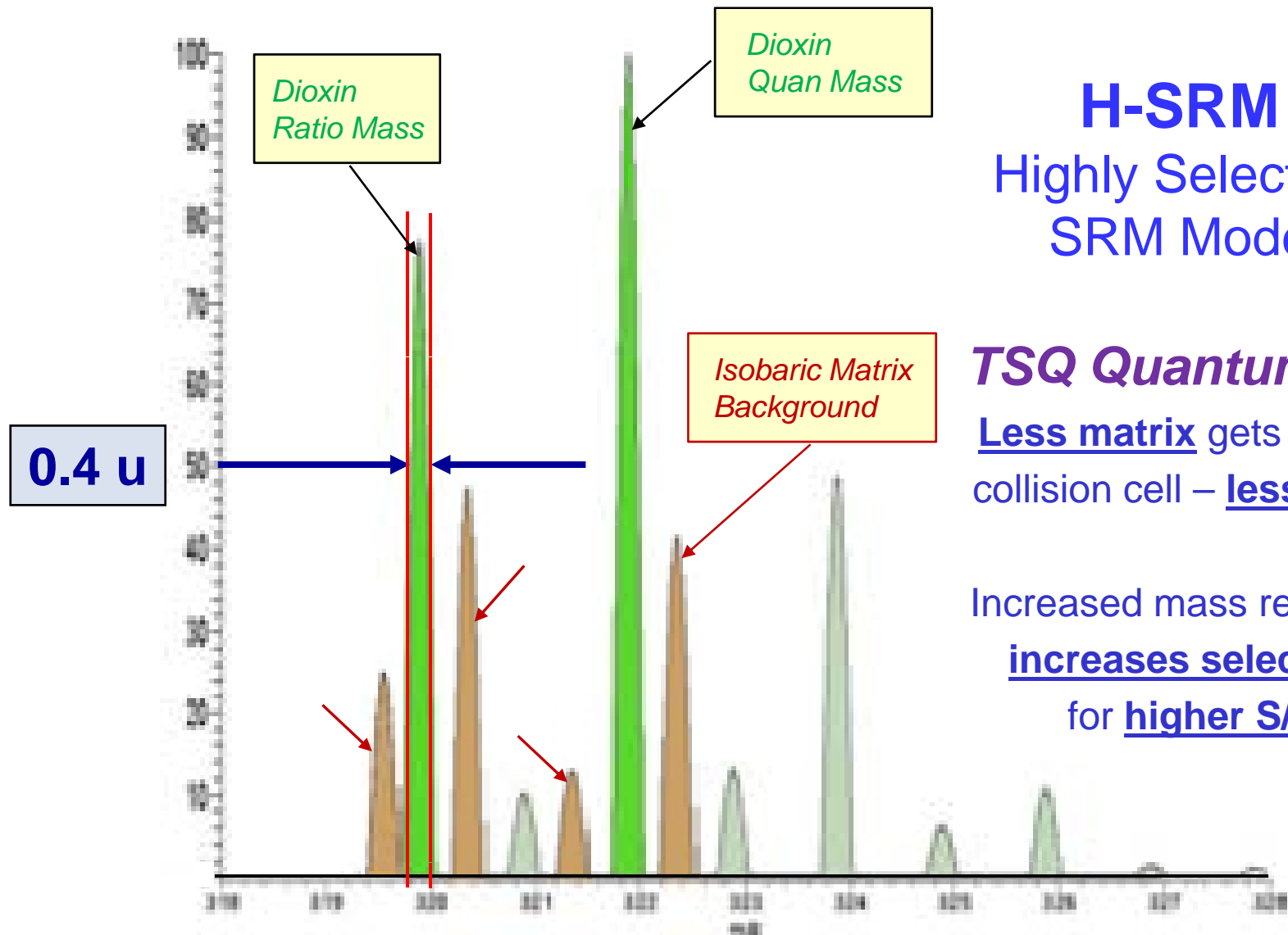


H-SRM
Highly Selective
SRM Mode

TSQ Quantum XLS
Less matrix gets into the
collision cell – less noise.

Increased mass resolution
increases selectivity
for higher S/N.

Higher Mass Resolution for More Selectivity



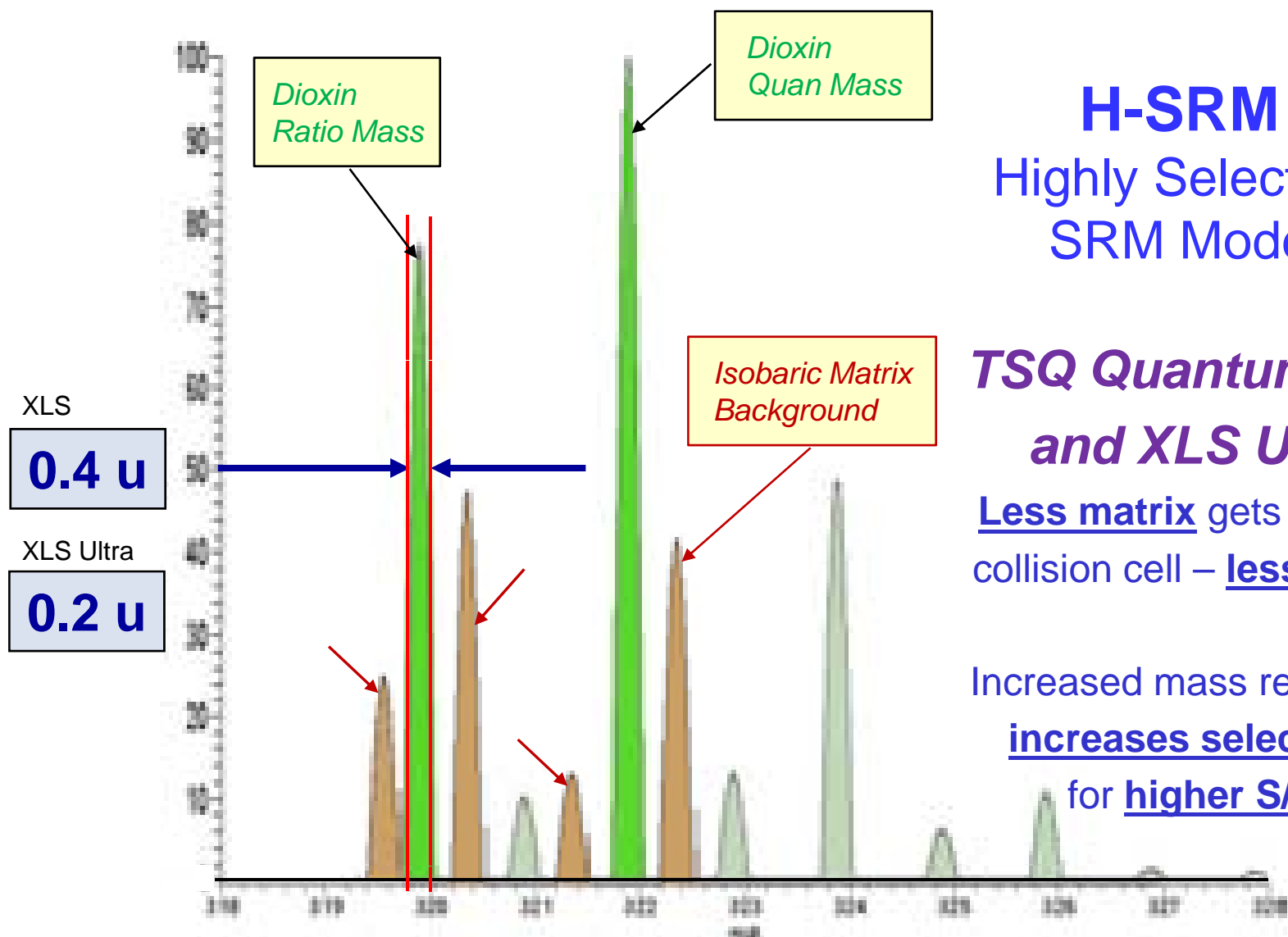
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Higher Mass Resolution for More Selectivity



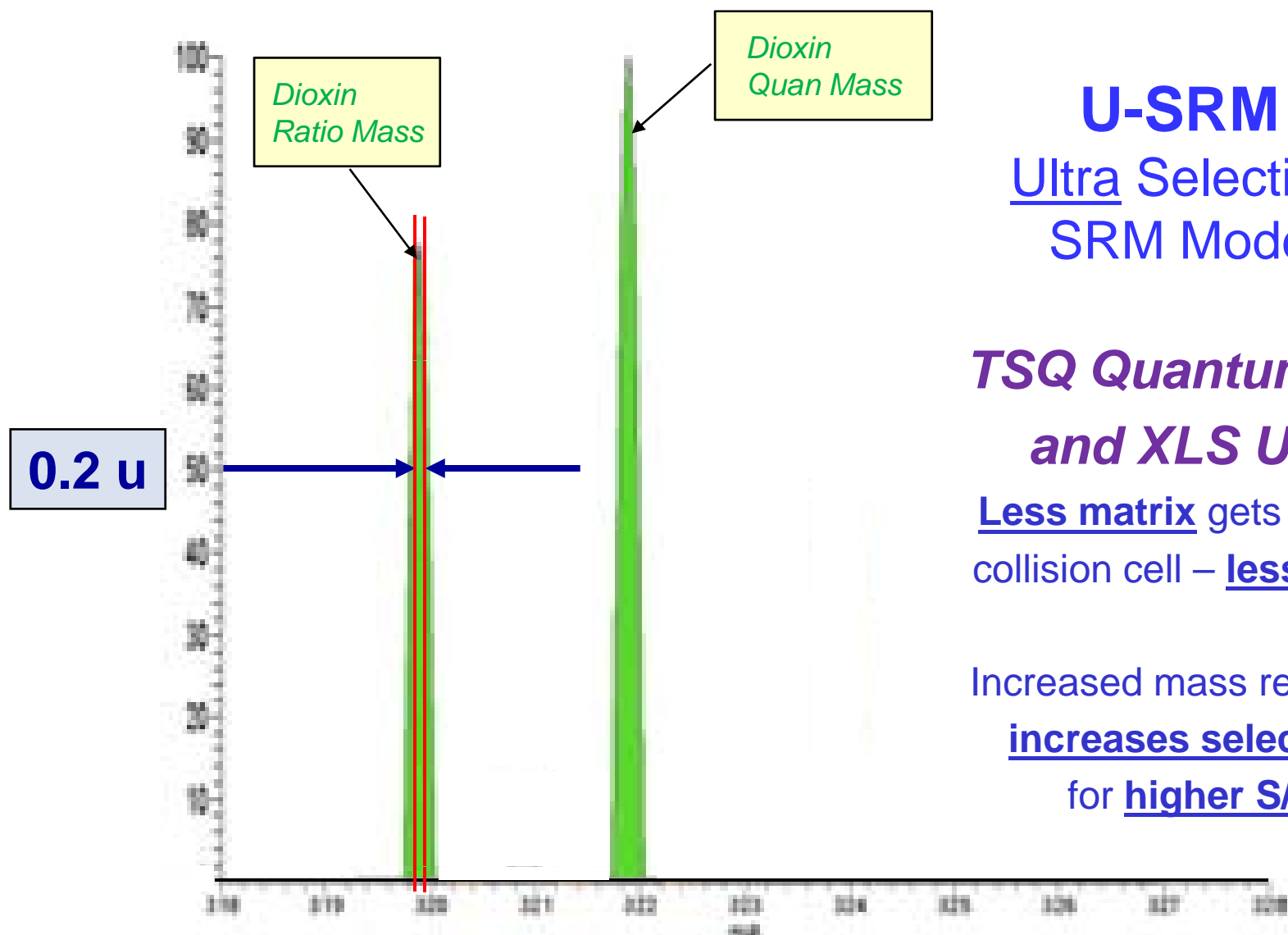
H-SRM
Highly Selective
SRM Mode

**TSQ Quantum XLS
and XLS Ultra**

Less matrix gets into the
collision cell – less noise.

Increased mass resolution
increases selectivity
for higher S/N.

Higher Mass Resolution for More Selectivity



U-SRM
Ultra Selective
SRM Mode

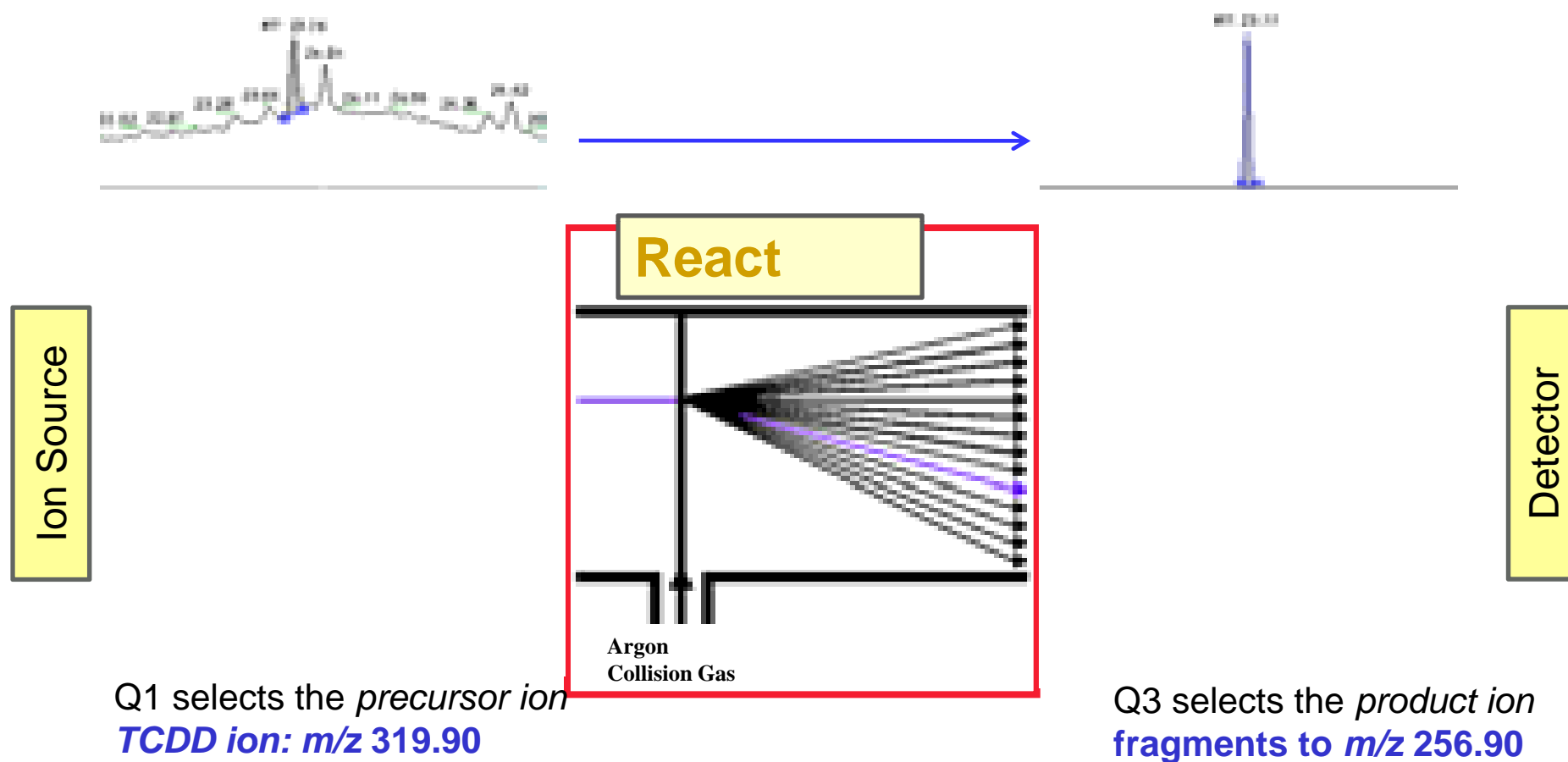
TSQ Quantum XLS
and XLS Ultra

Less matrix gets into the
collision cell – less noise.

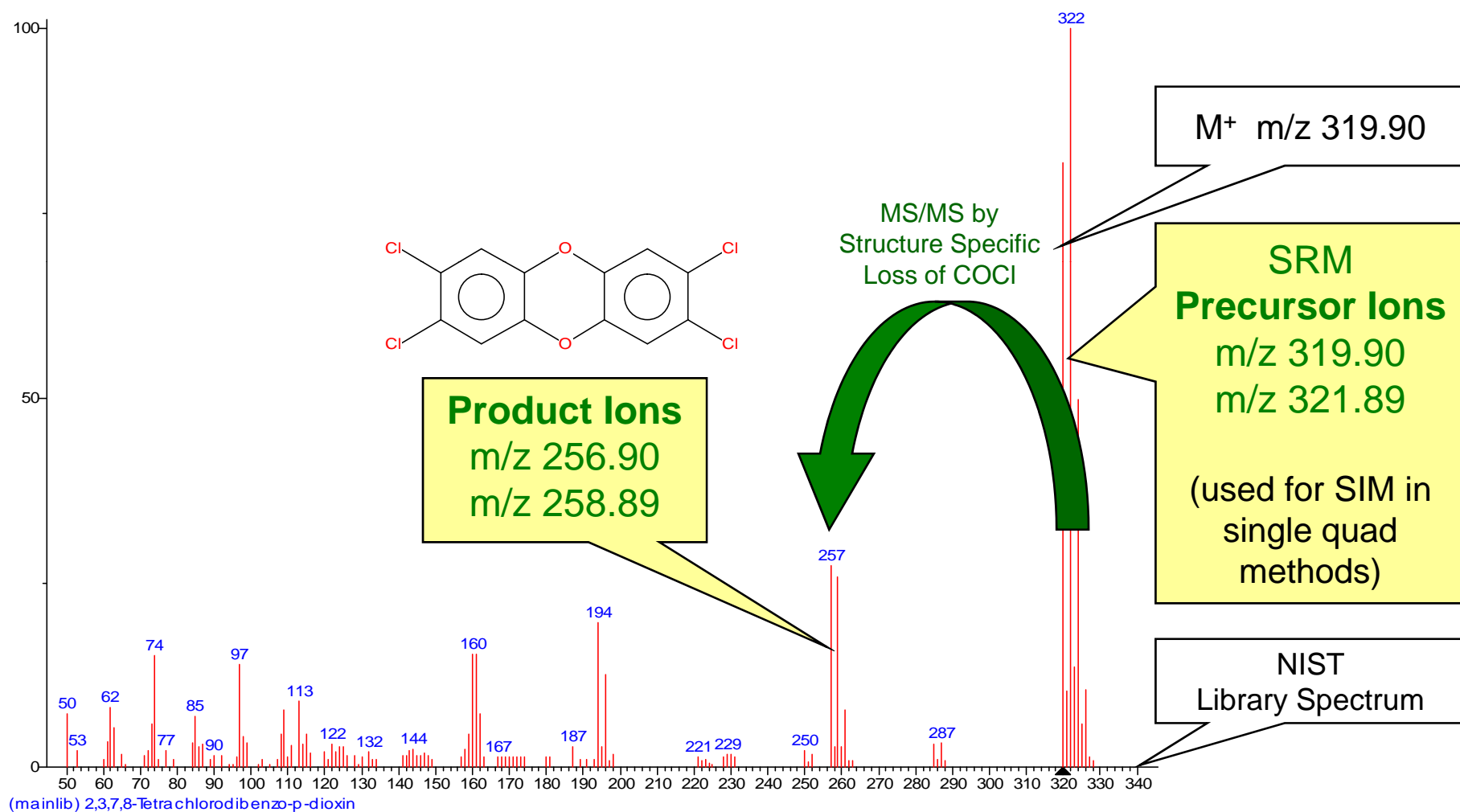
Increased mass resolution
increases selectivity
for higher S/N.

TSQ Quantum XLS - Selected Reaction Monitoring

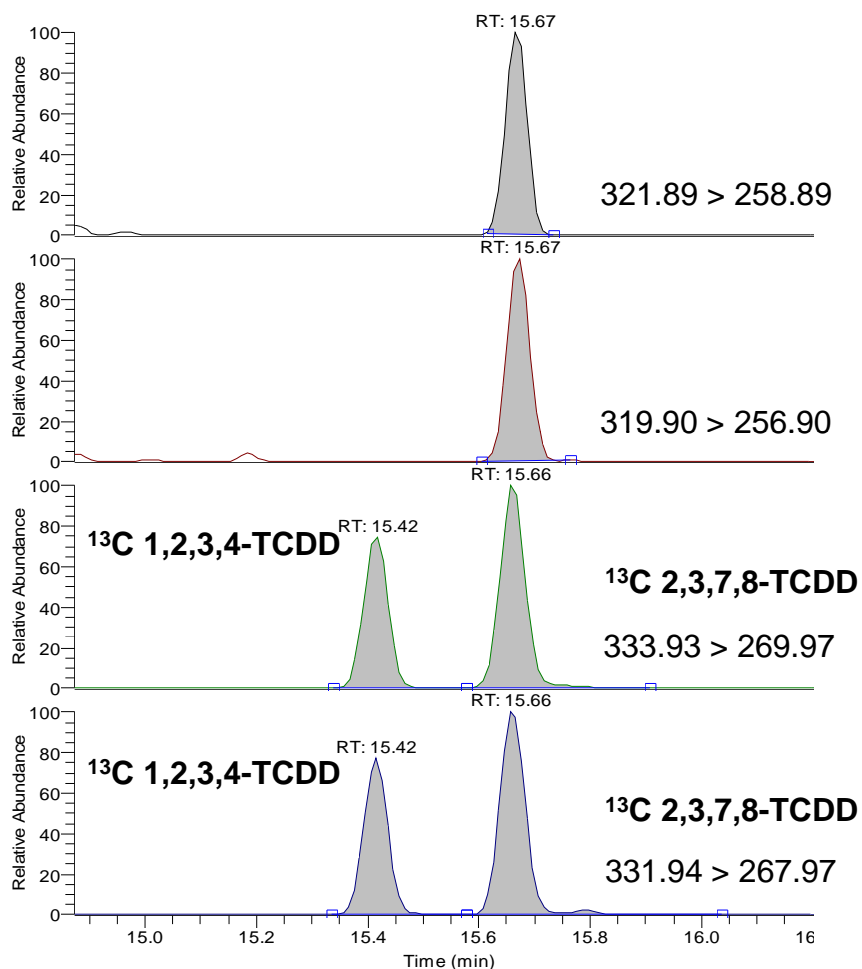
Quantitation of target compounds in matrix samples



Structure Specific Selectivity – TCDD, nominal M 320



TSQ Quantum XLS Ultra - Dioxin Screening



Native (in sample)
2,3,7,8 TCDD

Quantitation Trace

Confirmation Trace

Labelled Internal
Standards
(Recovery + Quantitation)

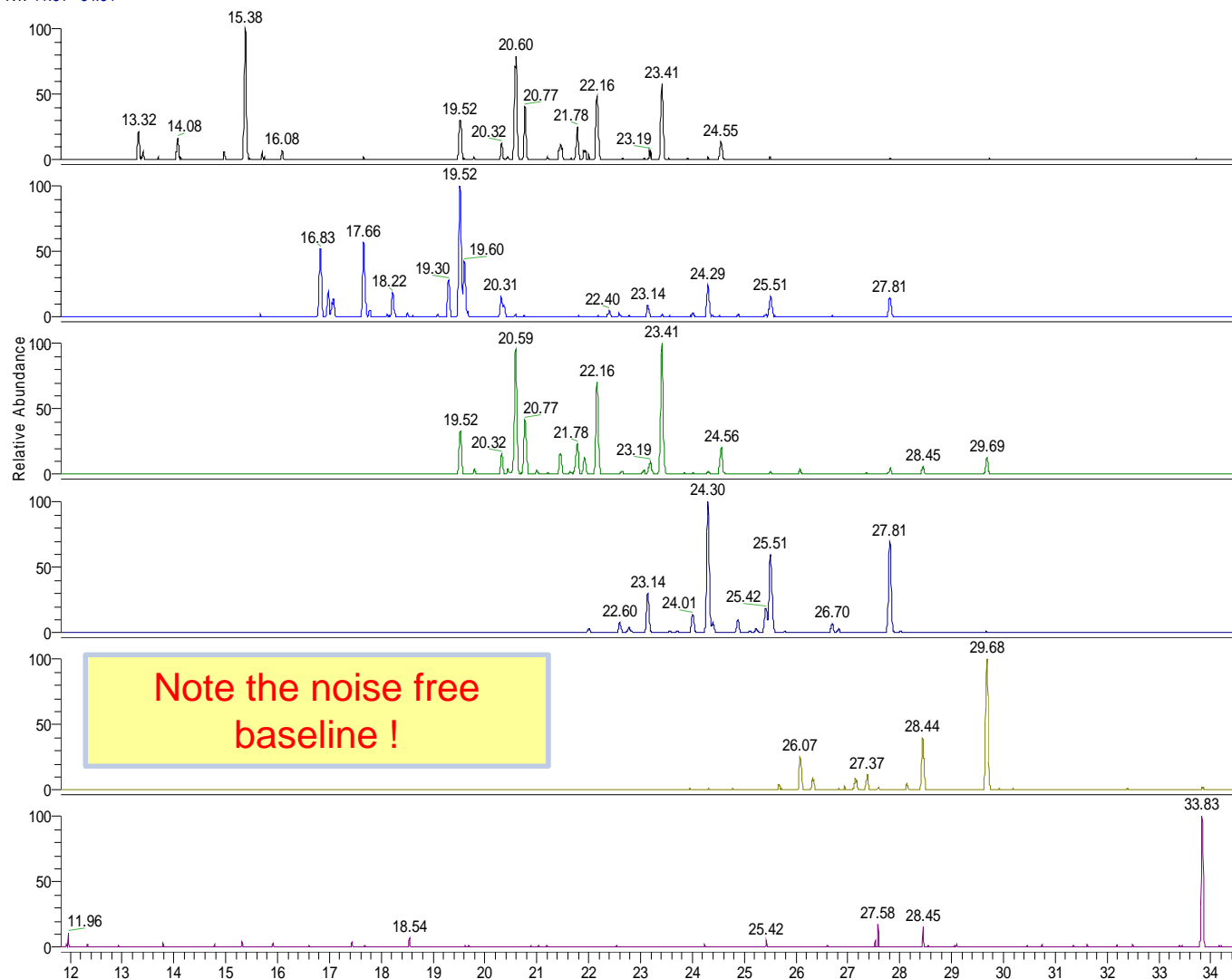
Quantitation Trace

Confirmation Trace

Animal fat sample with reduced sample clean-up
PCDD/F-TEQ value is 0.95 pg/g fat. 125 fg of 2,3,7,8-
TCDD injected.

TSQ Quantum XLS – Fish Extract with all PCBs

RT: 11.81 - 34.84



Tri-CBs:

m/z 255.95 \Rightarrow m/z 186.00

Tetra-CBs:

m/z 291.90 \Rightarrow m/z 222.00

Penta-CBs:

m/z 325.90 \Rightarrow m/z 255.90

Hexa-CBs:

m/z 359.85 \Rightarrow m/z 289.90

Hepta-CBs:

m/z 393.81 \Rightarrow m/z 358.80

Octa-CB:

m/z 463.75 \Rightarrow m/z 428.70

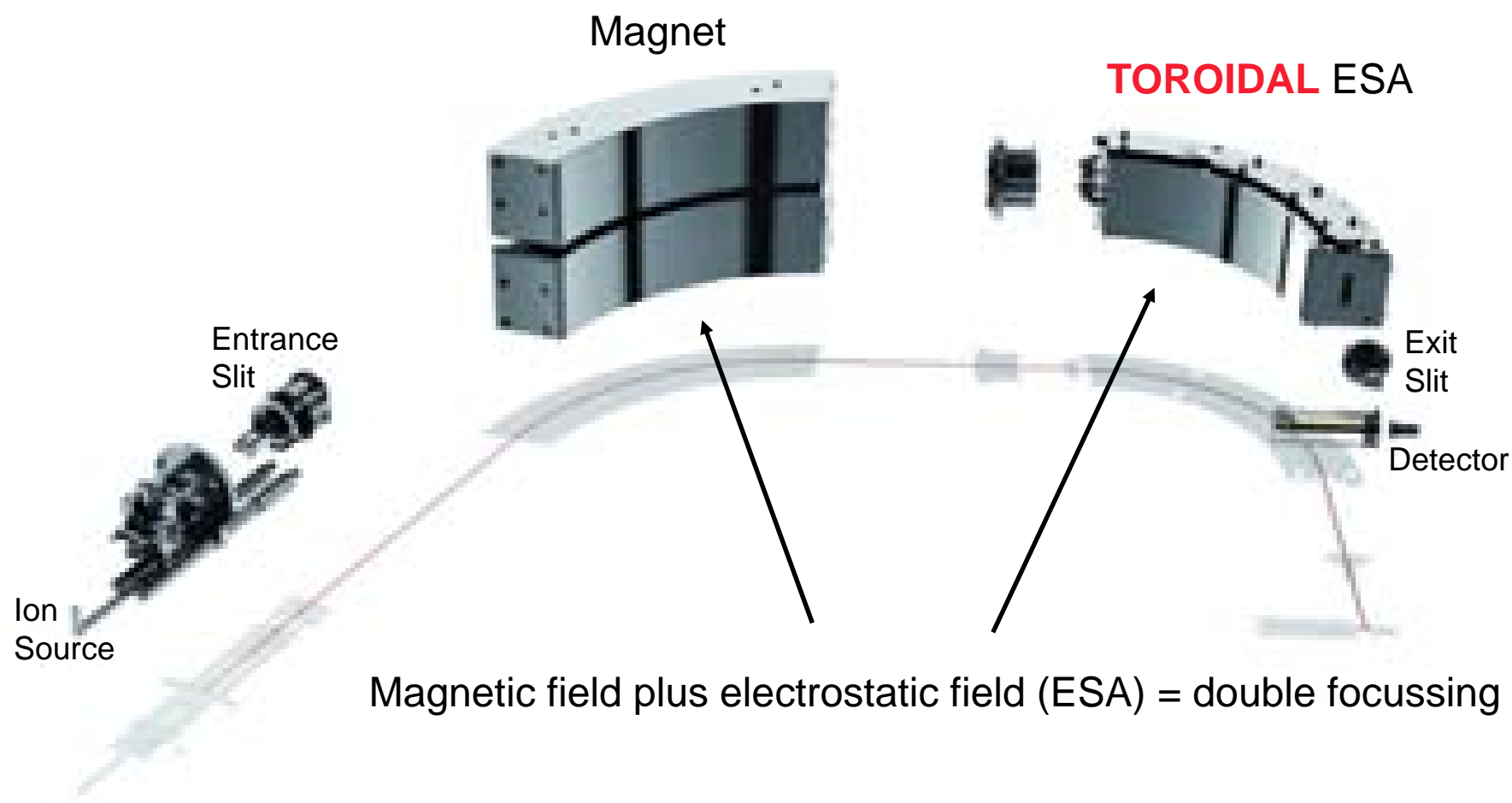
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Magnetic Sector HRMS: Thermo Scientific DFS

- **Benchtop-like** operation (autotune)
- Most **compact** instrument in its class
- **One unit**, moves on wheels
- **Toroidal ESA** for highest **sensitivity**
- **Low power** consumption
- Unique **Dual GC** configuration
- **Fit to purpose** data evaluation software

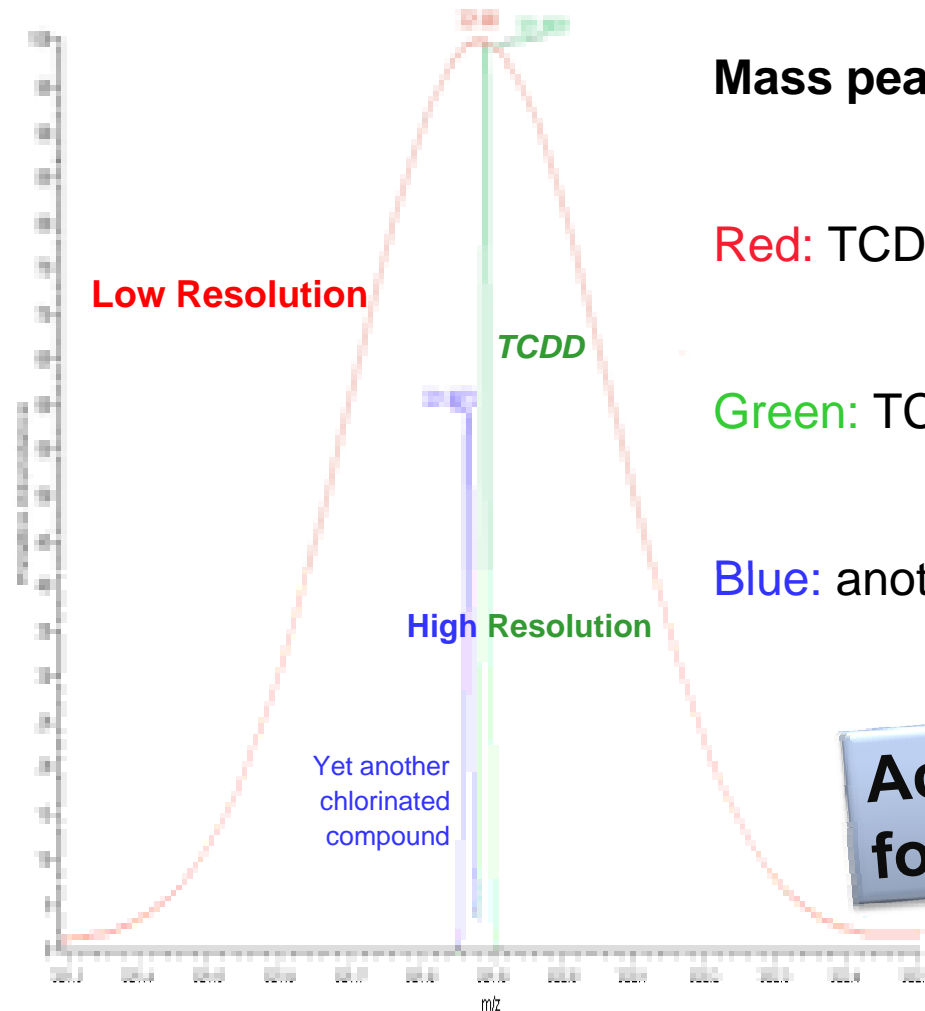


Magnetic Sector HRMS: Ion Optics of the DFS



Confirmation Analysis with „High Resolution“

CONFIRMATION ANALYSIS WITH „HIGH RESOLUTION“



Mass peaks at Different Resolution Settings:

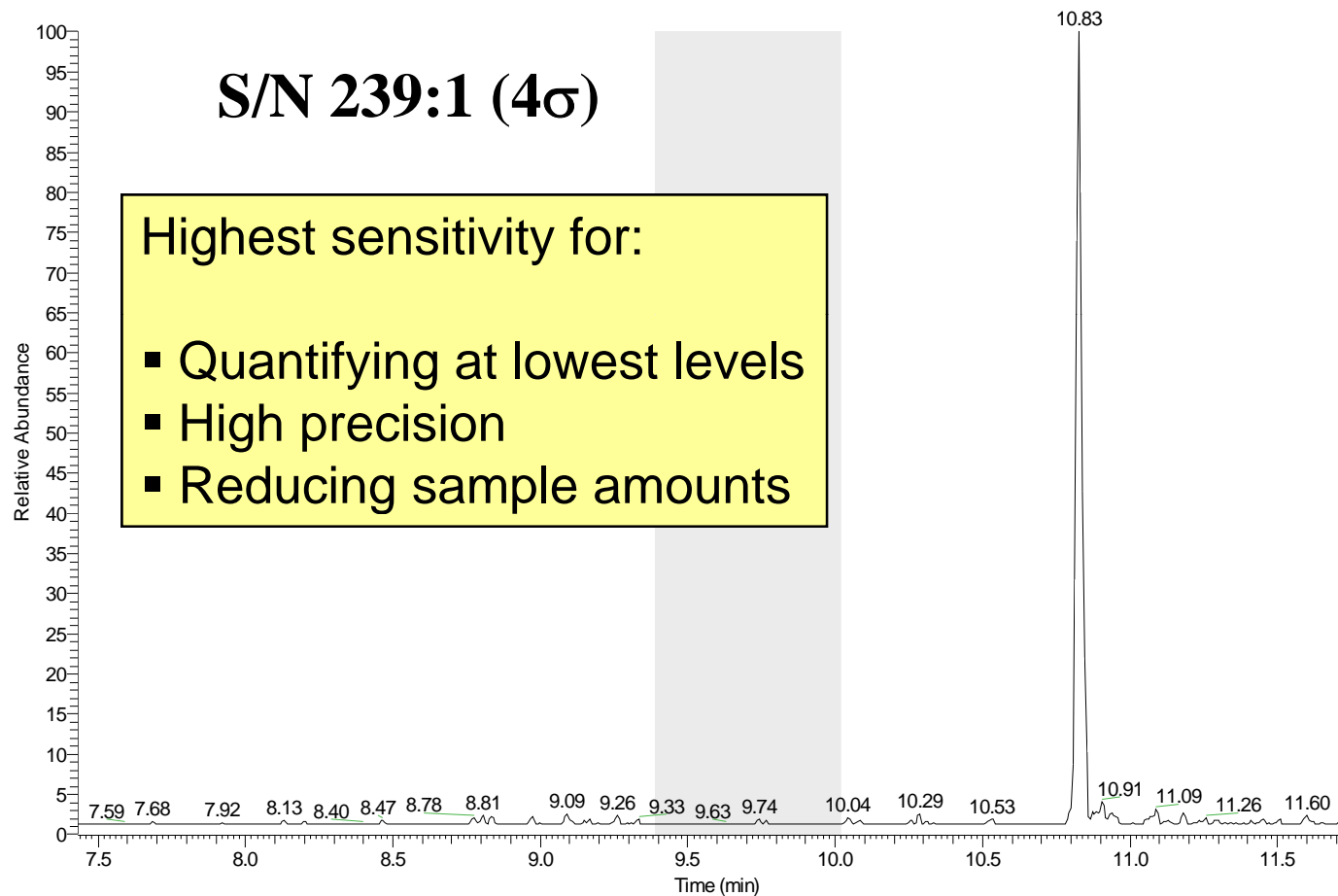
Red: TCDD mass trace at R 1.000

Green: TCDD mass trace at R 10.000

Blue: another chlorinated compound mass trace

Accurate masses are used for target compound analysis

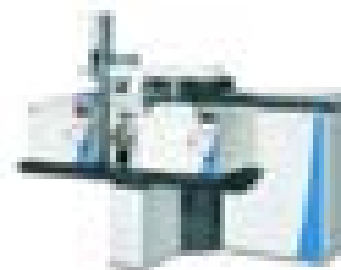
DFS Sensitivity: TCDD Specification **20 fg SN \geq 200:1(4 σ)**



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GC-MS/MS



GC-HRMS

Screening + Confirmation using MS/MS + HRMS

- **Dioxin Conference Presentation:**

- Triple-Quadrupole GC-MS/MS Technique for PCDD/F and DL-PCB Determination in Milk

- **Purpose:**

- Develop a method to analyze PCDDs, PCDFs and DL-PCBs by a triple quadrupole GC-MS/MS system for a fast screening of dioxin- positive milk samples



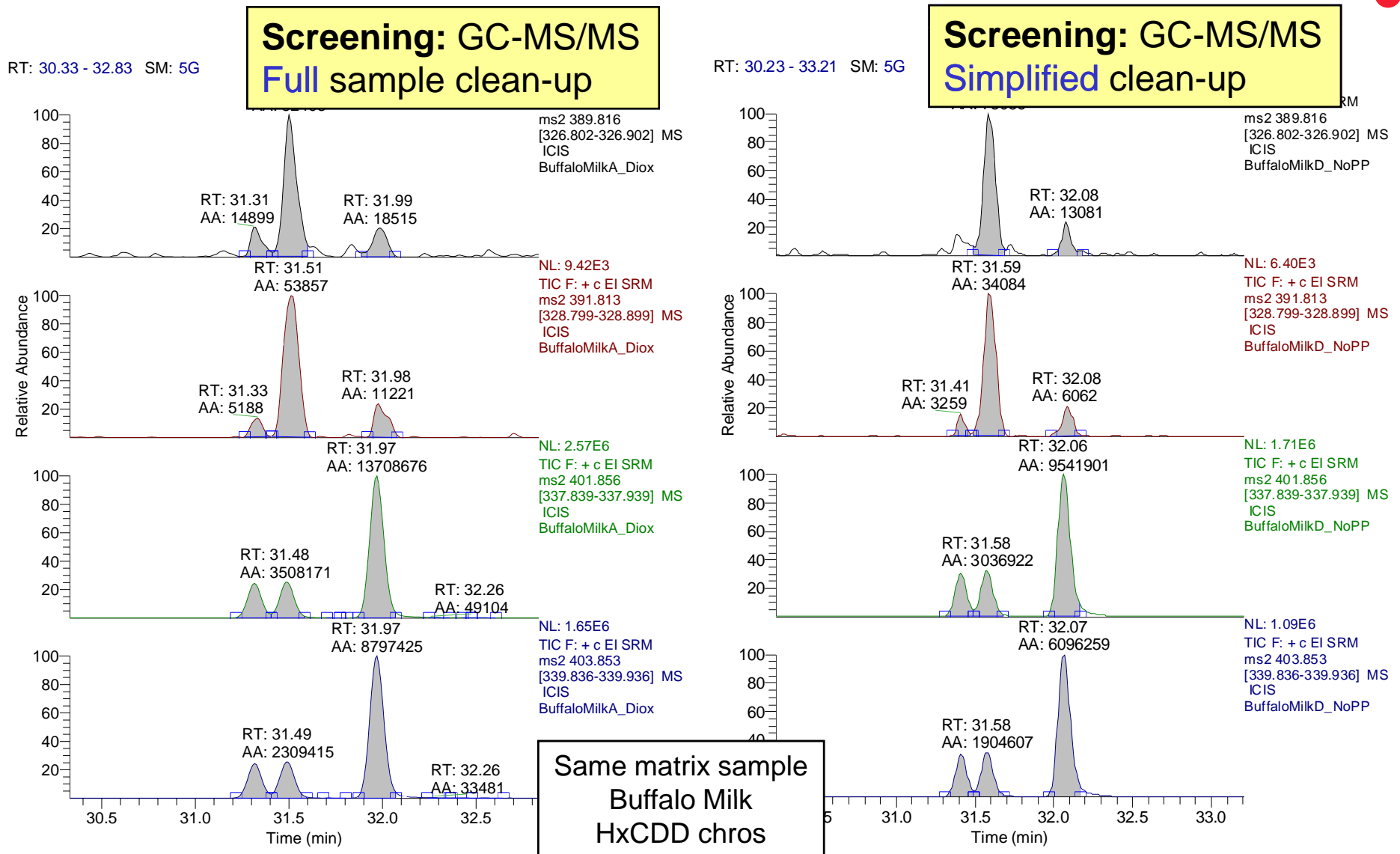
- **Collaboration with:**

- ISS Rome, the National Health Institute, Italy,
 - Nicola Iacovella, Anna Maria Ingelido, Elena De Felip, Gianfranco Brambilla

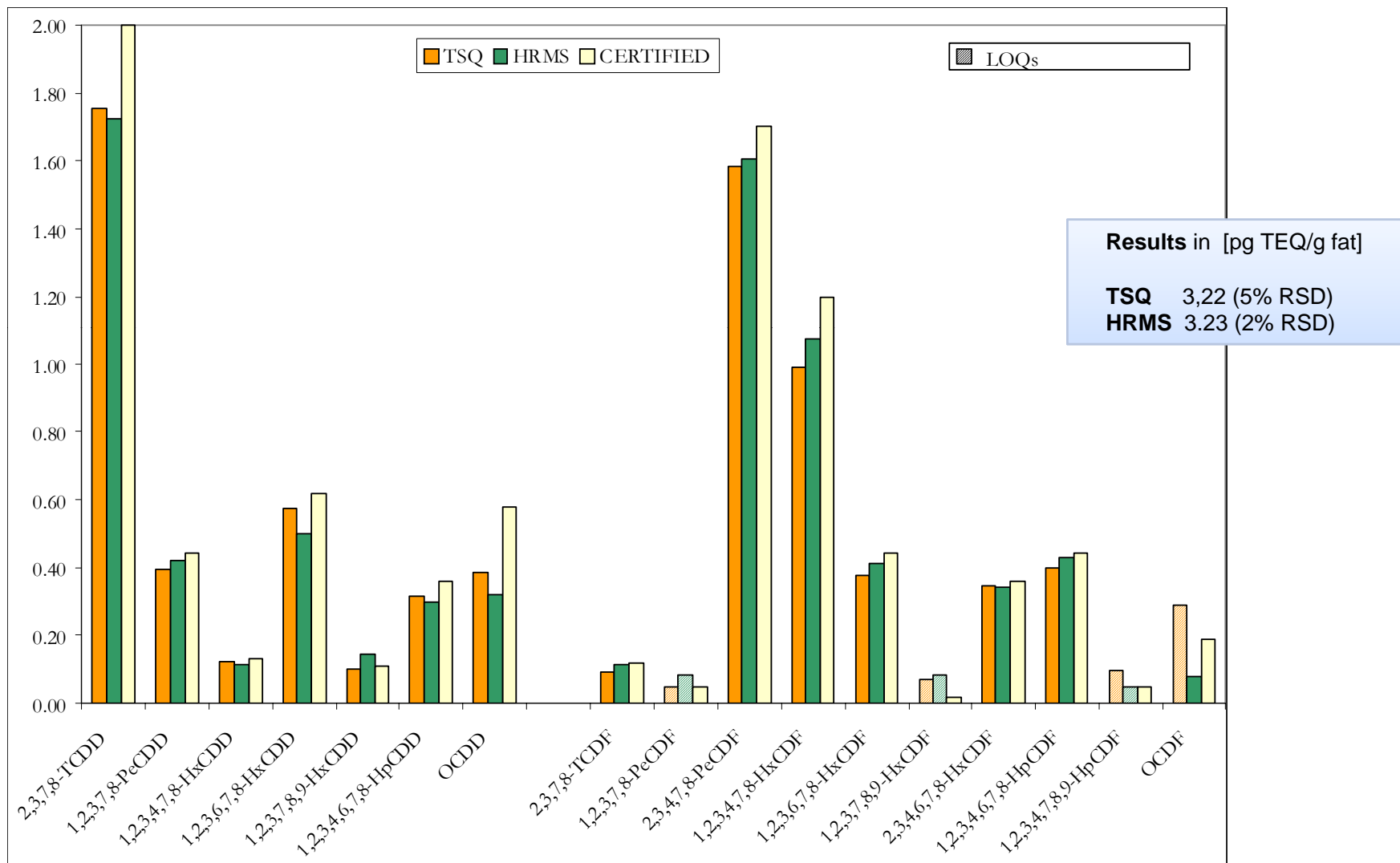
- **Posters presented at**

- Dioxin 2009, Beijing, China
- ISCC, Riva, Italy
- Dioxin 2010, San Antonio, TX, USA

Simplify Screening vs. Full Sample Preparation



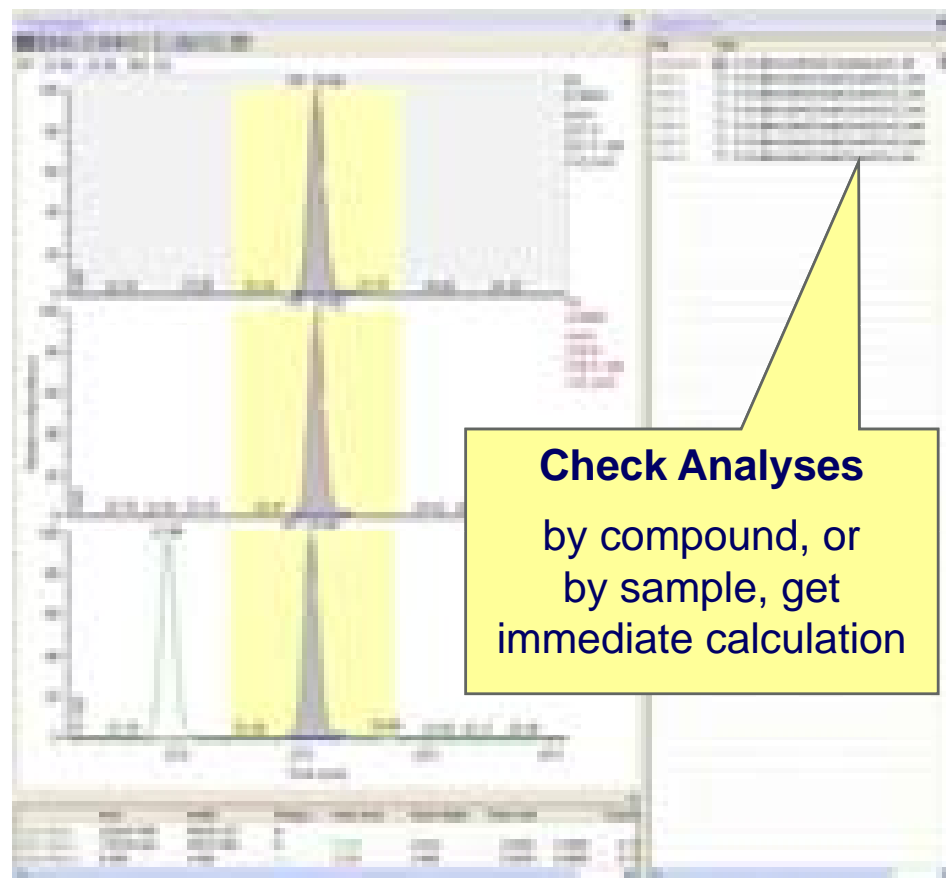
TSQ Quantum XLS vs. GC-HRMS analysis of CRM



TargetQuan – POPs Quantitation for TSQ and DFS

Features for dioxin applications:

- Toxicity equivalents (TEQ)
 - according to WHO definition
 - including lower, medium and upper boundary calculation
- User definable summation
 - Of calculated amounts or TEQs for reporting of sum TEQ values
- Isotopic ratio confirmation
 - One quantitation mass and up to two masses based on abundance
- EPA 1613, 1668 compliant
 - Allows quantification based on average response of selected compound Retention time correction

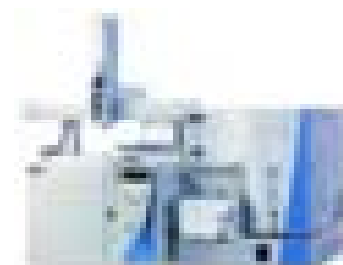


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Why MS/MS and HRMS for POPs?

- **GC-MS/MS** – The Screening Solution

- Well known instrumentation in every trace analysis lab
- Very high sensitivity and selectivity for matrix samples
- Provides TEQ results for PCDD/Fs and DL-PCBs
- Fast - with high throughput capability, automated runs



- **GC-HRMS** – The Confirmation Solution

- Compliant with international regulations
- Highest sensitivity and precision for low level analyses
- Highly productive routine method – high throughput operation



- **GC-MS/MS + GC-HRMS** – Work Seamlessly Together

- Use integrated sample prep workflow for screening and confirmation
- High precision data using labelled internal standards
- Common TargetQuan software platform

Complete Method Setup for DL-PCBs and PCDD/Fs

- For *TSQ Quantum XLS* Screening and *DFS GC-HRMS* Confirmation

Analysis of PCBs in Food and Biological Samples Using GC Triple Quadrupole

PCDD/F Screening in the Maximum Residue Limit for Food Safety Analysis using Highly Selective Triple Quadrupole GC/MS/MS

ThermoFisher Scientific | Application Note

The article describes a method for the screening of polychlorinated dibenz-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) in food samples. It highlights the use of a highly selective triple quadrupole GC/MS/MS system for accurate detection and quantification. The method involves sample extraction, cleanup, and analysis using a specific GC/MS/MS protocol. The article includes a table of detection limits and a list of compounds analyzed.

Compound	Detection Limit (pg/kg)
2,3,7,8-TCDF	0.1
2,3,7,8-TCDF	0.1
2,3,7,8-TCDF	0.1
2,3,7,8-TCDF	0.1
2,3,7,8-TCDF	0.1
2,3,7,8-TCDF	0.1
2,3,7,8-TCDF	0.1
2,3,7,8-TCDF	0.1
2,3,7,8-TCDF	0.1
2,3,7,8-TCDF	0.1

Real Time Acquisition for High Throughput Analysis of Polychlorinated Dioxin/Furans

Confirmation of Low Level Dioxins and Furans in Dirty Matrix Samples using High Resolution GC/MS

ThermoFisher Scientific | Application Note

This article focuses on the real-time acquisition and confirmation of low-level dioxins and furans in dirty matrix samples. It details the use of high-resolution GC/MS for accurate identification and quantification. The method involves sample preparation, cleanup, and analysis using a specific GC/MS protocol. The article includes a table of detection limits and a list of compounds analyzed.

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2,3,7,8-TCDF	0.1
2,3,7,8-TCDF	0.1
2,3,7,8-TCDF	0.1
2,3,7,8-TCDF	0.1
2,3,7,8-TCDF	0.1

Thermo opened the POPs Center of Excellence

POPs Center of Excellence

for Food, Feed and Environmental Analysis

Technical Resources

ThermoFisher Scientific has a strong commitment to supporting customers through with the task of researching and monitoring POPs. It ensures high productivity, added value solutions for some of the most challenging POPs determinations.

To reinforce our commitment, we have established a POPs Center of Excellence staffed with experts in POPs analysis. It can be used as a direct resource for our customers and associates.

Located in Sunnyvale, California, the POPs Center of Excellence is dedicated to support our international customers and associates, addressing the following requirements:

- Reference for POPs analysis
- Point for access to POPs methodology on Thermo Scientific products
- Resource for collaborative projects on the latest instrumentation
- Knowledge hub for Thermo Scientific product users who are working on POPs analysis





Thank you very much
for your attention.