Use of High Resolution Selected Reaction Monitoring (H-SRM) to Overcome Sample Matrix Interferences in Food Residue Analysis by LC-MS-MS

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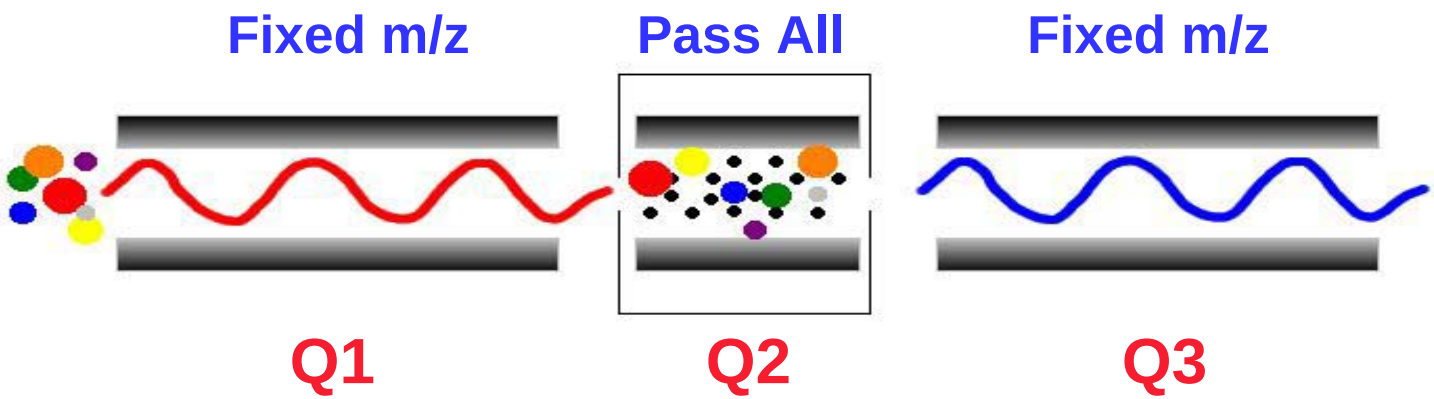
Outline

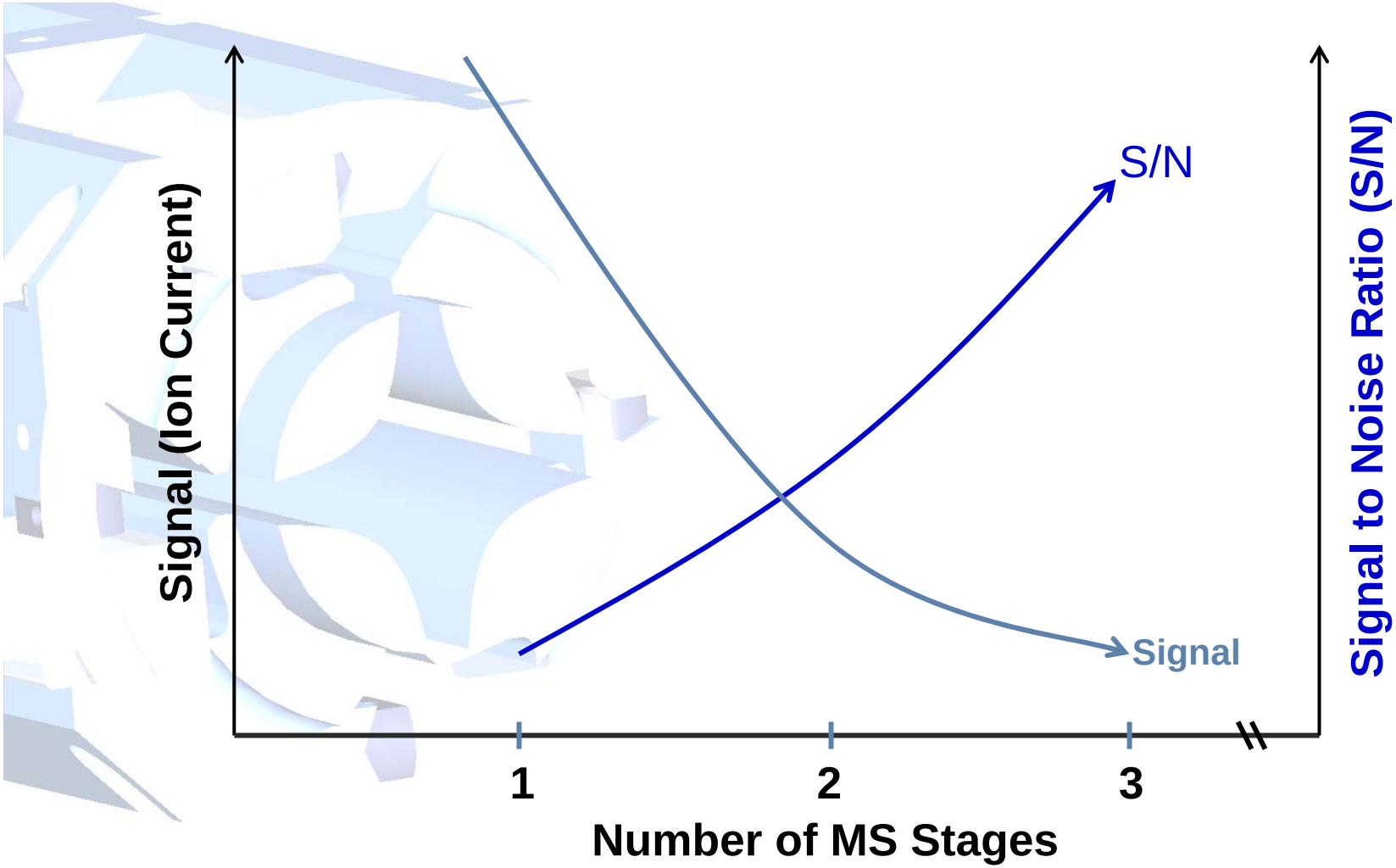
- ☑ *What is H-SRM?*
- ☑ *How does it work?*
- ☑ *Applications: Chloramphenicol, Pesticides, etc.*

Selected Reaction Monitoring (SRM)



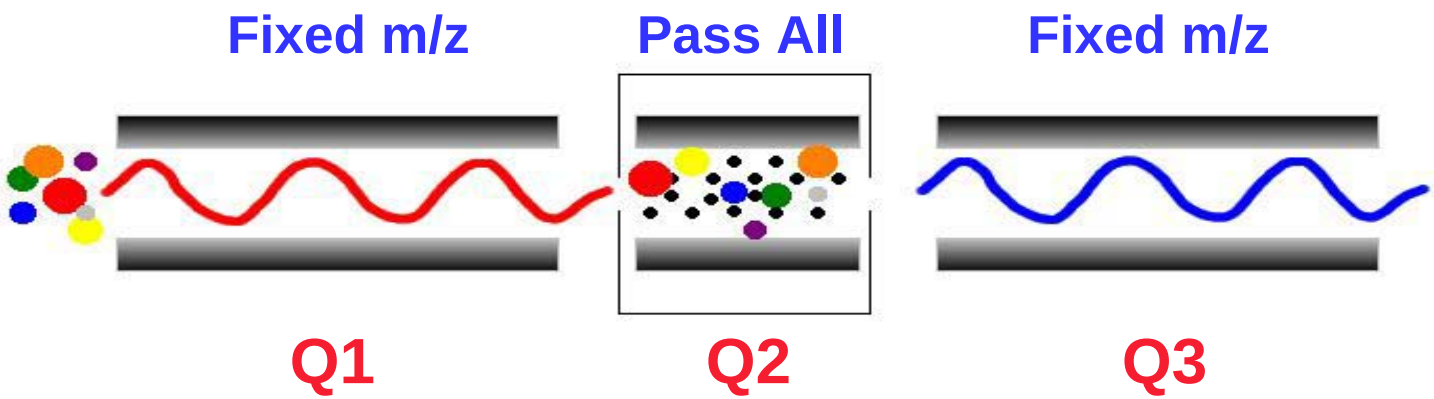
- An MS/MS Technique with High Selectivity and Sensitivity
- Used Mostly in Quantitative Analysis
- Less Chromatography Required

The Benefit of MS/MS



From McLafferty, circa 1980

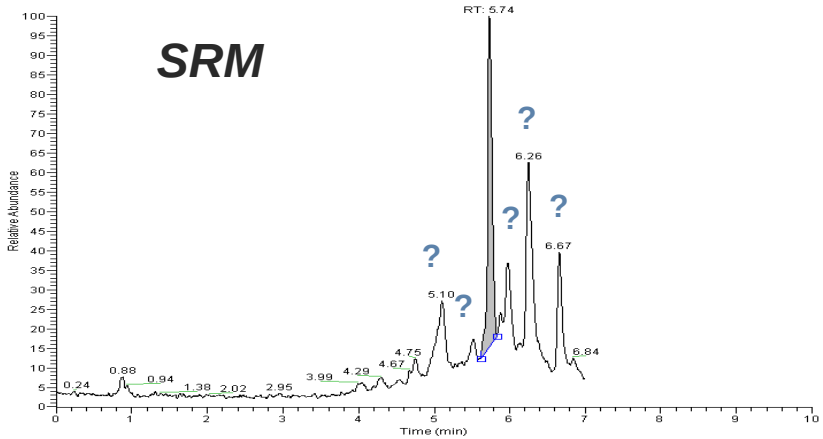
H-SRM: High Resolution SRM

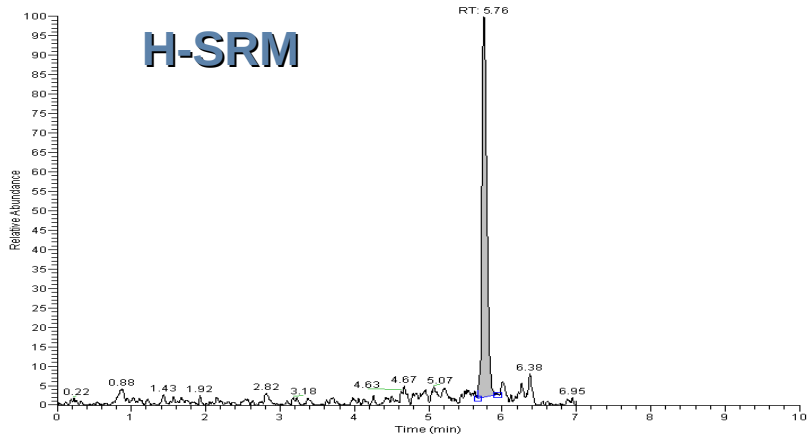


- **Narrow the Q1 Peak Width from 0.7 Da (unit) to 0.1-0.2 Da**
- **But keep the Q3 Peak Width at 0.7 Da**

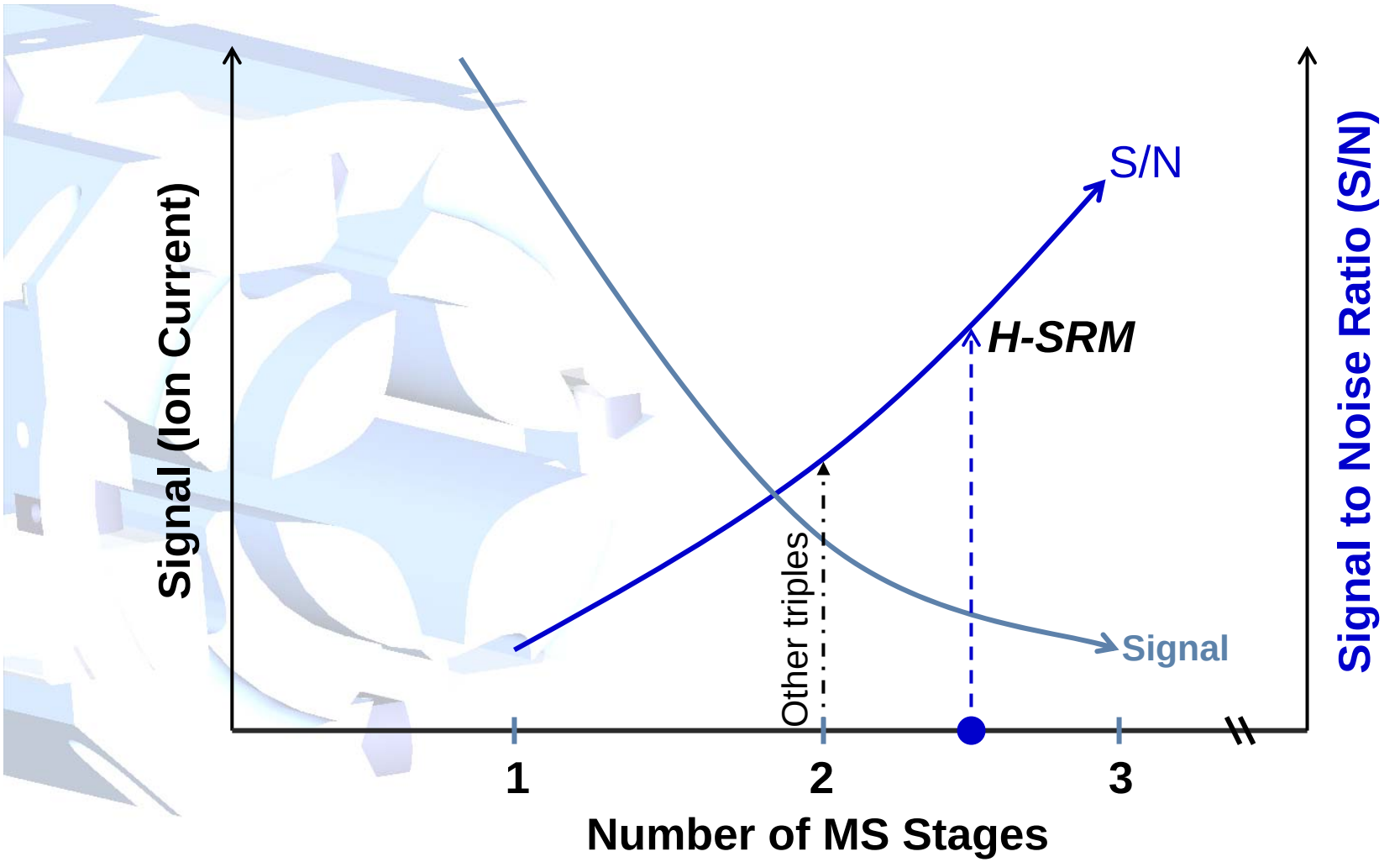
H-SRM : The Benefit

- Reduces “isobaric” chemical noise
- Increases confidence of analysis & improves detection limits





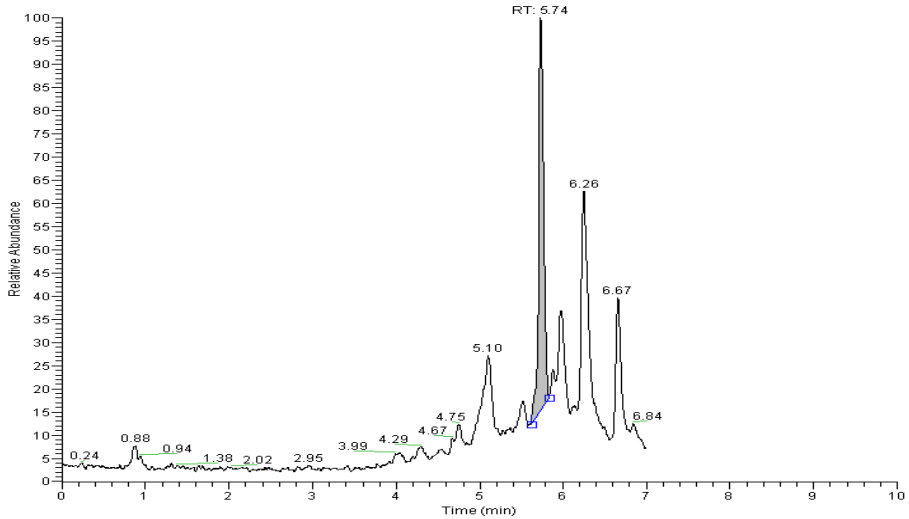
H-SRM Benefit: Improves S/N

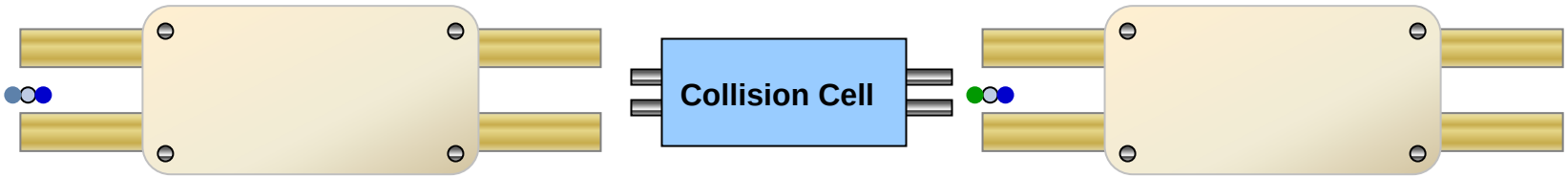


From McLafferty, circa 1980

Unit Resolution Operation

- ***Can have chemical noise in SRM trace***





= pre-cursor ion

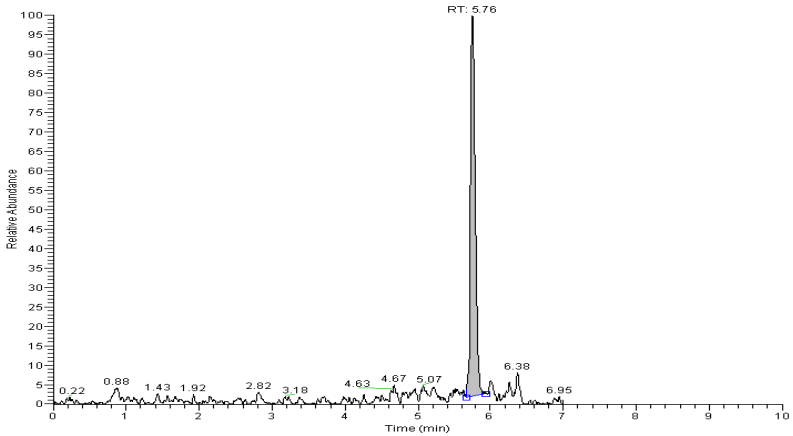
= ***Q1*** *chemical noise*

= product ion

= ***Q3*** *chemical noise*

H-SRM Operation – Enhancing Specificity

- ✓ *Lower chemical noise*
- ✓ *Lower detection limits*

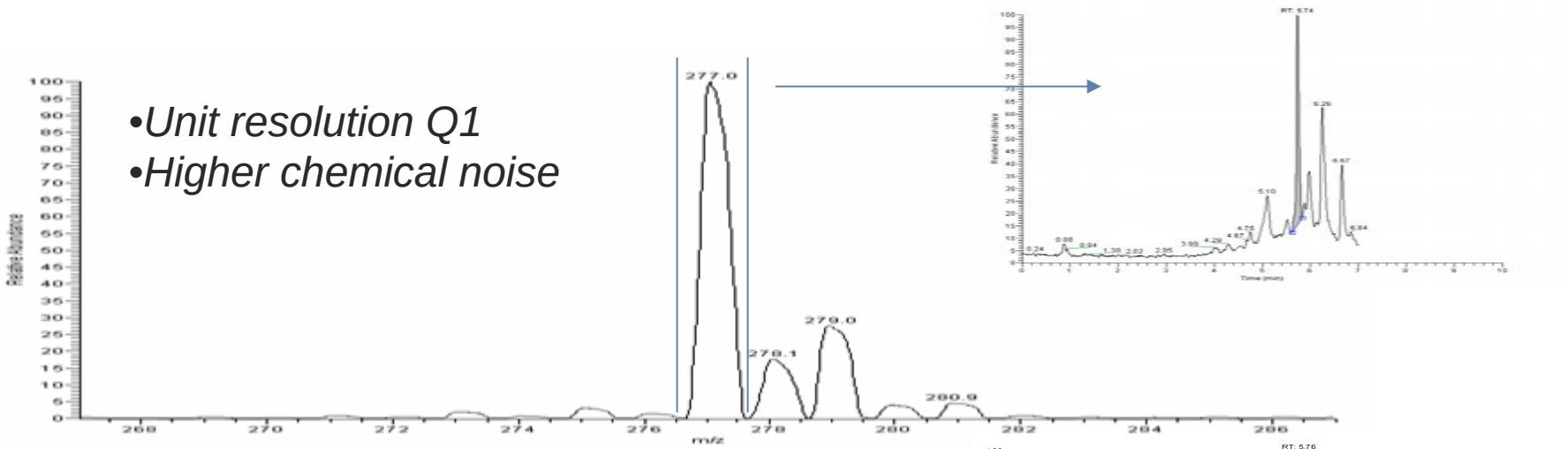


= only pre-cursor ion transmitted

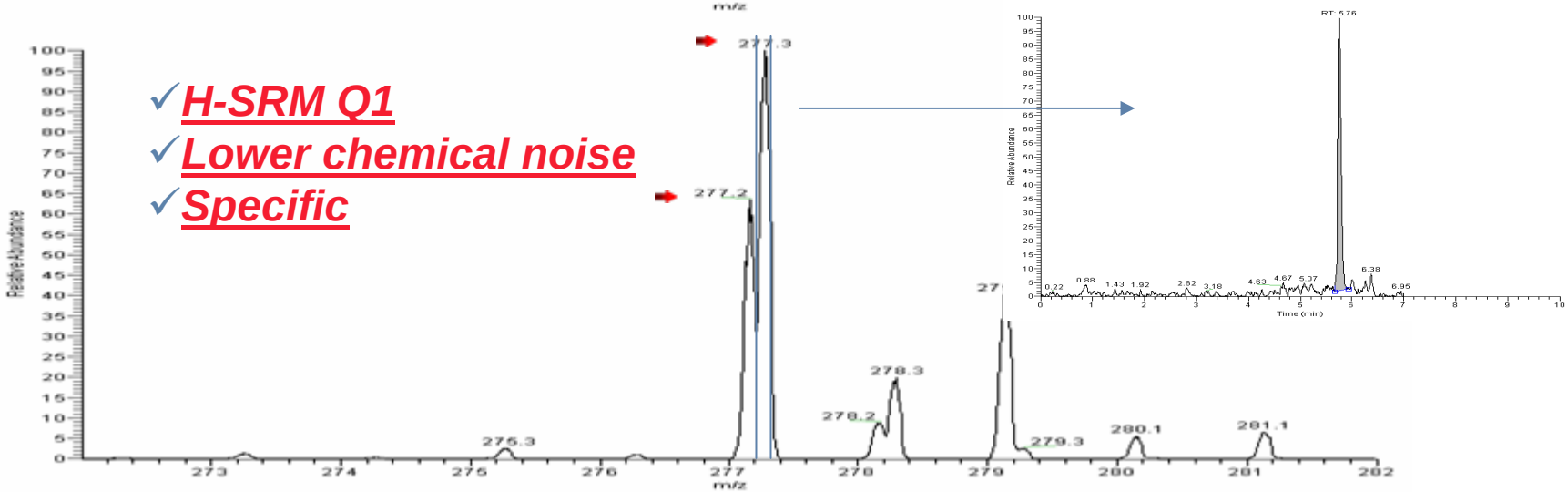
H-SRM : Eliminating Chemical Noises

Analysis of Clenbuterol (target) + 19-norandrosterone in human urine

- *Unit resolution Q1*
- *Higher chemical noise*



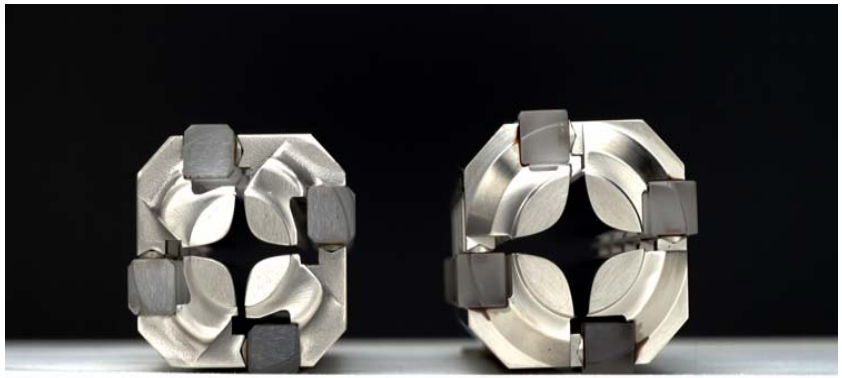
- ***H-SRM Q1***
- ***Lower chemical noise***
- ***Specific***



H-SRM: Quadrupole Design

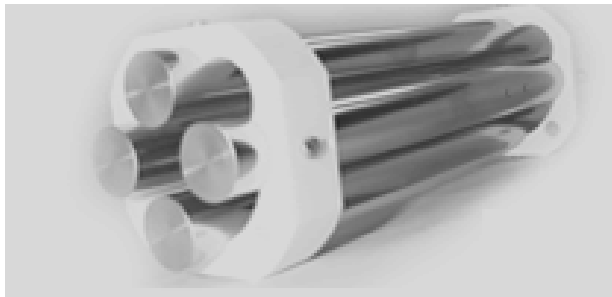
Hyperbolic Rods

- ***High Transmission*** **at High Resolution**



TSQ 700/7000 **Quantum**

Other Manufacturers' Quads



Round Rods

- ***Low Transmission at Increased Resolution***

H-SRM: Origin

GENERAL ELECTRIC

SCHENECTADY, NEW YORK

CLASS 1

MASS SPECTROSCOPY USING RADIO-FREQUENCY QUADRUPOLE FIELDS

by

P. H. Dawson and N. R. Whetten, General Physics Laboratory

Report No. 68-C-418

December 1968

7. Hyperbolic vs Circular Rods

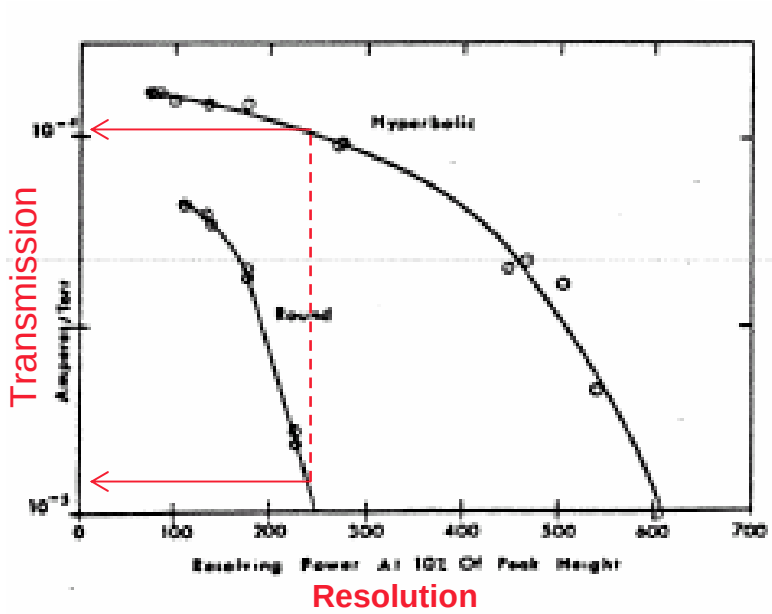
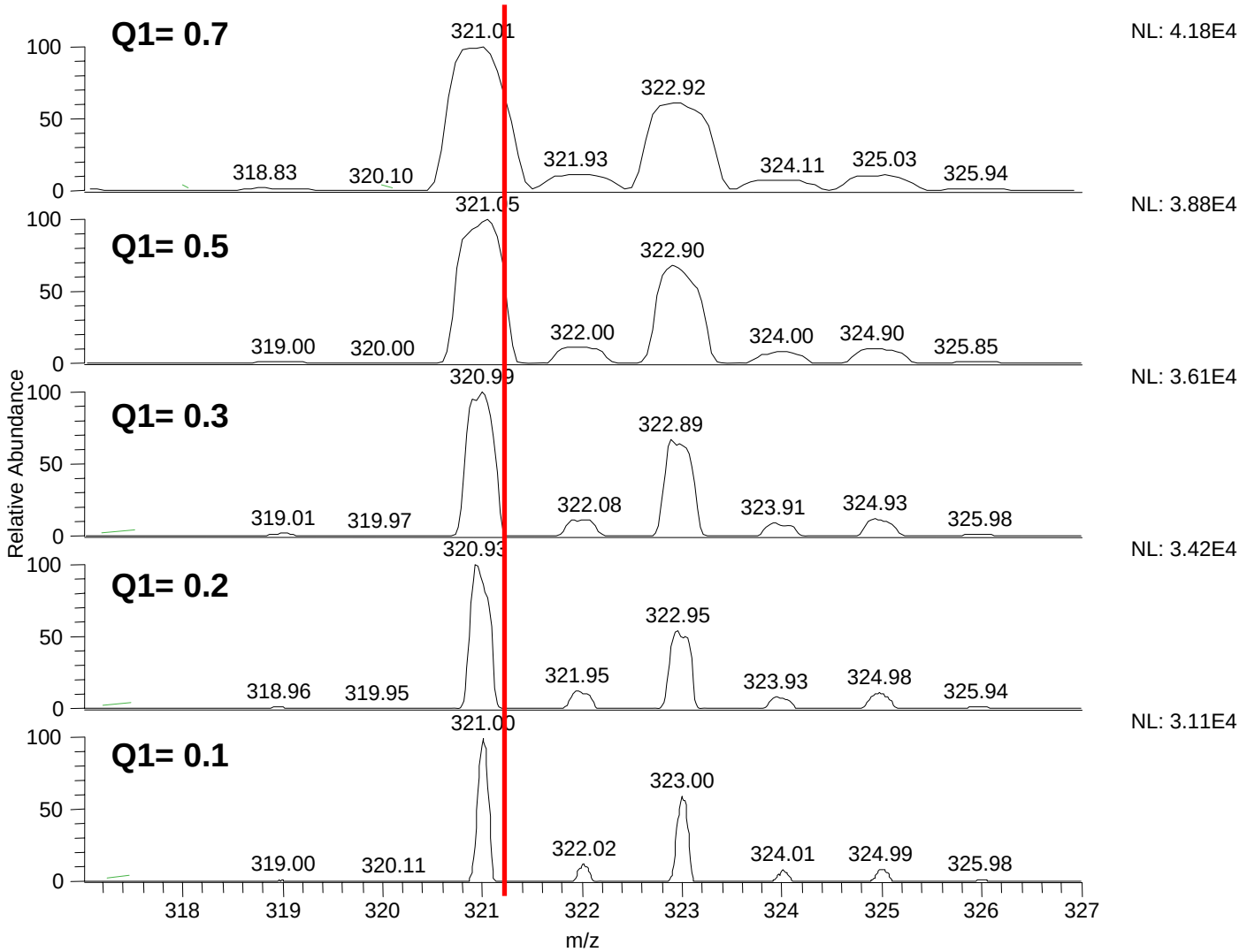


Fig. 40 Comparison of mass filter performance with round and hyperbolic rods. [42, 43]

Mass Spectra of Chloramphenicol at Various Q1 Resolution



SRM vs H-SRM: Stability

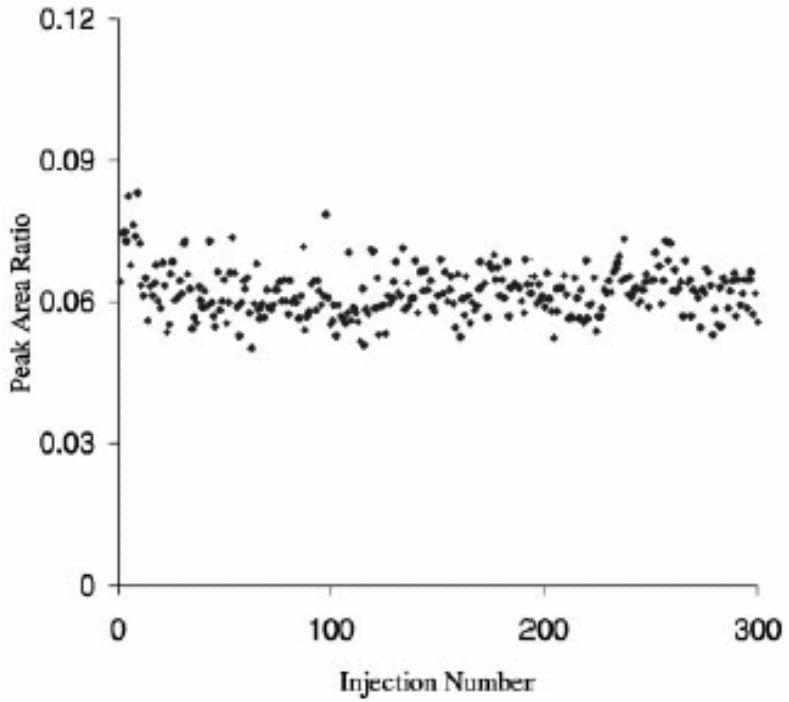


Figure 13. Intra-day reproducibility of compound A (50 ng/mL) on the Quantum at enhanced mass resolution (0.2 Da peak width, FWHM).

Table 2. Intra-day reproducibility for 50 ng/mL standard (compound A)

Source type	Mass resolution	Standard deviation (N = 300)
ESI	0.7 Da peak width, FWHM	0.2%
ESI	0.2 Da peak width, FWHM	0.5%

Table 3. Inter-day reproducibility for 2.5, 25 and 2500 ng/mL standards (compound A)

		Standard deviation (N = 9, 3/day for 3 days)		
Source type	Mass resolution	Std 2.5 (ng/mL)	Std 25 (ng/mL)	Std 2500 (ng/mL)
ESI	0.7 Da peak width, FWHM	0.2%	0.4%	1.4%
ESI	0.2 Da peak width, FWHM	0.1%	0.5%	5%

Rapid Commun. Mass Spectrom. 2003; 17: 832–837

Schering-Plough

Analyze • Detect • Measure • Control™

Thermo ELECTRON CORPORATION

H-SRM Using CCM

- H-SRM
  - *Calibration Correction Method Software*
    - Works “on the fly”
    - Provides a small correction to the peak position and width, ensuring that the system remains within a tight calibration window.
    - The correct analyte and calibrant masses of the charged analyte are imported into the method.
    - Calibration is only done for Q1
    - Extra QC at mid-to-high level required in sequence
  - *Using CCM, H-SRM analysis becomes routine without worrying about instrument drift*
  - *Available upon release on Quantum 1.3 software (Pittcon 06)*

Application: Chloramphenicol (CAP)

Quantum Discovery Max

Xu et al 53rd ASMS 2005

CAP Experimental: LC Condition

HPLC analysis was performed on the Finnigan Surveyor HPLC module with MS Pump and Autosampler

–Column: Thermo Hypersil Gold C18 (100×2.1 mm, 5μ)

–Mobile Phase: *A: Water; B: Acetonitrile*

–Column Temperature: 40 ^{o}C

–Gradient Program: 0.25 mL/min

Time (min)	% A	% B
0	80	20
2.5	20	80
3.0	20	80
3.1	80	20
5.0	80	20

–Injection: 20 uL with loop

Quantum Discovery Max Operation Conditions

Ion source:	**ESI**
Ion polarity:	**Negative**
Spray voltage:	**4000 V**
Sheath gas pressure:	**45**
Auxiliary gas pressure:	**15**
Ion transfer capillary temperature:	**300 ^{o}C**
Source CID:	**8 V**
Scan Type:	**SRM, 3 transitions of $[M-H]^-$ (m/z: 321) (321->152, 321->194 and 321->257)**
Q1 peak width	**0.7 Da in SRM or 0.2 Da in H-SRM**
Q3 peak width	**0.7 Da**
Collision Pressure:	**Ar at 1.3 mTorr**

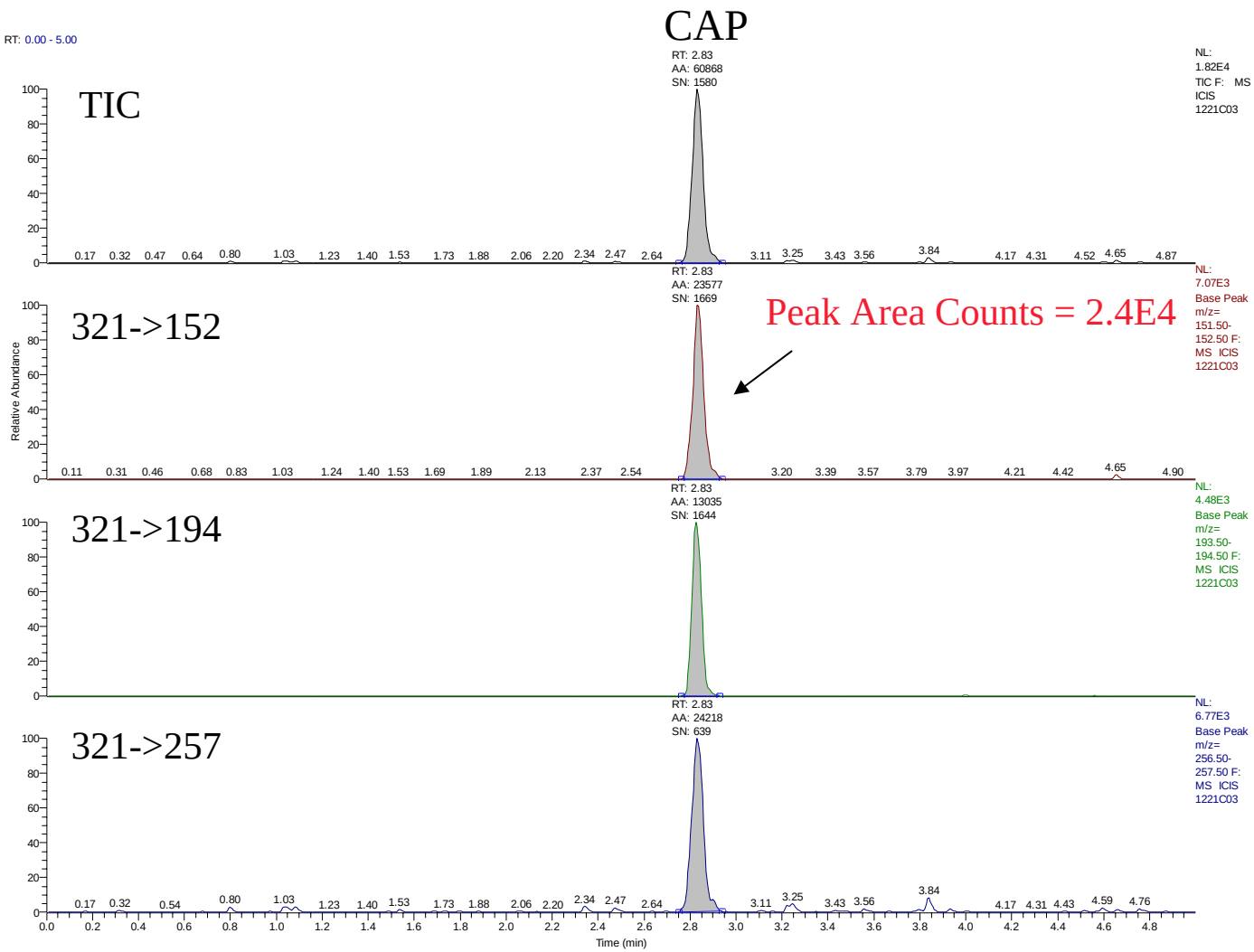
Chloramphenicol Results with SRM

SRM Results at Unit Resolution

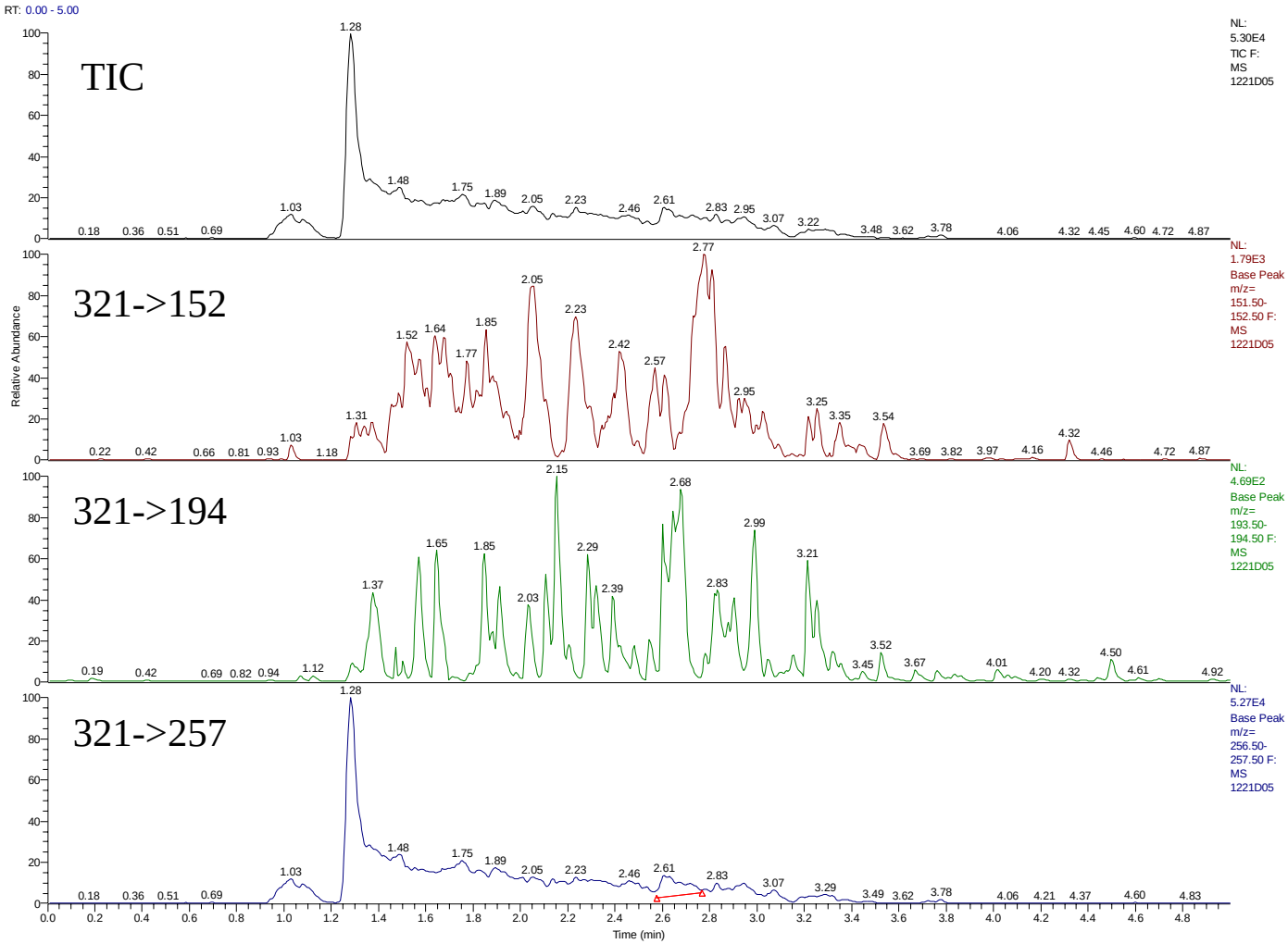
(Q 1 = 0.7 Da)

(Q 3 = 0.7 Da)

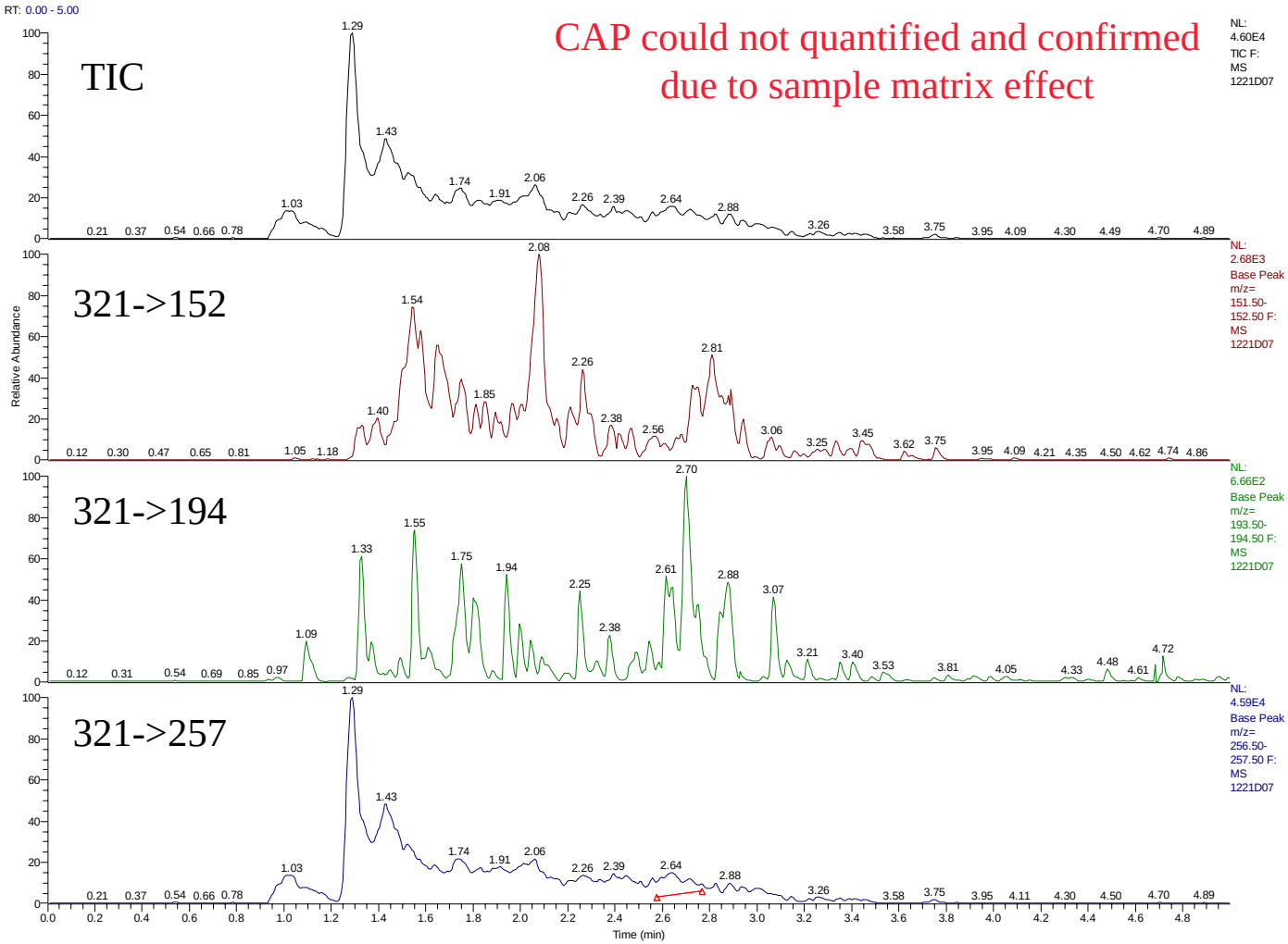
SRM Result: CAP Standard 0.5 ng/mL



SRM Result: Kidney Extract Blank



SRM Result: Kidney Spiked 0.03 ug/kg CAP



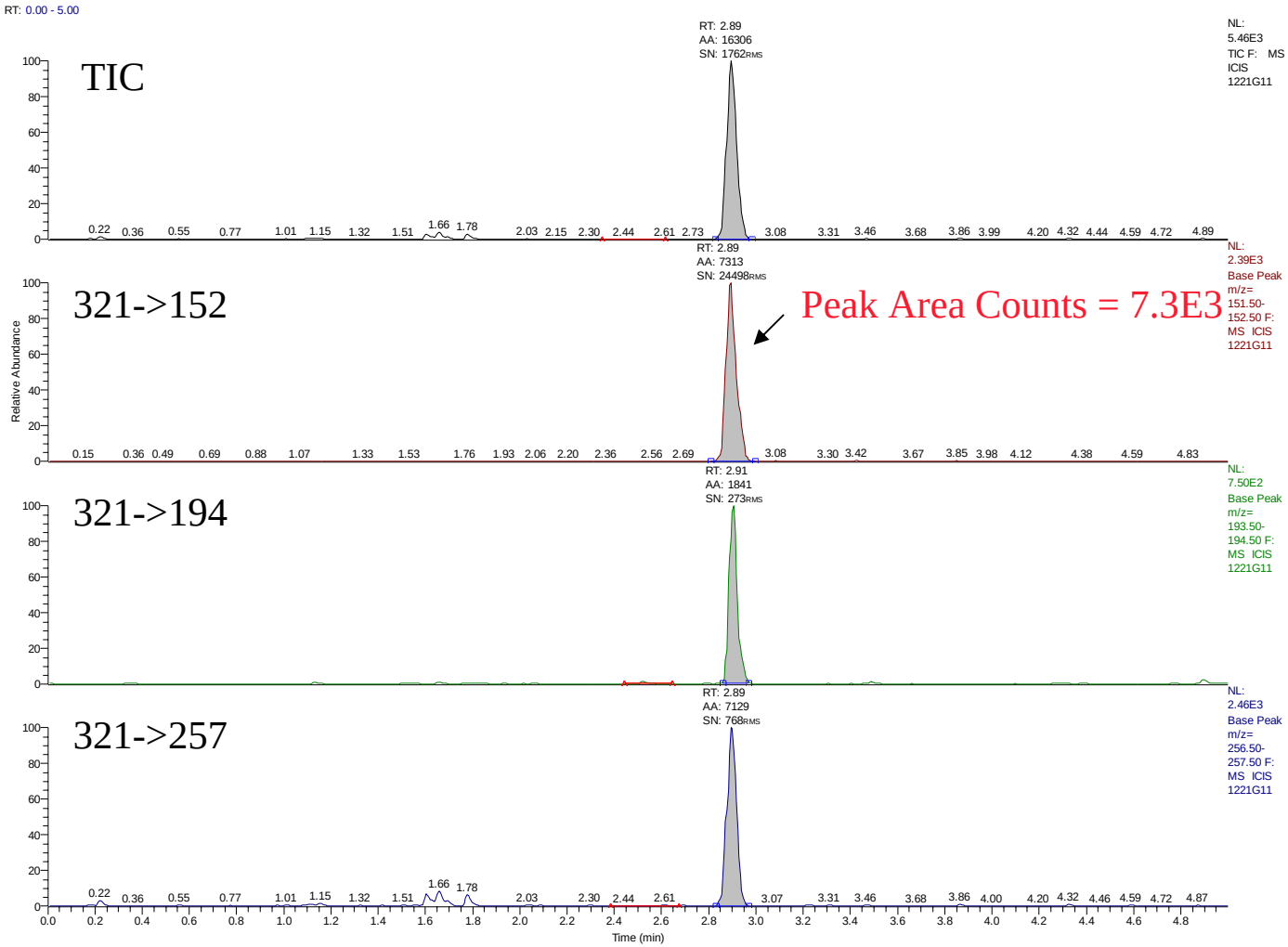
Chloramphenicol Results with H-SRM

H-SRM Results

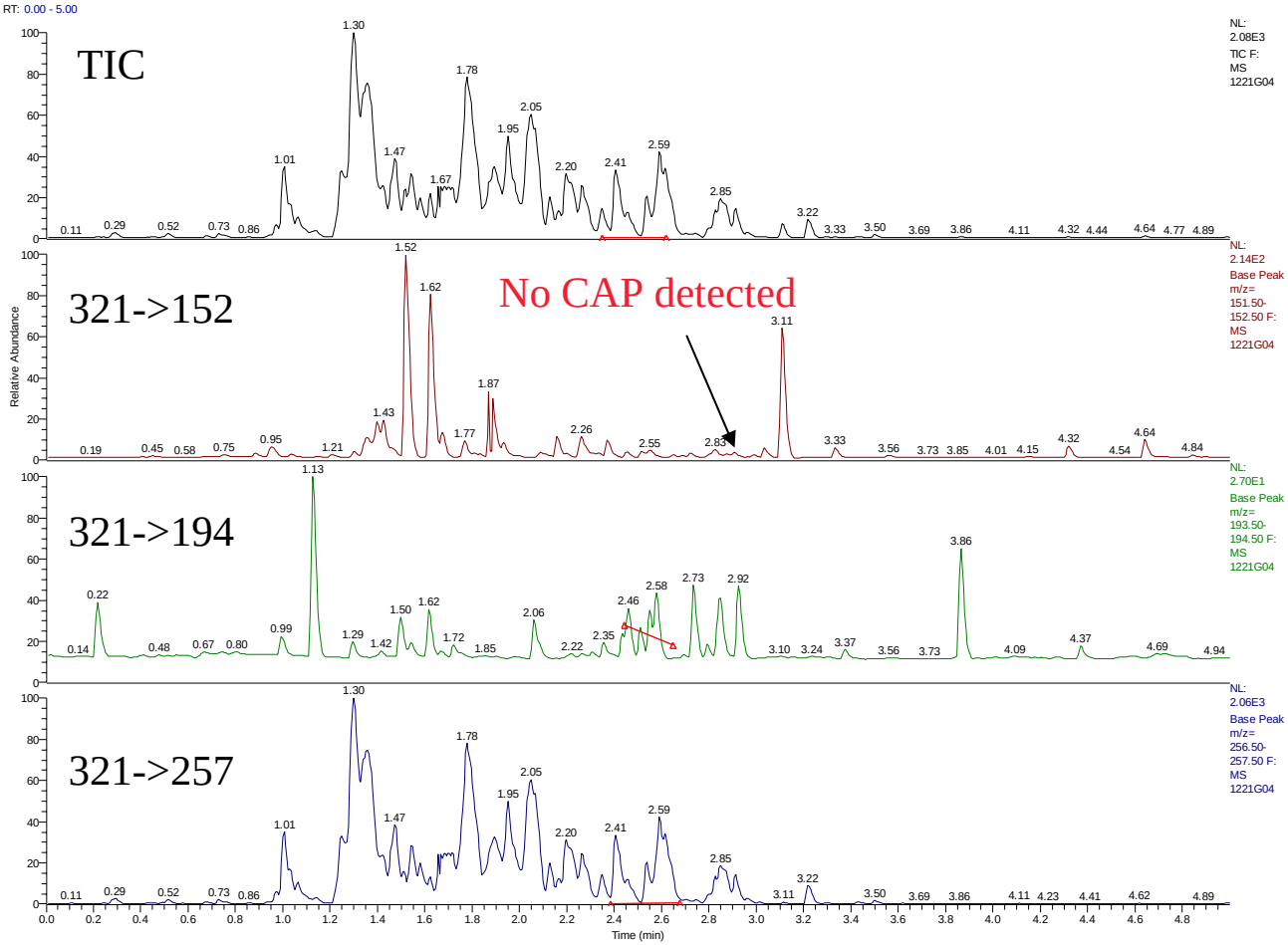
(Q 1 = 0.2 Da)

(Q 3 = 0.7 Da)

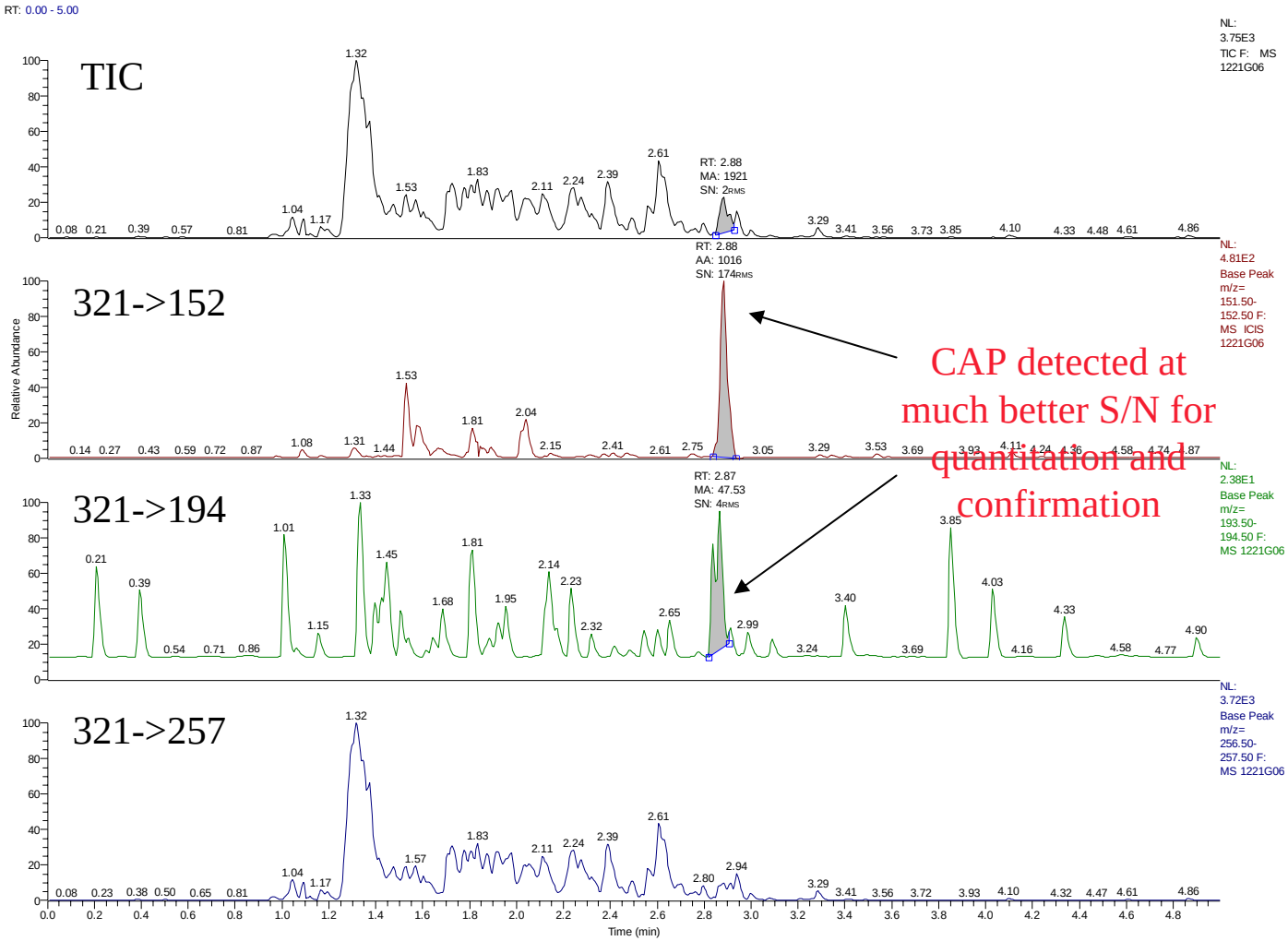
H-SRM Result: CAP Standard 0.5 ng/mL



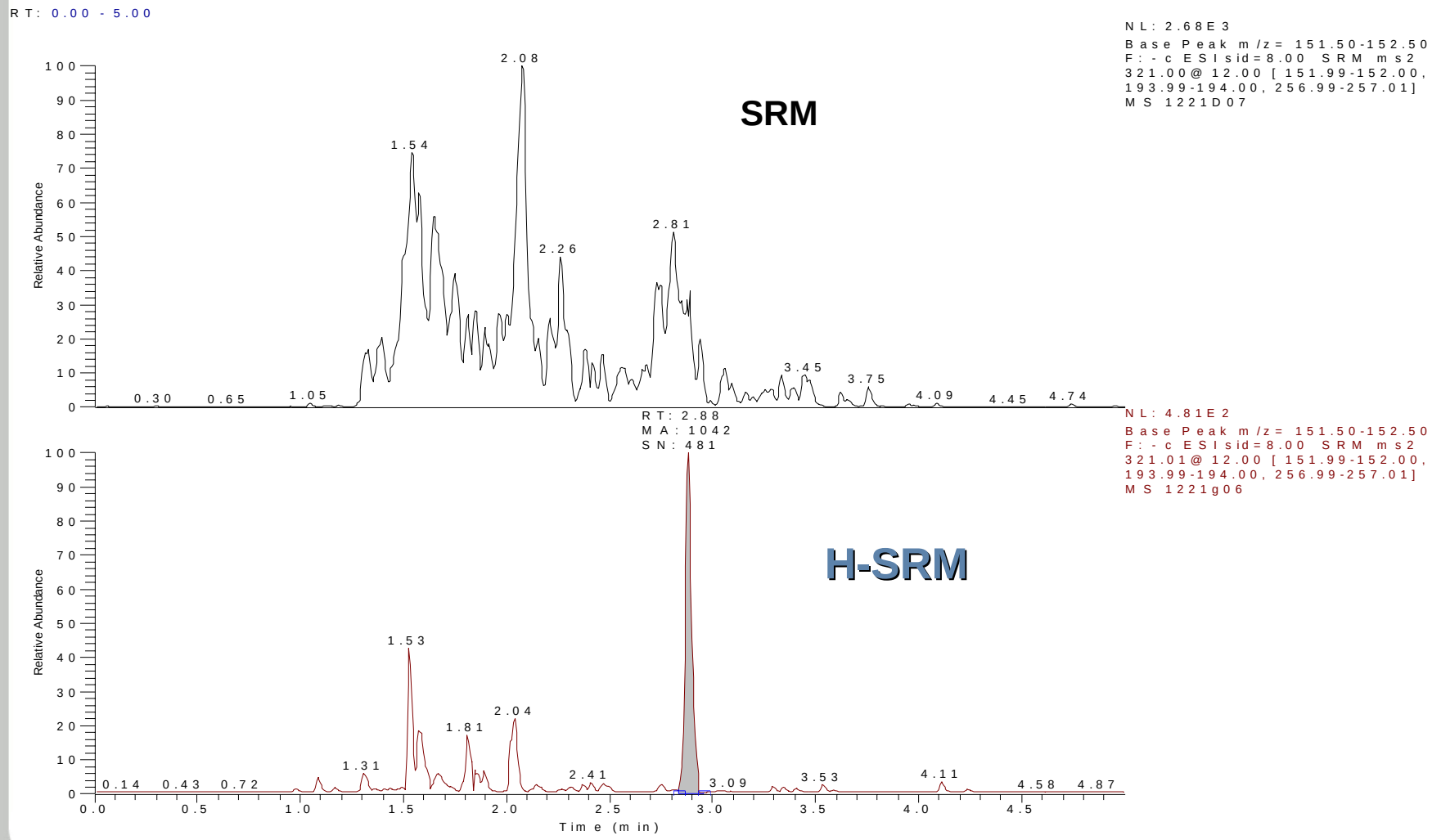
H-SRM Result: Kidney Blank



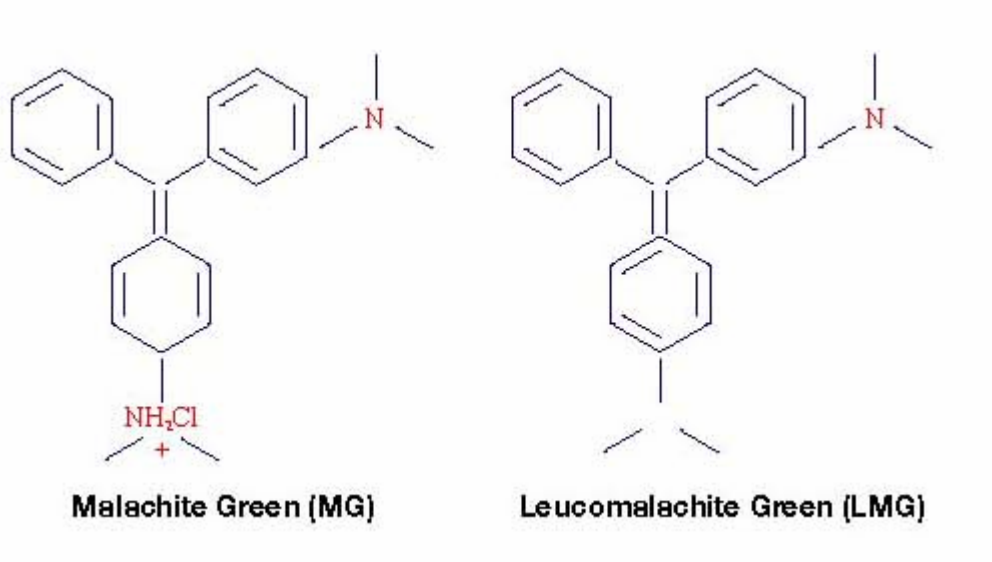
H-SRM Result: Kidney Spike 0.03 ug/kg



SRM and H-SRM (321→152) Chromatograms of Chloramphenicol in Spiked Pig Kidney Extract



Application: Malachite Green (MG) and Leuco-Malachite Green (LMG) in Roasted Eel

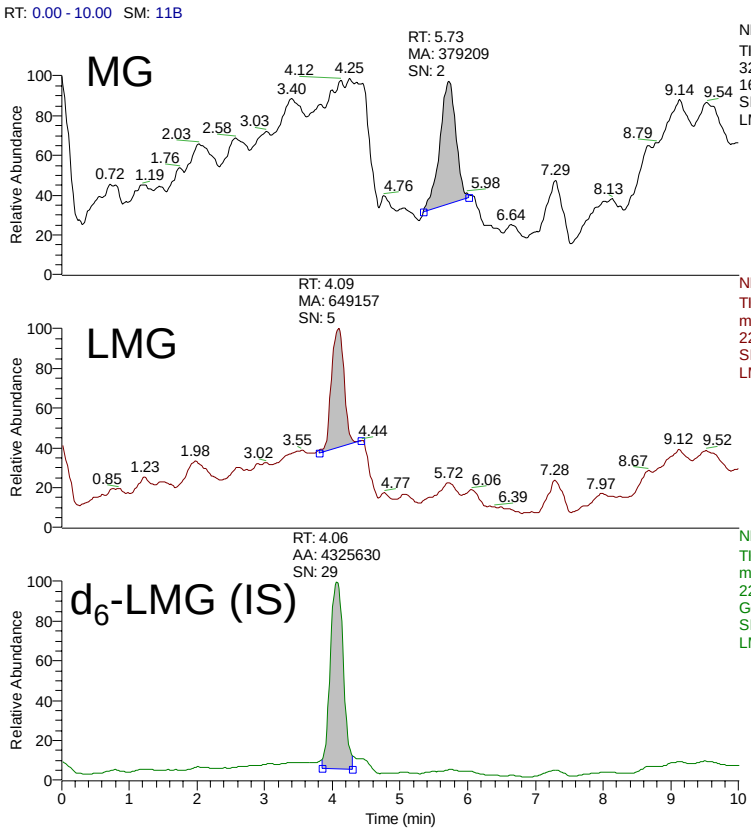


- TSQ Quantum Discovery Max in ESI+
- Thermo Hypersil Gold CN column, Acetonitrile:5mM Ammonium Acetate (60:40, v/v), 220 µ L/min.

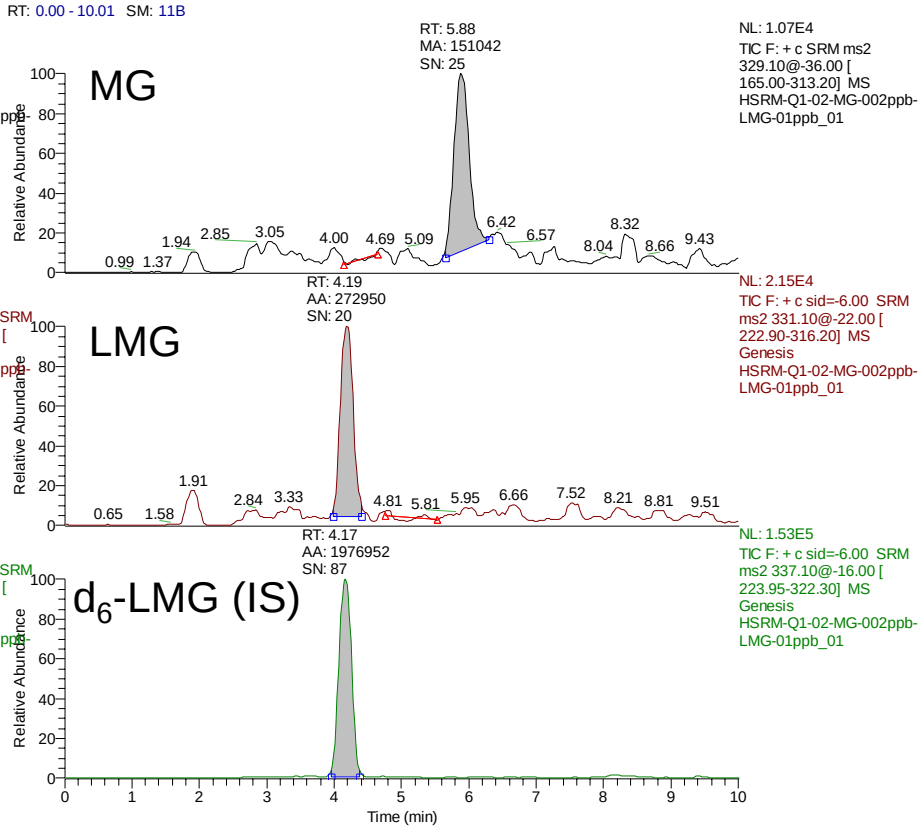
MG and LMG: SRM vs H-SRM

Final Conc.$_{MG}$=0.02 ppb, Conc.$_{LMG}$=0.1 ppb

SRM:Q1=0.7,Q3=0.7



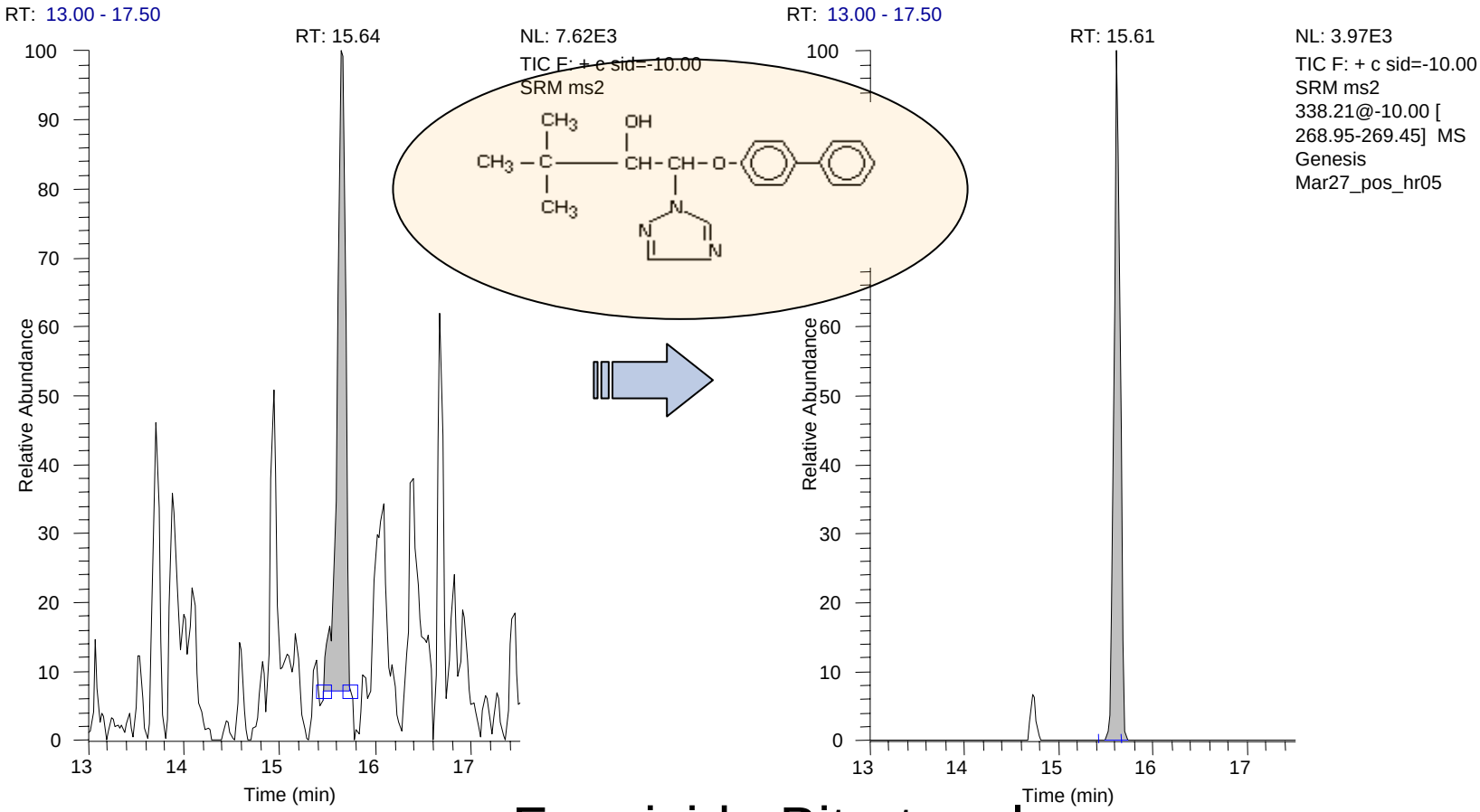
H-SRM: Q1=0.2,Q3=0.7



Application: Pesticides Analysis in Vegetable Extracts

SRM Mode

H-SRM Mode

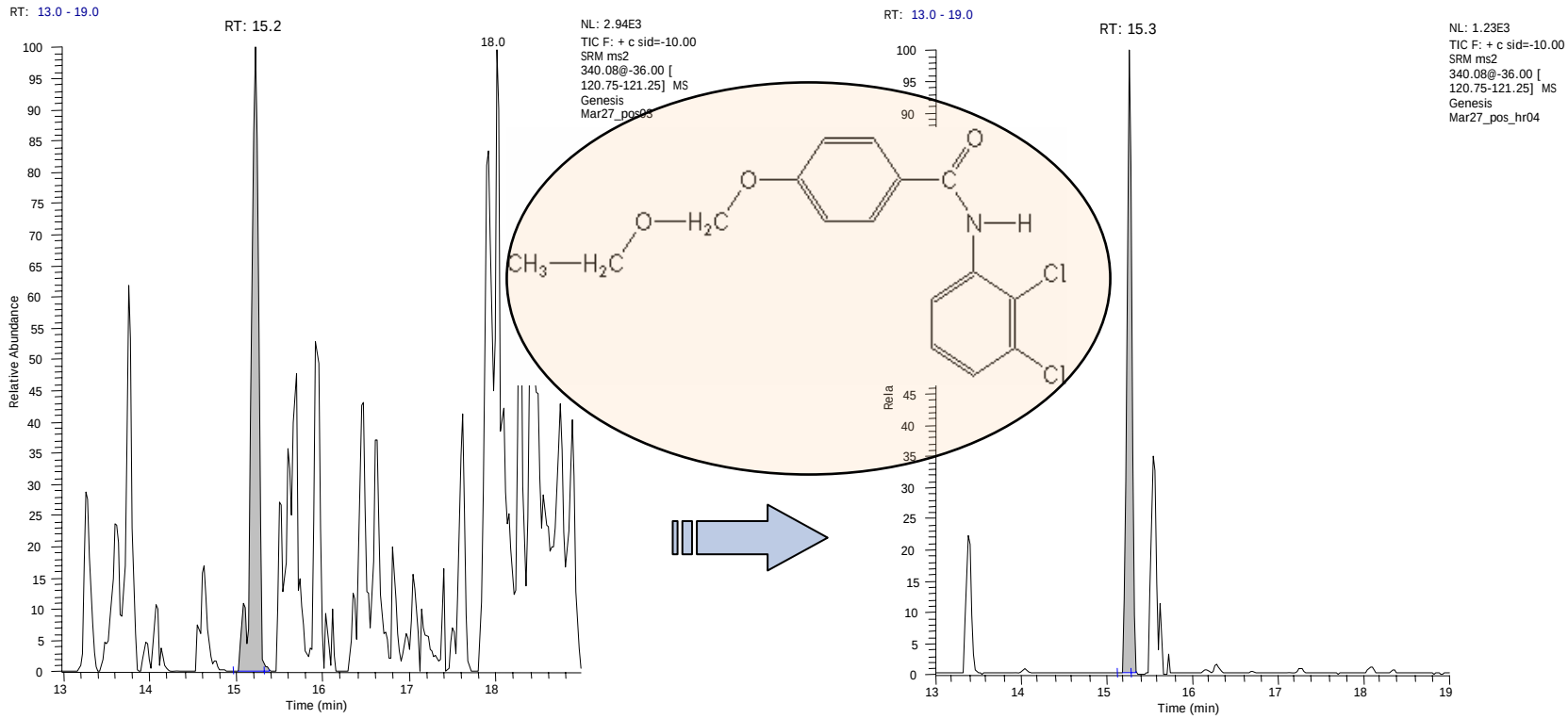


Fungicide Bitertanol

Pesticide Analysis: SRM vs H-SRM

SRM Mode

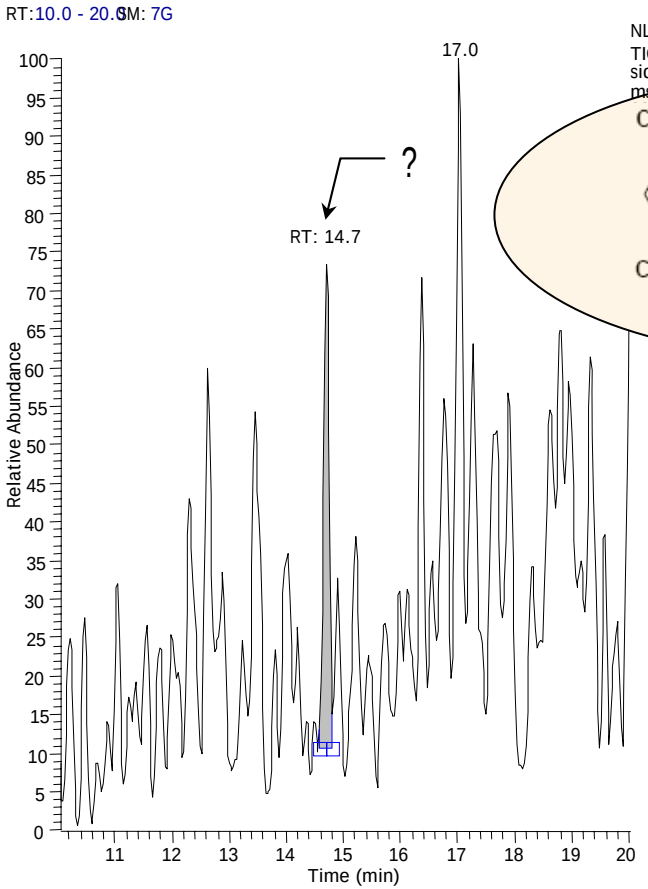
H-SRM Mode



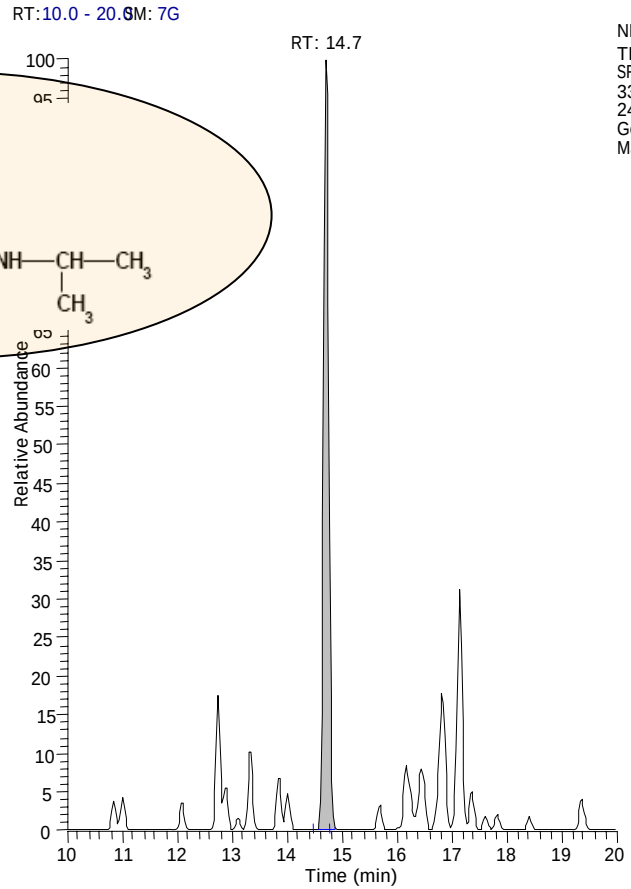
Herbicide Etobenzanid

Pesticide Analysis: SRM vs H-SRM

SRM Mode



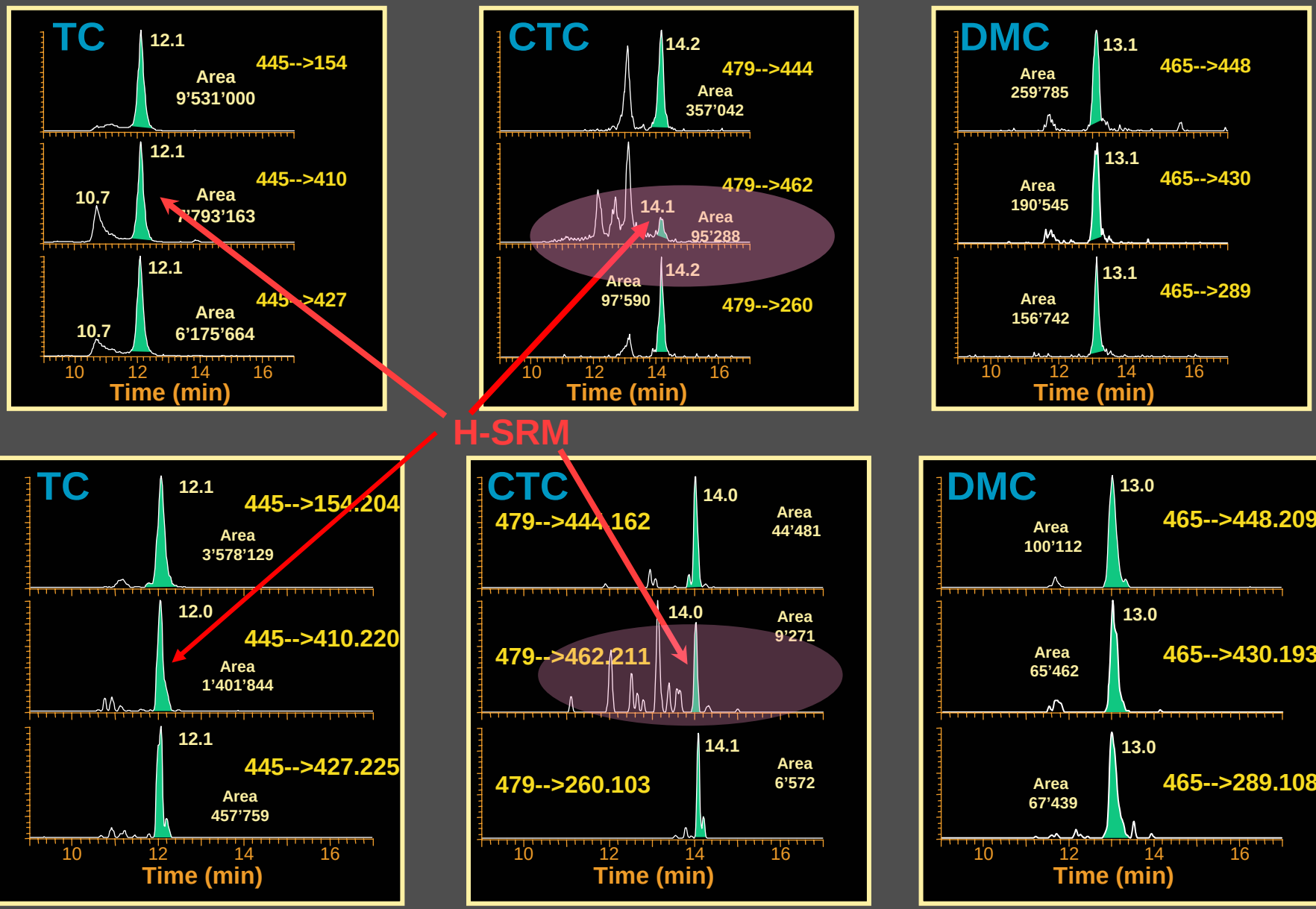
H-SRM Mode



Fungicide Iprodione

Effects of H-SRM

NRC Nestlé Research Center



Conclusions

- ✓ ***H-SRM: It works***
- ✓ ***H-SRM: It is on every Finnigan TSQ Quantum***
- ✓ ***H-SRM: It is simple to use***

Acknowledgement

- Agri-Food & Veterinary (AVA) Authority of Singapore
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- Yoko Yamagishi, Thermo Electron, Yokohama, Japan

Thank You & Any Questions?

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