Benefits and Characteristic Applications of High Resolution GC/MS and LC/MS.

Frank David

RIC and Ghent University

RIC | Research Institute for Chromatography

Mass Spectrometry

Structure Elucidation

Identification

Selective and Sensitive Detection

Target Compound Analysis

RIC | Research Institute for Chromatography

Mass Spectrometry

Structure Elucidation

Identification (NIST, Wiley)

Selective and Sensitive Detection

Target Compound Analysis

RIC | Research Institute for Chromatography

General Trend in Trace Analysis: from SIM (MS) to MRM (QqQ)

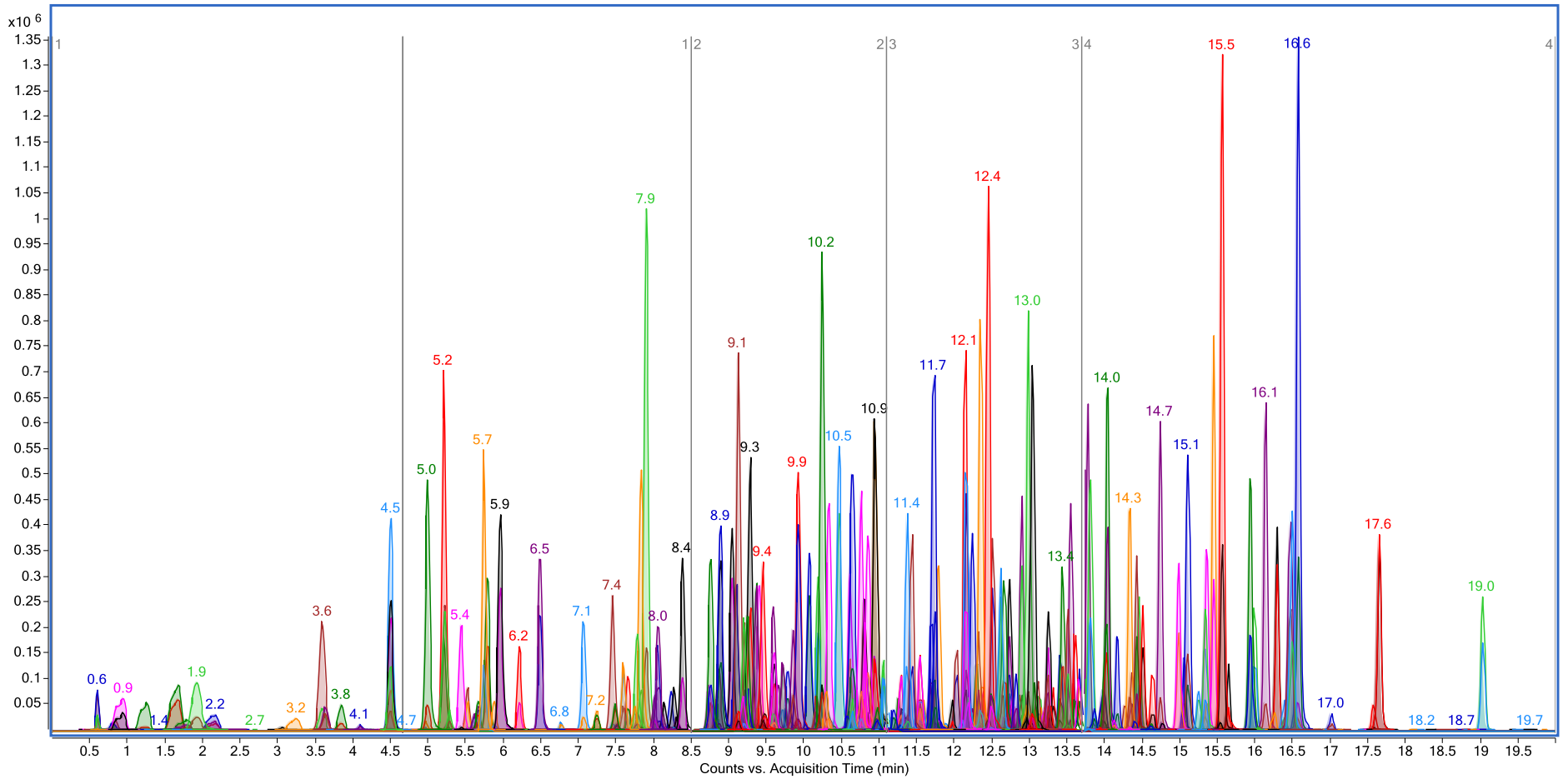
Application: pesticides in food

- Driven by EU regulation and SANCO guidelines:
  - Baby food: 10 µg/kg
  - SANCO: scan or 4 ions or 2 transitions (for confirmation)

- GC/LC-SCAN-MS: sensitivity?
- GC/LC-SIM-MS: interference on 1 or more ions
- GC/LC-MS/MS in MRM mode

RIC Research Institute for Chromatography

Dynamic MRM of 300 Pesticides - 2 Transitions Each



1290 UHPLC: **Eclipse Plus-C18, 2.1 x 100mm, 1.8 µm**

RIC | Research Institute for Chromatography

Mass Spectrometry

Structure Elucidation

Identification (NIST, Wiley)

Selective and Sensitive Detection

Target Compound Analysis by MS/MS

RIC | Research Institute for Chromatography

Mass Spectrometry

Structure Elucidation

Identification: accurate mass

"Open Methods"

Selective and Sensitive Detection

Target Compound Analysis by MS/MS

RIC | Research Institute for Chromatography

GC with high resolution TOF (Agilent 7200 GC/Q-TOF)

Accurate mass: high selectivity

High sensitivity (10 x MSD)

“Scan” mode: identification

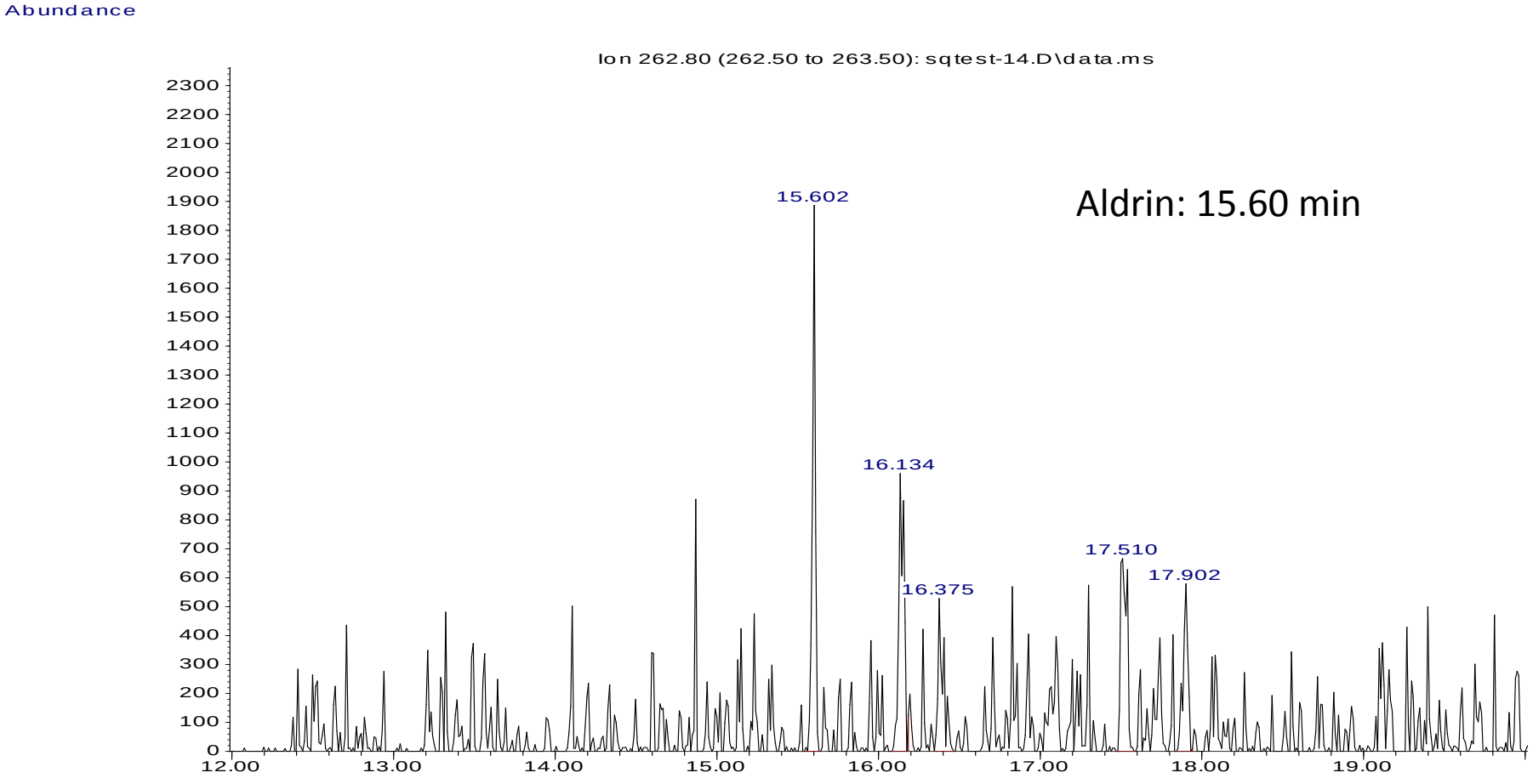
“EIC” at accurate mass: quantification

+ “retrospective” analysis (“open” methods)

RIC | Research Institute for Chromatography

Analysis by GC-MSD (5975C) - SCAN

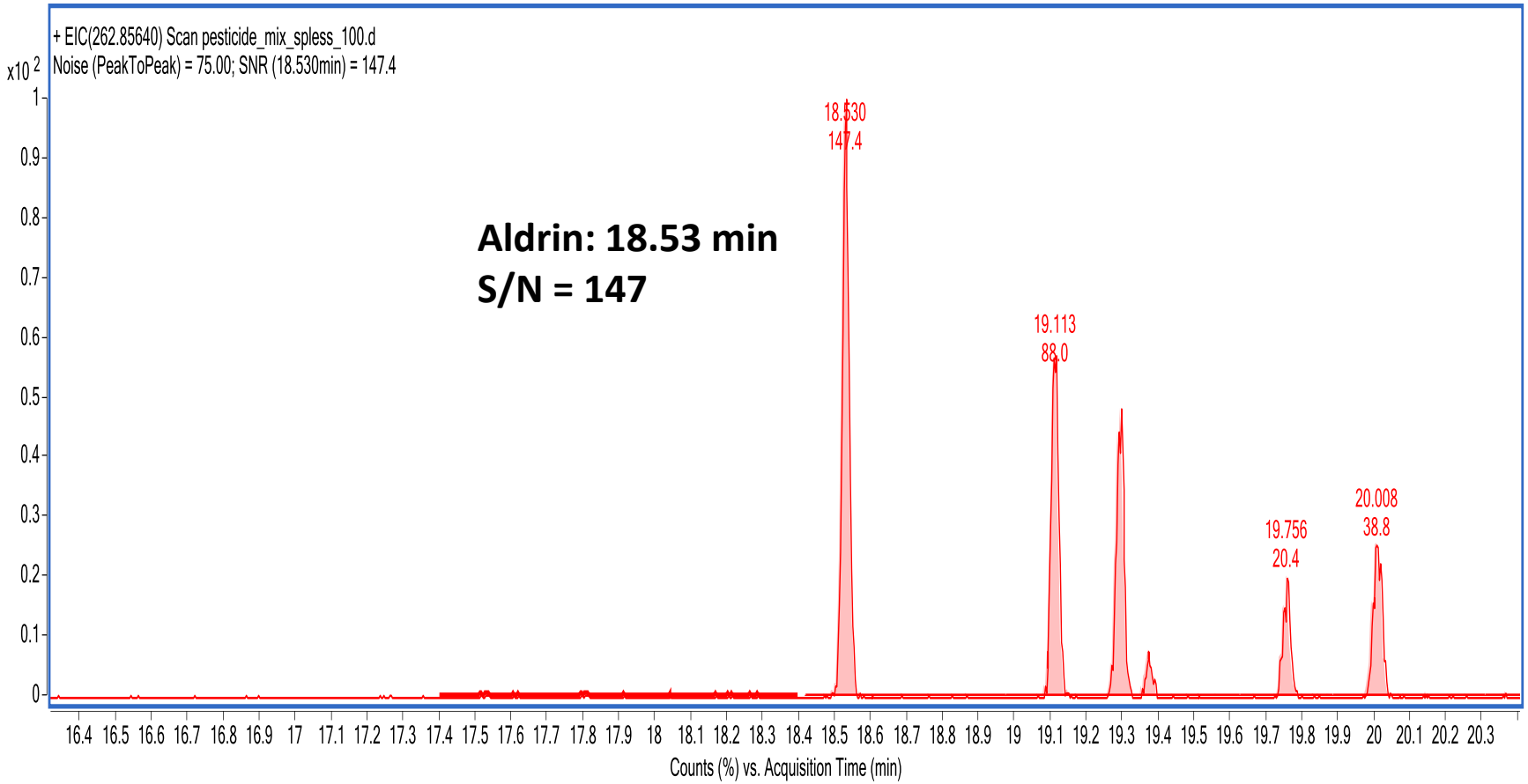
10 pg on-column - EIC (262.8)



RIC Research Institute for Chromatography

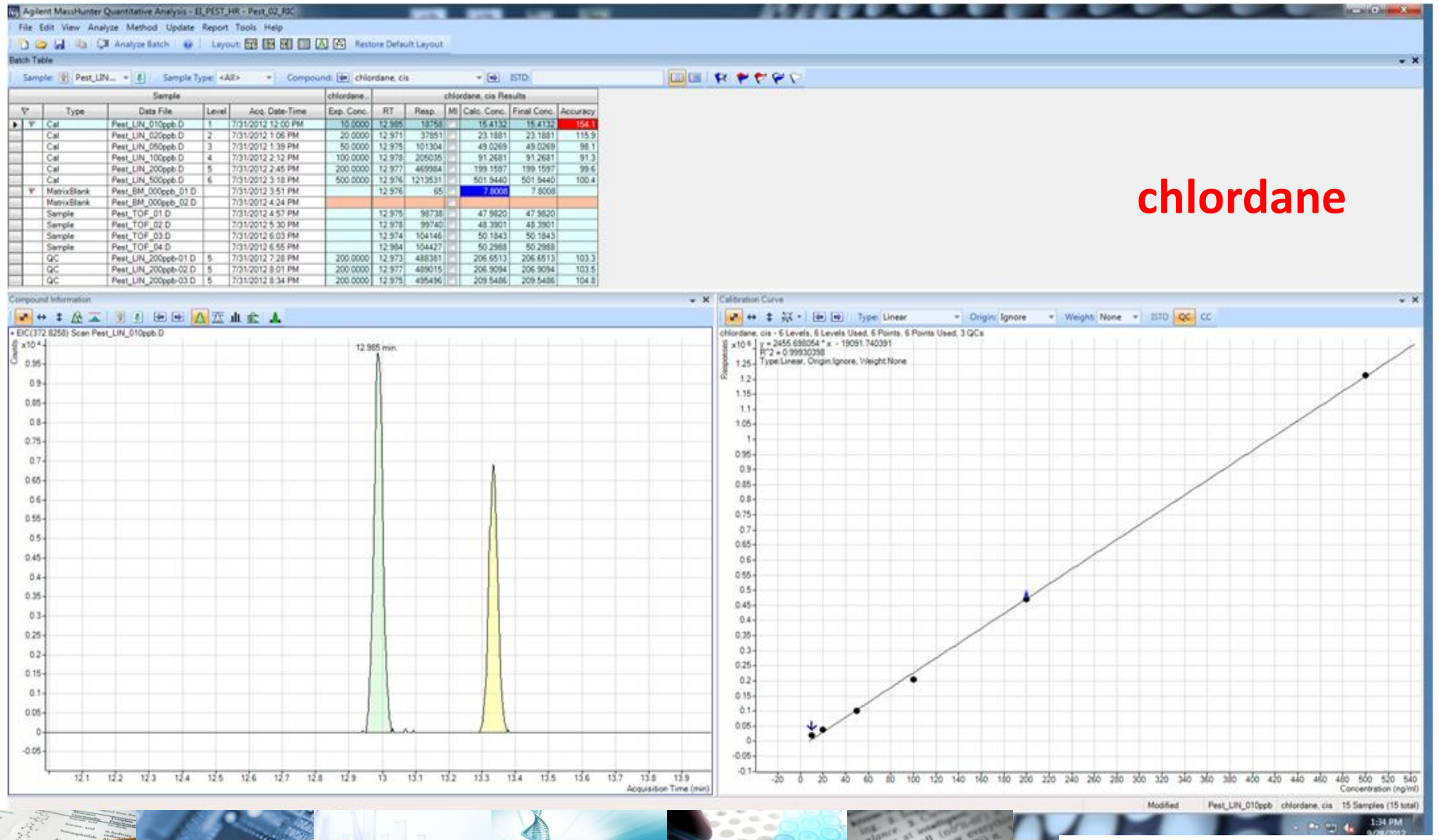
Analysis by GC-QTOF (7200)

10 pg on-column - EIC (262.8564)



RIC | Research Institute for Chromatography

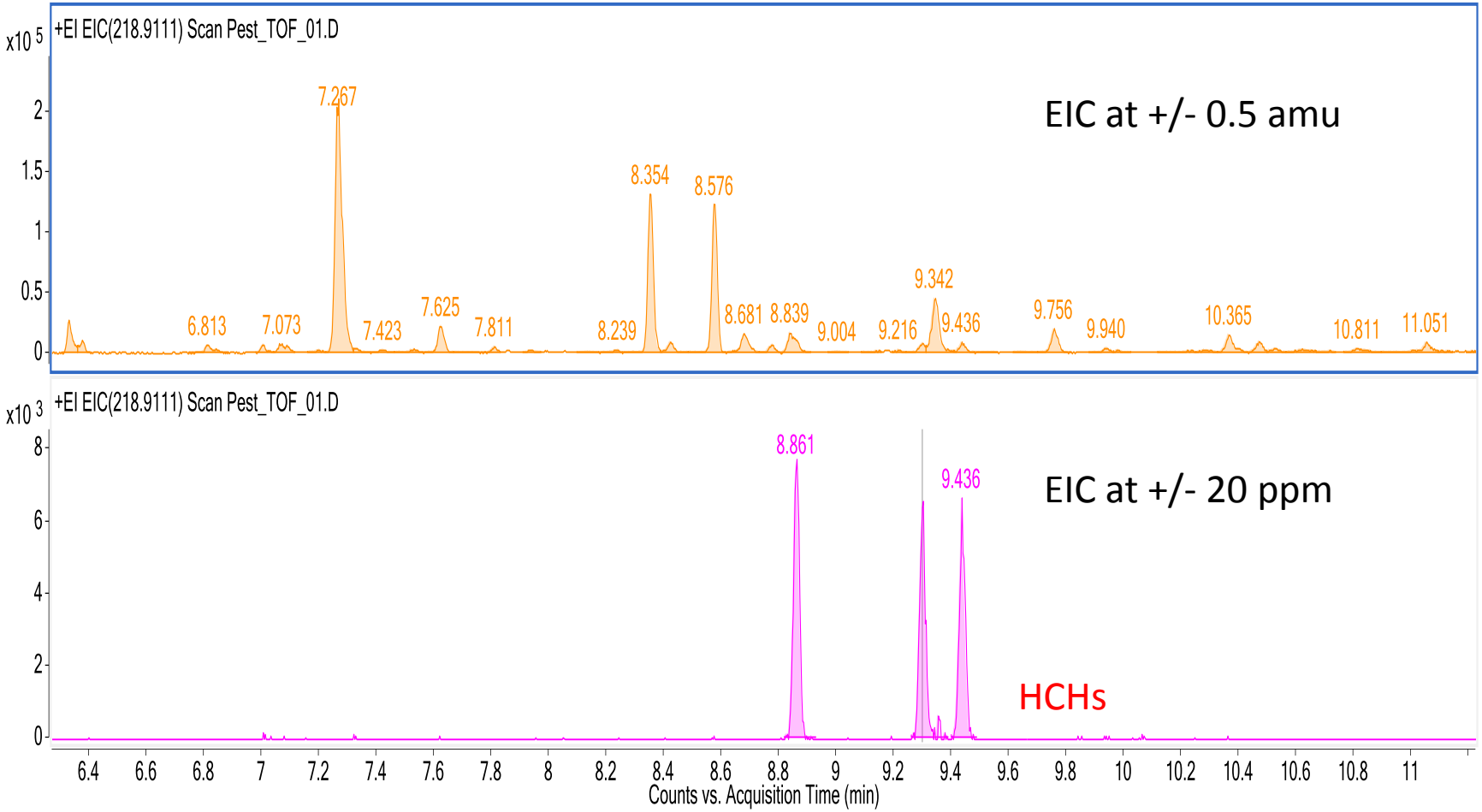
Pesticides in food (“Known knowns”): target compound analysis



RIC Research Institute for Chromatography

Pesticide in food ("Unknown knowns")

after target compound analysis:
are there other (non-target) pesticides?



RIC Research Institute for Chromatography

Why Q-TOF?

1. Extra Selectivity
2. Structural Elucidation (Product Ion Spectra) With Accurate Mass and Hi R

Precursor 1 (M^+) → All Fragment Product Ions
Precursor 2 (Fragment 1^+) → Product Ions

Precursor-Product Ion Relationship Is Documented
And Ion Molecular Formula Confirmed by Accurate Mass

Requires multiple analyses and much more sensitive than NMR
Will not replace NMR, But Will Complement Nicely

RIC | Research Institute for Chromatography

MS/MS accurate mass EIC

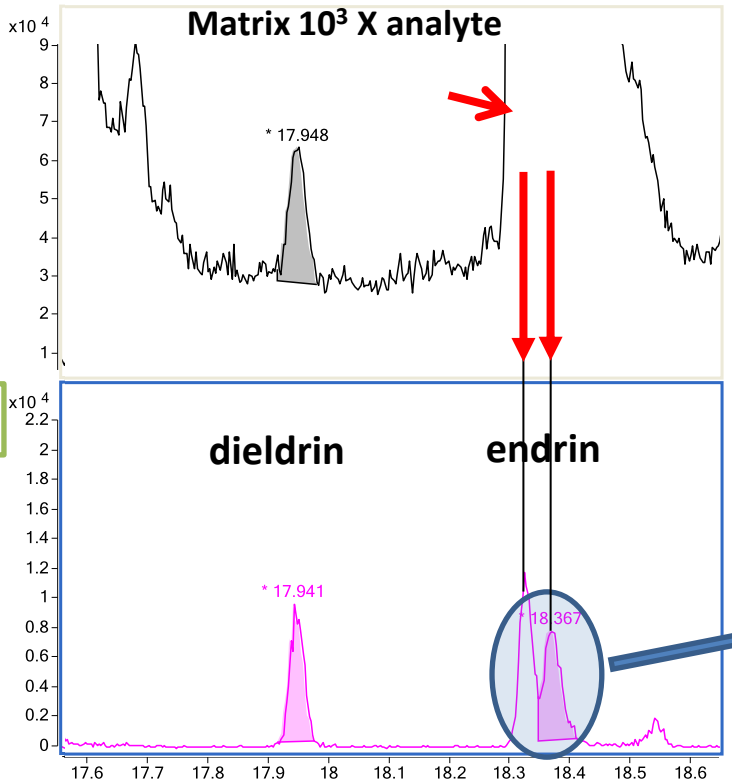
10 pg sample – EIC m/z = 262.8564

Full scan MS
High resolution/accurate mass

MS/MS
High resolution/accurate mass

EIC ±0.5 Da.
+/-1900 ppm

EIC ±20 ppm



EIC of product ion
263>192.9150 +/-20 ppm

Resolution and accurate mass are insufficient – MS/MS solves the problem

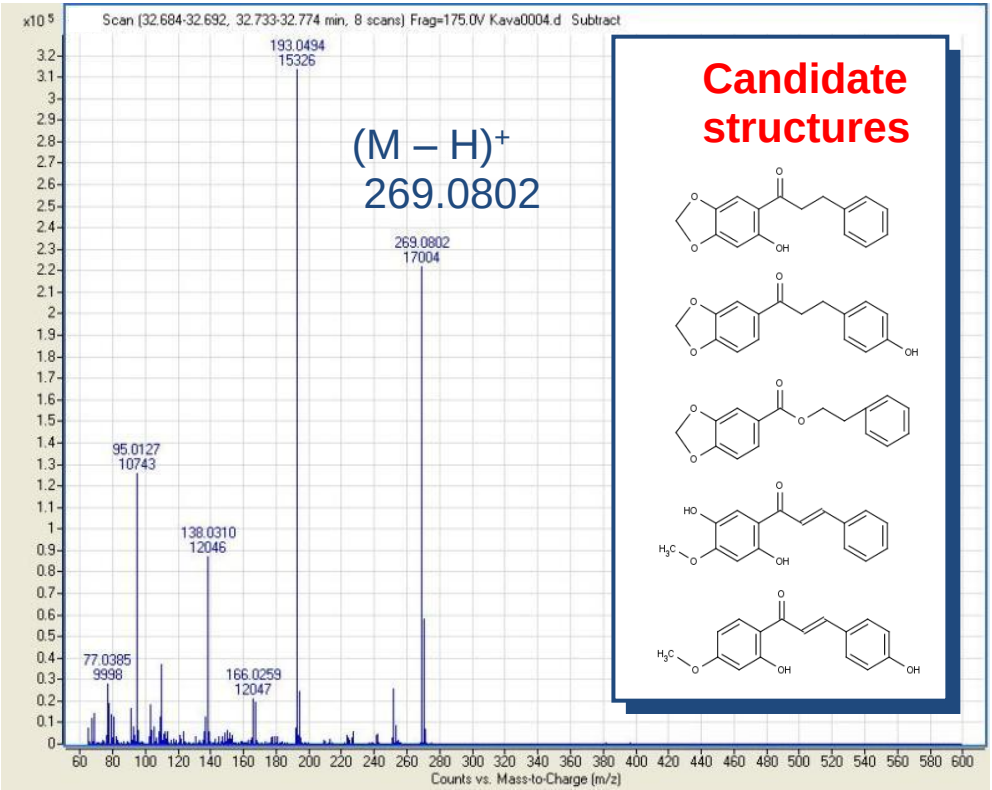
RIC Research Institute for Chromatography

MS/MS to Confirm Most Likely Structure

Kava Extract - Compound "B", $C_{16}H_{14}O_4$

(Rings + Double Bonds = 10)

EI Full Scan



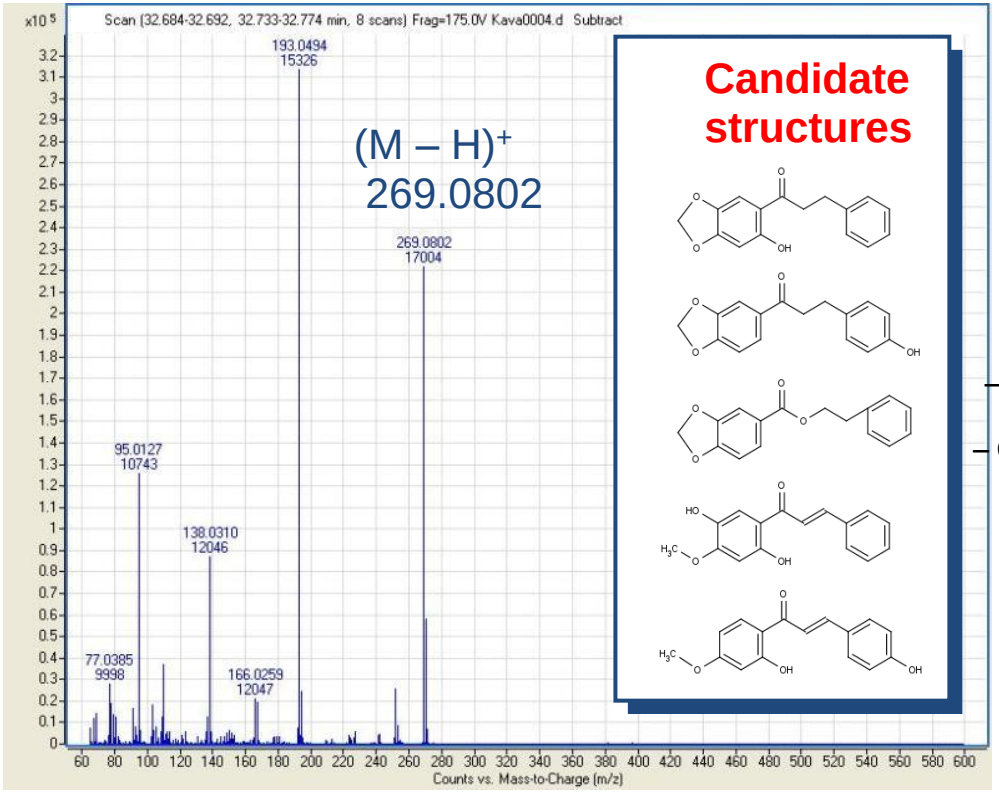
RIC Research Institute for Chromatography

MS/MS to Confirm Most Likely Structure

Kava Extract - Compound "B", $C_{16}H_{14}O_4$

(Rings + Double Bonds = 10)

EI Full Scan



MS/MS experimental measurements

	m/z (experimental)	Formula	Error (ppm)	Score
– H	**269.0802**	**$C_{16}H_{13}O_4$**	**2.2**	**80.7**
– C_6H_5	**193.0494**	**$C_{10}H_9O_4$**	**0.6**	**96.7**
– $CH{=}CH{-}C_6H_5$	**167.0334**	**$C_8H_7O_4$**	**3.0**	**N/A**
– $CH_2{=}CH{-}C_6H_5$	**166.0259**	**$C_8H_6O_4$**	**0.6**	**N/A**
– CO	**138.0310**	**$C_7H_6O_3$**	**1.1**	**98.1**
– CO	**110.0359**	**$C_6H_6O_2$**	**3.0**	**N/A**
– CH_3	**95.0127**	**$C_5H_3O_2$**	**0.9**	**99.5**

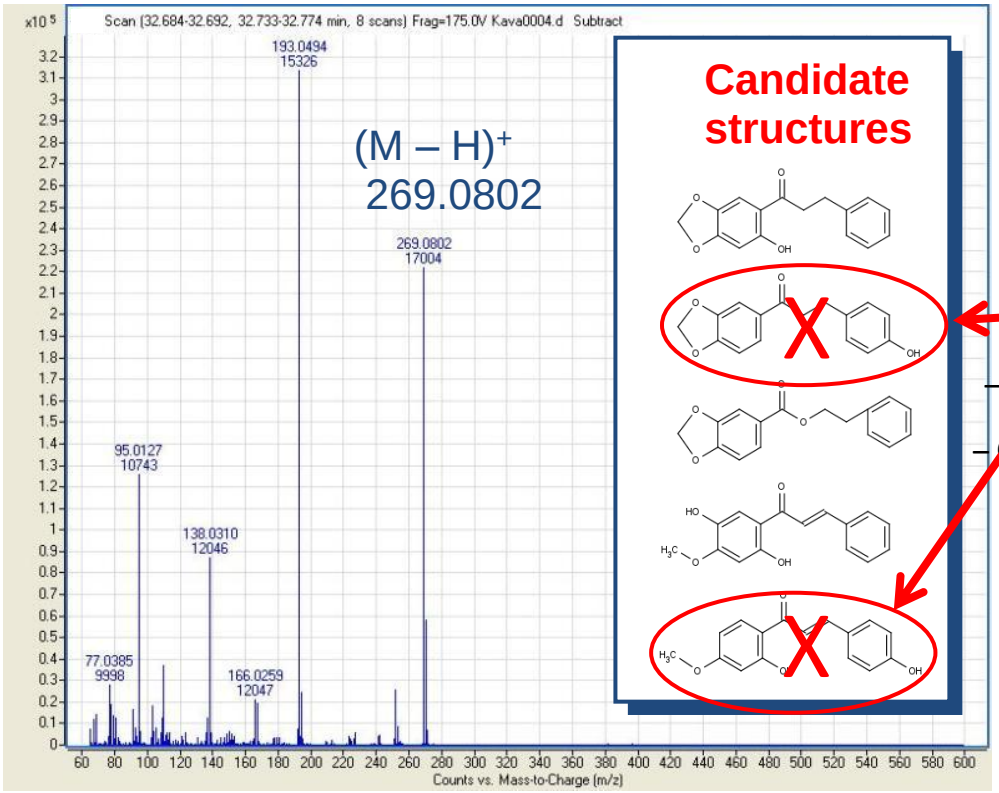
RIC Research Institute for Chromatography

MS/MS to Confirm Most Likely Structure

Kava Extract - Compound "B", $C_{16}H_{14}O_4$

(Rings + Double Bonds = 10)

EI Full Scan



MS/MS experimental measurements

m/z (experimental)	Formula	Error (ppm)	Score
269.0802	**$C_{16}H_{13}O_4$**	**2.2**	**80.7**
193.0494	**$C_{10}H_9O_4$**	**0.6**	**96.7**
167.0334	**$C_8H_7O_4$**	**3.0**	**N/A**
166.0259	**$C_8H_6O_4$**	**0.6**	**N/A**
138.0310	**$C_7H_6O_3$**	**1.1**	**98.1**
110.0359	**$C_6H_6O_2$**	**3.0**	**N/A**
95.0127	**$C_5H_3O_2$**	**0.9**	**99.5**

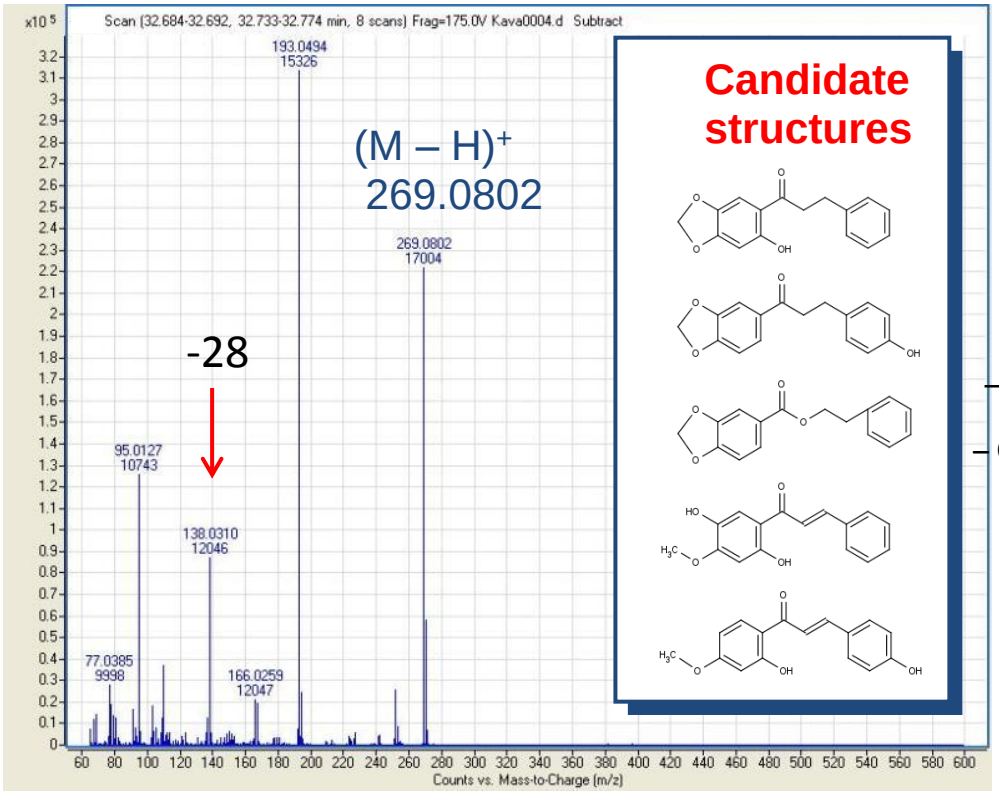
RIC Research Institute for Chromatography

MS/MS to Confirm Most Likely Structure

Kava Extract - Compound "B", $C_{16}H_{14}O_4$

(Rings + Double Bonds = 10)

EI Full Scan

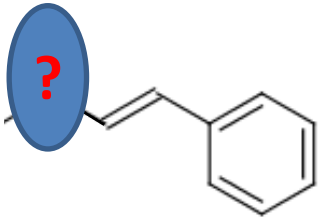


MS/MS experimental measurements

	m/z (experimental)	Formula	Error (ppm)	Score
– H	**269.0802**	**$C_{16}H_{13}O_4$**	**2.2**	**80.7**
– C_6H_5	**193.0494**	**$C_{10}H_9O_4$**	**0.6**	**96.7**
– $CH{=}CH{-}C_6H_5$	**167.0334**	**$C_8H_7O_4$**	**3.0**	**N/A**
– $CH_2{=}CH{-}C_6H_5$	**166.0259**	**$C_8H_6O_4$**	**0.6**	**N/A**
– CO	**138.0310**	**$C_7H_6O_3$**	**1.1**	**98.1**
– CO	**110.0359**	**$C_6H_6O_2$**	**3.0**	**N/A**
– CH_3	**95.0127**	**$C_5H_3O_2$**	**0.9**	**99.5**

RIC Research Institute for Chromatography

MS/MS to Confirm Most Likely Structure



Mass at 138 is consistent with:

loss of COCH=CH-C_6H_5 (131.04969) from 269.08020

or

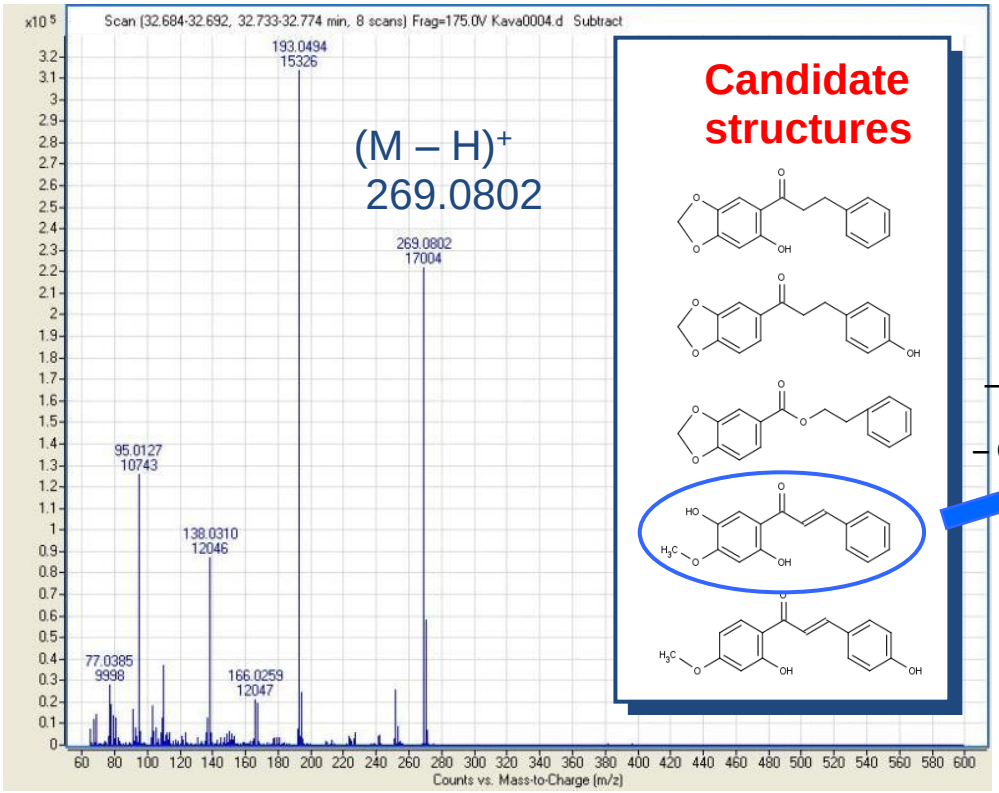
loss of C_2H_4CH=CH-C_6H_5 (131.086075) from 269.08020.

However, measured value of 269.0802 - 138.0310 = 131.04920 is consistent only with **COCH**=CH- C_6H_5.

RIC Research Institute for Chromatography

Confirm Most Likely Structure

EI Full Scan



MS/MS experimental measurements

	m/z (experimental)	Formula	Error (ppm)	Score
	269.0802	**$C_{16}H_{13}O_4$**	**2.2**	**80.7**
– H	**193.0494**	**$C_{10}H_9O_4$**	**0.6**	**96.7**
– C_6H_5	**167.0334**	**$C_8H_7O_4$**	**3.0**	**N/A**
– $CH{=}CH{-}C_6H_5$	**166.0259**	**$C_8H_6O_4$**	**0.6**	**N/A**
– $CH_2{=}CH{-}C_6H_5$	**138.0310**	**$C_7H_6O_3$**	**1.1**	**98.1**
– CO	**110.0359**	**$C_6H_6O_2$**	**3.0**	**N/A**
– CO	**95.0127**	**$C_5H_3O_2$**	**0.9**	**99.5**
– CH_3				

For the 5 candidate structures, only one fit the losses identified by CID experiments on multiple precursor ions

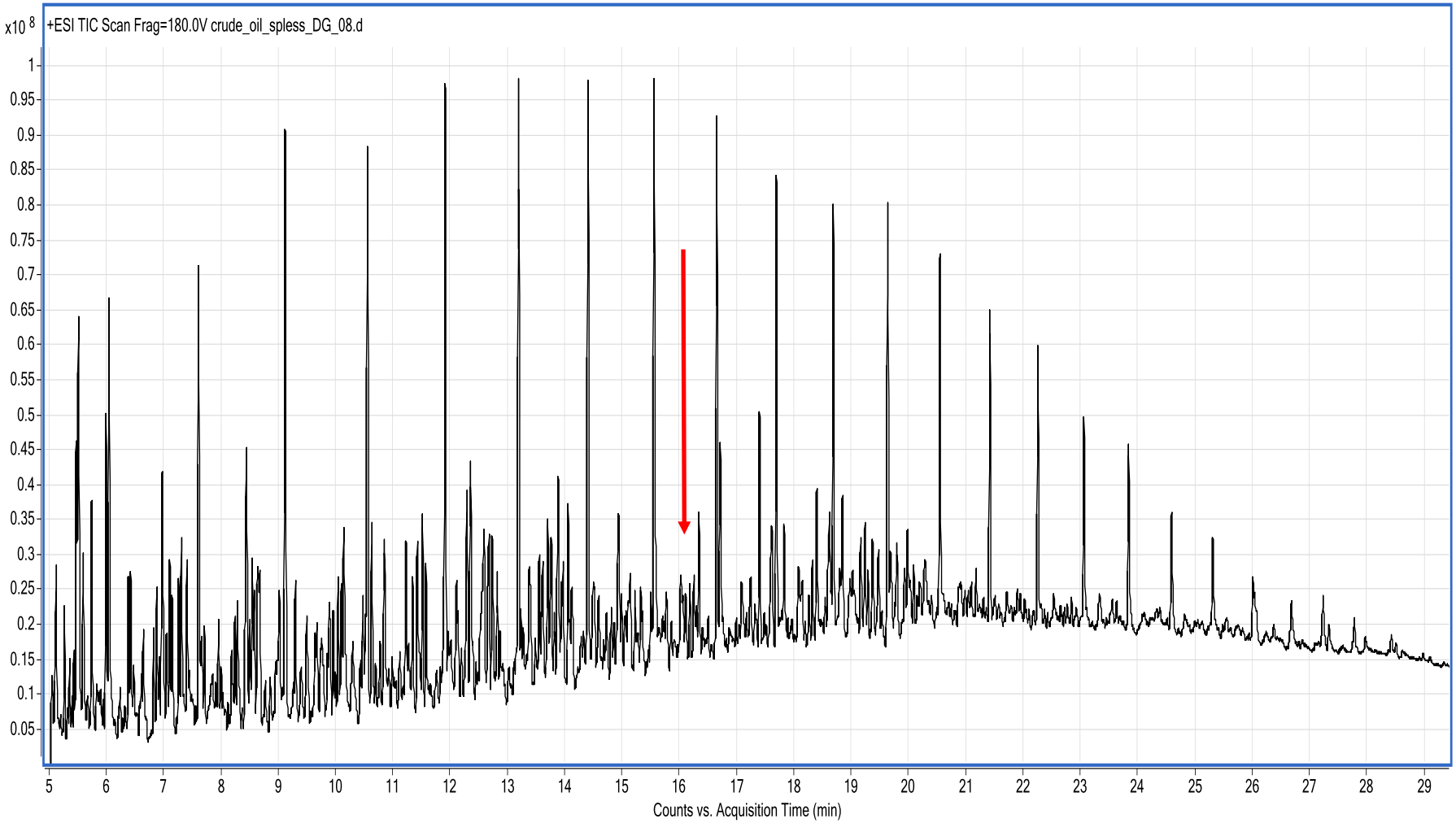
RIC Research Institute for Chromatography

Analysis of Petrochemical Biomarkers

- Biomarkers: more stable against weathering
- Include: (heterocyclic) PAHs, hopanes, steranes (can be > 100 analytes)
- Analysed for oil characterization, oil spillage identification,…

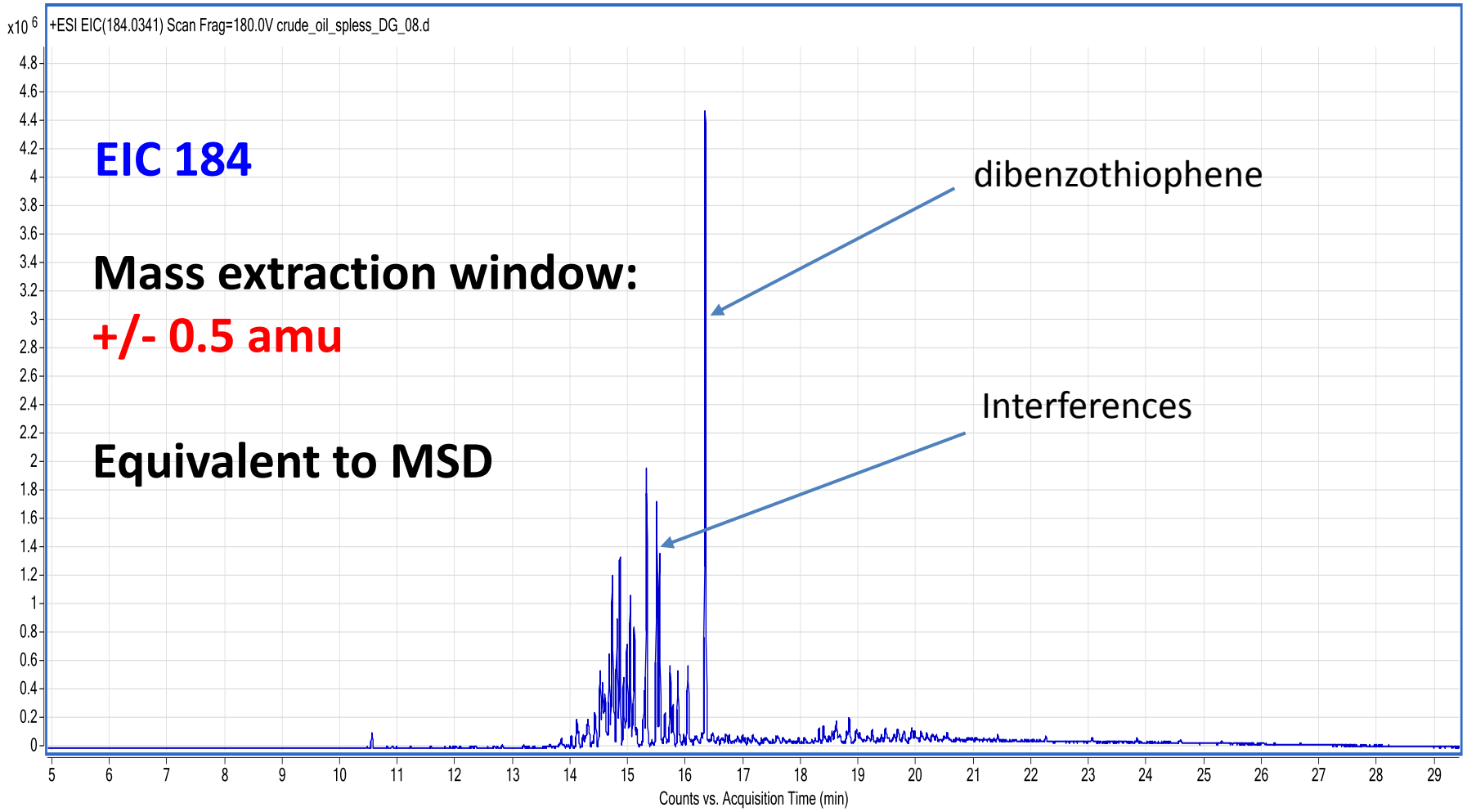
RIC Research Institute for Chromatography

Analysis of Biomarkers in Crude Oil



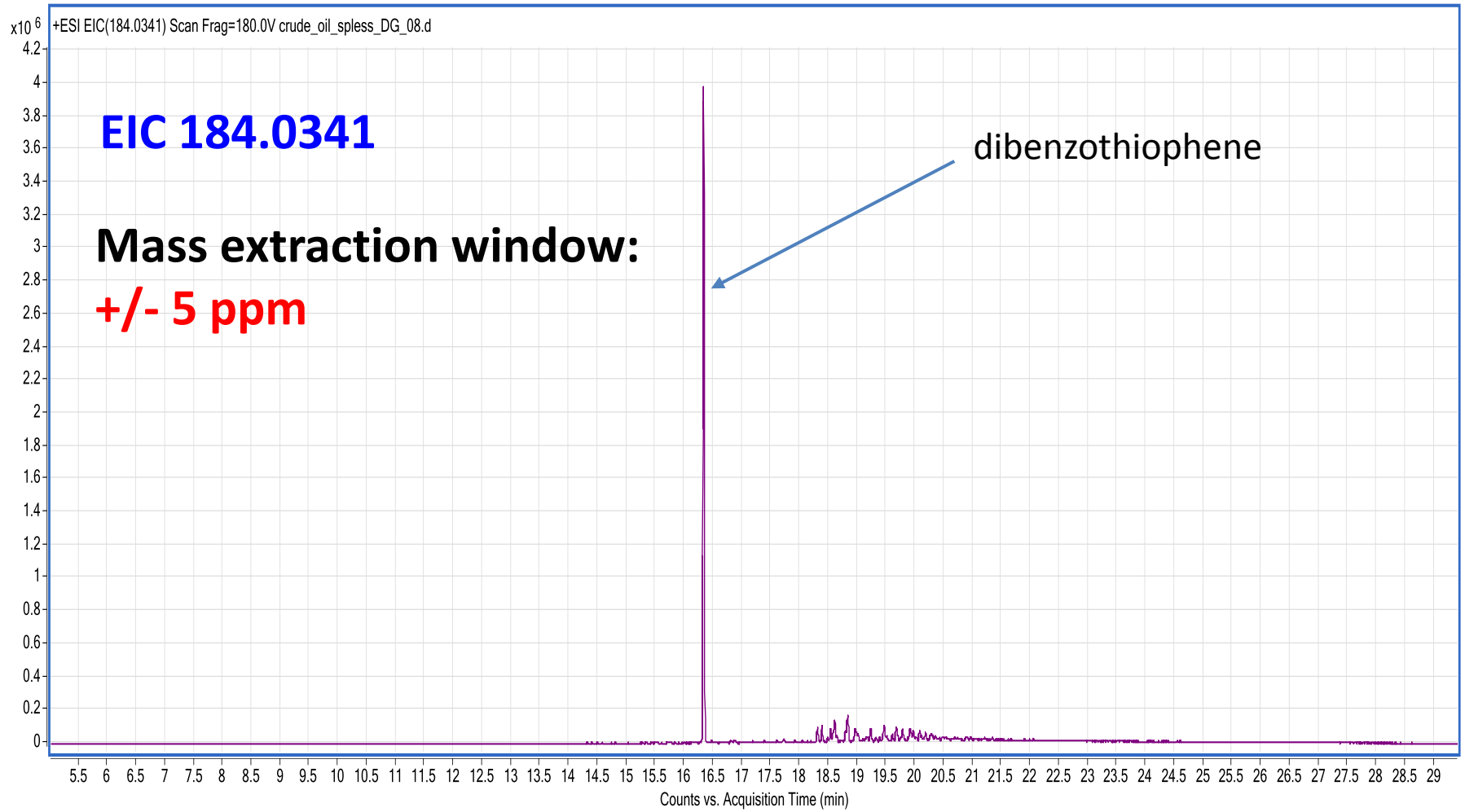
RIC | Research Institute for Chromatography

Analysis of Biomarkers in Crude Oil



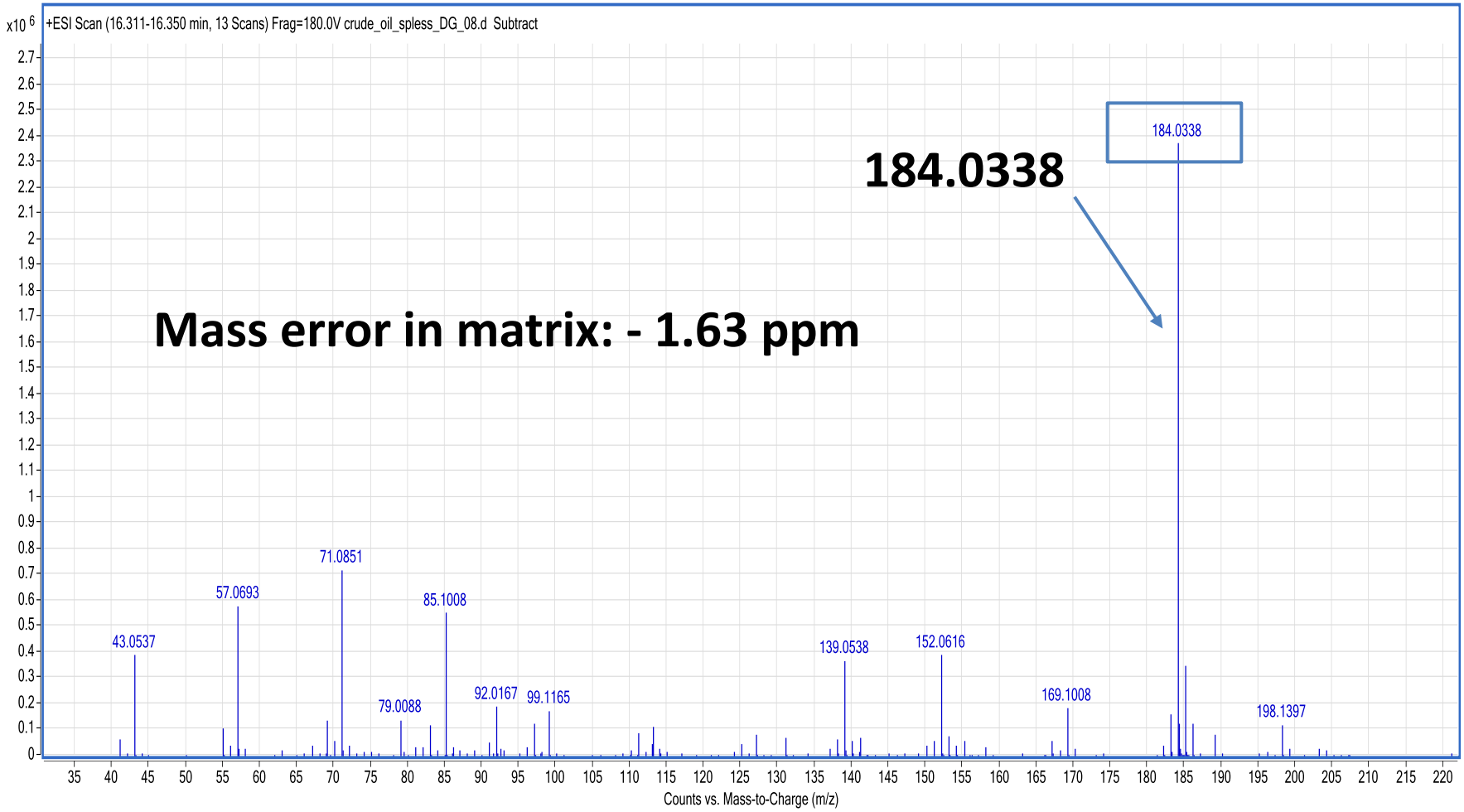
RIC Research Institute for Chromatography

Analysis of Biomarkers in Crude Oil



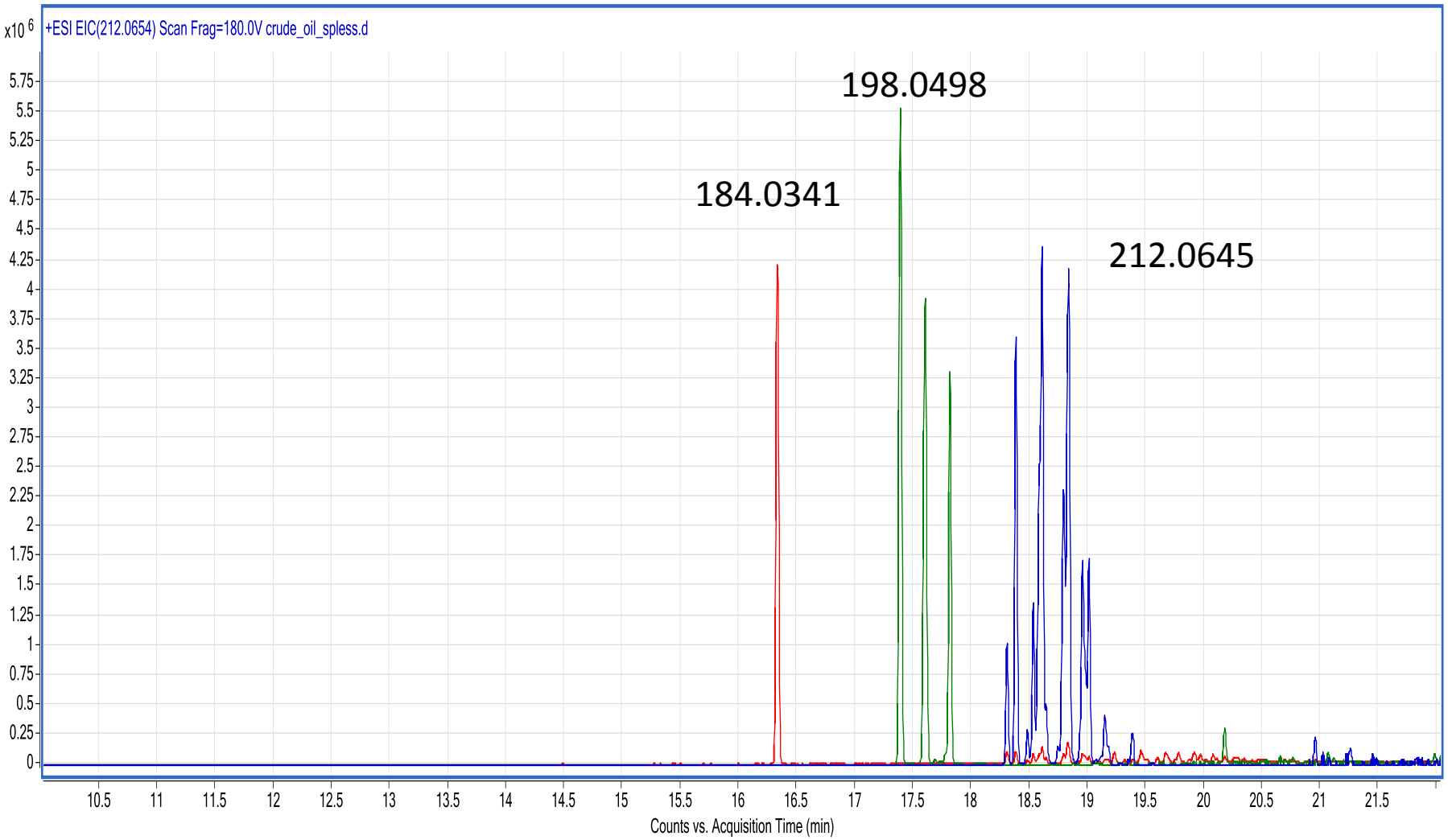
RIC Research Institute for Chromatography

Analysis of DBT – Mass Accuracy (in matrix)



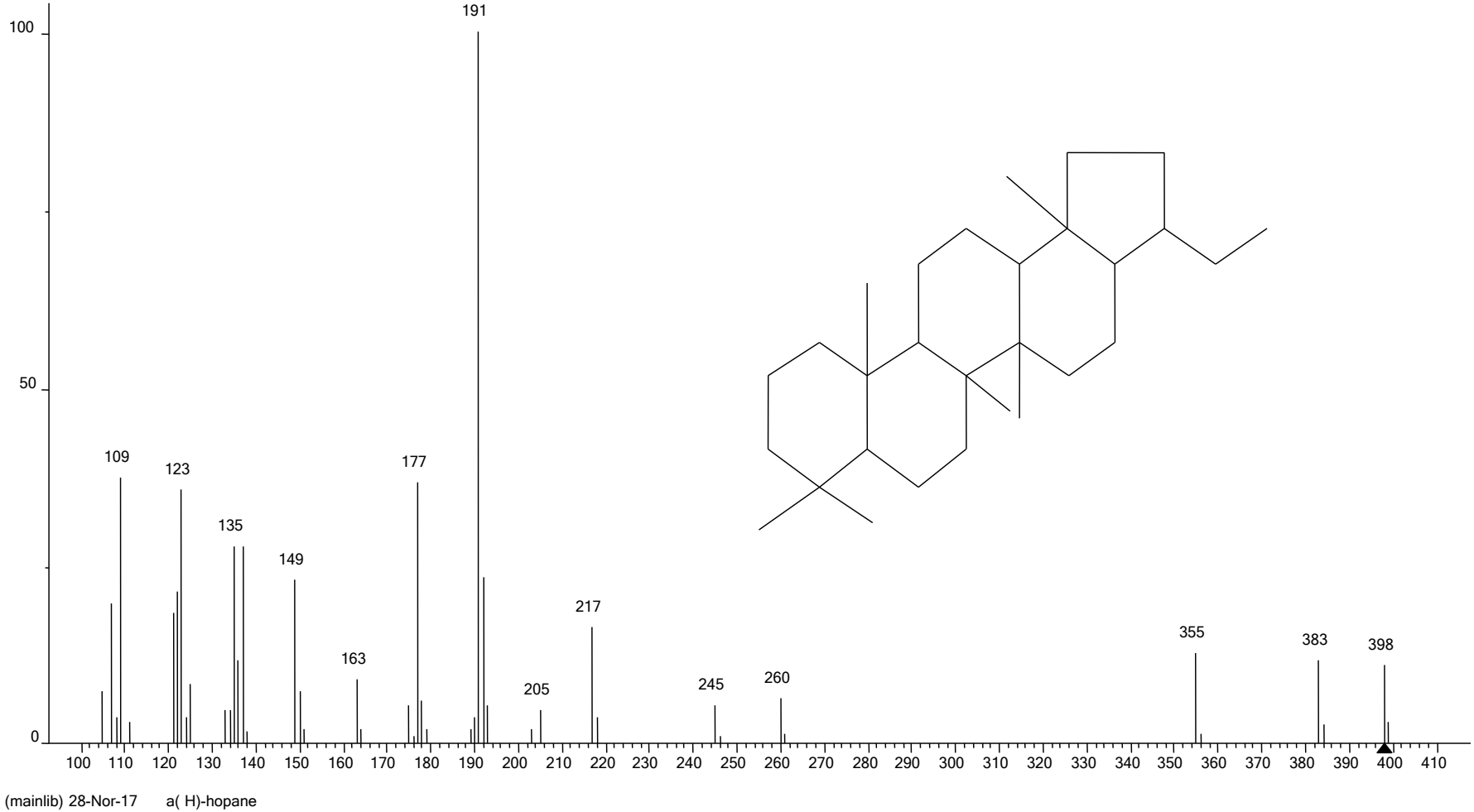
RIC Research Institute for Chromatography

DBT, mono- and dimethyl DBTs



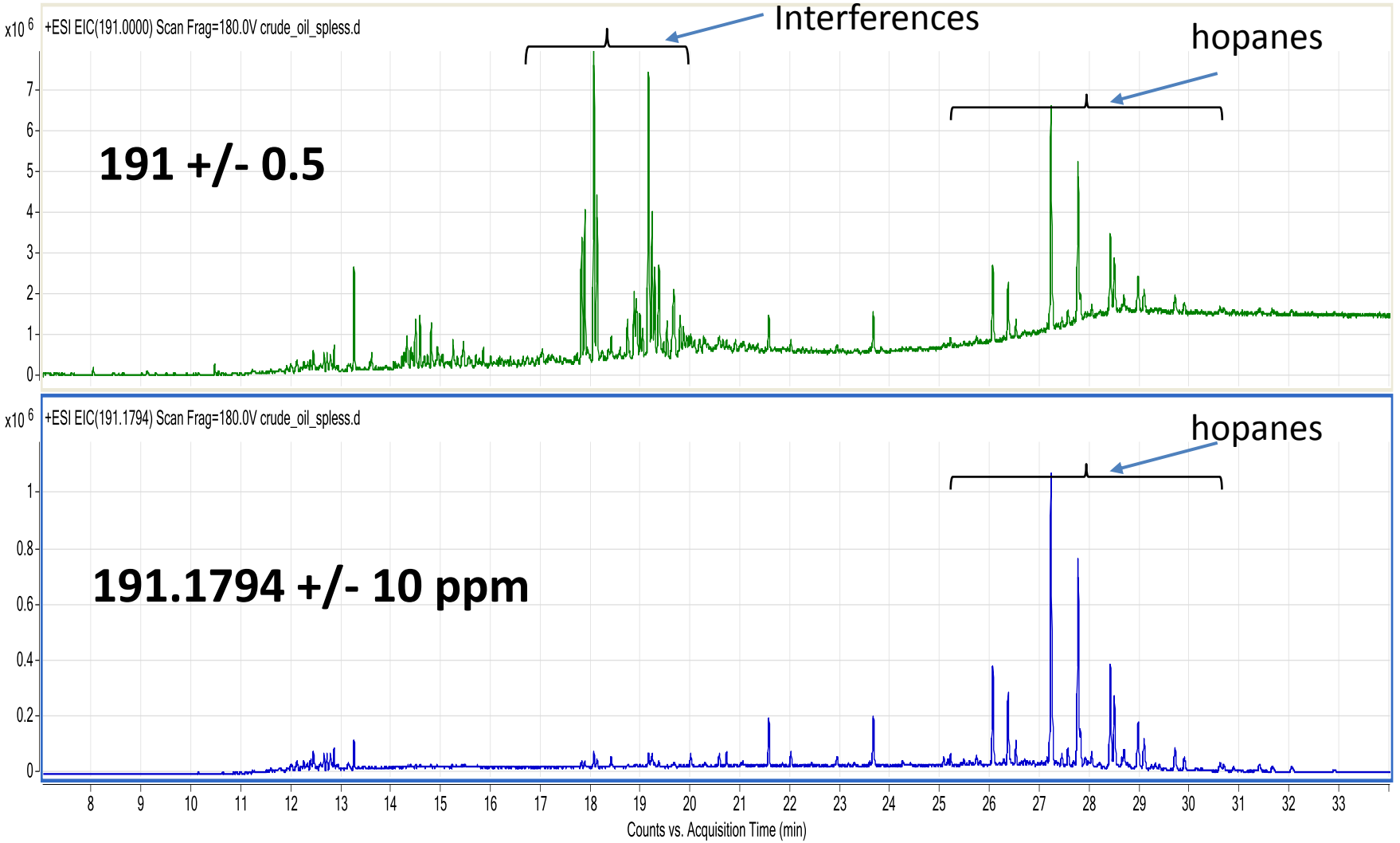
RIC Research Institute for Chromatography

Hopanes



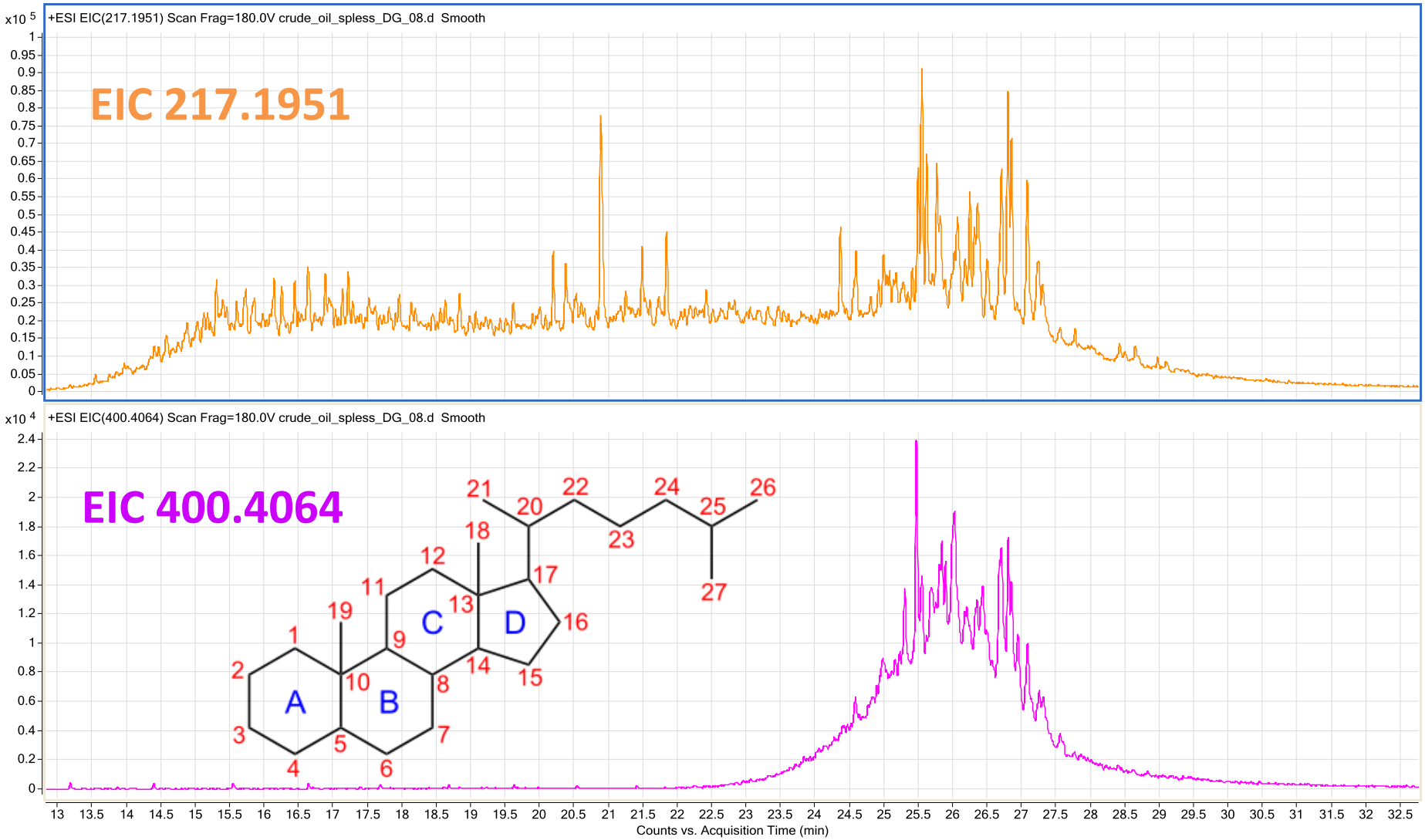
RIC Research Institute for Chromatography

Analysis of Hopanes in Crude Oil



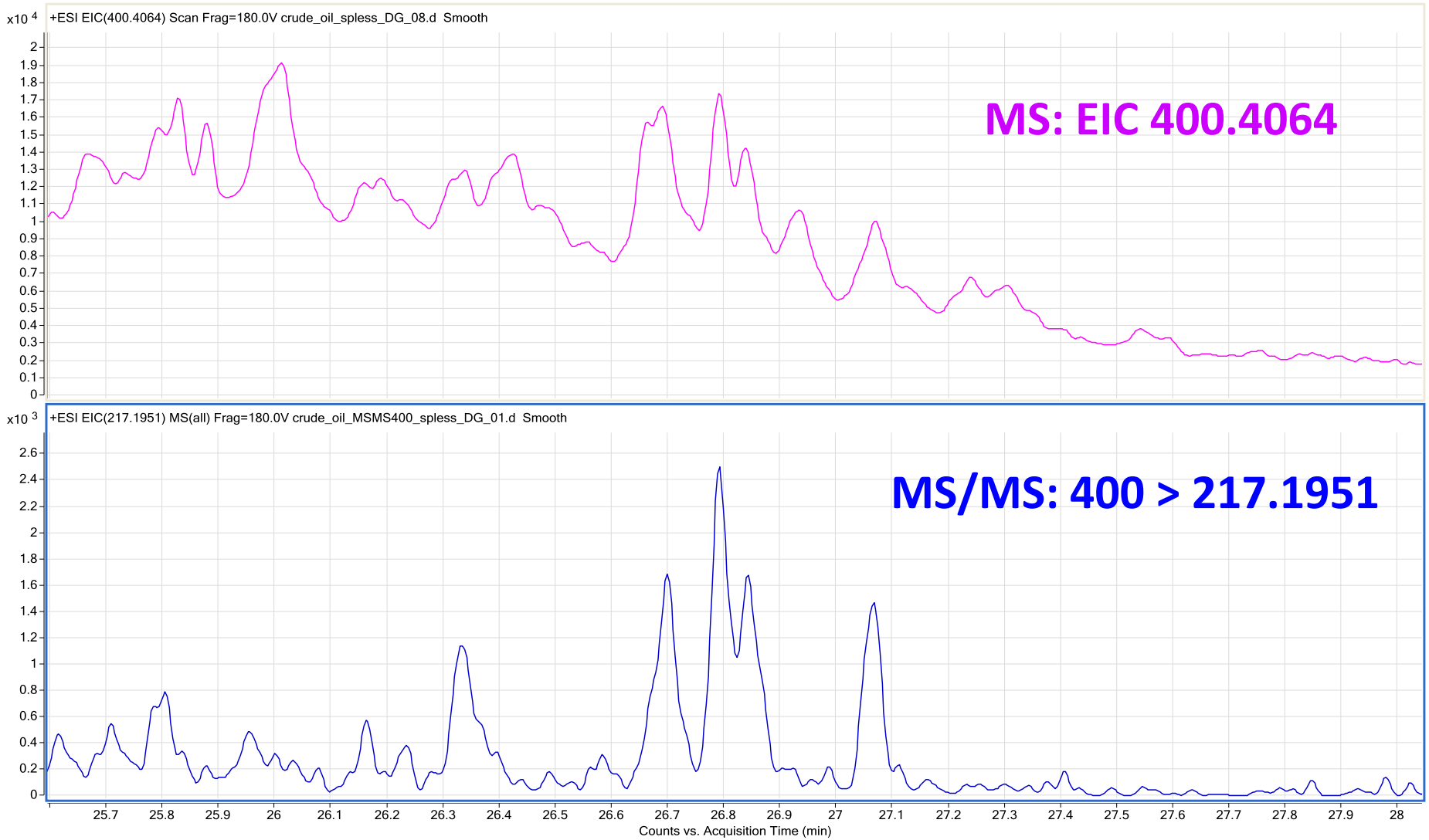
RIC Research Institute for Chromatography

Analysis of Steranes in Crude Oil



RIC | Research Institute for Chromatography

Steranes in Crude Oil by GC-Q-TOF - MS/MS mode



RIC | Research Institute for Chromatography

GC-MS in Natural Product Characterization

MW

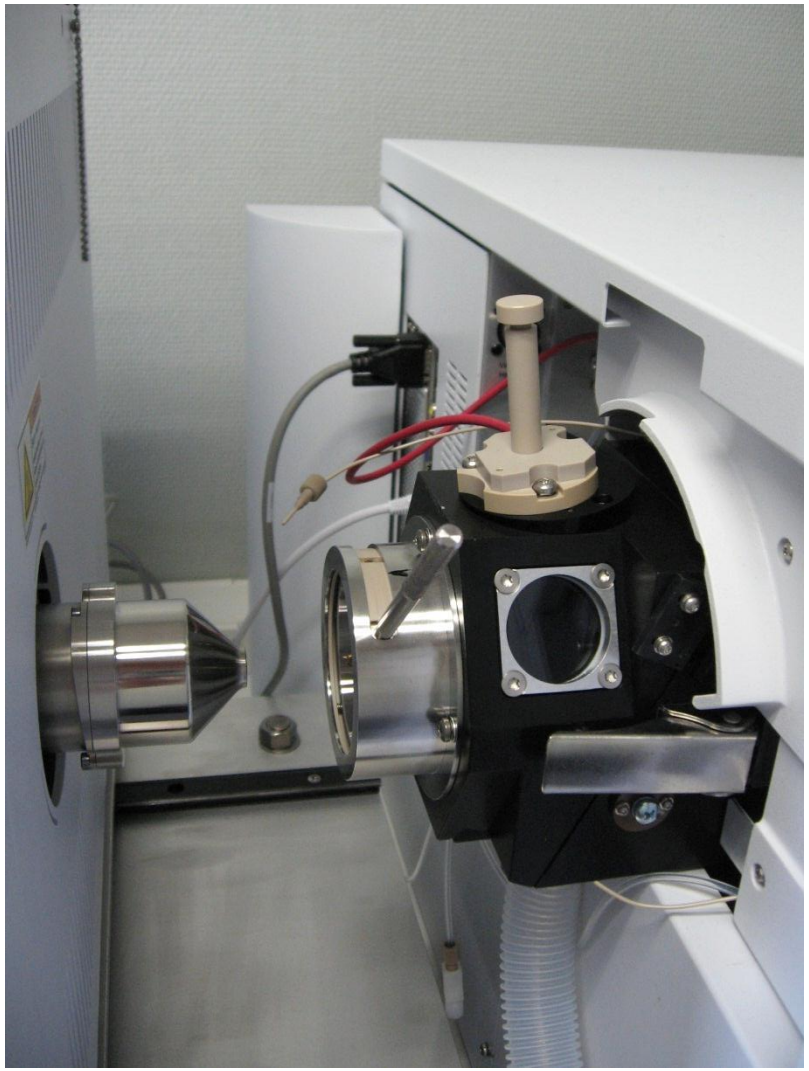
Apolar C10-C40 fraction Generic GC-EI-MS **Alkanes, alcohols…** **EI: strong fragmentation**	**Apolar > C40 fraction** LC-MS **HTGC-MS** **Low beta column, high flow**
Polar fraction (amino acids, sugars, acids,…) **Derivatization GC-MS** **Strong fragmentation**	Starch, proteins, oligosaccharides LC-MS (CE)

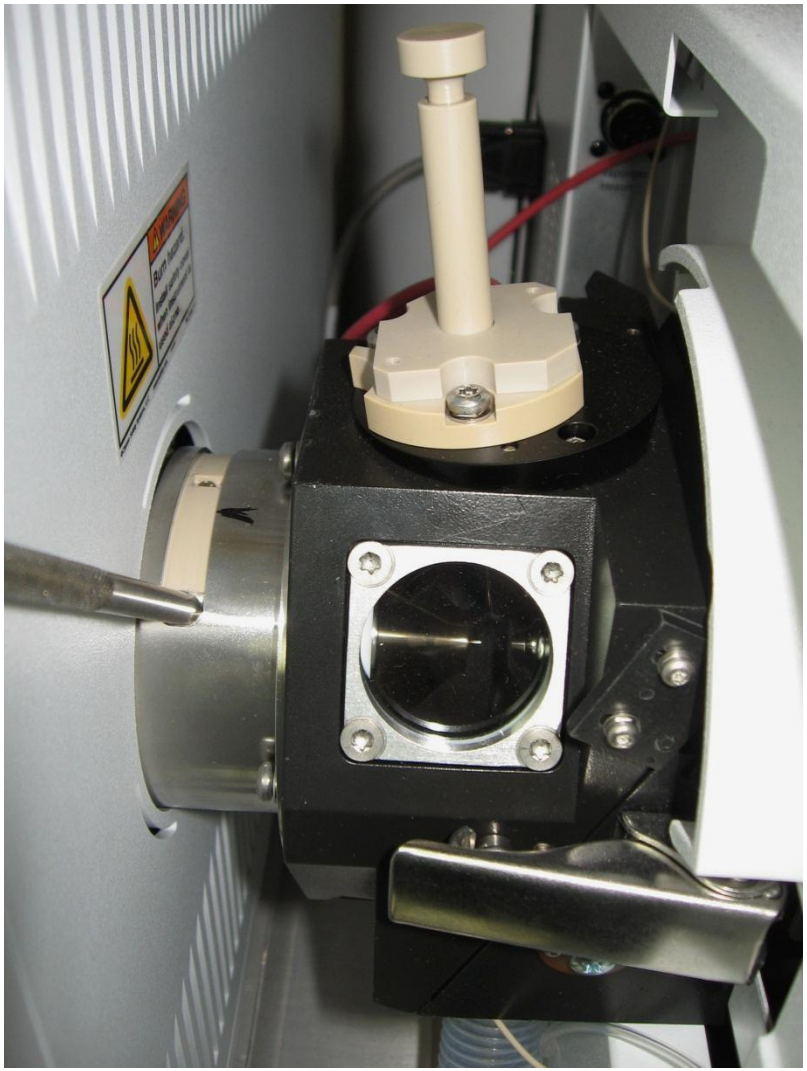
Polarity

GC combined with APCI and (LC) TOF

RIC | Research Institute for Chromatography

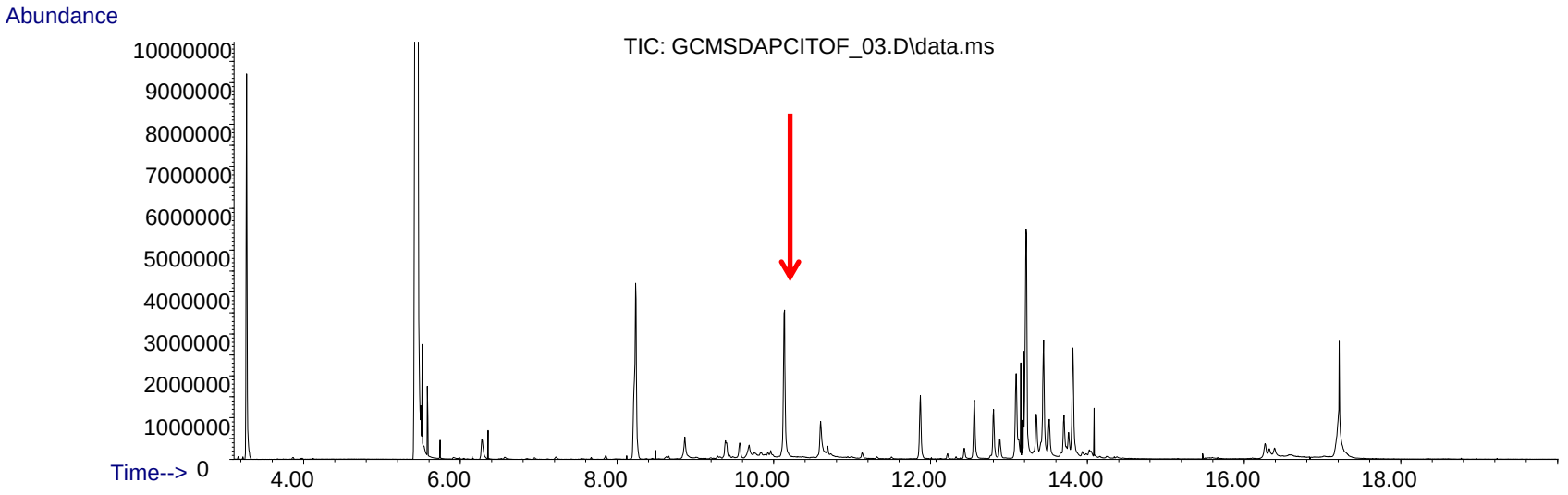
GC combined with APCI and (LC) TOF

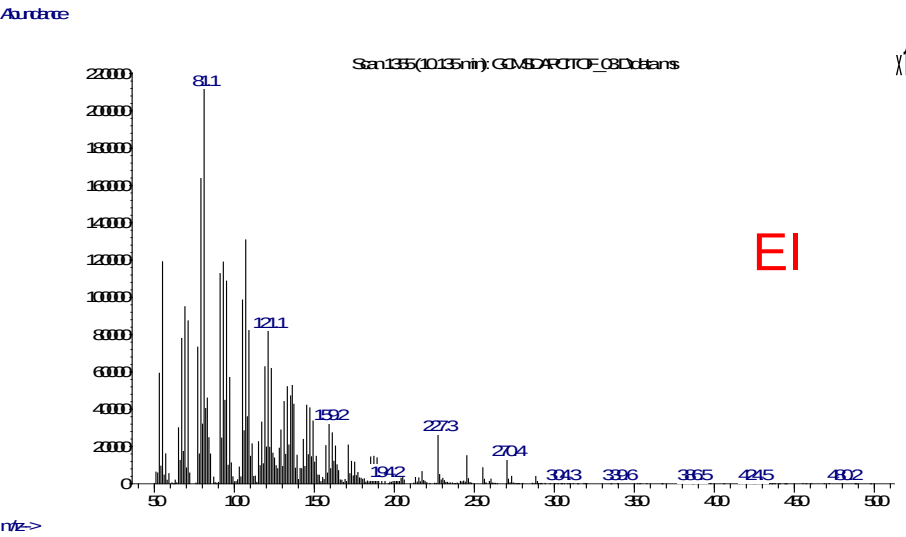




RIC | Research Institute for Chromatography

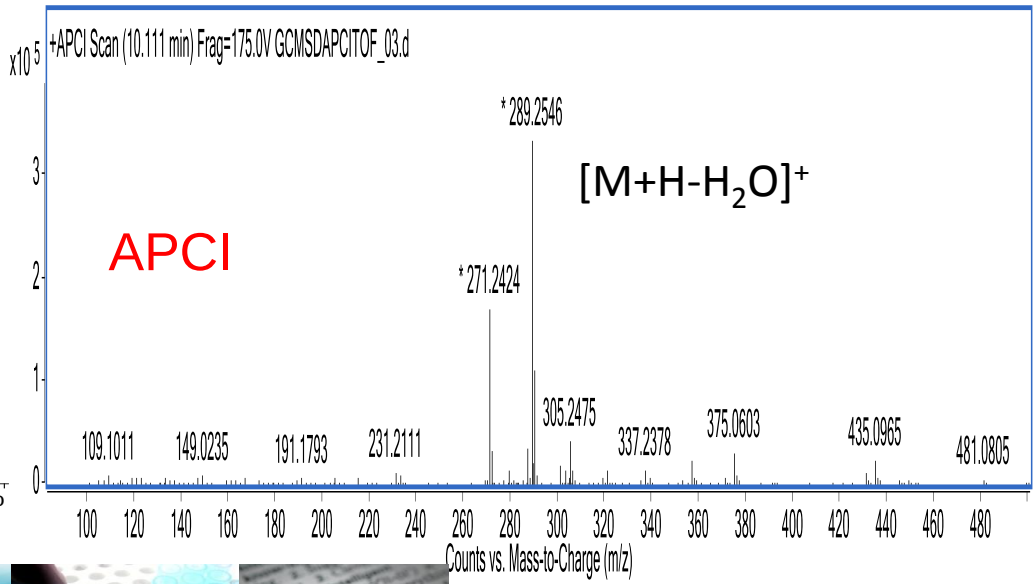
GC-APCI-TOF: detection & identification of 4,8,13-duvatriene-1,3-diol ($C_{20}H_{34}O_2$, MW= 306)





EI

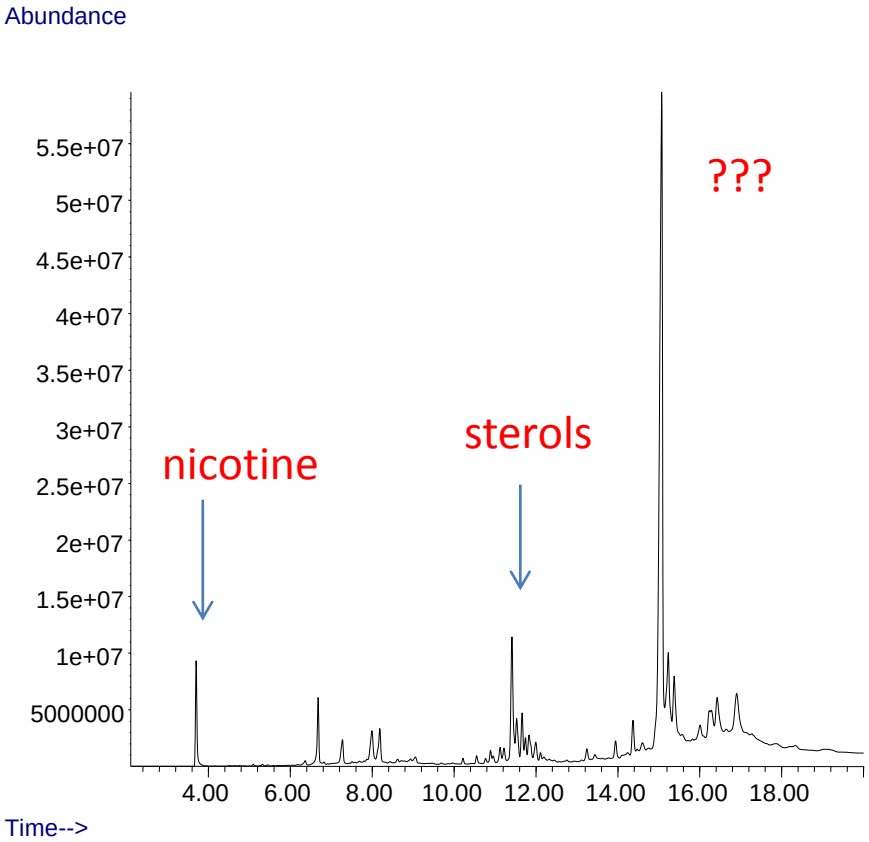
APCI



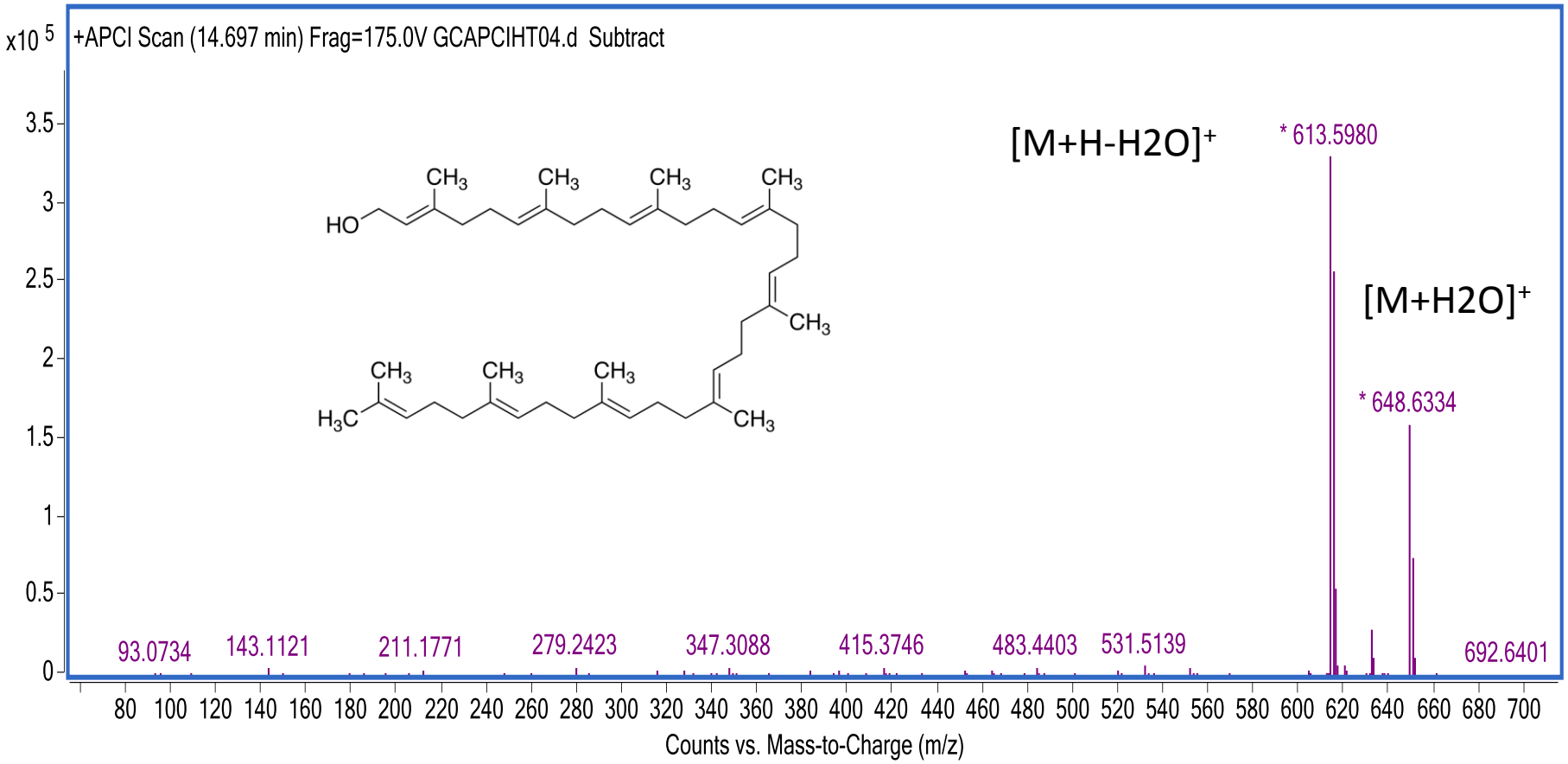
RIC Research Institute for Chromatography

HTGC-MS using APCI-MS

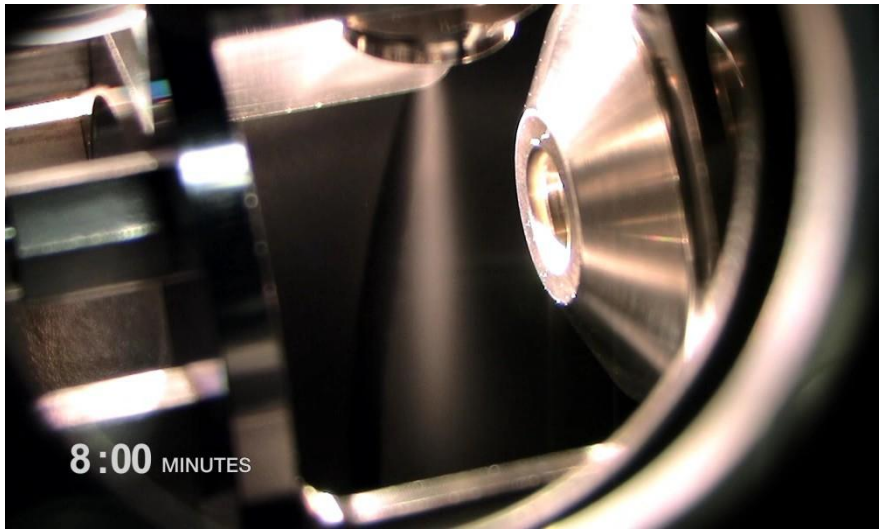
- 15 m x 0.53 mm ID x 0.15 µm HP-SIMDIST
- 1 µL COC
- 10 mL/min helium
- 40°C (1 min) – 20°C/min – 350°C (7 min)
- MS range: 40 – 800

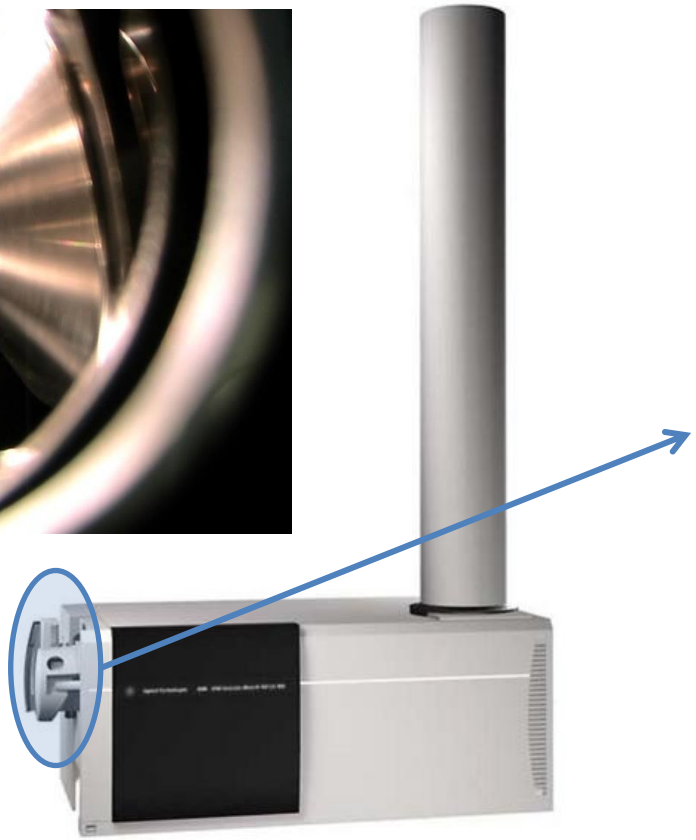


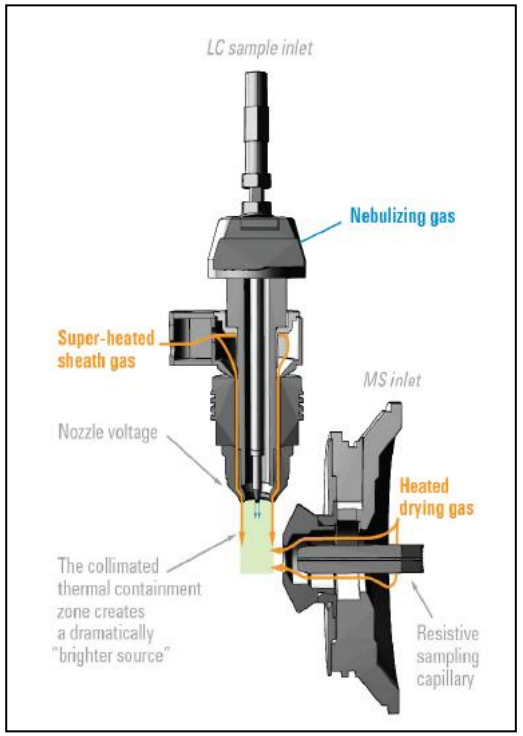
Mass Spectrum of Solanesol by APCI



UHPLW 6540 Q-TOF with Jetstream ESI





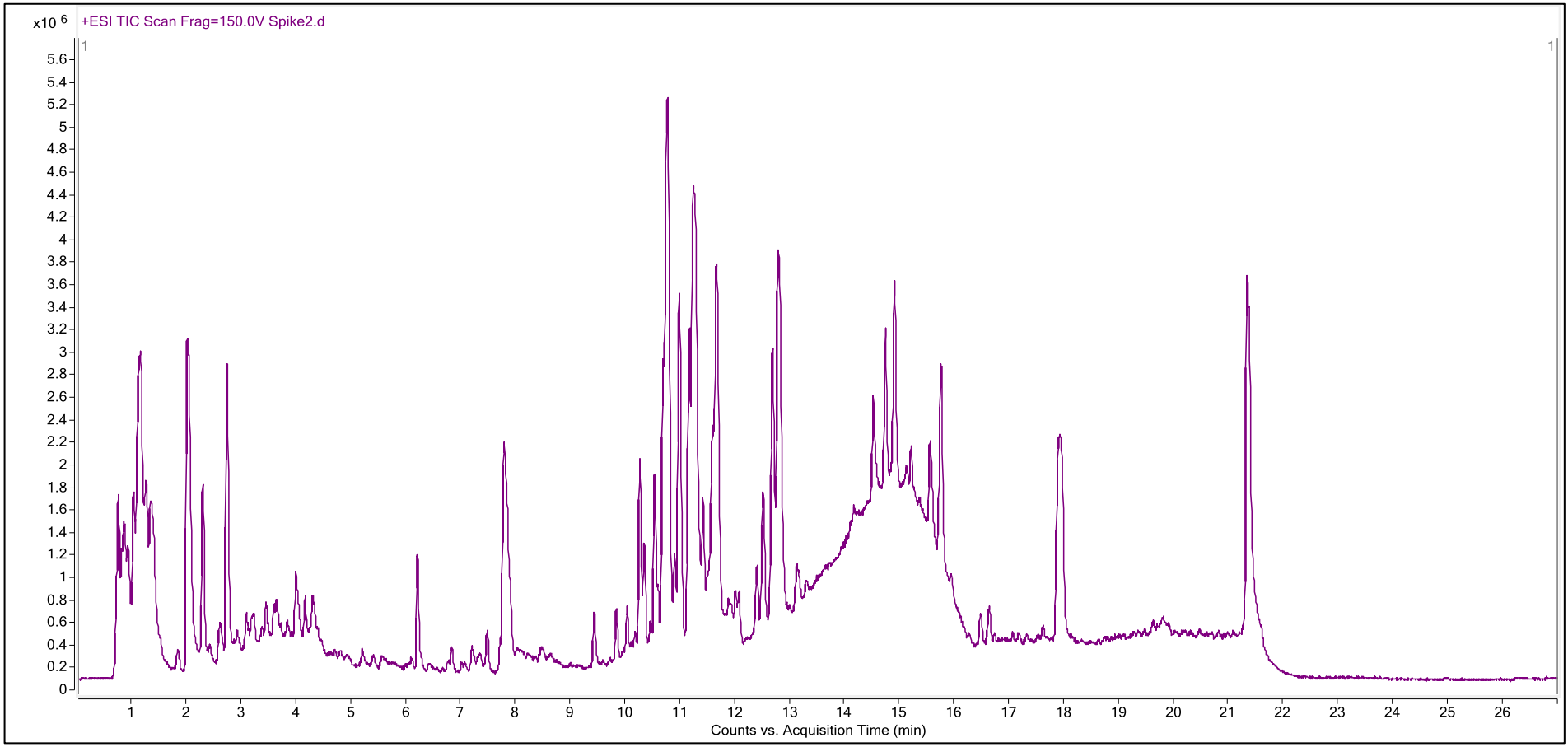


Sensitivity increase (up to 10x) compared to standard ESI source

RIC | Research Institute for Chromatography

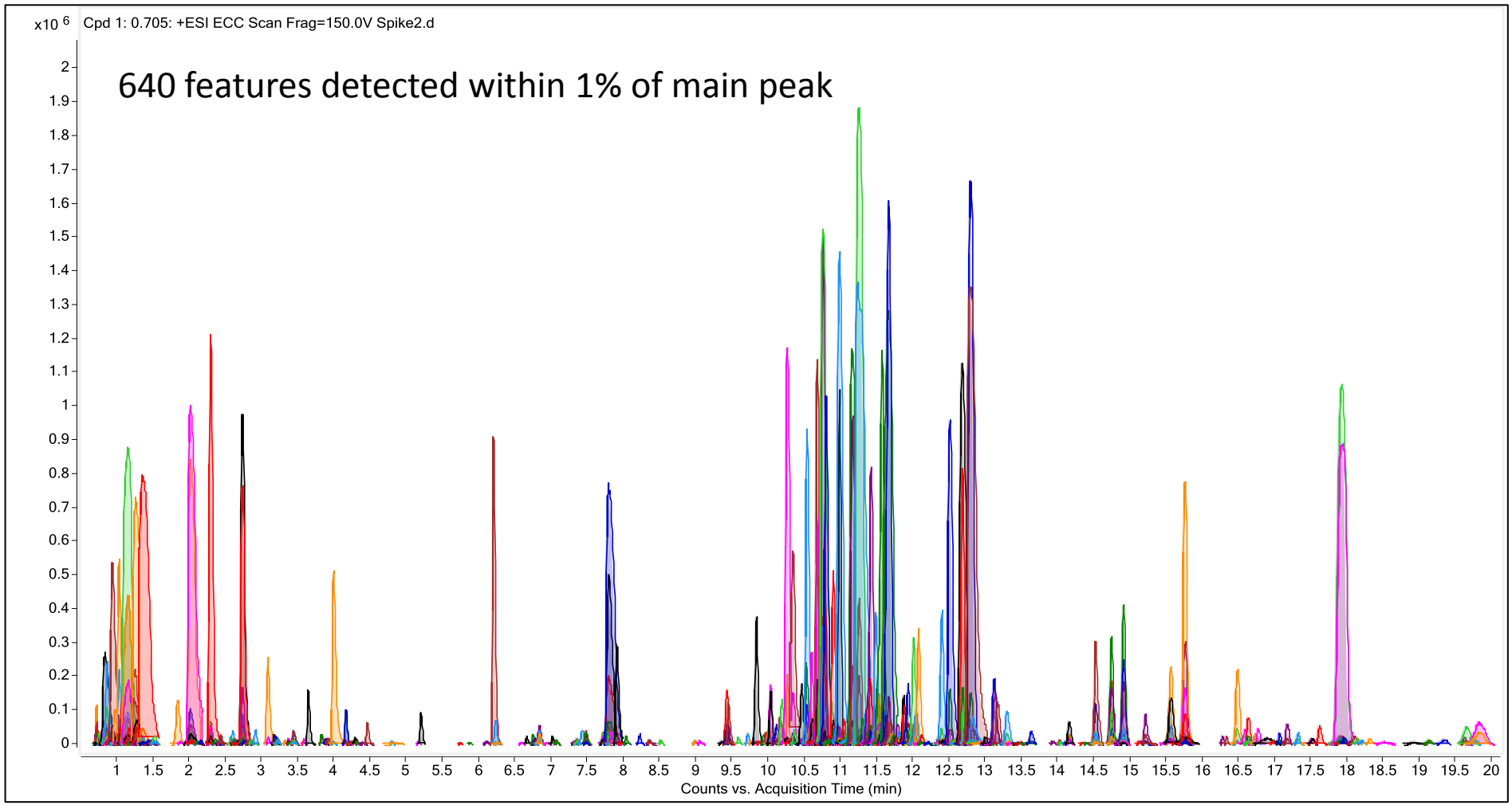
Analysis of Egg extract by LC-ESI-QTOF (+)

(spiked antibiotics, mycotoxins)



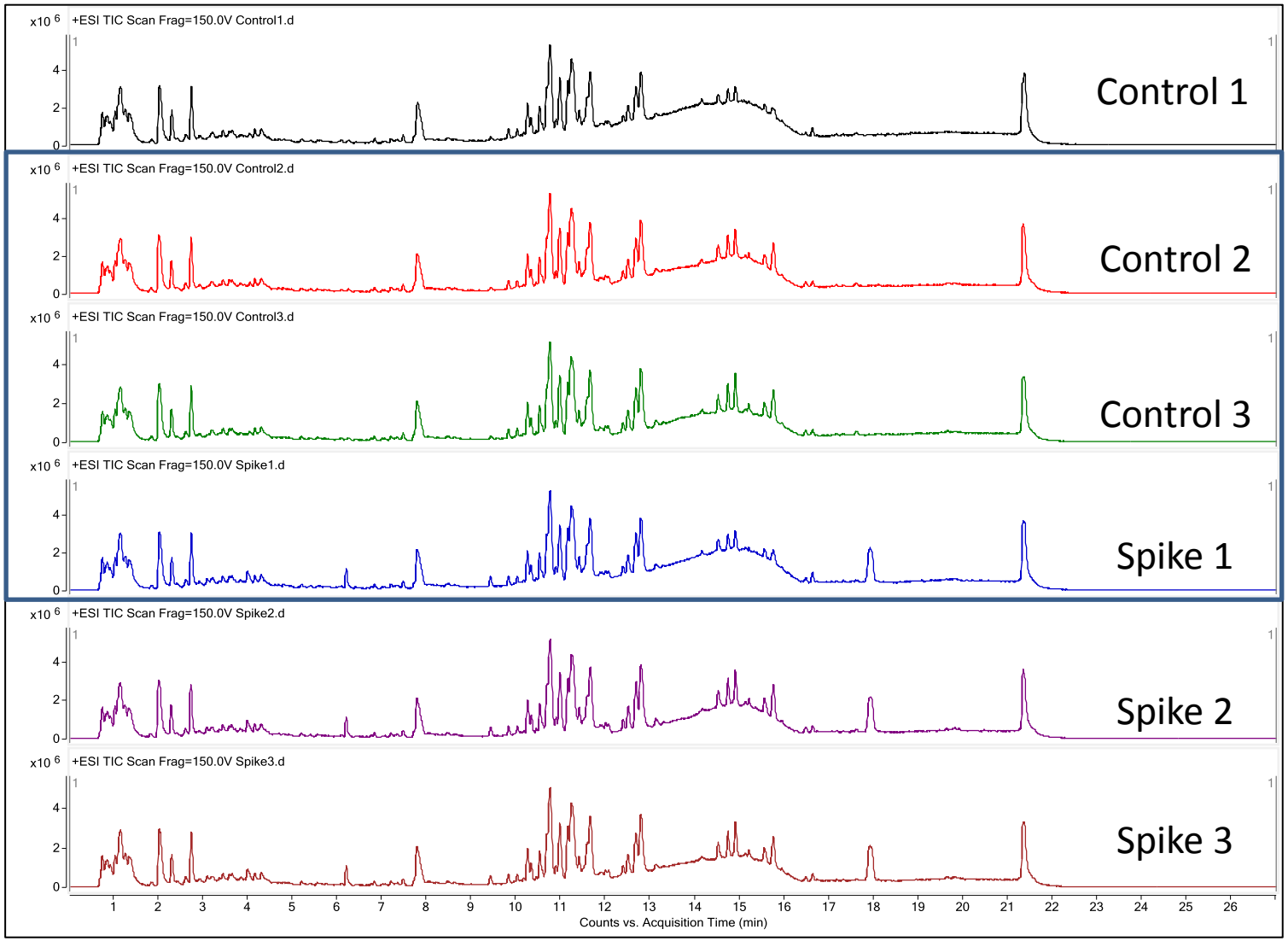
RIC | Research Institute for Chromatography

Feature extraction – Positive mode



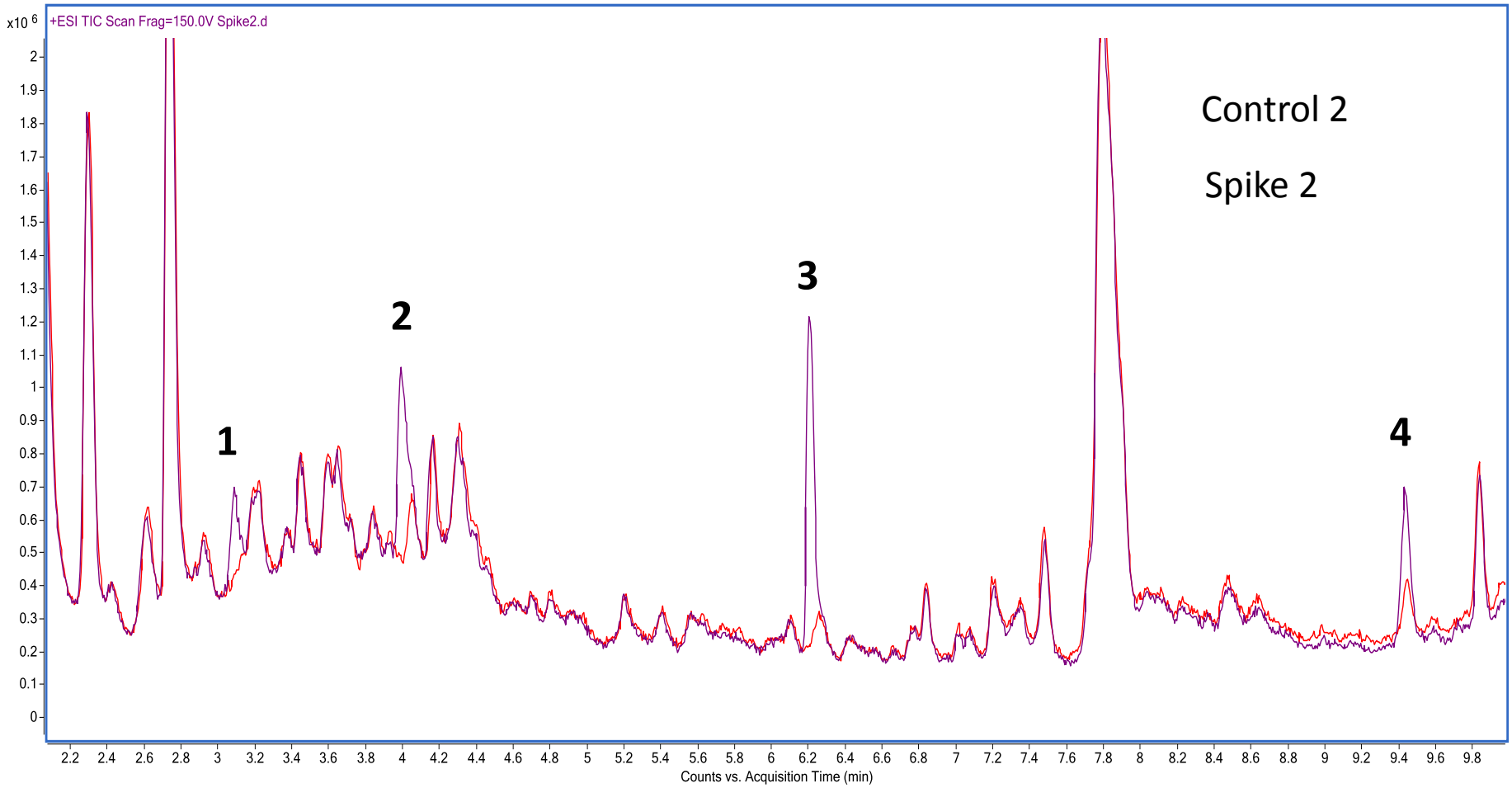
RIC | Research Institute for Chromatography

Differential analysis (3 x control – 3 x spike)



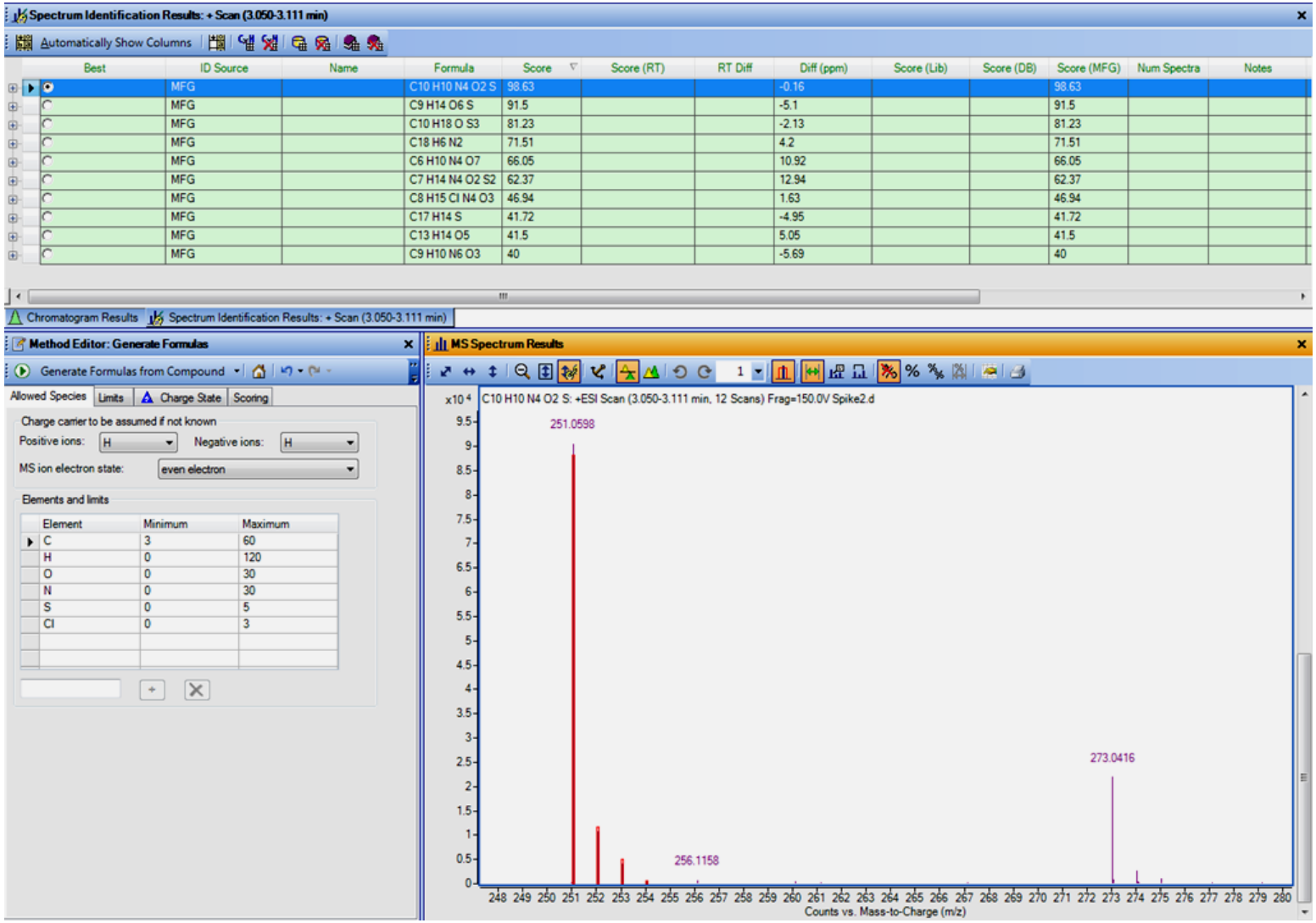
RIC | Research Institute for Chromatography

Differential analysis – Positive mode



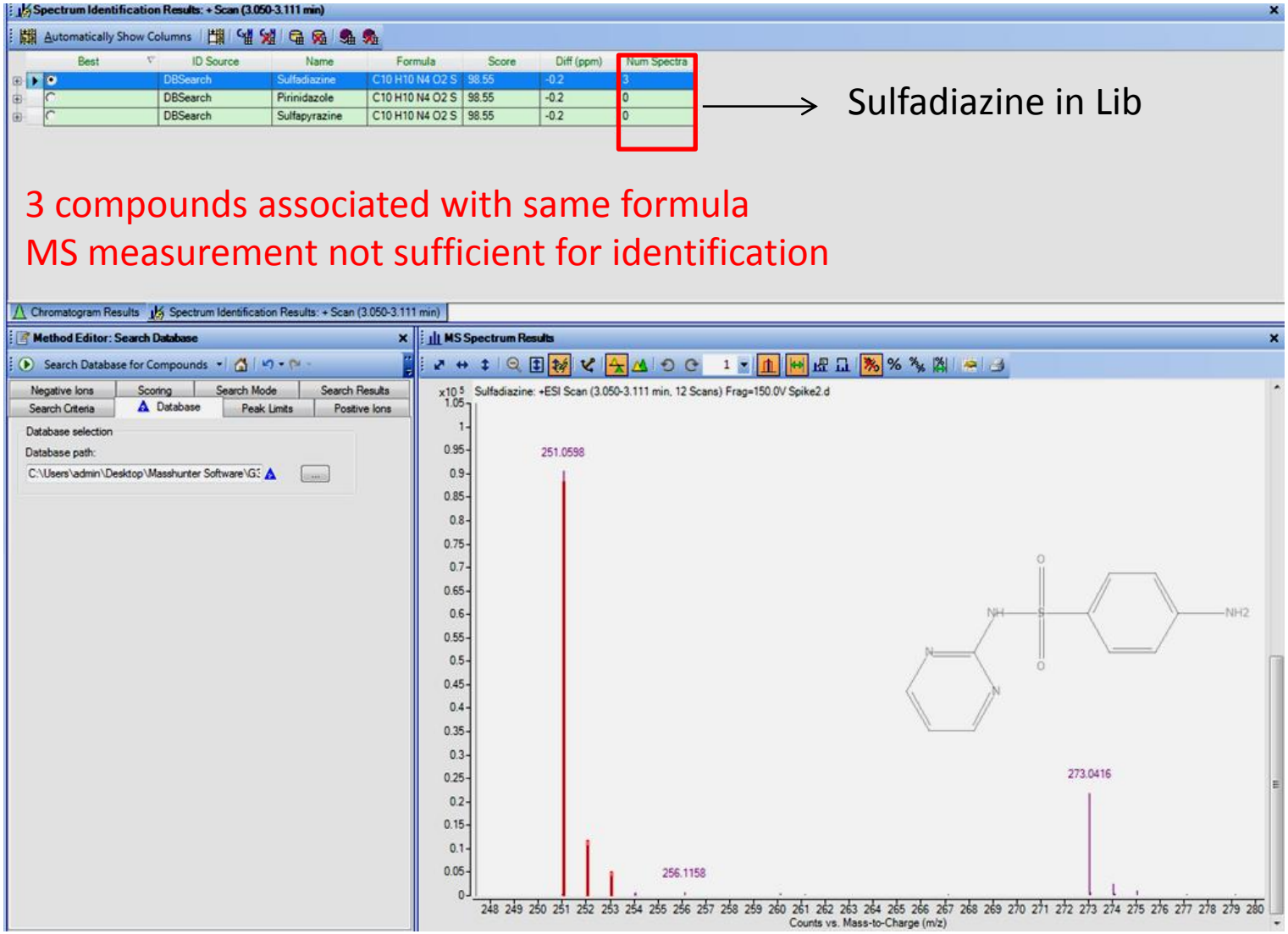
RIC | Research Institute for Chromatography

Peak 1 - ID – Molecular Formula Generation



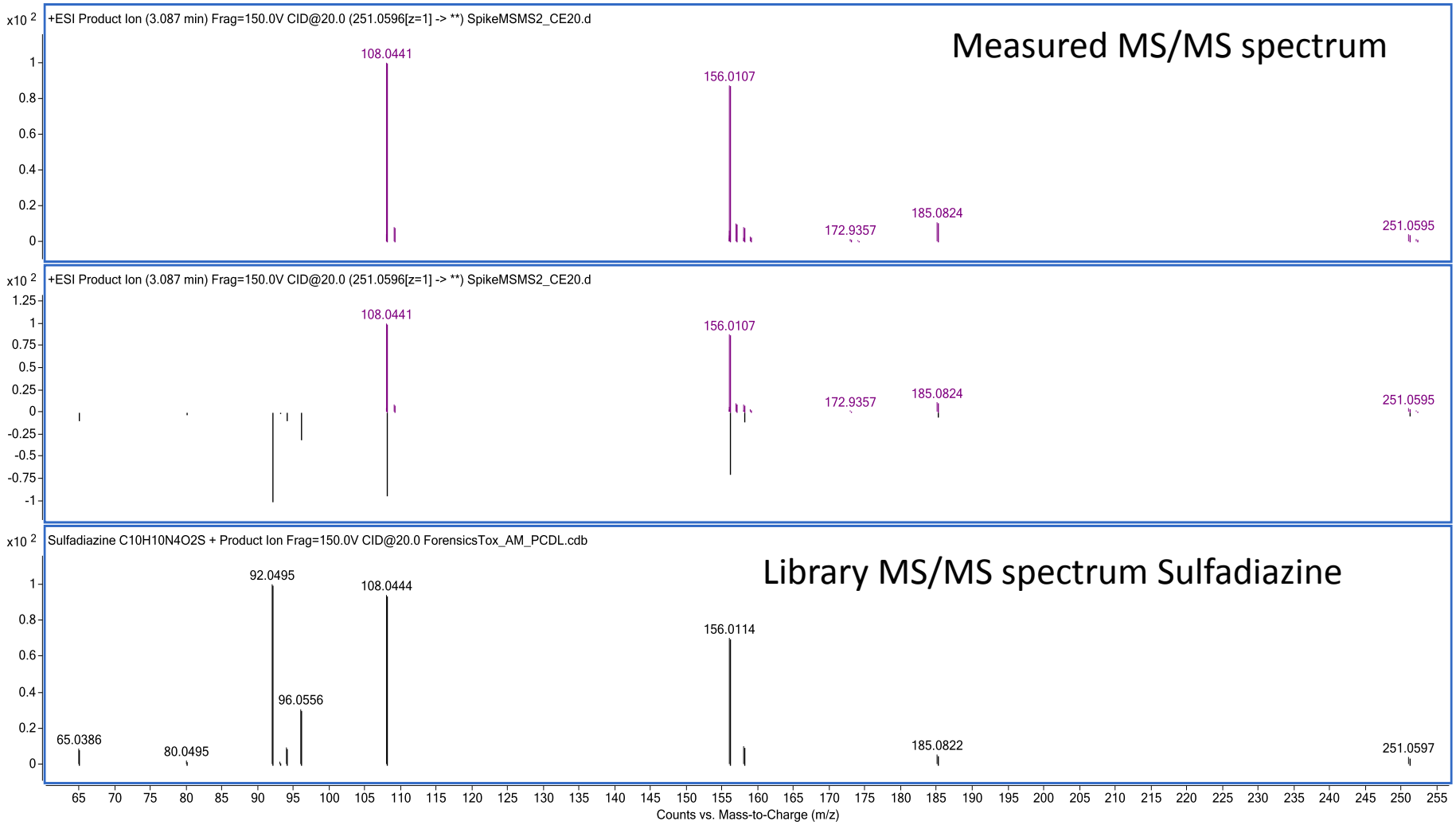
RIC | Research Institute for Chromatography

Peak 1 - ID – Database Search



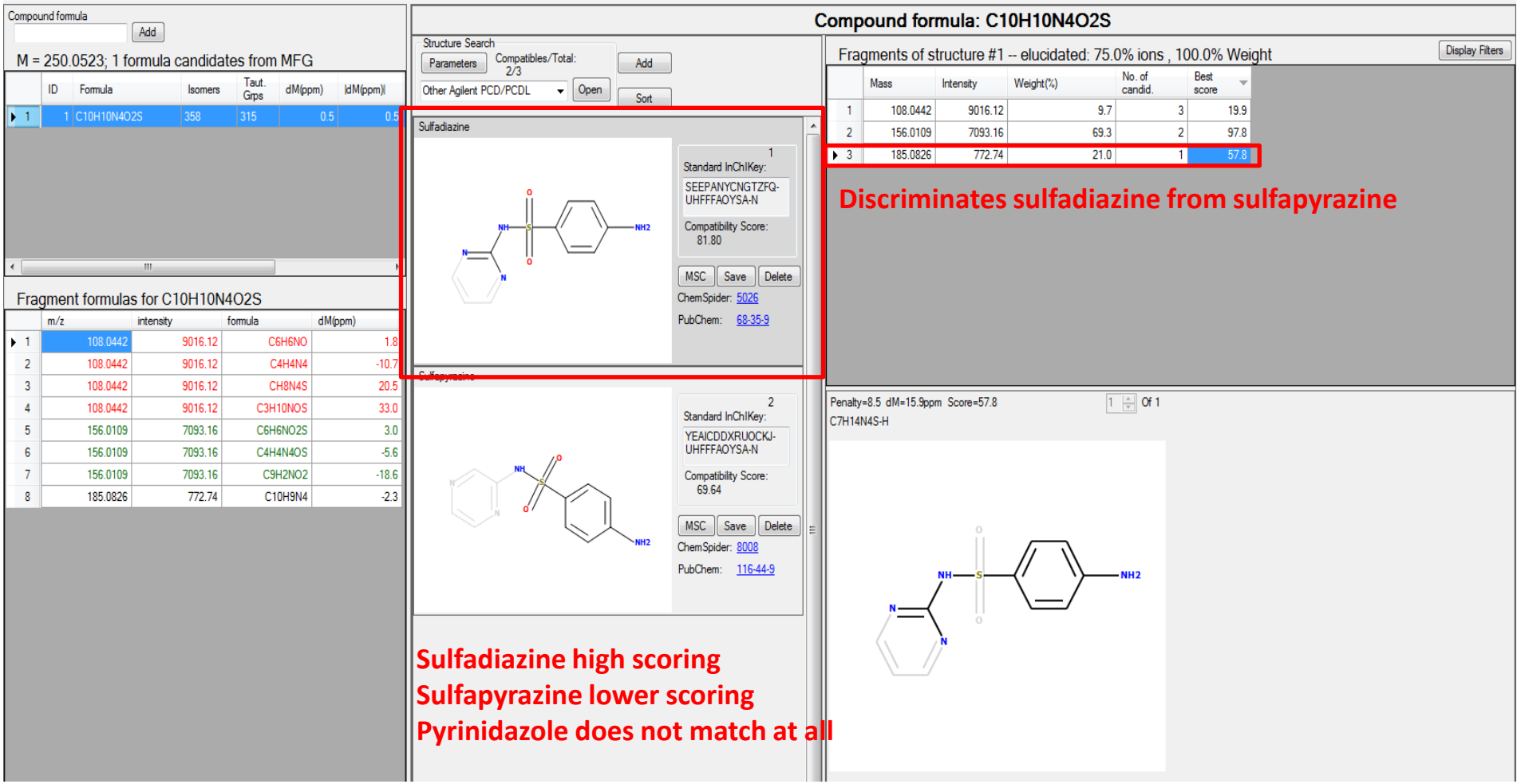
RIC Research Institute for Chromatography

Peak 1 - ID – MS/MS



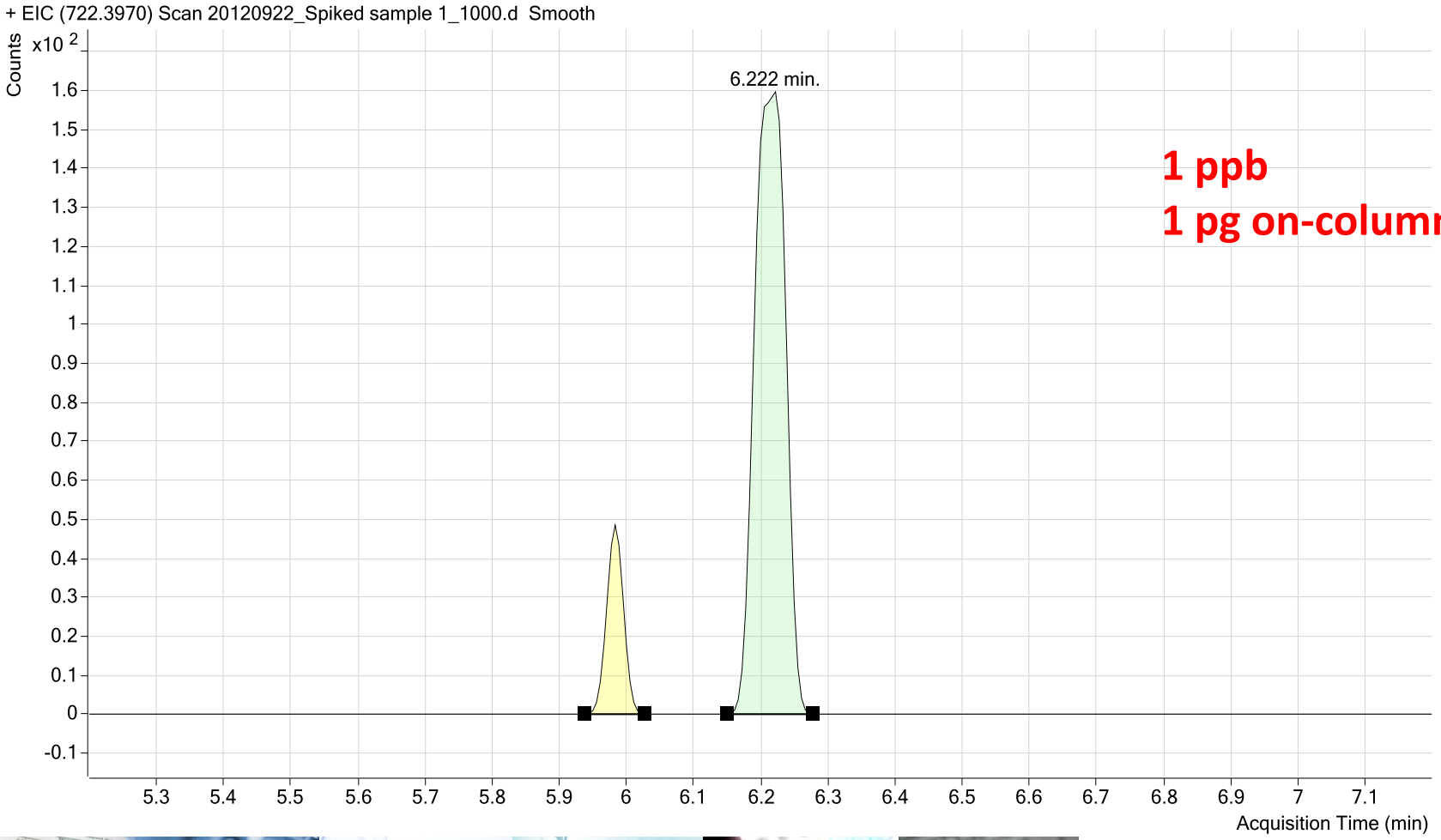
RIC | Research Institute for Chromatography

Peak 1 - ID – Molecular Structure Correlator (MSC)



RIC Research Institute for Chromatography

Quantitative analysis (+ mode) of Mycotoxin (fumonisin B1)

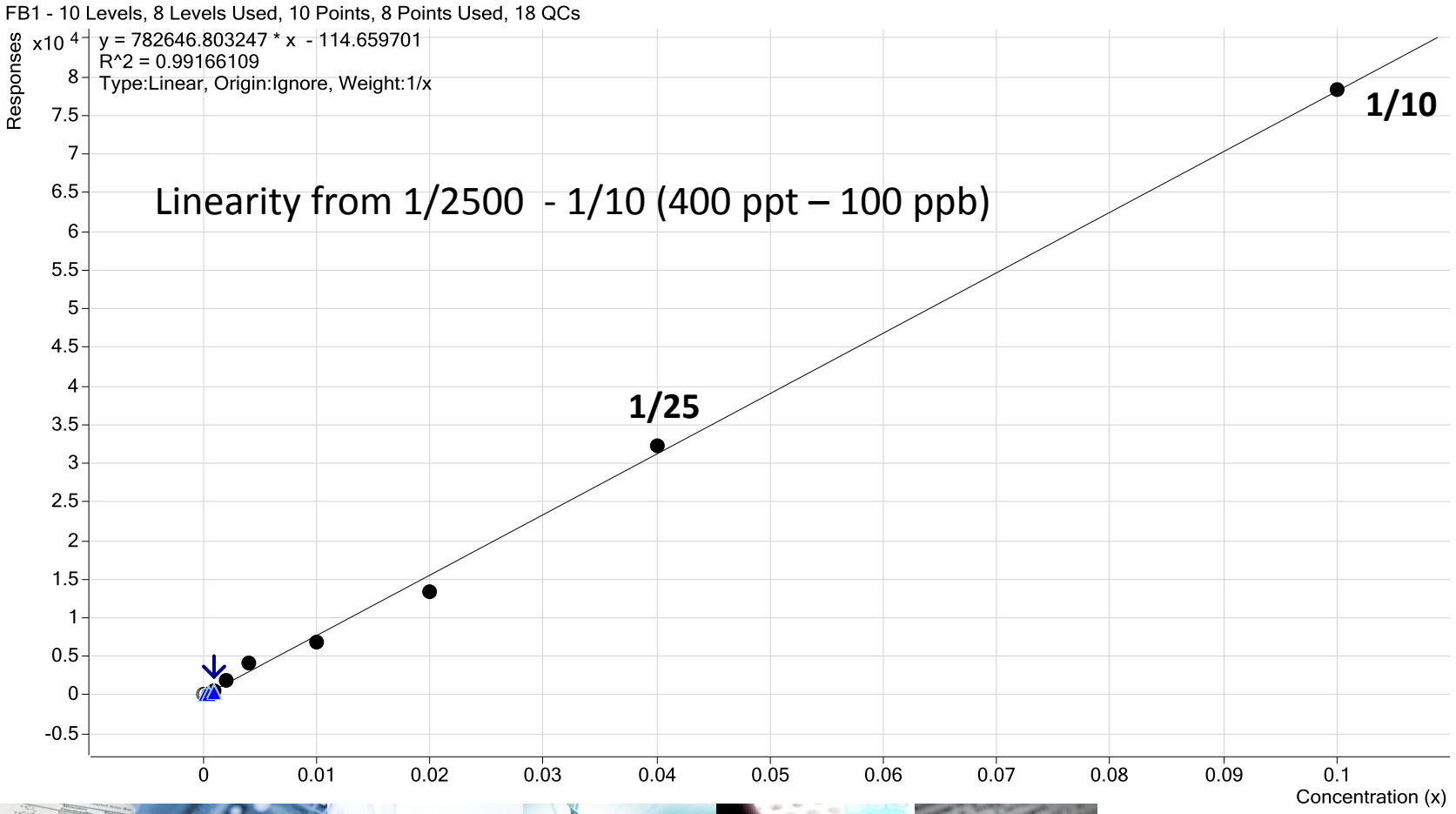


1 ppb

1 pg on-column

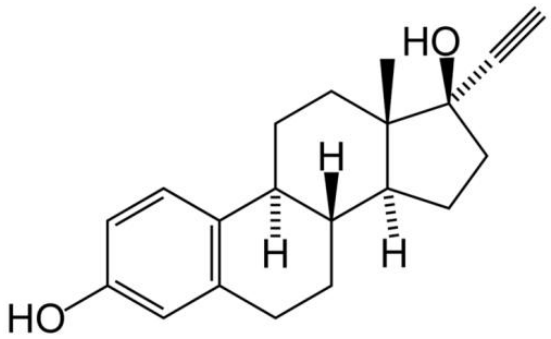
RIC | Research Institute for Chromatography

Quantitative analysis (+ mode) of Mycotoxin (fumonisin B1)



RIC | Research Institute for Chromatography

Case Study: the fate of a hormone (ethinylestradiol) upon chlorination (water treatment)



Non-doped extract (3*)

Doped extract (3*)

LC-Q-TOF-MS

Feature extraction

Differential analysis

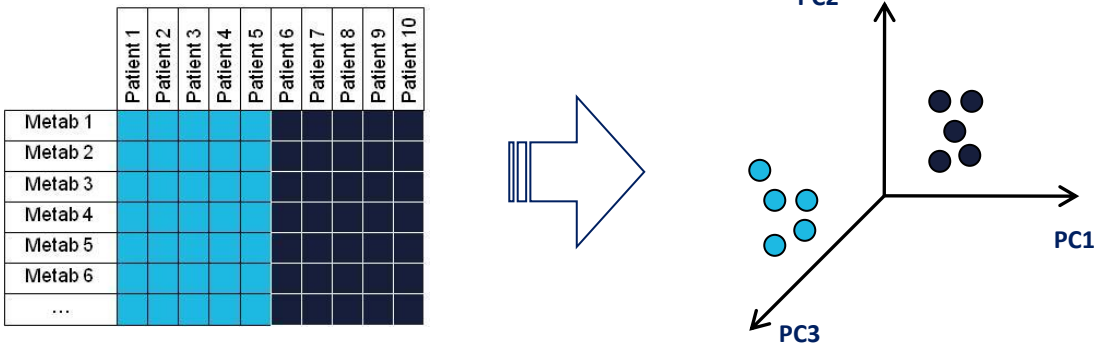
List of differential features

Formula generation

RIC | Research Institute for Chromatography

Un-targeted data processing

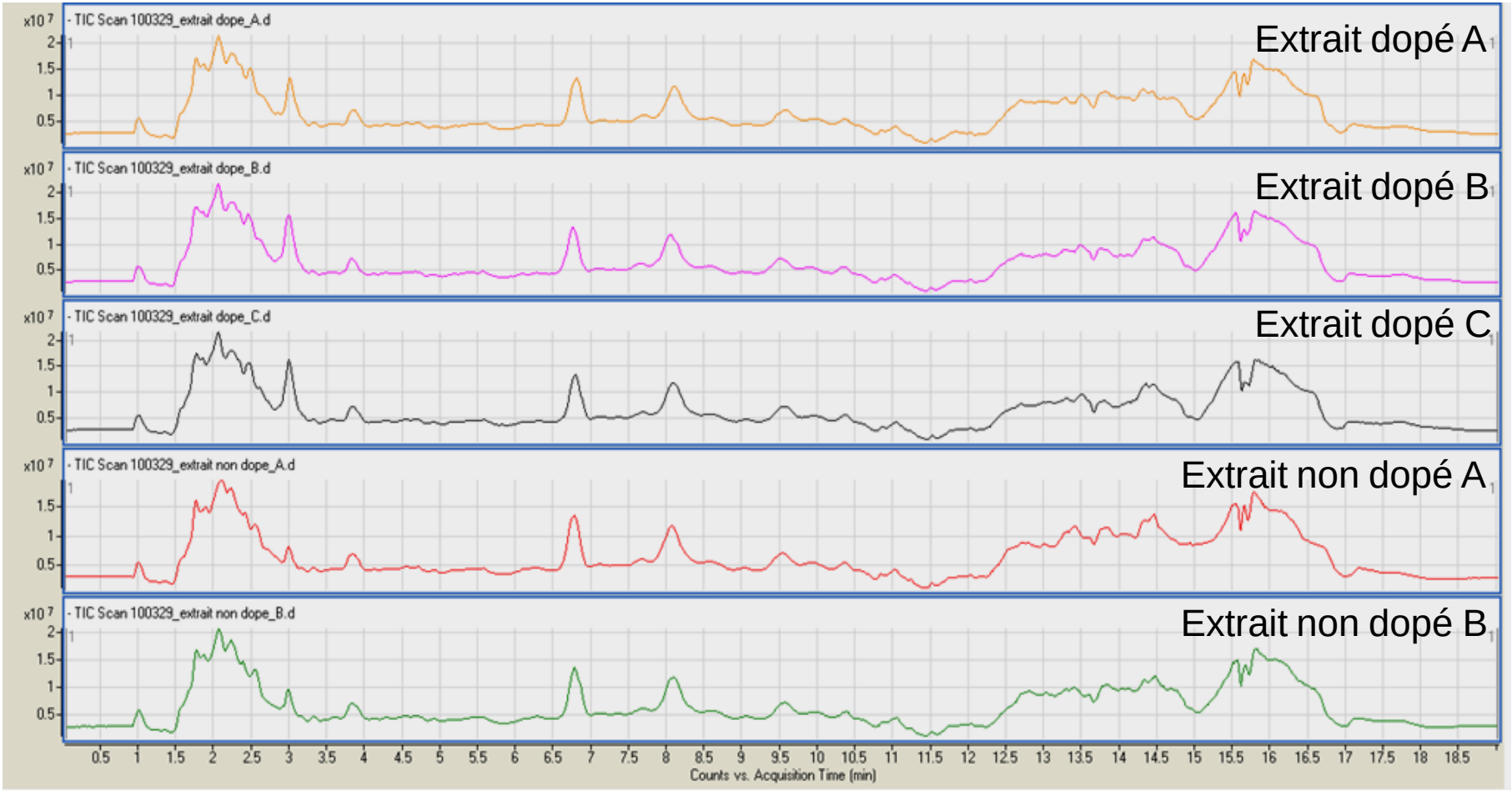
- Extract all features (*m/z*-retention time pairs) out of the raw data ➔ data matrix (tR, *m/z*, intensity)
- Statistical analysis
  - Multivariate analysis (PCA, PLS-DA ...) (Mass Profiler Professional)
- Identification of down- or up-regulated features



RIC | Research Institute for Chromatography

Water Sample Profiling by LC-QTOF

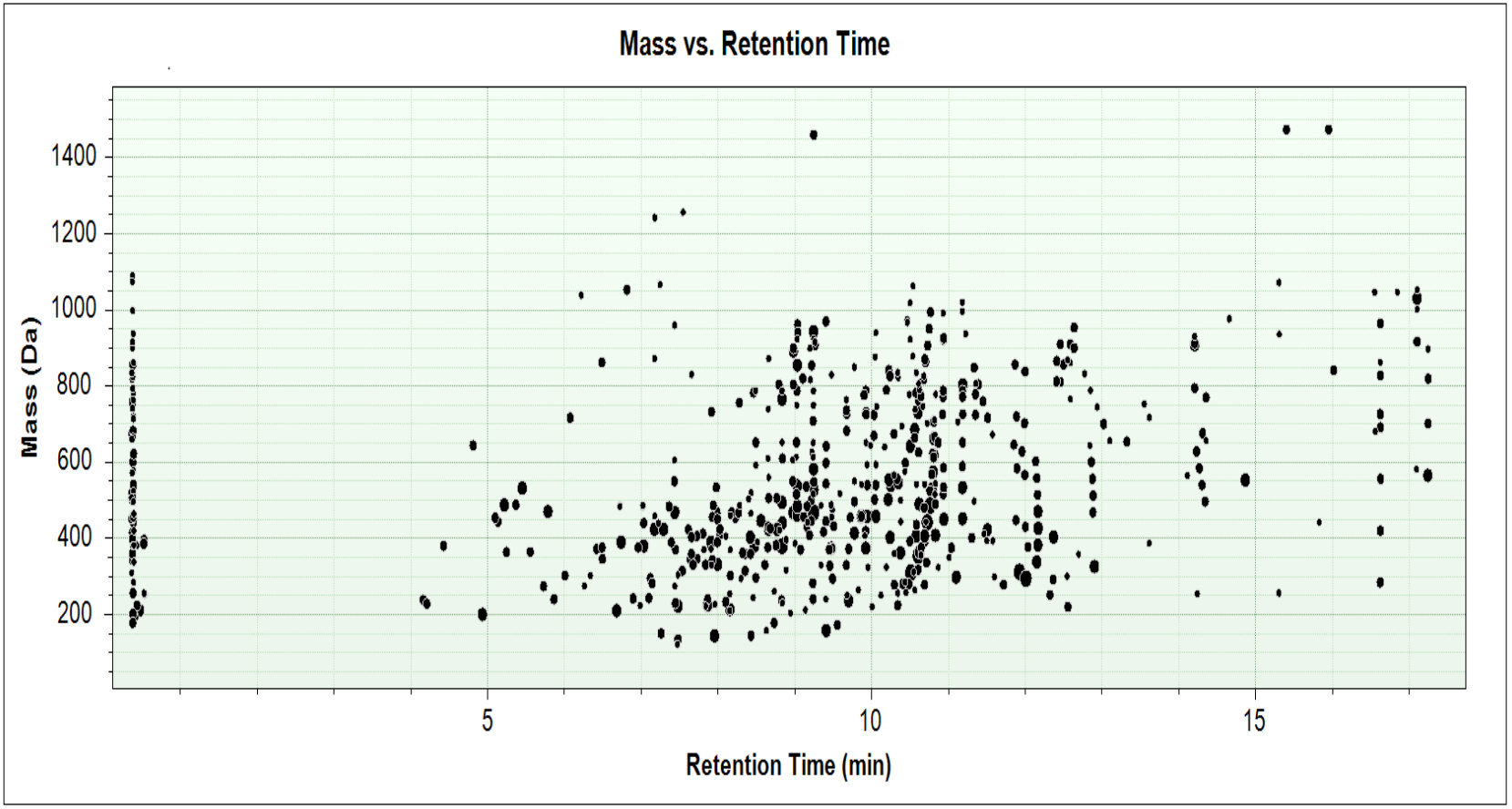
TIC



RIC | Research Institute for Chromatography

Water Sample Profiling by LC-QTOF

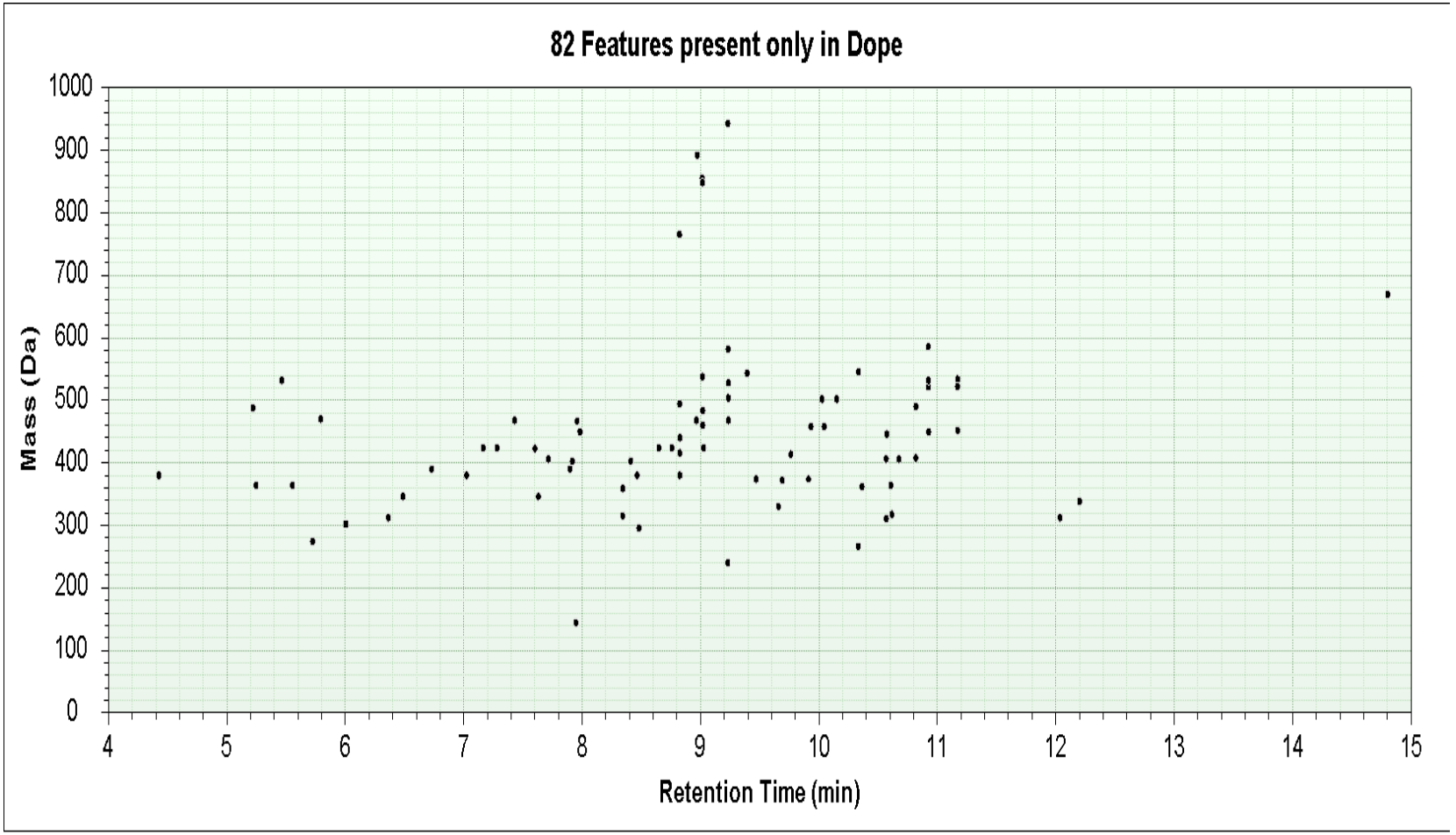
Data interpretation by Molecular Feature Extraction (MFE)



RIC | Research Institute for Chromatography

Water Sample Profiling by LC-QTOF

Sample Comparison



RIC | Research Institute for Chromatography

Formula generation

m/z 466.98653 - settings: C (3-60), H (0-120), O (0-30), N (0-5), S (0-5), Cl (0-5), Br (0-5)

MS Formula Results: - Scan (9.247 min)

m/z	Ion	Formula	Abundance
466.98653	(M-H)-	C20 H21 Br2 O3	466042.6

Best	Formula (M)	Ion Formula	Score	Cross Score	Calc m/z	Diff (ppm)	Mass Match	Abund Match	Spacing Match
☑	C20 H22 Br2 O3	C20 H21 Br2 O3	98.67		466.98629	0.21	99.95	98.6	96.18
☐	C12 H26 Br2 N2...	C12 H25 Br2 N2 O...	92.9		466.98564	-1.44	97.89	79.62	98.88
☐	C18 H23 Br Cl2...	C18 H22 Br Cl2 O...	88.73		466.98556	-2.31	94.66	71.54	97.49
☐	C14 H23 Br Cl2...	C14 H22 Br Cl2 O8	81.57		466.98806	3.16	90.24	54.78	96.35
☐	C15 H27 Br Cl2...	C15 H26 Br Cl2 O...	80.72		466.98893	4.71	79.61	67.8	98.45
☐	C15 H21 Cl5 N2...	C15 H20 Cl5 N2 O4	80.38		466.98712	-0.02	100	33.17	97.79
☐	C17 H26 Br2 O3...	C17 H25 Br2 O3 S	77.79		466.98966	7.22	58.5	93.74	97.23
☐	C16 H26 Br2 N2...	C16 H25 Br2 N2 S2	77.05		466.98314	-6.91	61.17	85.11	99.12
☐	C15 H23 Br2 Cl...	C15 H22 Br2 Cl N...	76		466.98544	-2.1	95.58	24.18	99.06
☐	C13 H20 Cl4 N4...	C13 H19 Cl4 N4 O...	75.25		466.98866	3.19	90.07	30.4	99.42
☐	C15 H19 Br Cl2...	C15 H18 Br Cl2 N...	73.97		466.9894	5.95	69.49	60.44	99.18
☐	C16 H22 Br Cl N...	C16 H21 Br Cl N2...	73.62		466.9871	1.08	98.8	10.28	99.27
☐	C9 H19 Cl3 N2...	C9 H18 Cl3 N2 O13	73.02		466.988	2.17	95.28	14.76	98.44
☐	C16 H16 Cl4 N4...	C16 H15 Cl4 N4 O4	72.61		466.98529	-3.83	86.01	28.39	98.86
☐	C22 H11 Cl3 N4...	C22 H10 Cl3 N4 O2	72.13		466.98748	1.21	98.49	6.13	98.61
☐	C22 H19 Cl3 O...	C22 H18 Cl3 O S2	72.11		466.98701	-0.1	99.99	4.2	97.87
☐	C16 H24 Cl4 O3...	C16 H23 Cl4 O3 S2	71.81		466.98482	-5.04	76.98	41.37	98.01
☐	C21 H15 Cl3 O6	C21 H14 Cl3 O6	71.74		466.98614	-1.57	97.51	8.13	96.54
☐	C11 H18 Cl2 N4...	C11 H17 Cl2 N4 O...	71.73		466.98703	-0.18	99.97	1.36	99.69
☐	C12 H22 Cl2 N4...	C12 H21 Cl2 N4 O...	71.5		466.9879	1.19	98.57	3.37	99.11
☐	C15 H18 Br Cl N...	C15 H17 Br Cl N2...	71.39		466.98623	-0.33	99.89	1.78	97.93
☐	C18 H16 N2 O5...	C18 H15 N2 O5 S4	71.35		466.98693	0.09	99.99	0	99.7
☐	C21 H13 Cl N4...	C21 H12 Cl N4 O...	71.23		466.98672	-0.47	99.78	0	99.62
☐	C14 H23 Cl3 N2...	C14 H22 Cl3 N2 O...	70.92		466.98636	-1.89	96.4	4.78	99.31
☐	C25 H13 Br N2...	C25 H12 Br N2 O S	70.87		466.98592	0.19	99.96	0	97.7
☐	C20 H9 Cl N4 O...	C20 H8 Cl N4 O6 S	70.79		466.98586	-0.97	99.04	0	99.24
☐	C12 H21 Br O12...	C12 H20 Br O12 S	70.4		466.98643	0.88	99.2	0	97.28
☐	C21 H16 Cl4 N2...	C21 H15 Cl4 N2 O2	70.32		466.98931	4.89	78.19	34.42	97.65
☐	C14 H28 Br2 Cl...	C14 H27 Br2 Cl2...	70.06		466.98727	1.57	97.49	0.97	98.09

RIC Research Institute for Chromatography

Formula generation

m/z 466.98653 - settings: C (3-60), H (0-120), O (0-30), N (0-5), S (0-5), Cl (0-5), Br (0-5)

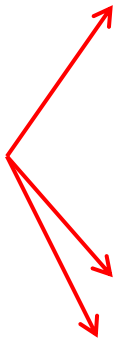
MS Formula Results: - Scan (9.247 min)

Best	Formula (M)	Ion Formula	Score	Cross Score	Calc m/z	Diff (ppm)	Mass Match	Abund Match	Spacing Match
✓	C20 H22 Br2 O3	C20 H21 Br2 O3	98.67		466.98629	0.21	99.95	98.6	96.18
	C12 H26 Br2 N2...	C12 H25 Br2 N2 O...	92.9		466.98564	-1.44	97.89	79.62	98.88
	C16 H26 Br2 N2...	C16 H25 Br2 N2 S2	77.05		466.98314	-6.91	61.17	85.11	99.12
	C15 H23 Br2 Cl...	C15 H22 Br2 Cl N...	76		466.98544	-2.1	95.58	24.18	99.06
	C13 H20 Cl4 N4...	C13 H19 Cl4 N4 O...	75.25		466.98866	3.19	90.07	30.4	99.42
	C15 H...								99.18
	C16 H...								99.27
	C9 H...								98.44
	C16 H...								98.86
	C22 H...								98.61
	C22 H...								97.87
	C16 H...								98.01
	C21 H...								96.54
	C11 H...								99.69
	C12 H...								99.11
	C15 H...								97.93
	C18 H...								99.7
	C21 H...								99.62
	C14 H...								99.31
	C25 H...								97.7
	C20 H...								99.24
	C12 H21 Br O12...	C12 H20 Br O12 S	70.4		466.98643	0.88	99.2	0	97.28
	C21 H16 Cl4 N2...	C21 H15 Cl4 N2 O2	70.32		466.98931	4.89	78.19	34.42	97.65
	C14 H28 Br2 Cl...	C14 H27 Br2 Cl2...	70.06		466.98727	1.57	97.49	0.97	98.09

C20H22Br2O3
Score: 98.67 (>> 92.9 for 2nd)
Diff (ppm): 0.21
Mass match: 99.95
Abund match: 98.6
Spacing match: 96.18

RIC Research Institute for Chromatography

Formula generation for all features



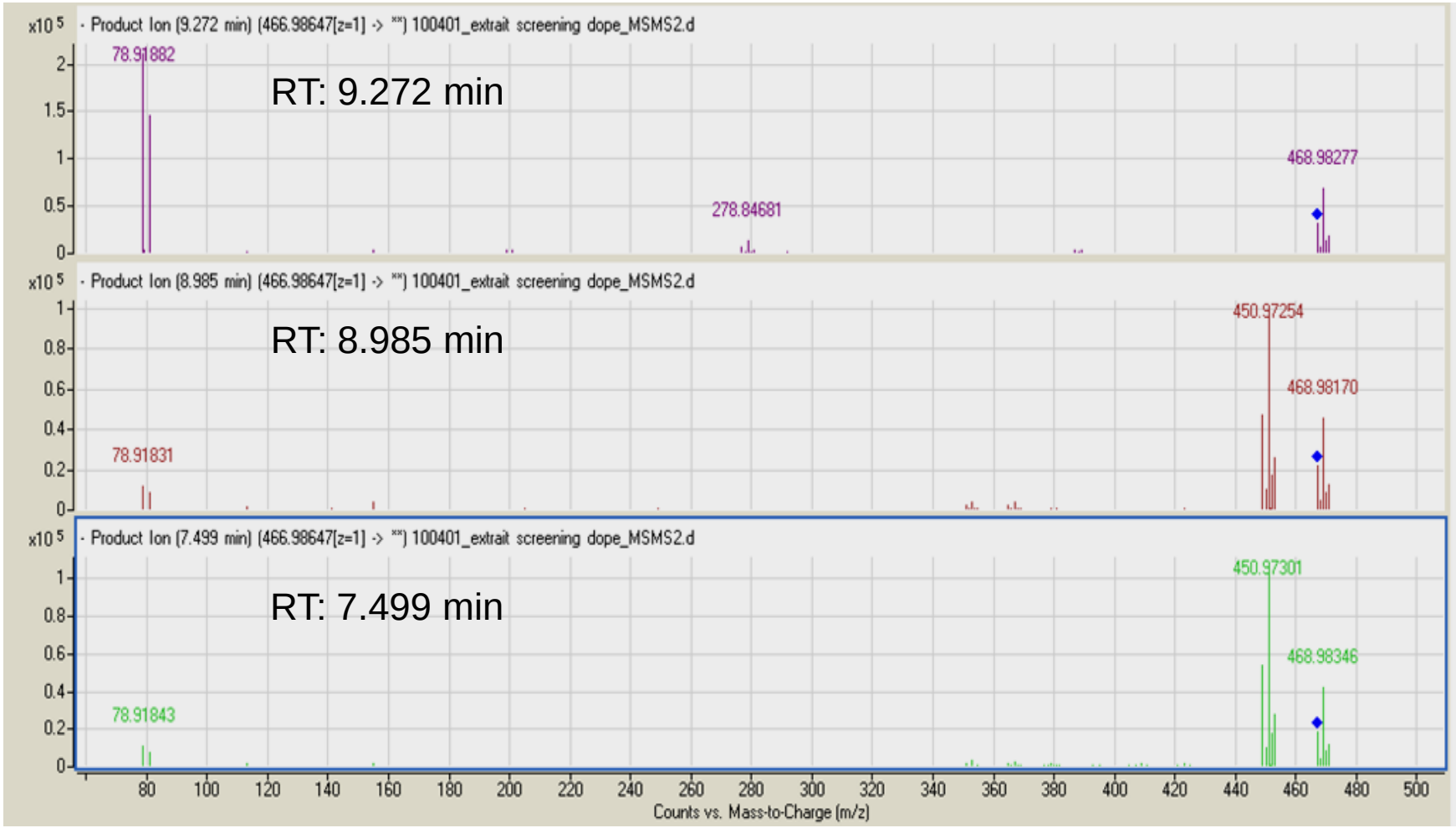
467.9935 = C20H22Br2O3 (3 x ???)

ethinylestradiol

Mass	Retention Time	Abundance	Formula	Mass	Retention Time	Abundance	Formula
467,9942	9,233	23943030	C20H22Br2O3	312,173	6,364	567390	C20H24O3
848,0886	9,016	16087590	C40H44Br2Cl2O6	364,1003	10,609	543875	C20H22Cl2O2
380,0955	8,824	14349780	C20H22Cl2O3	534,0007	11,17	542713	C19H24Br2N2O6
424,0458	9,026						C20H22BrClO3
451,9996	11,171						C20H21BrCl2O3
449,9841	10,926						C20H23Cl3O3
408,0503	10,818	2884004	C20H22BrClO2	545,9054	10,333	455787	C20H21Br3O3
406,0345	10,568	1657978	C20H20BrClO2	338,2126	12,201	417926	C16H34O5S
484,0653	9,018	1646482	C22H26BrClO5	423,0607	7,601	375988	C20H23BrClNO2
467,9946	7,428	1593335	C20H22Br2O3	531,9744	10,925	364172	C20H22Br2O7
424,0458	7,164	1424033	C20H22BrClO3	494,0875	8,823	351181	C19H24Cl2N2O9
467,9935	8,963	1324857	C20H22Br2O3	266,2916	10,331	350051	C12H26O4S
440,1165	8,825	1145927	C22H26Cl2O5	144,115	7,948	340101	C8H16O2
362,0852	10,364	1124280	C20H20Cl2O2	346,1352	6,486	328292	C20H23ClO3
528,0143	9,235	1047905	C22H26Br2O5	490,0518	10,819	326672	C19H24BrClN2O6
406,0343	10,676	1017551	C20H20BrClO2	374,0888	9,469	314224	C20H23BrO2
390,0849	6,729	1011866	C20H23BrO3	585,957	10,923	277448	C21H20Br2N2O8
424,045	8,648	1004305	C20H22BrClO3	501,9518	10,026	276945	C15H21Br2ClN2O5
538,0367	9,017	995718	C19H24BrClN2O9	359,1295	8,342	273291	C20H22ClNO3
460,0206	9,018	993316	C20H23BrCl2O3	446,1549	10,572	260336	C18H26N2O11
581,9857	9,232	918908	C19H24Br2N2O9	310,3298	10,568	250356	C14H29O5S
403,0794	8,41	902334	C20H22BrNO3	372,0728	9,69	245611	C20H21BrO2
380,0957	8,464	874822	C20H22Cl2O3	544,0087	9,393	244247	C22H26Br2O6
390,0843	7,897	854135	C20H23BrO3	315,1395	8,343	228329	C19H22ClNO
501,9539	10,15	792275	C20H21Br2ClO3	346,1347	7,629	226145	C20H23ClO3
458,0056	10,044	776216	C20H21BrCl2O3	402,3099	7,916	217614	C21H42N2O5
380,0962	7,023	772586	C20H22Cl2O3	330,1391	9,658	203717	C20H23ClO2
374,089	9,911	764030	C20H23BrO2	522,1083			31BrO10
424,0452	7,281	738988	C20H22BrClO3	450,1047			27BrO5
503,97	9,234	727172	C20H23Br2ClO3	240,1359			H20O4
467,01	7,957	679208	C20H23Br2NO2	406,1559	7,716	111636	C22H27ClO5
414,0564	9,763	593193	C20H21Cl3O3	**296,1778**	**8,48**	**95050**	**C20H24O2**

3 isomers of $C_{20}H_{22}Br_2O_3$

⇒ Role of Q-TOF (MS/MS)



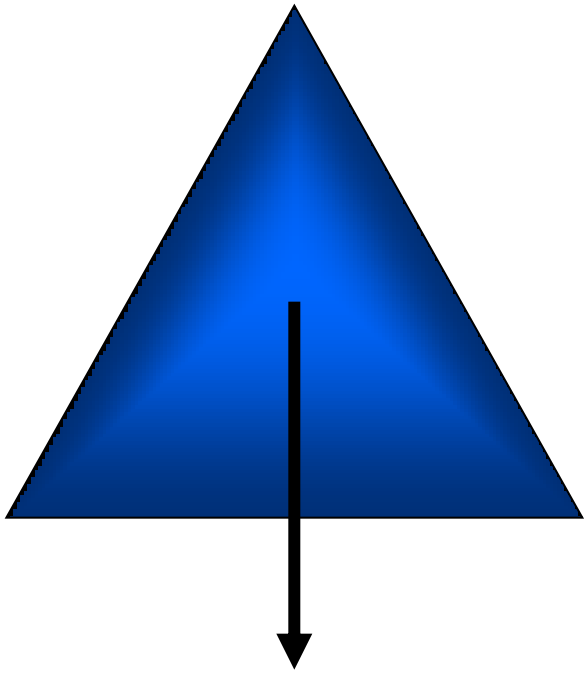
RIC | Research Institute for Chromatography

GC and LC - QTOF

- Full spectral data capture at **high resolution**, **high mass accuracy**, **high sensitivity** and **high speed**
- Ideal for **untargeted** screening
- **Identification** tool due to its mass accuracy, accurate read-out of isotopic information and MS/MS capabilities
- **Quantification** tool via MS or MS/MS (MRM mimic) with selectivity offered by mass accuracy and MS/MS filtering
- Q-TOF **MassHunter** software equipped with powerful data mining and analysis tools and complemented with packages such as **Mass Profiler (Pro)** to detect differences in an untargeted manner and databases/libraries (*Toxicology, Pesticide and Metlin*) for identification

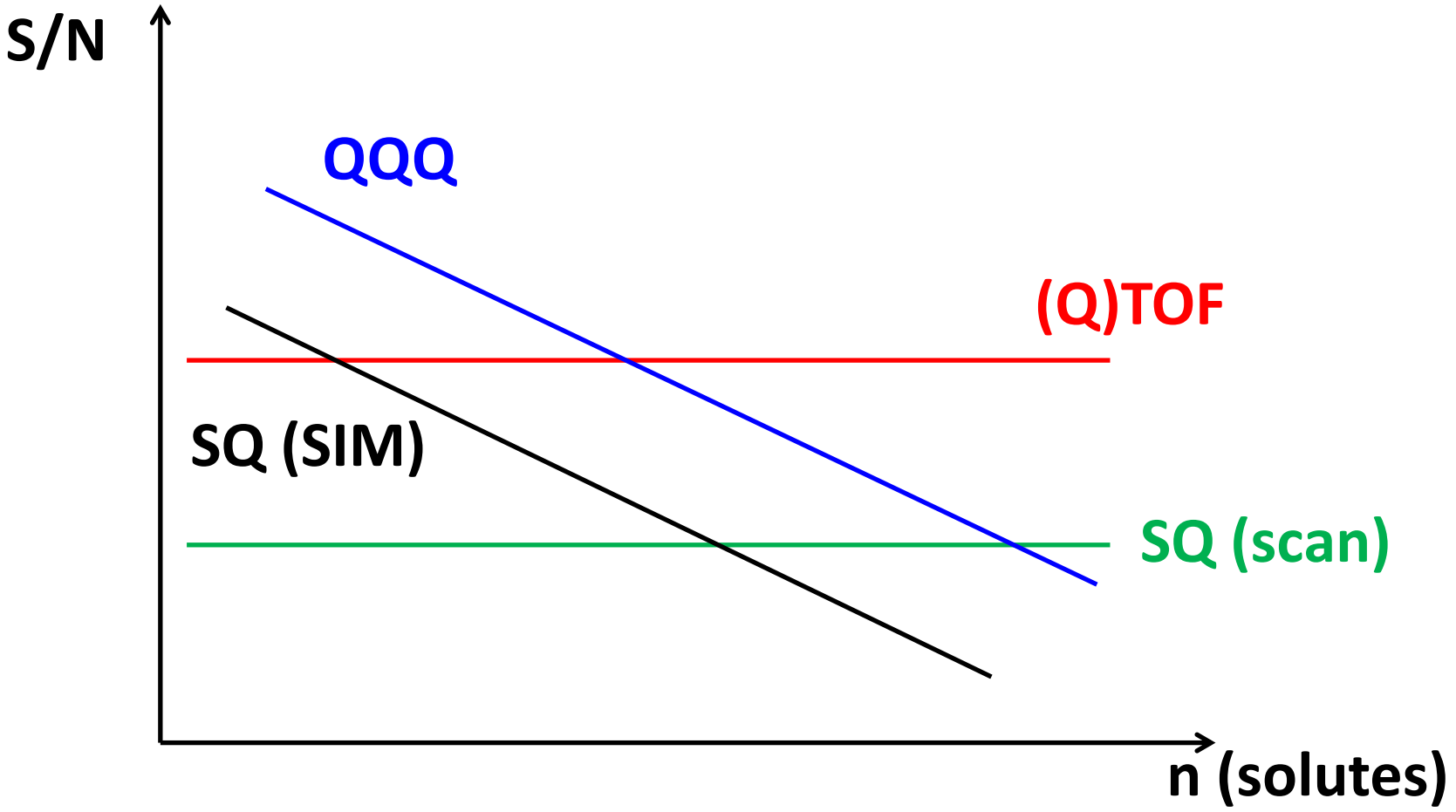
RIC | Research Institute for Chromatography

The (GC/LC) MS compromise triangle



RIC | Research Institute for Chromatography

“Scan” versus “SIM” (MRM)



RIC | Research Institute for Chromatography