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Excellence in Science

Development of a New Method to Determine Pesticides and Polychlorinated Biphenyl Congeners in Aqueous Solution Using New Triple Quadrupole GCMS-TQ8050

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3. Eurofins Eaton Analytical, Monrovia, CA

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Background

- Pesticides-PCBs – what they are and why are we still interested in them
- Toxicity
- How do we analyze them – Analytical Methods
- What does Not Detected mean?
- Limitations to Pesticides-PCB analysis
- Advantages of GC-MS/MS triple quadrupole technique

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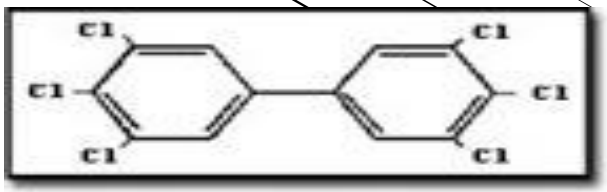
PCBs Chemical Structure



- 209 possible congeners – mono thru decachlorobiphenyl
- Twelve are considered dioxin like and have been assigned Toxic Equivalency Factors (TEF)

- MonoCB 3 congeners
- DiCB 12 congeners
- TriCB 24 congeners
- TetraCB 42 (2) congeners
- PentaCB 46 (5) congeners
- HexaCB 42 (4) congeners
- HeptaCB 24 (1) congeners
- OctaCB 12 congeners
- NonaCB 3 congeners
- DecaCB 1 congeners

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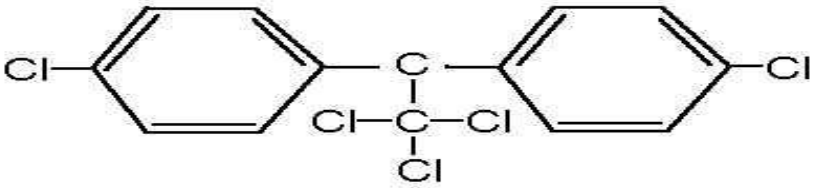
Pesticides Chemical Structure



SOME PESTICIDES PERSIST AND BIOCONCENTRATE

PERSISTENT ORGANIC POLLUTANTS (POPs)

- Low water solubility
- Persist in the environment
- Accumulate in the food-chain
- Lypophilic
- Travel long distances
- Concentrate in marine animals
- May produce toxic effects PESTIC

PESTICIDES
Aldrin
Dieldrin
Chlordane
DDT
Endrin
Heptachlor
Mirex Toxaphene

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Analytical Methods

GC/HRMS Methods
-EPA 1668
- Contemporary Samples
- Analysis on GC-HRMS
- Two GC columns
  - PCBs/OCPs (~ 1hr/sample)
  - PBDEs (~ ½ hr/sample)
- Total time:
  ~ 1.5 hrs. or more/sample

- GC/MS Methods
  EPA 8270

Federally Funded Projects

GC/ECD Methods
-EPA 8081, 8082, 608, 508
- Archived Samples (1960s)
  and Contemporary Samples
- Analysis on GC-ECD
- Dual Column Method
  - Run concurrently
  - PCBs/OCPs
- Total time:
  ~ 2.5 hrs./or more /sample

- Upgraded to GC-tandem MS

Pesticide and PCBs Analysis Overview

- Currently, all analyses for routine organochlorine (OC) pesticides and PCBs target list are run by Method 8081 and Method 608 on GC/ECD.

- ECD is a highly sensitive detector for compounds containing electronegative atoms or functional groups (halogens, organometallics, nitrites, nitro groups), and is capable of achieving (and exceeding) the low reporting limits required for target list OC pesticides.

- These methods perform very well if sample extracts are relatively free of interferences. Unfortunately, environmental sample extracts rarely meet this criterion.

- As a non-specific detector, target compound identification is achieved via agreement between sample chromatographic peak retention time (RT) and its expected retention time as determined during calibration. This must be confirmed by a second dissimilar stationary phase column or other qualitative technique (e.g. GC/MS).

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Compounds of Interest

Measured on GC-ECD (two columns)

Organochlorine Pesticides (OCPs)

- Alpha-BHC
- Gamma-BHC (Lindane)
- Beta-BHC
- Delta- BHC
- Heptachlor
- Aldrin
- 4,4'Dibromobiphenyl (SURR)
- Heptachlor Epoxide (isomer B)
- Trans-Chlordane
- Cis-Chlordane
- Endosulfan I
- 4,4'-DDE
- p-Terphenyl-d14 (IS)
- Diledrin
- Endrin
- 4,4'-DDD
- Endosulfan II
- Endrin Aldehyde
- 4,4'-DDT
- Endosulfan Sulfate
- Methoxychlor
- Endrin Ketone

Polychlorinated Biphenyls (PCBs

- Decachlorobiphenyl (BZ#209) (IS)
- Decafluorobiphenyl (IS)
- 2-Chlorobiphenyl (#1)
- 2,3-Dichlorobiphenyl (#5)
- 2,2',5-Trichlorobiphenyl (#18)
- 2,4',5-Trichlorobiphenyl (#31)
- 2,2',5,5'-Tetrachlorobiphenyl (#52)
- 2,2'3,5'-Tetrachlorbiphenyl (#44)
- 2,3'4,4'-Tetrachlorobiphenyl (#66)
- 2,2',4,5,5'-Petachlorobiphenyl (#101)
- 2,2',3,4,5'-Pentachlorbiphenyl (#87)
- 2,3,3'4',6-Pentachlorobiphenyl (#110)
- 2,2',3,5,5',6-Hexachlorobiphenyl (#151)
- 2,2',4,4',5,5'-Hexachlorobiphenyl (#153)
- 2,2',3,4,5,5'-Hexachlorbiphenyl (#141)
- 2,2',3,4,4',5'-Hexachlorobiphenyl (#138)
- 2,2',3,4',5,5',6-Heptachlorobiphenyl (#187)
- 2,2'3,4,4'5',6- Heptachlorobiphenyl (#183)
- 2,2',3,4,4',5,5'-Heptachlorobiphenyl (#180)
- 2,2'3,3'4,4'5- Heptachlorobiphenyl (#170)
- 2,2'3,3'4,4'5,5',6-Nonachlorobiphenyl (#206)

Measured on GC-HRMS

Measured on one column

Polybrominated Diphenyl Ethers (PBDEs)

- BDE17
- BDE28
- BDE47
- BDE66
- BDE85
- BDE99
- BDE100
- BDE153
- BDE154
- BDE183
- BDE196
- BDE197
- BDE201
- BDE202
- BDE203
- BDE206
- BDE207
- BDE208
- BDE209

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Pesticide and Semi-volatile analysis requires two extractions and two analyses

- Pesticides → 1000 ml → $MeCL_2$ → Hexane
  - 608
  - 8080
- Semi-Volatiles → 1000 ml → $MeCL_2$
  - 625
  - 8270

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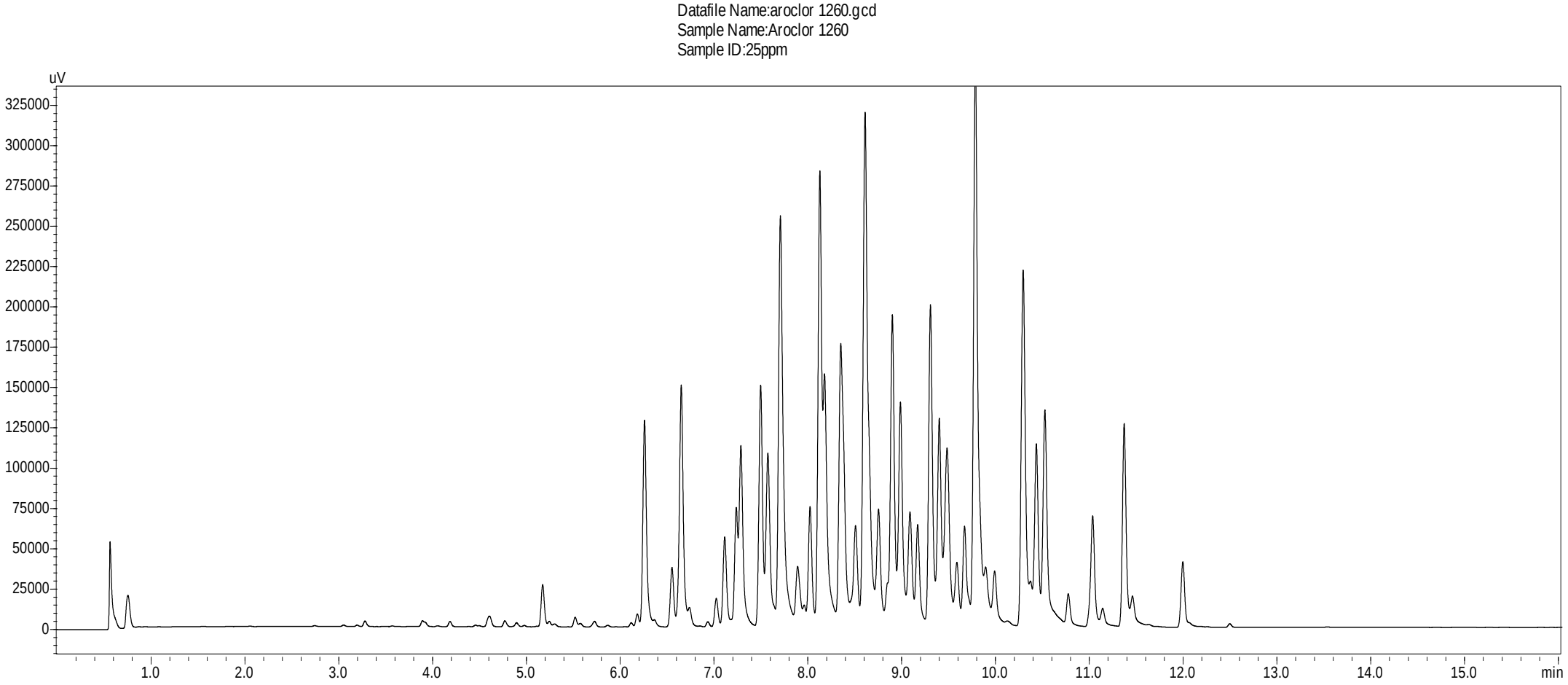
Pesticide requires GC-ECD with dual column confirmation, PCB require pattern recognition



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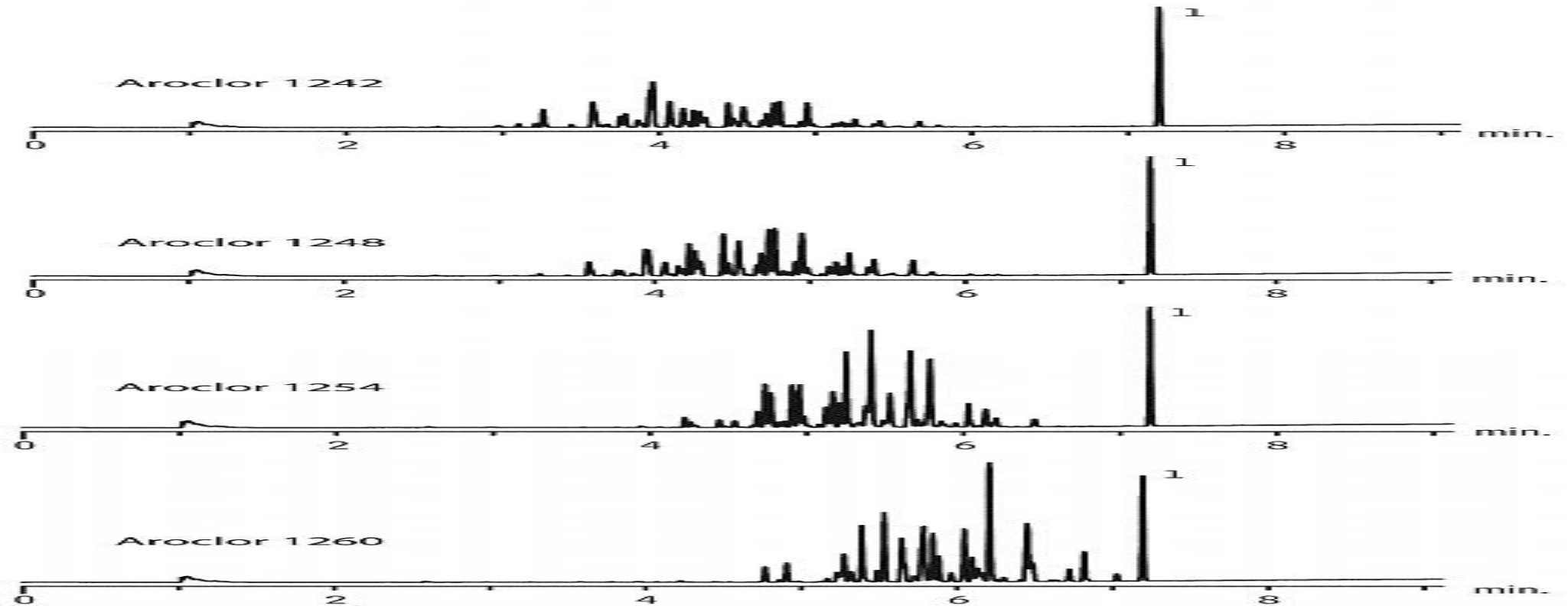
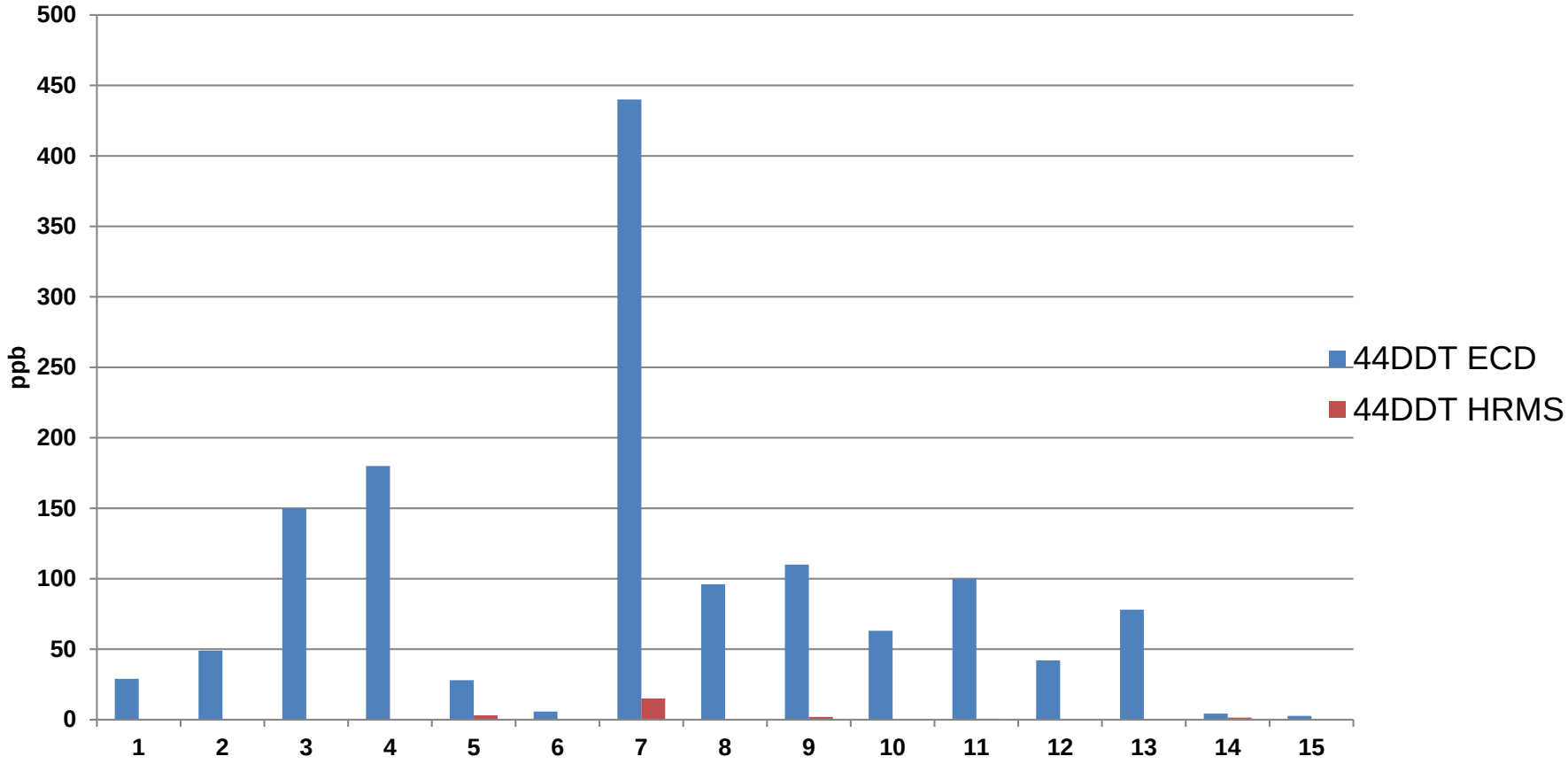
Pattern Recognition

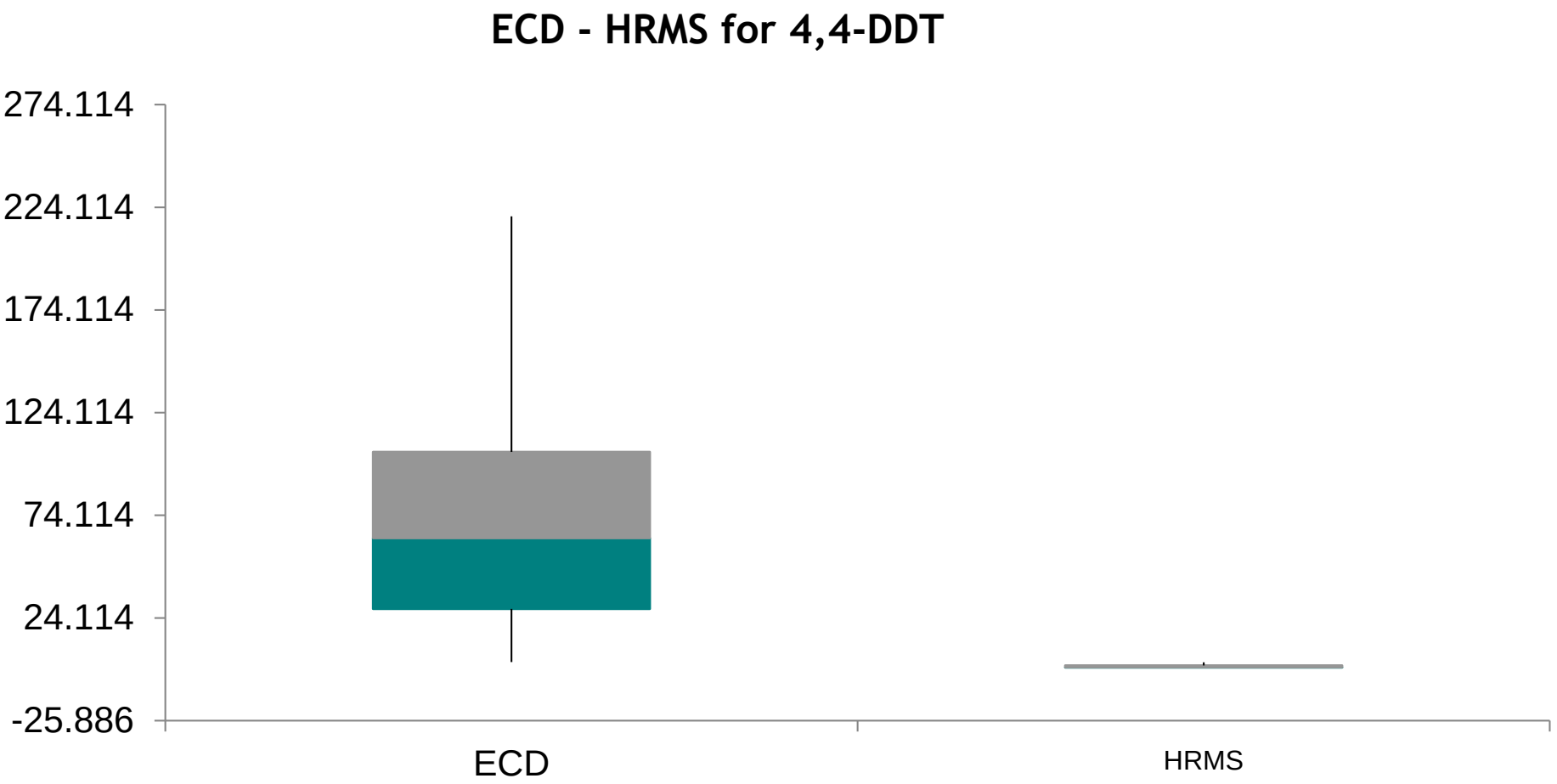


Interference of 4,4 DDT in PCB contaminated Soil using Method 8081



James J McAteer, Jr., and Erin Carroll Hughes; Bias in Organochlorine Pesticide Data, Comparison of Analysis by GC/ECD and HRGC/MSMS; NEMC 2014

Significant positive bias for DDT by GC-ECD



Method Development

- EPA 8270
  -Generic catch-all method
  -Uses GC/MS for ultimate identification, but causes a greatly reduced sensitivity (increased detection limit)
  -Detection limits 10 ug/L (Aroclor equivalents)
- Homolog Total Methods
  -Uses GC/MS in SIM for greater sensitivity and selectivity
  -Internal Standard for each level of chlorination
  -PCB defined as having peaks in QM channels and being within 15% of theoretical mass ratio
  -Quantifies on congener basis but not identified
  -Detection limits 0.001 ug/L on a congener basis

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Method Development

- New Method
  -Transfer method from GC-ECD to GC-triple quadrupole mass spectrometer (GC/MS/MS)

- Goals for Method Transfer:

  – All analytes from GC-ECD (PCBs/OCPs)

  – Shorter analysis time than GC-ECD

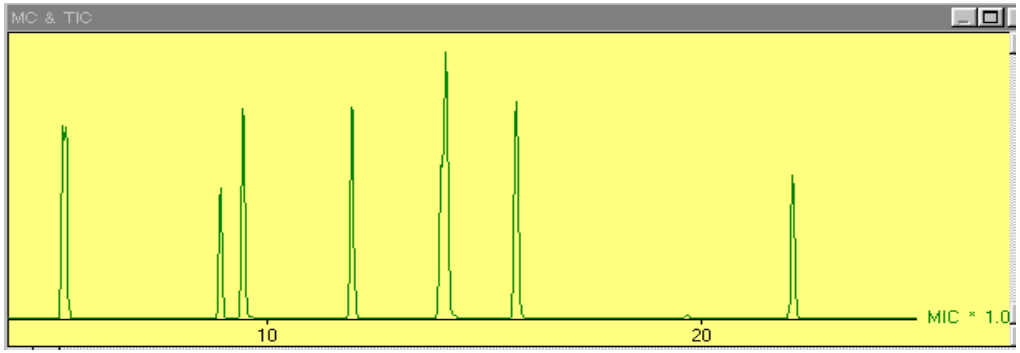
  - To decrease the total work required by the analyst for the analysis of so many compounds

  - Eliminate Matrix Interferences

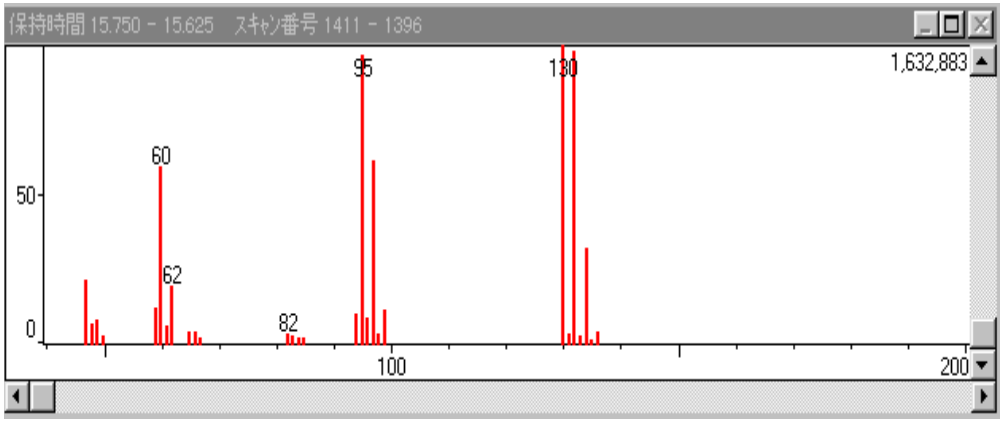
  - Provide Qualitative Information about the detected analytes, and

  - Achieve GC-ECD detection limits or better

Single quadrupole GCMS (full scan) is used for semi-volatile analysis



Gas Chromatograph: separation on a capillary column, identification by RT, and quantitation



Mass Spectrometer: positive identification by matching to a library

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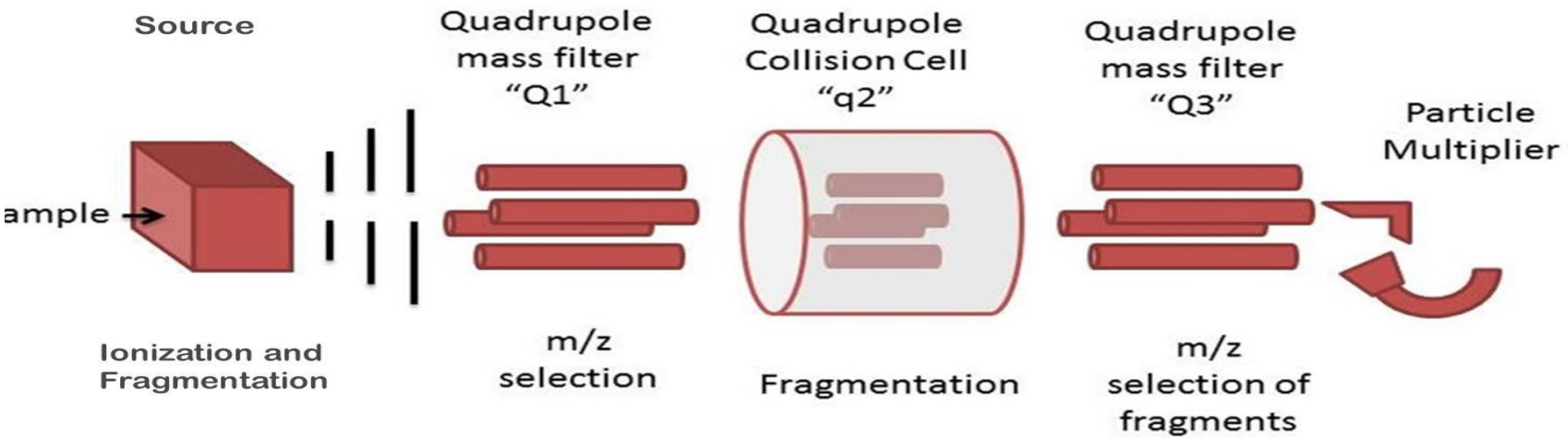
Triple Quadrupole GC-MS/MS



- Triple quadrupole GC-MS/MS a new technology is an obvious choice for targeted environmental analyses, due to high level of selectivity that the detector configuration is able to achieve.

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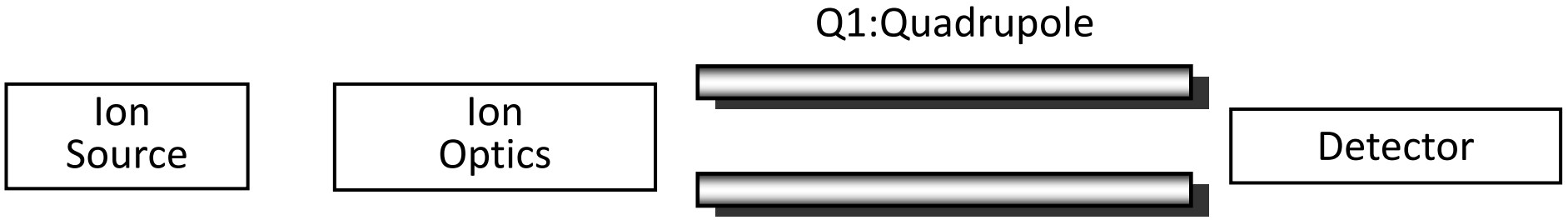
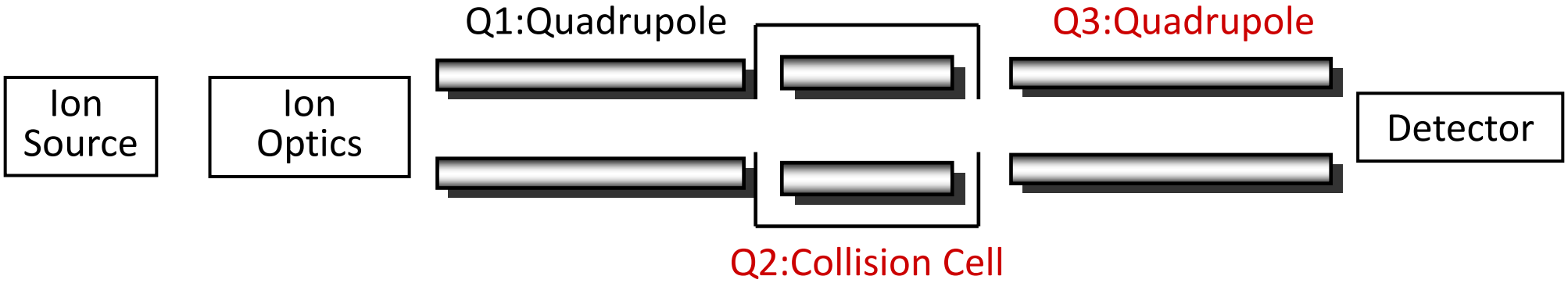
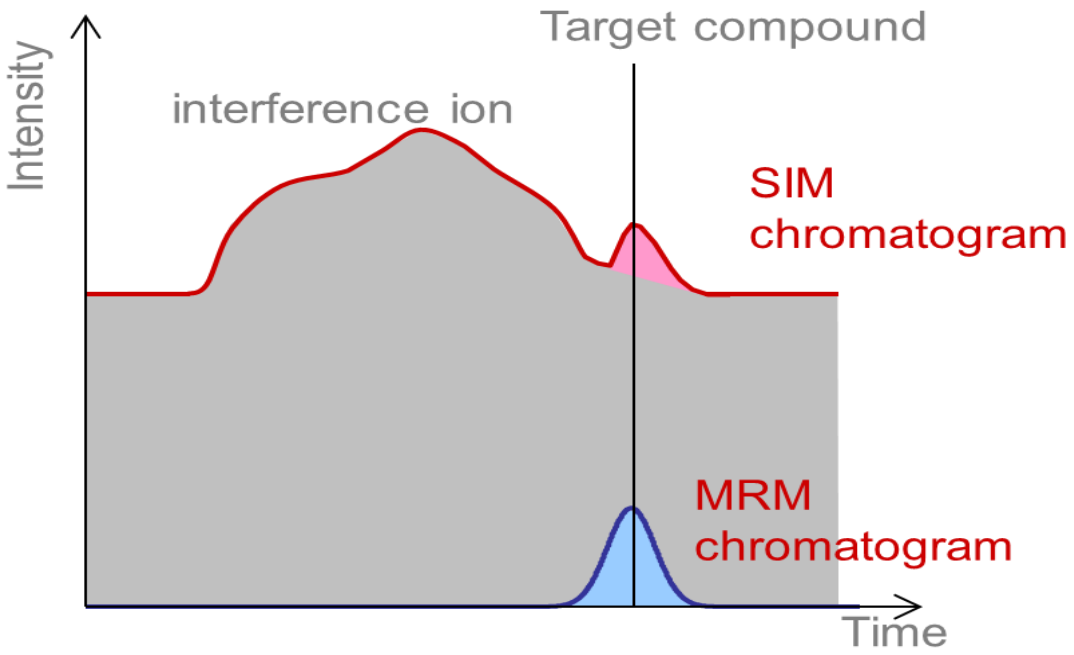
A triple Quadrupole adds a collision cell and another quadrupole





Benefits of MS/MS Detection Technique



Example of a SIM and MRM chromatogram showing improvement of MRM S/N ratio

- Chemical noise in the chromatography is virtually eliminated
- Greatly improving S/N and detector sensitivity

- RT selectivity (e.g. ECD)
- m/z selectivity (e.g. MS-SIM)
- **structure related selectivity** (e.g. …MSMS)

- **10x lower MDL than SIM**
  Extended linear range

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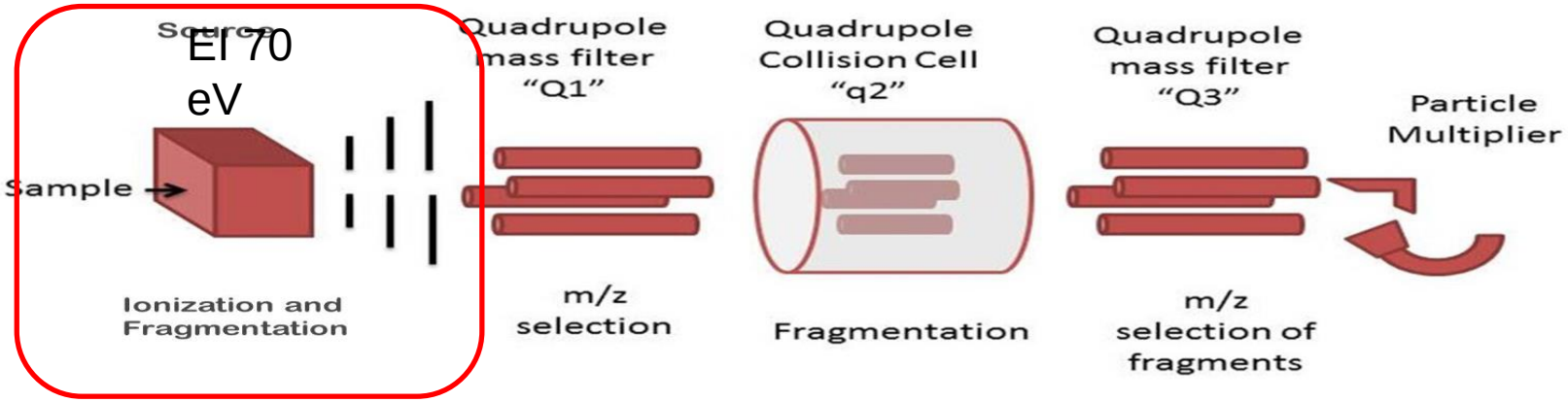
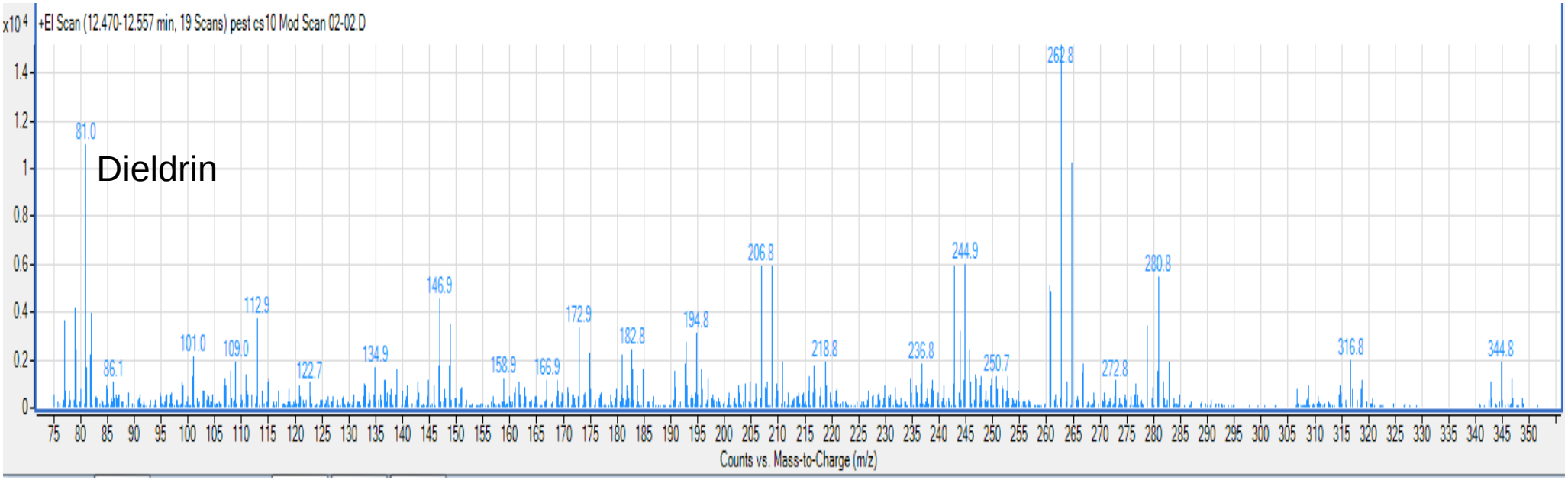
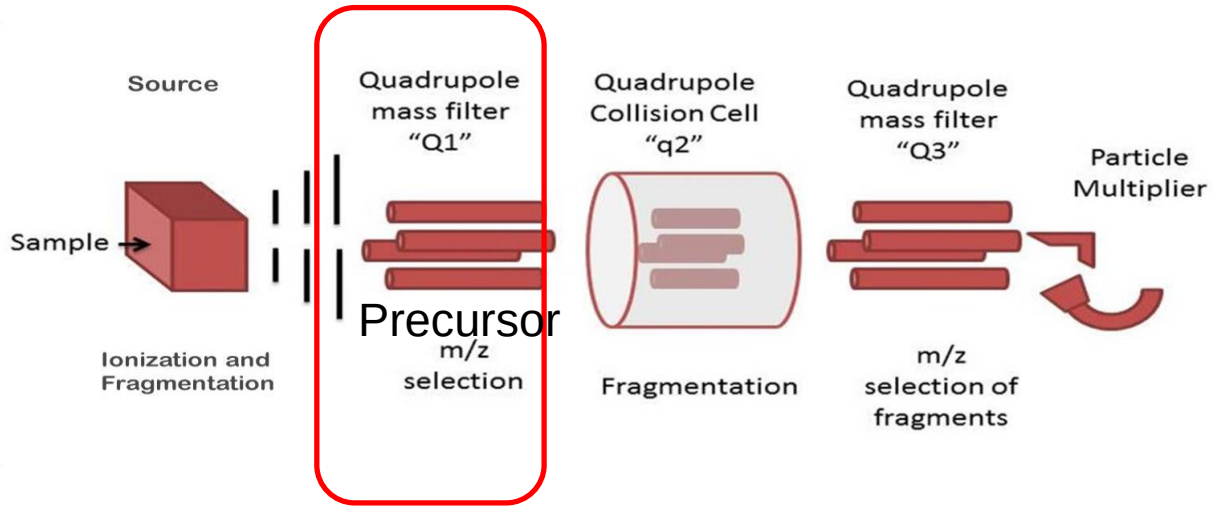
GC-MSMS Overview

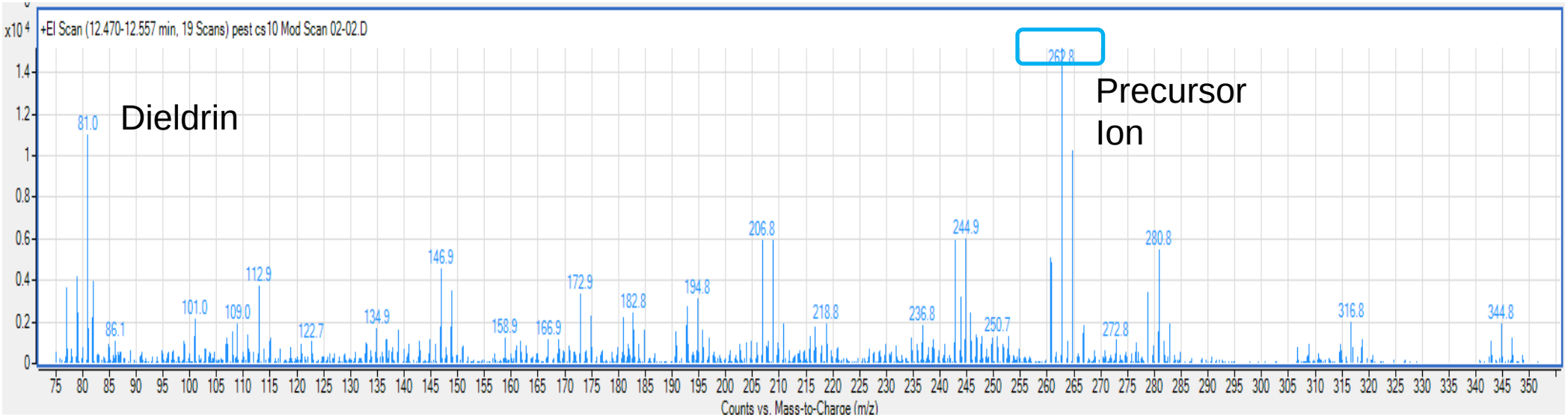




GC-MSMS Overview

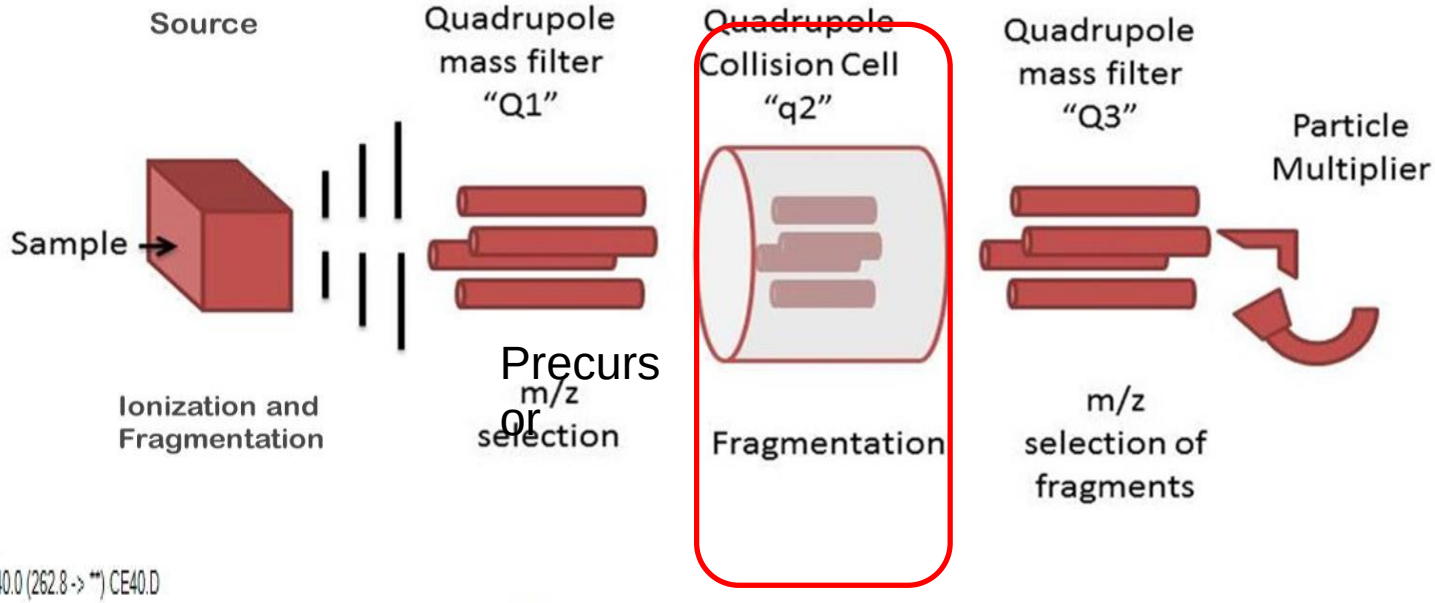
Structure Related Selec

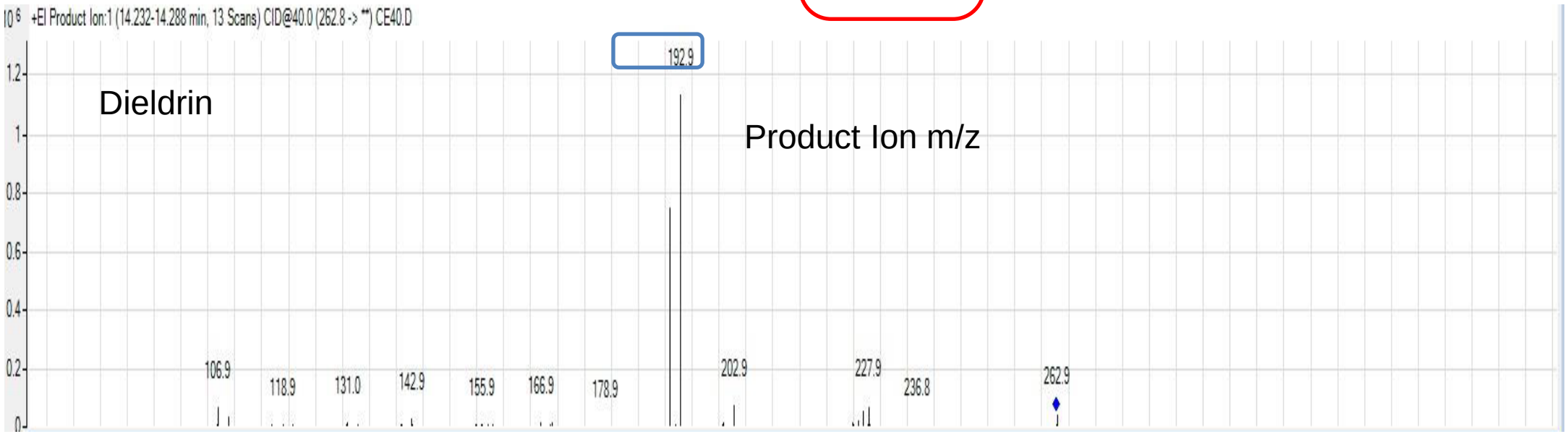




GC-MSMS Overview

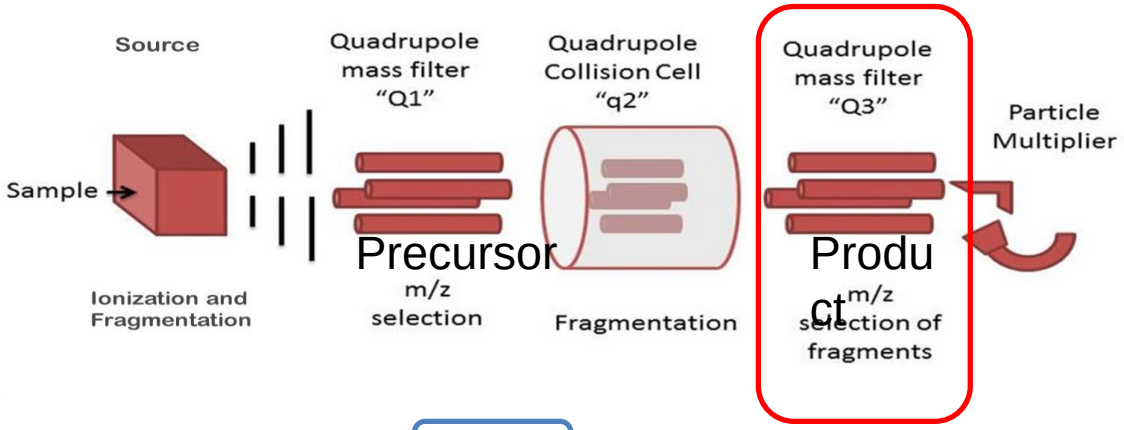
Structure Related Selectivity

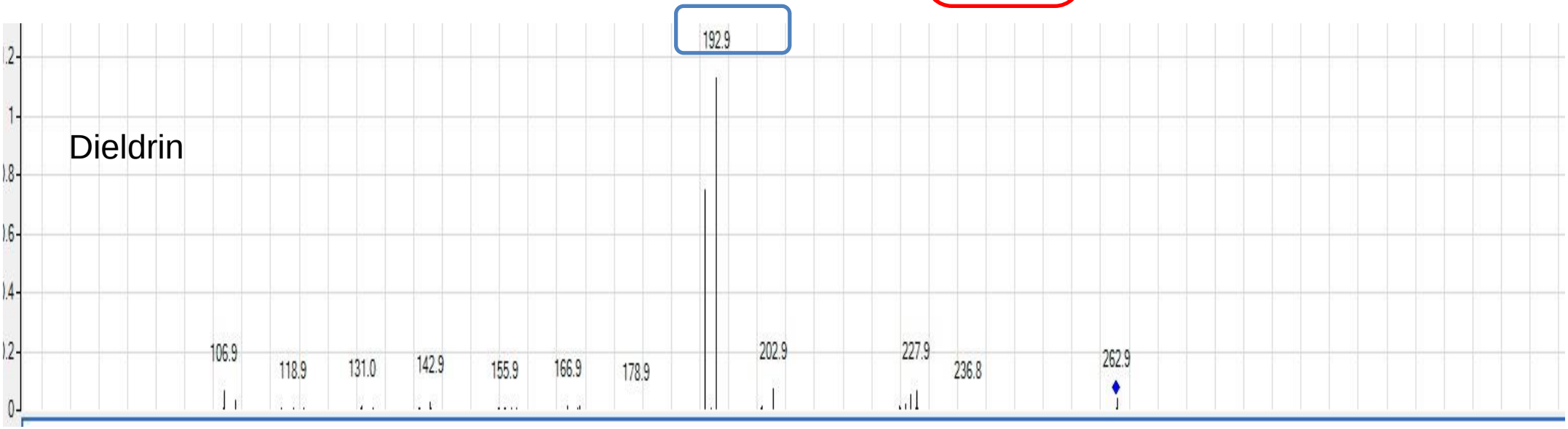




GC-MSMS Overview

Structure Related Selecti





MRM

GCMSMS Multiple Reaction Monitoring allows us to see lower concentrations with large dynamic range with less interference

Shimadzu Triple Quadrupole GC-MS/MS

2016
GCMS-TQ8050

S/N ≥ 40,000
IDL ≤ 0.5 fg

2014
GCMS-TQ8040

S/N ≥ 8,000
IDL ≤ 4 fg

2012
GCMS-TQ8030

S/N ≥ 5,000
IDL ≤ 6 fg

24

Features of GCMS-TQ8050

Enhanced Sensitivity

Durable Hardware

Superior Performance

Reliable Operation

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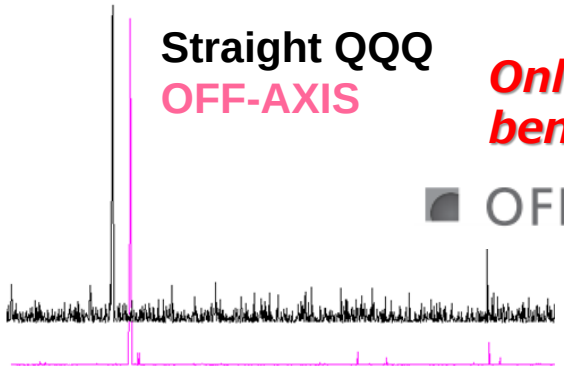
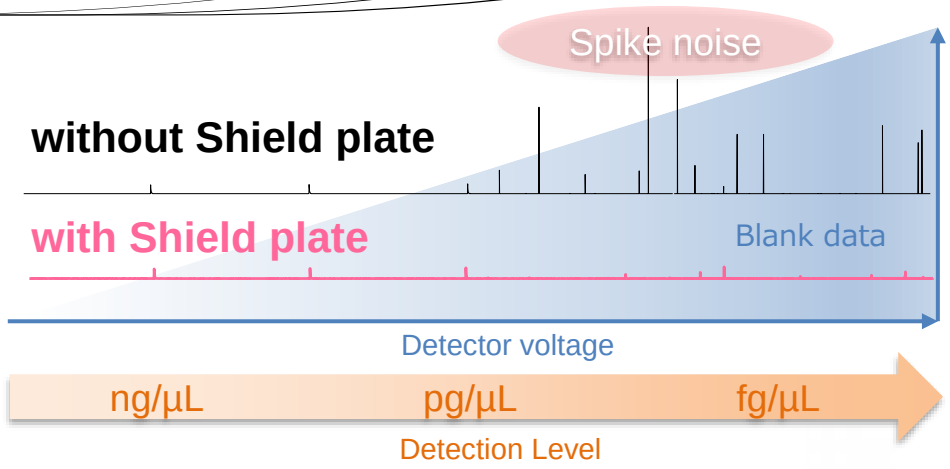
Enhanced Sensitivity with *UF-Transmission*

3 Novel Noise Reduction Systems makes lower baseline than ever!



Straight QQQ
OFF-AXIS

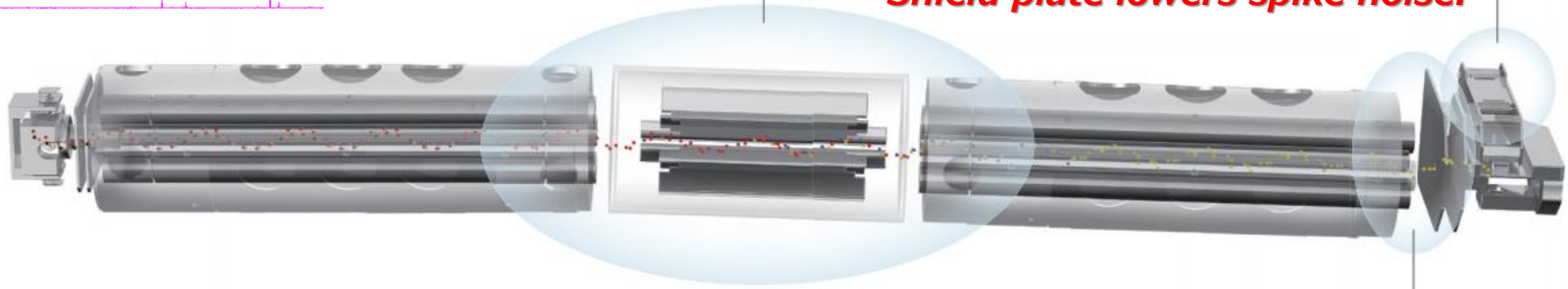
Only ions can pass through bended quadrupoles.

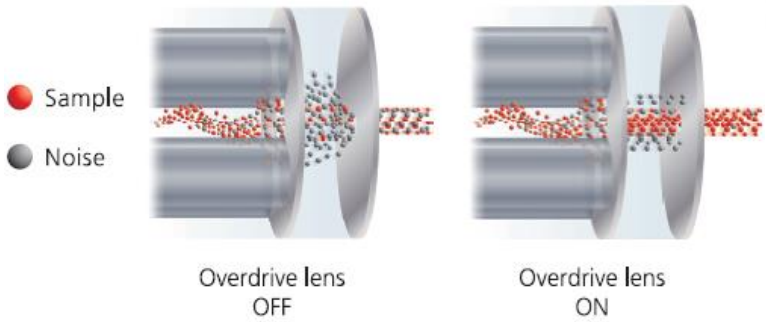


■ OFF-AXIS Ion Optics

■ Shielded Detector

Shield plate lowers spike noise.



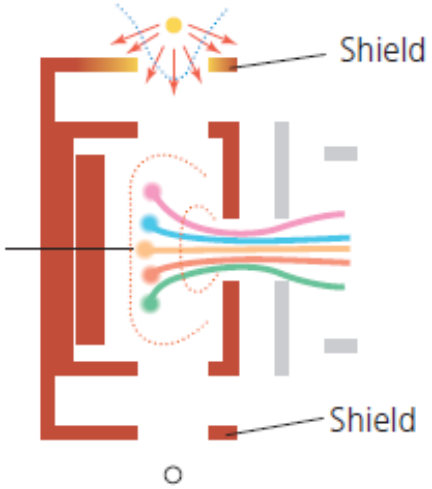


■ Overdrive Lens

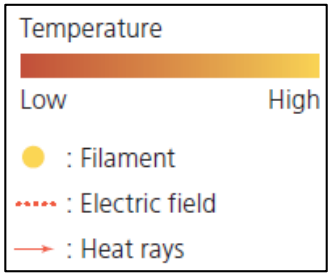
Overdrive lens takes out more noise.

Durable Hardware

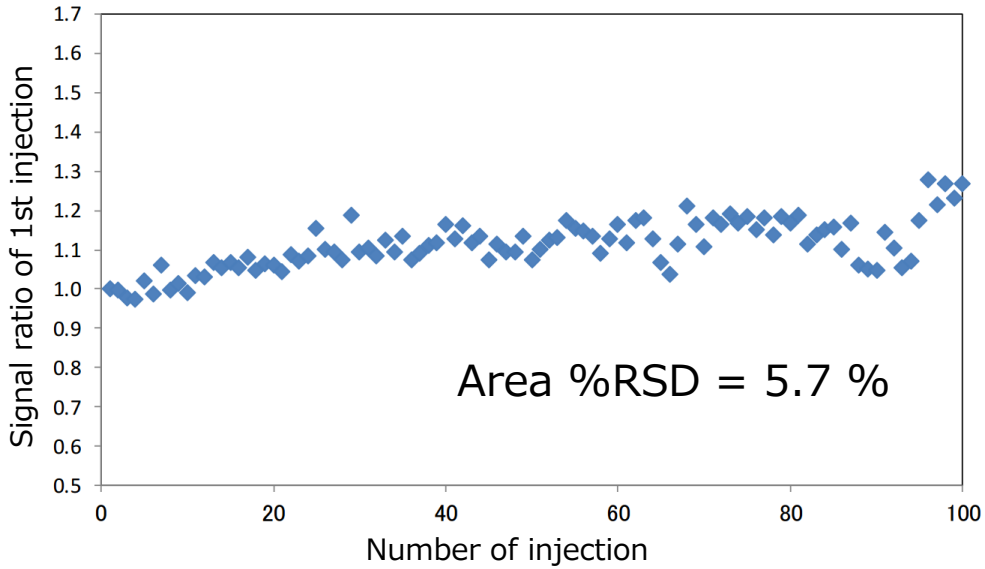
Patented Ion Source is hard to be contaminated!



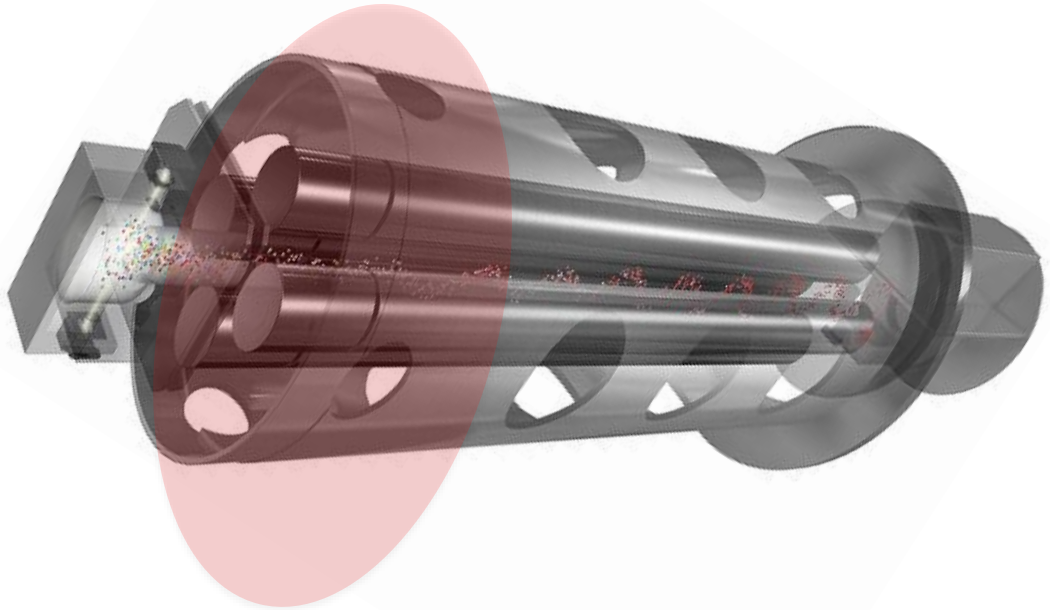
Shield in ion source prevents the box from contamination.



Fenitrothion 50ppb in Sesame oil extract



Quadrupoles are protected by Pre-Rods from contamination!



It's not necessary to change pre-rods. They are able to rotate and face to clean side.

Triple Quadrupole GC-MSMS Operating Conditions

Shimadzu GC-2010 Plus

– Injector
  - Pulsed Splitless
  - 275°C

- Column
  - Flow Rate: 1mL/min
  - Carrier Gas: Helium
  - Constant linear velocity, 43.5 cm/sec

Shimadzu GCMS-TQ 8050 Series

- Source
  - Electron Impact Extractor
  - 70eV

- Collision Cell
  - Argon (200kpa <1.0mL/min)
  - Helium (~1.1mL/min)
- Q1 and Q3
  - 230°C
  - MS1 resolution: 0.8amu
  - MS2 resolution: 0.8amu
- Detector:
  - Electron multiplier

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GC-MSMS Operating Conditions

Shimadzu GCMS-TQ8050

-Column -SH-Rxi-5 MS, 30 m x 0.25 mm x 0.25 µm

-Oven Program -50oC, hold 0.5 minute, 28oC/minute to 265oC, 3oC/minute to 285oC,
25oC/minute to 330oC, hold 1.0 minute

-Injector -Pulse Splitless (250kPa for 1.50 minutes, 2µL 275°C)

-Carrier Gas -Helium
-Constant linear velocity mode, 43.5 cm/sec

-Total Flow 30 mL/min, Column Flow = 1.44 mL/min Purge Flow 3.0 mL/min

-Interface Temperature -290 °C

Mass Spectrometer GCMS-TQ8050

-Ion Source Temperature -230 °C

-MS Operating Mode -Acquisition Mode, MRM

-CID gas, Argon

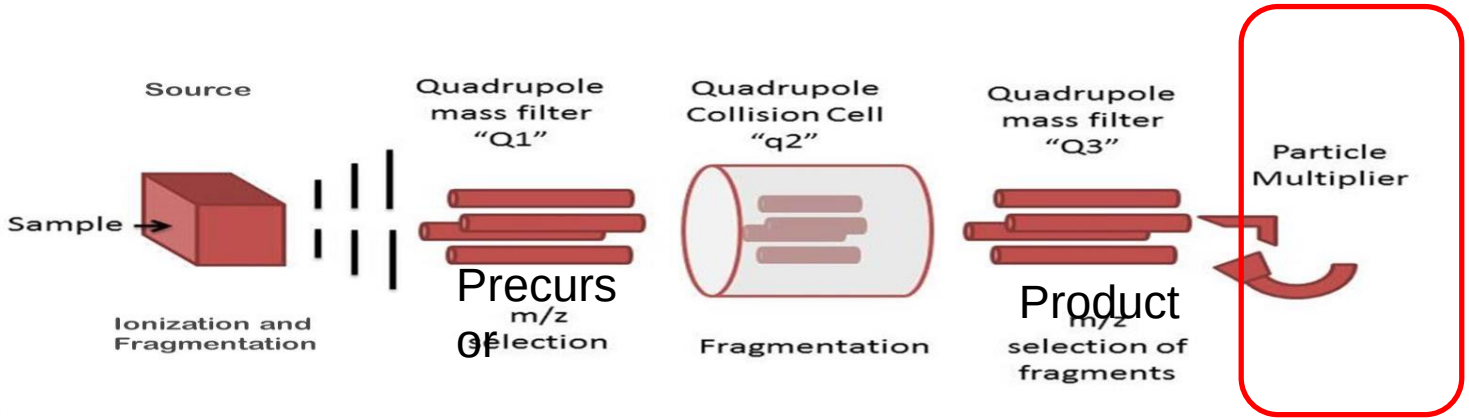
-Solvent cut Time, 1.35 minutes

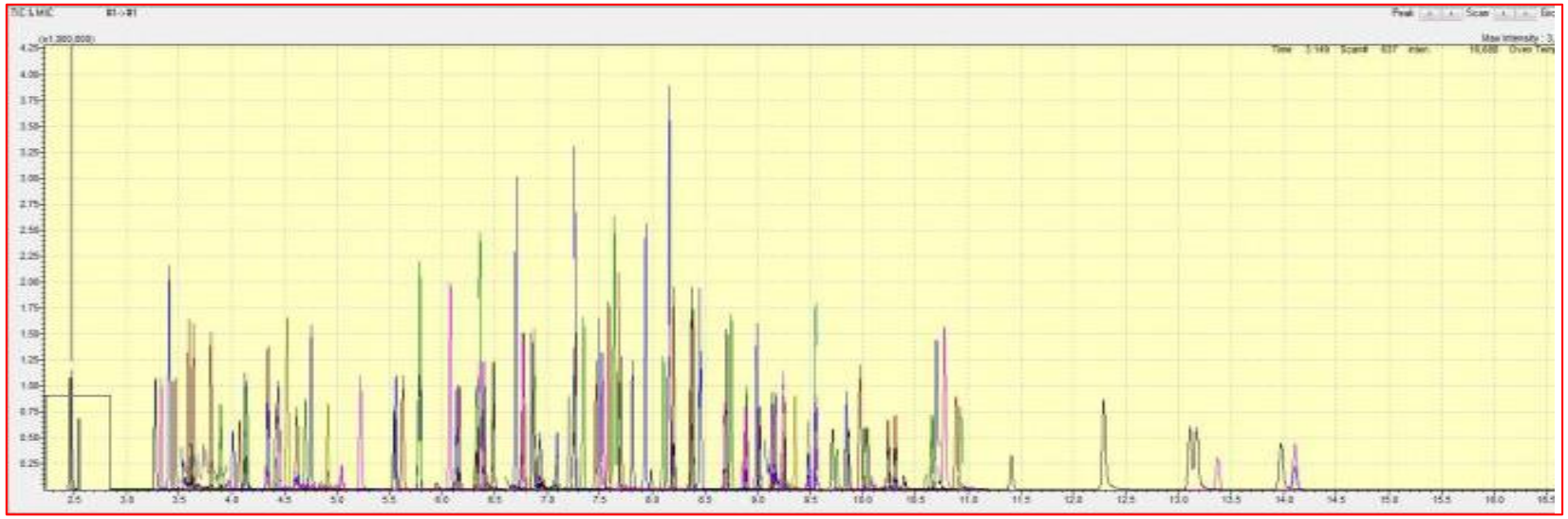
-Detector voltage Relative to Tune , 0.550kV

-Threshold = 0

-Analysis Times - 17.65 minutes
-GC Cycle Time - 22.00 minutes
-

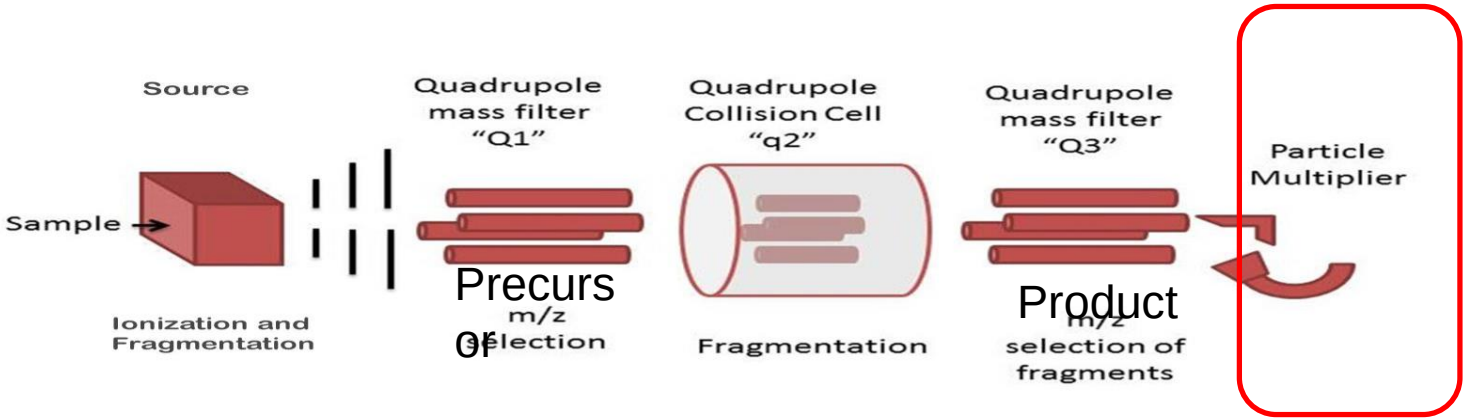
GC-MSMS Overview

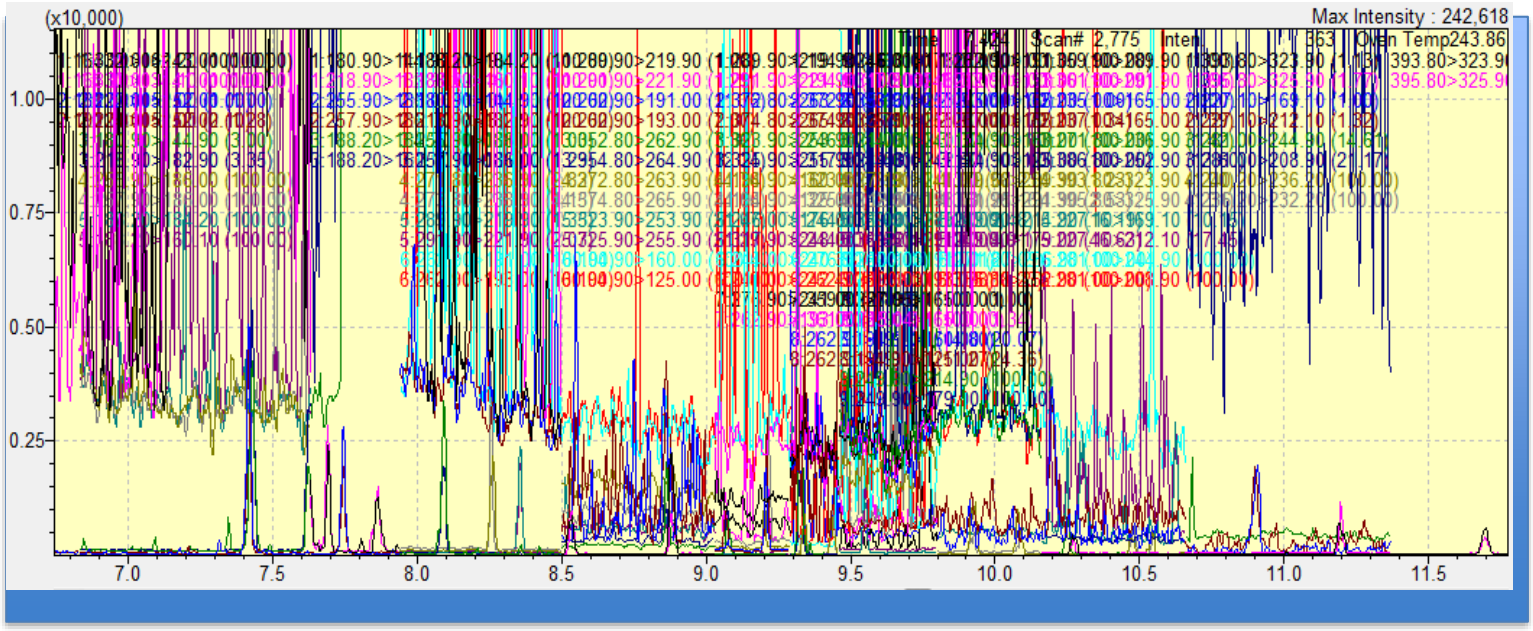




TQ8040 Mid-point standard MRM chromatogram from the 50ug/L calibration standard

GC-MSMS Overview





TQ8050
Low-point standard MRM chromatogram from the 0.10ug/L calibration standard

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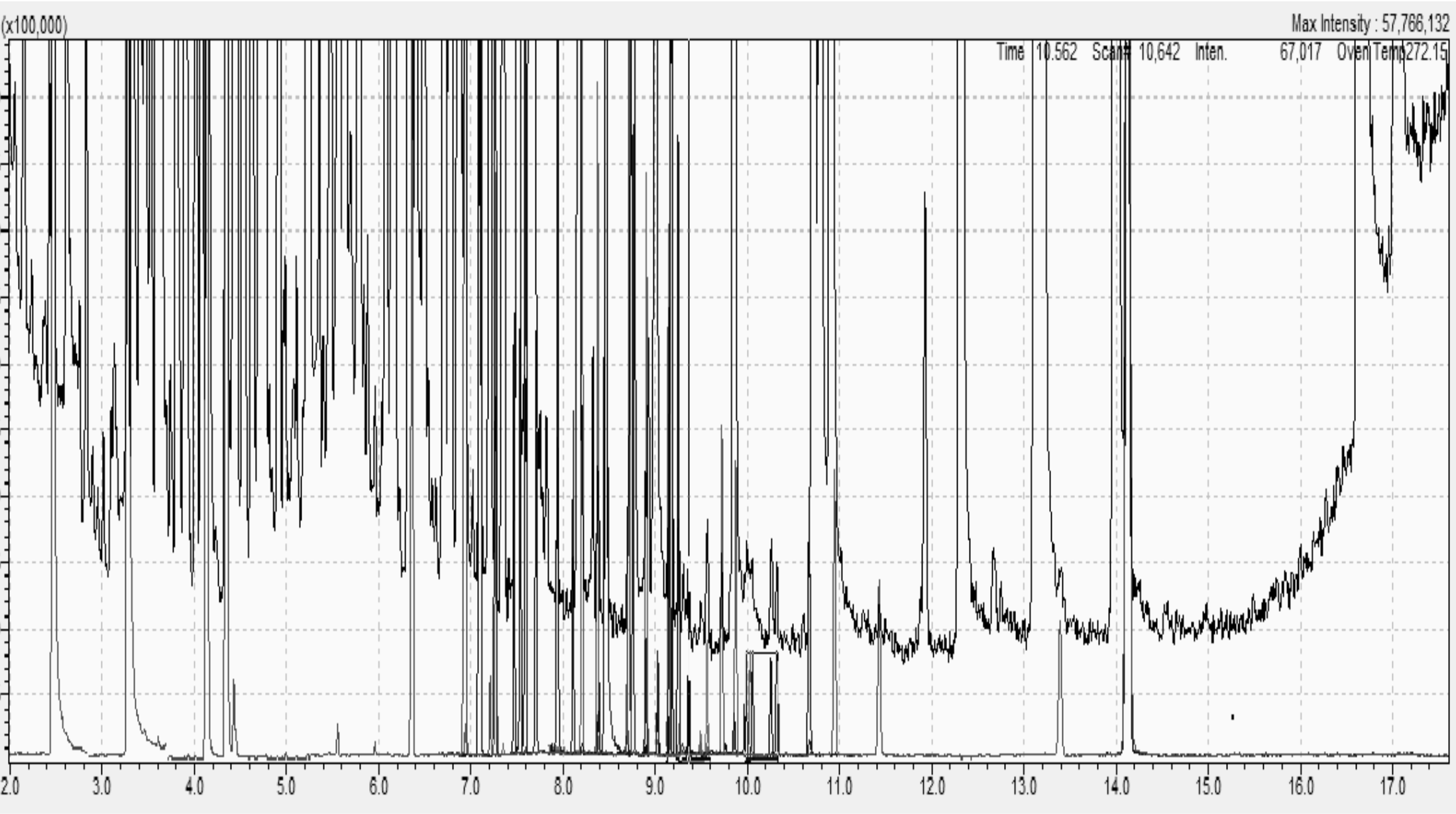
MRM peaks buried in matrix showing matrix removal by TQ technique



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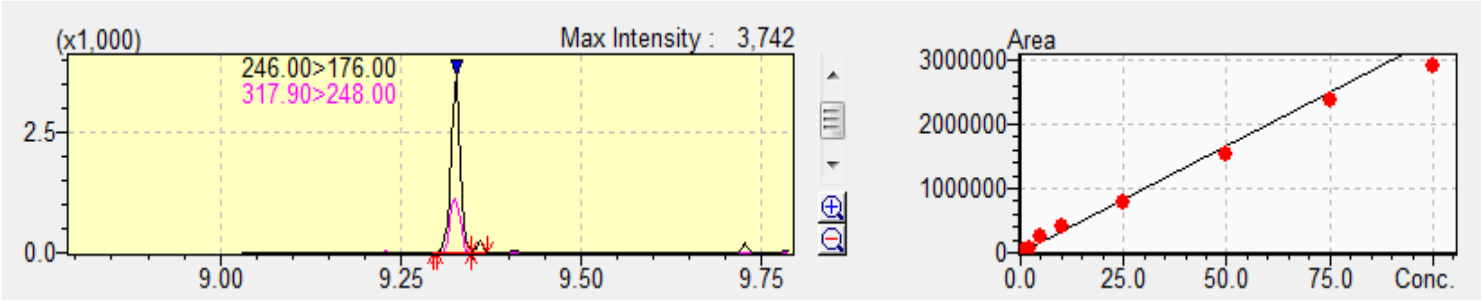
Advantages and disadvantages for MRM analysis of pesticides

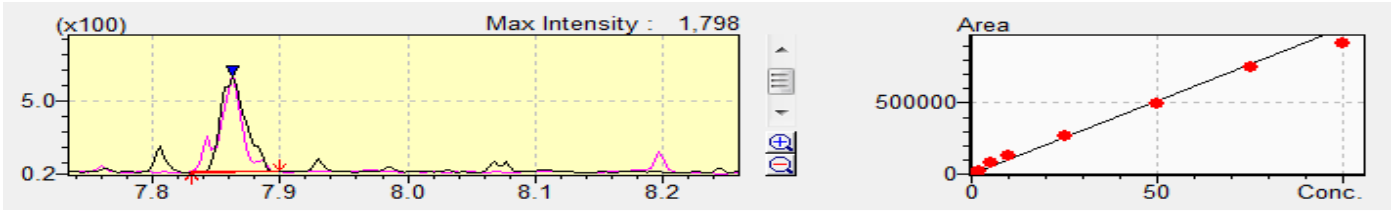
Advantages	Disadvantages
More Selective than ECD for pesticides analysis	Not EPA approved
Low detection limits	No ILS data
Run Pesticides from Semi-volatile extract	

TQ8050:Calibration Curves For Pesticides For a Selection of Target Compounds 0.1 ppb Standard



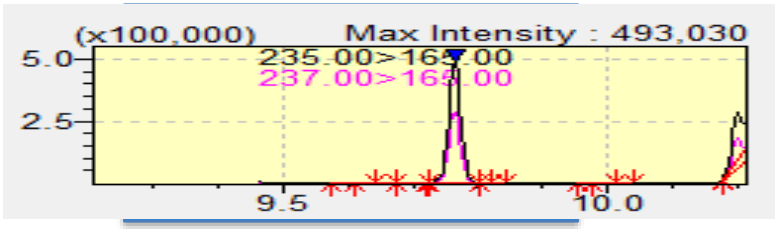
4,4'-DDE

RF %RSD : 21.735

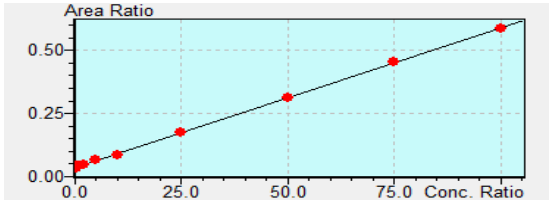


delta-BHC

RF %RSD : 24.79648



4,4'-DDD



RF %RSD = 20.291

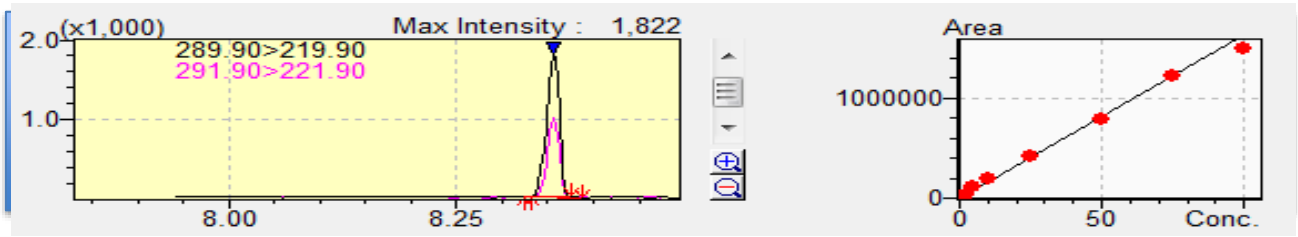
Calibration Curves

Calibration curves were produced in the range of 0.1 µg/L to 100 µg/L for the OCPs and PCBs.

All curves had a regression coefficient higher than 0.995. Curves for a selection of target compounds are plotted

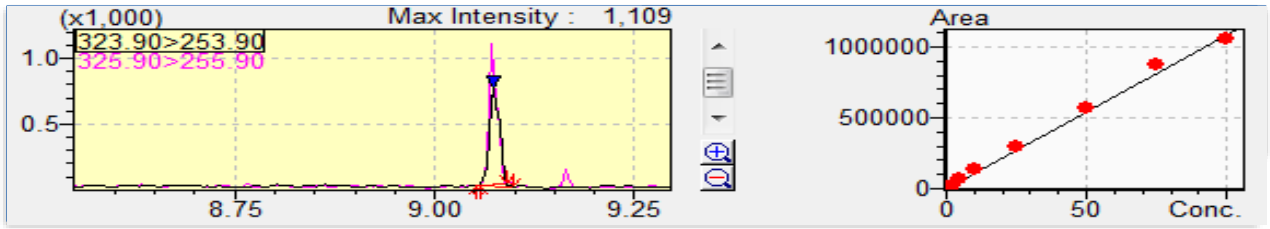
Level	Concentration
1	0.1
2	0.2
3	0.5
4	1.0
5	2.0
6	5.0
7	10.0
8	25
9	50
10	75
11	100

TQ8050:Calibration Curves For PCB Congeners For a Selection of Target Compound 0.1 ppb Standard



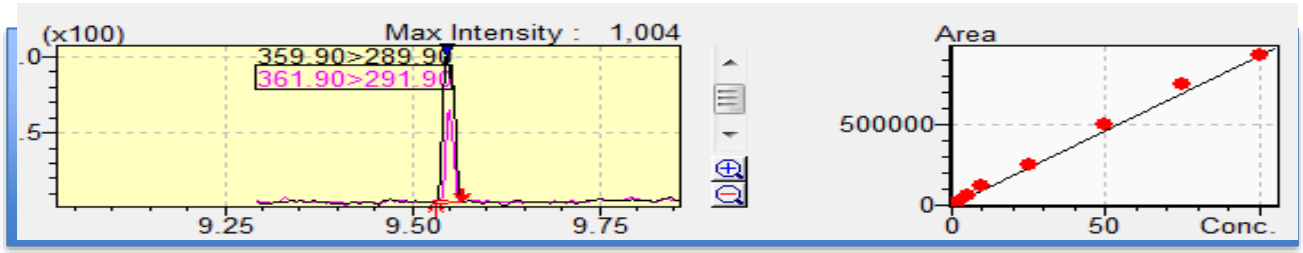
2,2',3,5'-Tetrachlorobiphenyl (#44)

RF %RSD=14.19



2,2',4,5,5'-Pentachlorobiphenyl (#101)

RF %RSD = 20.91



2,2',3,5,5',6-Hexachlorobiphenyl (#151)

RF %RSD = **22.81**

Calibration Curves

Calibration curves were produced in the range of 0.1 µg/L to 100 µg/L for the PCBs.

All curves had a regression coefficient higher than 0.995. Curves for a selection of target compounds are plotted

Level	Concentration
1	0.1
2	0.2
3	0.5
4	1.0
5	2.0
9	50
10	75
11	100

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TQ8050 : Method Detection Limit (MDL) Study Results

MDL compared to method 608 MDL For Organochlorine Pesticides

Component	Estimated MDL (assumes 1000ml/1mL) ug/L		
	0.5 ppb	1 ppb	Method
	TQ8050	TQ8050	608 MDL ug/L
alpha-BHC	0.0001	0.0003	0.003
gamma-BHC (Lindane)	0.0002	0.0006	0.004
beta-BHC	0.0001	0.0002	0.006
delta-BHC	0.0001	0.0004	0.009
Heptachlor	0.0001	0.0003	0.003
Aldrin	0.0002	0.0006	0.004
Heptachlor epoxide (isomer B)	0.0002	0.0005	0.083
trans-Chlordane	0.0002	0.0006	0.014
cis-Chlordane	0.0002	0.0005	0.014
Endosulfan I	0.0003	0.0010	0.014
4,4'-DDE	0.0002	0.0005	0.011
Dieldrin	0.0004	0.0013	0.002
Endrin	0.0004	0.0012	0.006
4,4'-DDD	0.0002	0.0005	0.012
Endosulfan II	0.0003	0.0009	0.004
Endrin aldehyde	0.0003	0.0008	0.023
4,4'-DDT	0.0001	0.0002	0.004
Endosulfan sulfate	0.0001	0.0003	0.004
Methoxychlor	0.0001	0.0003	
Endrin ketone	0.0002	0.0005	

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TQ8050: Method Detection Limit (MDL) Study Results

MDL compared to method 608 MDL For PCB Congeners

Component	Estimated MDL (assumes 1000ml/1mL) ug/L		
	0.5 ppb	1	Method
	TQ8050	TQ8050	608 MDL ug/L
2-Chlorobiphenyl (#1)	0.0001	0.0002	0.065*
2,3-Dichlorobiphenyl (#5)	0.0001	0.0002	
2,2',5-Trichlorobiphenyl (#18)	0.0001	0.0002	
2,2',5,5'-Tetrachlorobiphenyl (#52)	0.0001	0.0003	
2,4',5-Trichlorobiphenyl (#31)	0.0001	0.0003	
2,2',3,5'-Tetrachlorobiphenyl (#44)	0.0001	0.0003	
2,3',4,4'-Tetrachlorobiphenyl (#66)	0.0001	0.0003	
2,2',4,5,5'-Pentachlorobiphenyl (#101)	0.0001	0.0004	
2,2',3,4,5'-Pentachlorobiphenyl (#87)	0.0001	0.0004	
2,3,3',4',6-Pentachlorobiphenyl (#110)	0.0001	0.0003	
2,2',3,5,5',6-Hexachlorobiphenyl (#151)	0.0002	0.0006	
2,2',4,4',5,5'-Hexachlorobiphenyl (#153)	0.0001	0.0004	
2,2',3,4,5,5'-Hexachlorobiphenyl (#141)	0.0001	0.0004	
2,2',3,4,4',5'-Hexachlorobiphenyl (#138)	0.0001	0.0004	
2,2',3,3',4,4',5-Heptachlorobiphenyl (#170)	0.0001	0.0002	
2,2',3,4,4',5,5'-Heptachlorobiphenyl (#180)	0.0001	0.0003	
2,2',3,4,4',5',6-Heptachlorobiphenyl (#183)	0.0002	0.0005	
2,2',3,4',5,5',6-Heptachlorobiphenyl (#187)	0.0002	0.0006	
2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl (#206)	0.0001	0.0003	

*MDL given in method 608 for Aroclor (PCB-1242)

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TQ8050: %REC Results of Spiked PCB Congeners

Component				
	1 ppb	2 ppb	25 ppb	50 ppb
	TQ8050			
	%REC	%REC	%REC	%REC
2-Chlorobiphenyl (#1)	87	86	109	102
2,3-Dichlorobiphenyl (#5)	85	88	101	99
2,2',5-Trichlorobiphenyl (#18)	91	90	101	99
2,2',5,5'-Tetrachlorobiphenyl (#52)	95	91	103	97
2,4',5-Trichlorobiphenyl (#31)	88	93	98	95
2,2',3,5'-Tetrachlorobiphenyl (#44)	84	95	106	100
2,3',4,4'-Tetrachlorobiphenyl (#66)	92	96	98	93
2,2',4,5,5'-Pentachlorobiphenyl (#101)	88	107	108	104
2,2',3,4,5'-Pentachlorobiphenyl (#87)	94	92	109	108
2,3,3',4',6-Pentachlorobiphenyl (#110)	93	96	104	101
2,2',3,5,5',6-Hexachlorobiphenyl (#151)	85	98	109	108
2,2',4,4',5,5'-Hexachlorobiphenyl (#153)	90	89	104	100
2,2',3,4,5,5'-Hexachlorobiphenyl (#141)	80	99	110	106
2,2',3,4,4',5'-Hexachlorobiphenyl (#138)	96	100	108	105
2,2',3,3',4,4',5-Heptachlorobiphenyl (#170)	84	88	104	99
2,2',3,4,4',5,5'-Heptachlorobiphenyl (#180)	87	86	105	98
2,2',3,4,4',5',6-Heptachlorobiphenyl (#183)	90	94	110	105
2,2',3,4',5,5',6-Heptachlorobiphenyl (#187)	92	103	112	109
2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl (#206)	88	91	122	108

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TQ8050 : %REC of Spiked Organochlorine Pesticides

Component	1 ppb	2 ppb	25 ppb	50
	%REC	%REC	%REC	%REC
alpha-BHC	88	92	94	87
gamma-BHC (Lindane)	82	88	102	93
beta-BHC	86	91	97	90
delta-BHC	87	95	104	95
Heptachlor	90	100	99	92
Aldrin	100	90	89	79
Heptachlor epoxide (isomer B)	96	99	104	94
trans-Chlordane	99	93	97	92
cis-Chlordane	97	82	93	85
Endosulfan I	98	84	104	90
4,4'-DDE	78	96	96	91
Dieldrin	104	65	96	92
Endrin	108	97	96	87
4,4'-DDD	86	85	91	86
Endosulfan II	105	78	100	94
Endrin aldehyde	106	62	102	101
4,4'-DDT	76	84	96	91
Endosulfan sulfate	84	100	108	98
Methoxychlor	80	83	102	100
Endrin ketone	50	91	106	97

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ASTM will validate MRM analysis of pesticides in $MeCL_2$, and compare to Method 608

1. Minimum 9 matrices
2. MS/MSD
3. MDL
4. Comparison to ECD

5. See if acid-base neutral recoveries equal as received recoveries.
6. Multiple laboratory study of 608 list + PCBs and congeners

IDL’s are low enough that bigger split or less sample may be extracted – increasing life of liner and column.

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Advantages of MRM quantitation of Method 608 Pesticides and PCBs

- Fewer interferences
- Detection limits similar to GC-ECD
- No need to exchange to hexane
- Can run 625 and pesticides/PCB on one instrument
- May be possible to use acid / BN extraction for pesticides

Conclusions

- A sensitive and reliable GC/MS/MS method has been developed on Shimadzu GCMS-TQ8050
- No dual column needed to confirm the identity of peaks.
- Interferences eliminated through careful selection of precursor and product ions in the MSMS method. Targets masked in ECD chromatograms by interference could be identified.
- One set of calibration standards containing all the target compounds is needed.
- New method therefore would increase productivity by combining three separate analysis of OCPs, PAHs and PCBs into one, from three injections down to one injection.
- New method provides required MDLs and precision for analysis of 48 OCPs and PCBs studied in this application.
- Method is rapid and robust, with a run time as little as 17 minutes and a GC cycle time of 20 minutes

Summary

- In summary-Quantitative performance of Shimadzu GCMS-TQ8050 and methodology was excellent with a good level of linearity, excellent sensitivity, and high precision and is a suitable alternative to Organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) analysis by GC-ECD.

- Shimadzu GCMS-TQ8050 is a great instrument for targeted analysis in a variety of environmental matrices and the GC/MSMS Pesticides Method may be scheduled for inclusion in the next round of routine performance test analysis, with the intention of adding to laboratory's accreditation in the near future.

- For more detailed information please refer my app note published recently, see below

  -Determination of Organochlorine Pesticides and Polychlorinated Biphenyls Using GC/MS/MS Operated in the MRM (TQ8040) Shimadzu App Note No. GCMS-1601

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