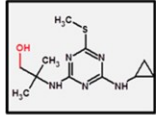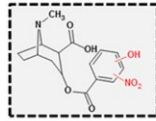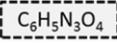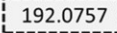# The Screening and Identification of Marine and Freshwater Toxins in Food and Feed Using a State-of-the-Art High Resolution Mass Spectrometry Technique

Mirjam Klijnstra

# Screening, identification and confirmation

- Nowadays LC-MS/MS golden standard for confirmation (level 1)

- High-resolution MS shows potential, however…

Identification level 5

		Minimum data requirements
	Level 1: Confirmed structure by reference standard	MS, MS², RT, Reference Std.
	Level 2: Probable structure a) by library spectrum match	MS, MS², Library MS²
	b) by diagnostic evidence	MS, MS², Exp. data
	Level 3: Tentative candidate(s) structure, substituent, class	MS, MS², Exp. data
$C_6H_5N_3O_4$	**Level 4: Unequivocal molecular formula**	MS isotope/adduct
192.0757	**Level 5: Exact mass of interest**	MS

- Found a *m/z* 842.50491

- Potential structures (based on C, H, N and O)

Formula	Mass	RDB	Delta [ppm]
$C_{47}H_{72}O_{12}N$	842.5049	12.5	0.008
$C_{46}H_{66}O_7N_8$	842.5049	18	0.015
$C_{33}H_{74}O_{18}N_6$	842.50541	0	-0.594
$C_{60}H_{64}ON_3$	842.50439	30.5	0.617
$C_{31}H_{72}O_{17}N_9$	842.50407	0.5	0.999
$C_{48}H_{68}O_8N_5$	842.50624	17.5	-1.579
$C_{45}H_{70}O_{11}N_4$	842.50356	13	1.602
$C_{34}H_{70}O_{14}N_{10}$	842.50675	5	-2.182
$C_{35}H_{76}O_{19}N_3$	842.50675	-0.5	-2.188
$C_{58}H_{62}N_6$	842.50305	31	2.211
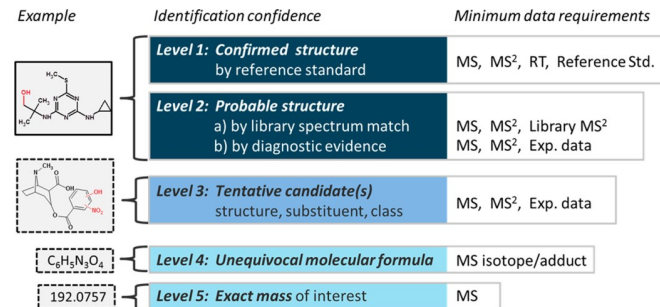
Identification level 4

Found m/z 842.50491 + isotopic pattern
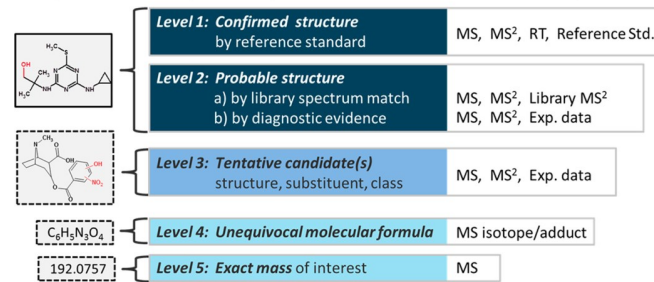
$C_{47}H_{71}NO_{12}$ still 25 structural possibilities (i.e. Chemspider)

ID	Structure	Molecular Formula	Molecular Weight	# of Data Sources ▼	# of References	# of PubMed	# of RSC
29394109 - 20/20 defined		$C_{47}H_{71}NO_{12}$	842.0661	9	9	0	0
9460900 - 20/20 defined		$C_{47}H_{71}NO_{12}$	842.0661	6	3	0	0
9724424 - 20/20 defined		$C_{47}H_{71}NO_{12}$	842.0661	4	3	0	0
10216857 - 20/20 defined		$C_{47}H_{71}NO_{12}$	842.0661	3	3	0	0

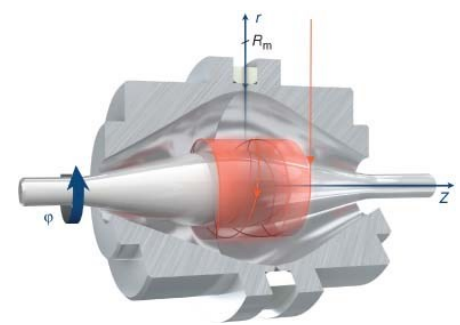| Example	Identification confidence		Minimum data requirements
	Level 1: Confirmed structure by reference standard		MS, MS², RT, Reference Std.
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	Level 3: Tentative candidate(s) structure, substituent, class		MS, MS², Exp. data
$C_6H_5N_3O_4$	**Level 4:** Unequivocal molecular formula		MS isotope/adduct
192.0757	**Level 5:** Exact mass of interest		MS

Then the challenge starts

- Often many candidates

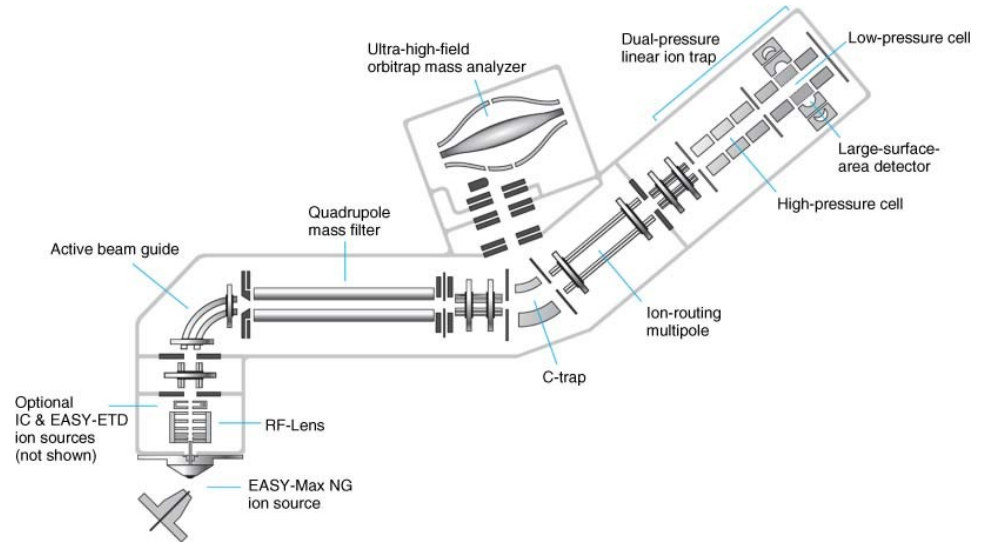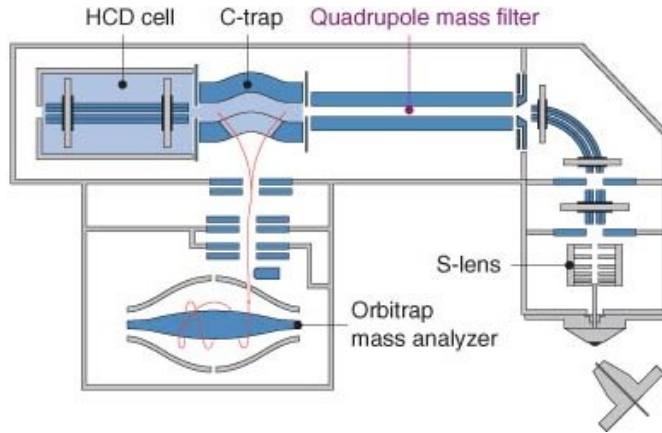- Generating MS^2 spectra
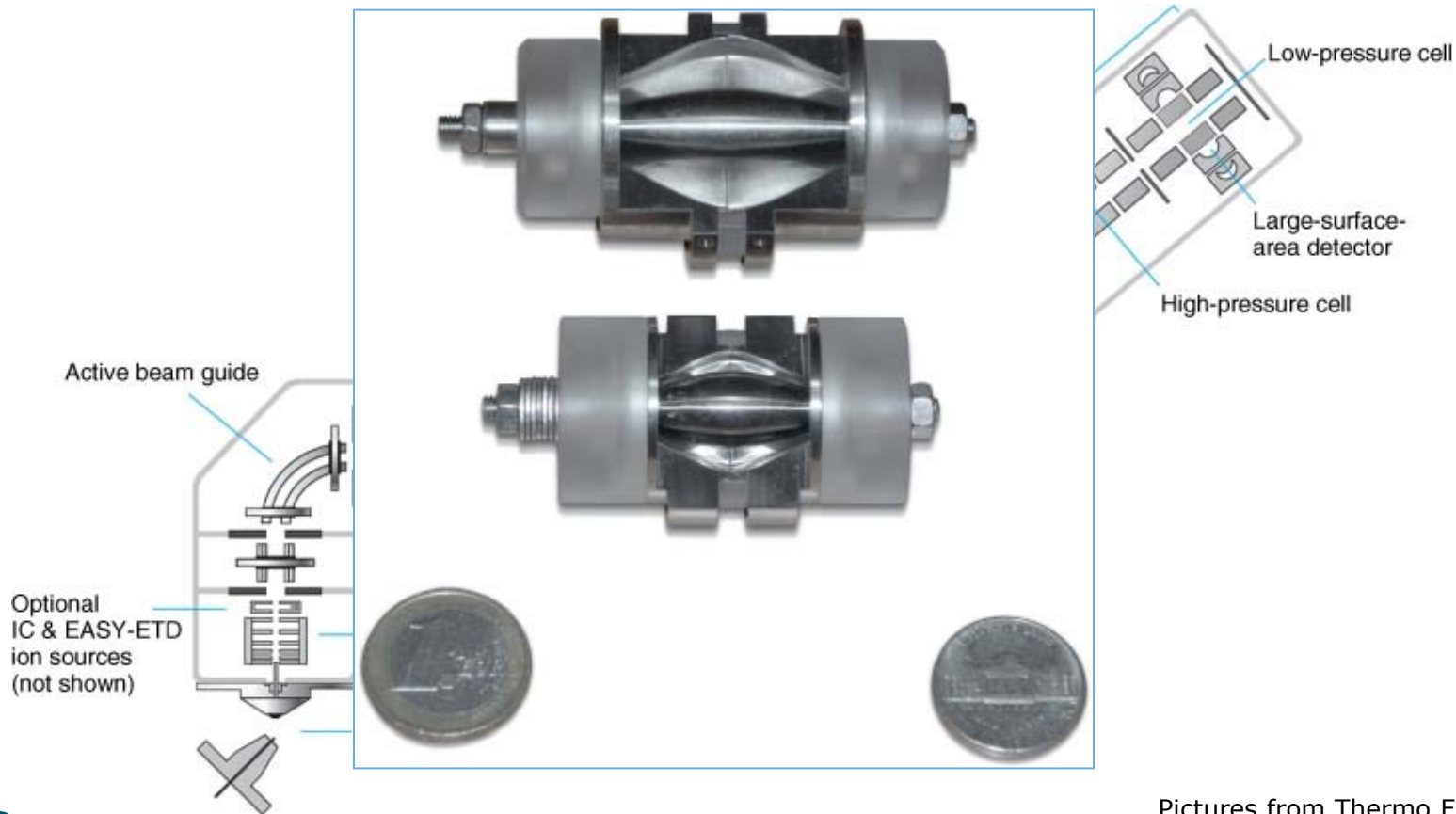
- Additional experiments and data analysis

Example	Identification confidence	Minimum data requirements
	Level 1: Confirmed structure by reference standard	MS, MS^2, RT, Reference Std.
	Level 2: Probable structure a) by library spectrum match b) by diagnostic evidence	MS, MS^2, Library MS^2 MS, MS^2, Exp. data
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$C_6H_5N_3O_4$	**Level 4: Unequivocal molecular formula**	MS isotope/adduct
192.0757	**Level 5: Exact mass** of interest	MS

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Approach we used previously

- Q-Exactive Orbitrap

- Generating Full Scan and fragmentation data in a single run

Full Scan m/z 100 – 1,500 Resolution 70,000		
All ion fragmentation m/z 100 – 500 Fragments: m/z 50 – 500 Resolution 17,500	All ion fragmentation m/z 500 – 1,000 Fragments: m/z 50 – 1,500 Resolution 17,500	All ion fragmentation m/z 1,000 – 1,500 Fragments: m/z 50 – 1,500 Resolution 17,500

Moving towards a tribrid Orbitrap MS

Pictures from Thermo Fisher
https://www.thermofisher.com/

The Orbitrap IQ-X

Pictures from Thermo Fisher
https://www.thermofisher.com/

Improved resolution

Resolution 60,000

Resolution 240,000

Resolution 500,000

Improved measurement strategy with AcquireX

Automatically updated run-to-run inclusion/exclusion lists
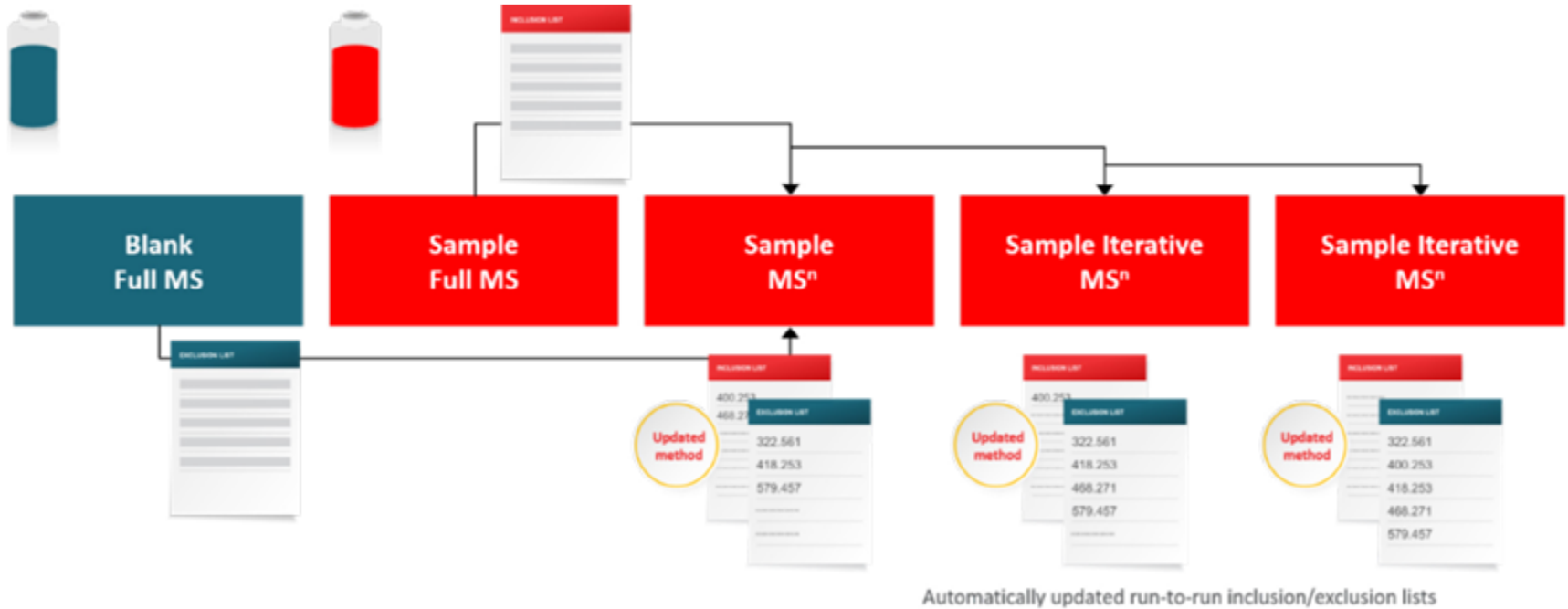
Improved measurement strategy with AcquireX
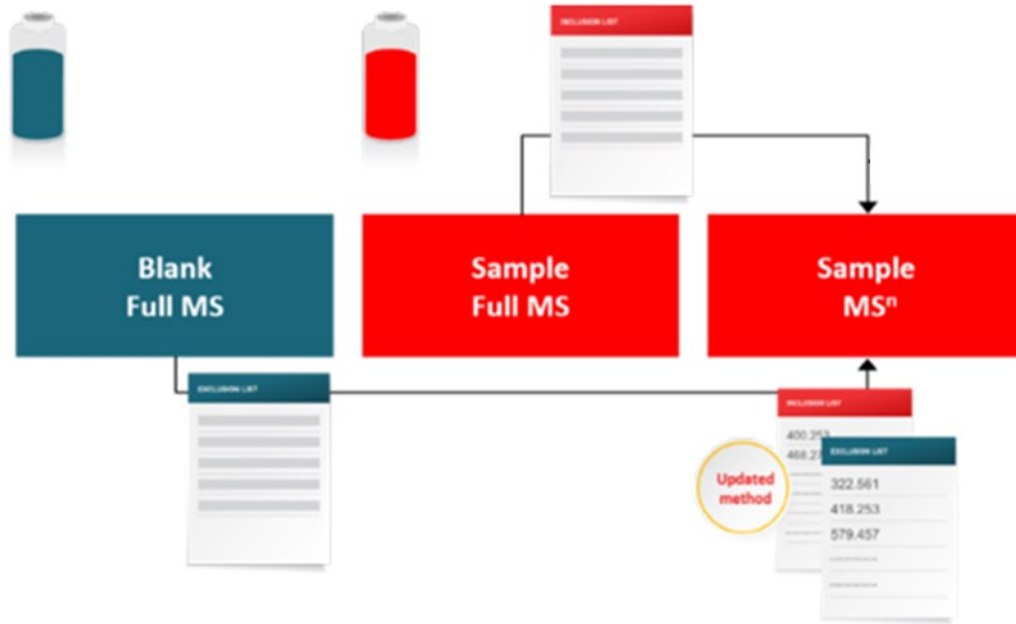
- We used a chemical blank

- Used various optimized collision energies

Using the blank to identify background ions
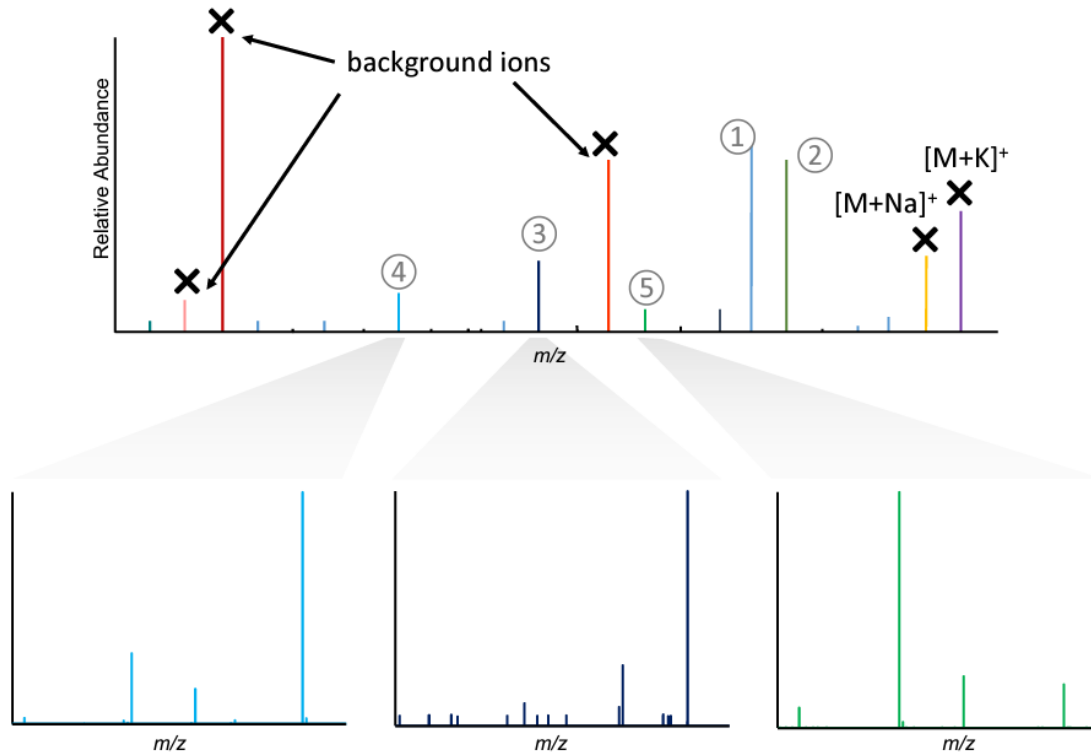
One single run!

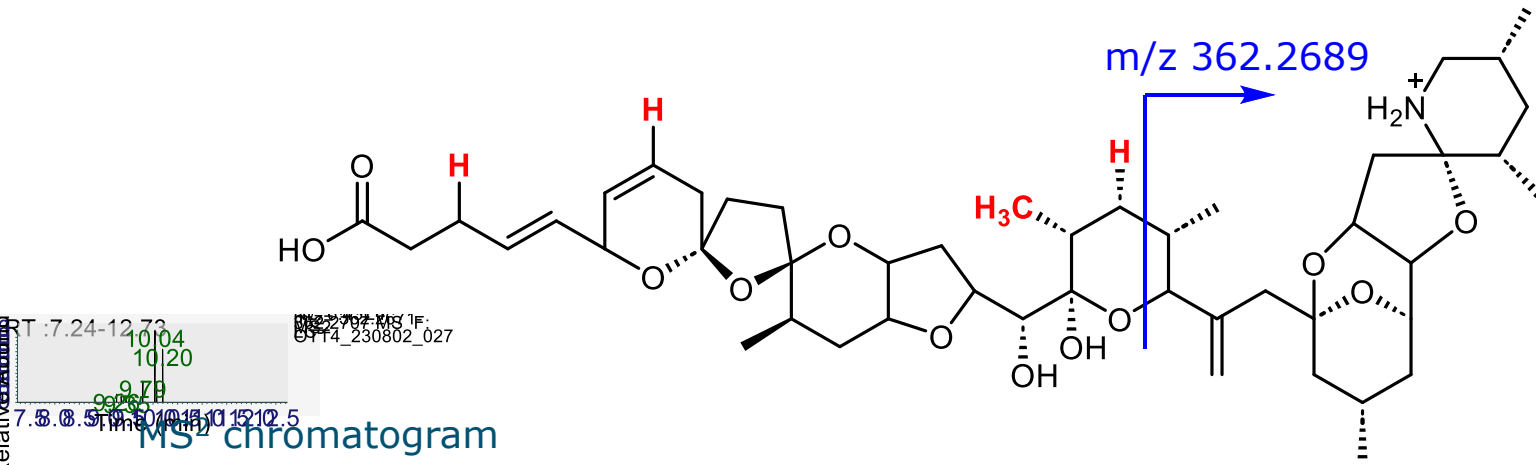- Used a 30, 60 stepped NCE

- Generic acid chromatography of total 20 min

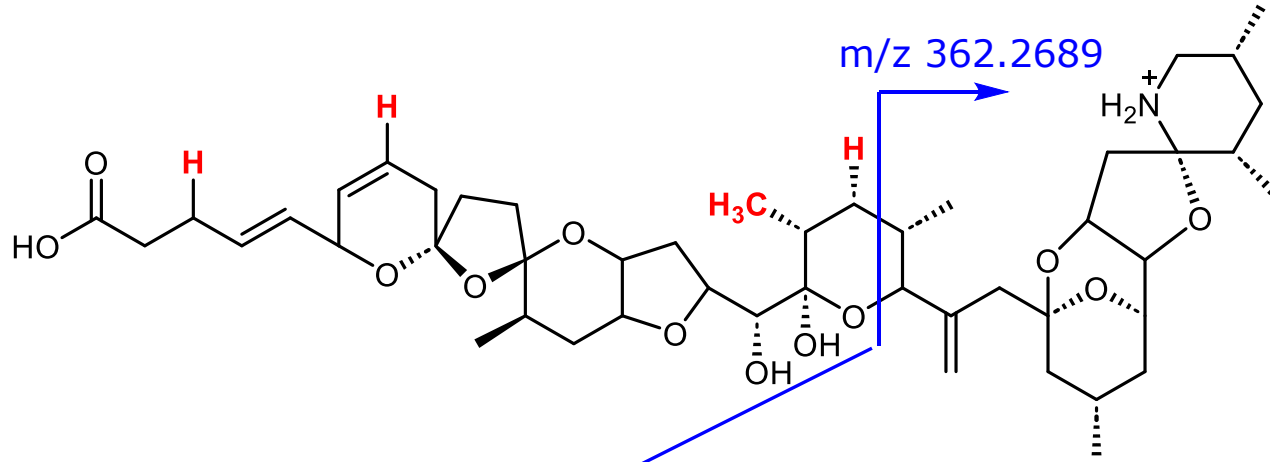Full scan chromatogram

MS^2 triggered chromatogram

General fragmentation (fragment ion flagging)

$[M+H]^+$

m/z 824 + R_1 + R_2 + R_3 + R_4

m/z 655 + R_3 + R_4

m/z 362

m/z 446 + R_3 + R_4

General fragmentation (fragment ion flagging)

m/z 362.2689

MS² chromatogram

Azaspiracid-1 fragmentation

m/z 362.2689

Azaspiracid-1 fragmentation

m/z 462.3214

Azaspiracid-1 fragmentation

m/z 672.4106

Other AZA's in the sample
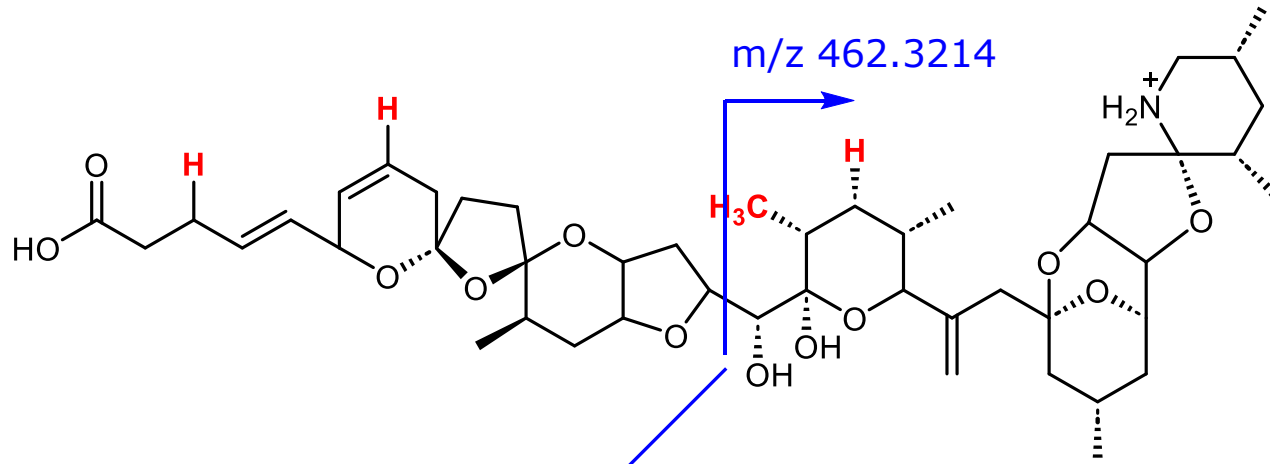
LC-OTT4_230802_027 #6204 RT: 10.20 AV: 1 NL: 1.10E6
FTMS + p ESI d Full ms2 836.5201@hcd45.00 [74.0000-867.0000]

AZA2

LC-OTT4_230802_027 #5928 RT: 9.79 AV: 1 NL: 3.38E5
FTMS + p ESI d Full ms2 828.4895@hcd45.00 [73.0000-839.0000]

AZA3

Other AZA's in the sample

LC-OTT4_230802_027 #5700 RT: 9.46 AV: 1 NL: 4.54E4
T: FTMS + p ESI d Full ms2 674.4841@hcd45.00 [73.0000-855.0000]

AZA4

LC-OTT4_230802_027 #5704 RT: 9.46 AV: 1 NL: 1.13E5
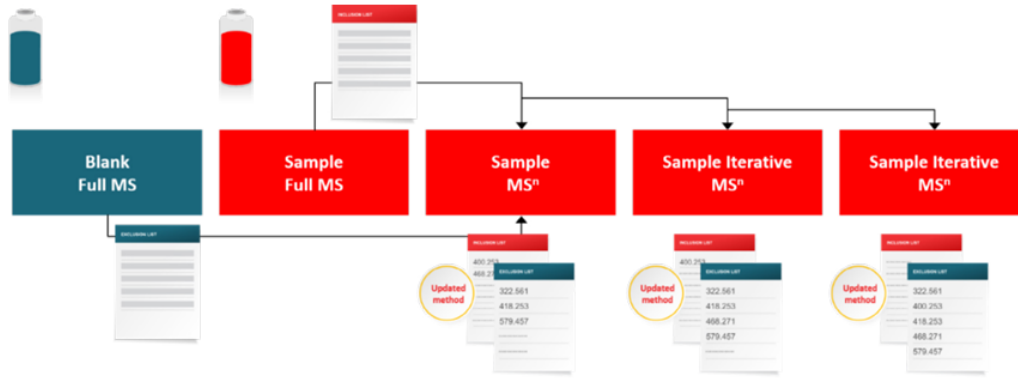T: FTMS + p ESI d Full ms2 658.4979@hcd45.00 [74.0000-869.0000]

AZA9

Next steps and possibilities

- Direct integration in the workflow of spectral databases
  - Home build or available

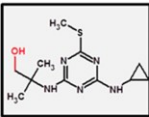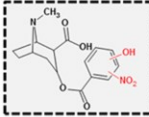- If only partial hit > automatic generation of spectral trees (MS^3)

Automatically updated run-to-run inclusion/exclusion lists

Conclusion

- Technique added value for discovering and rapidly identify compounds with a high level of confidence.

- Important in food and feed safety incidents.

Example	Identification confidence	Minimum data requirements
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Thank you!

Aknowledgement:

Paul Zomer

Marco Blokland

Arjen Gerssen

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