

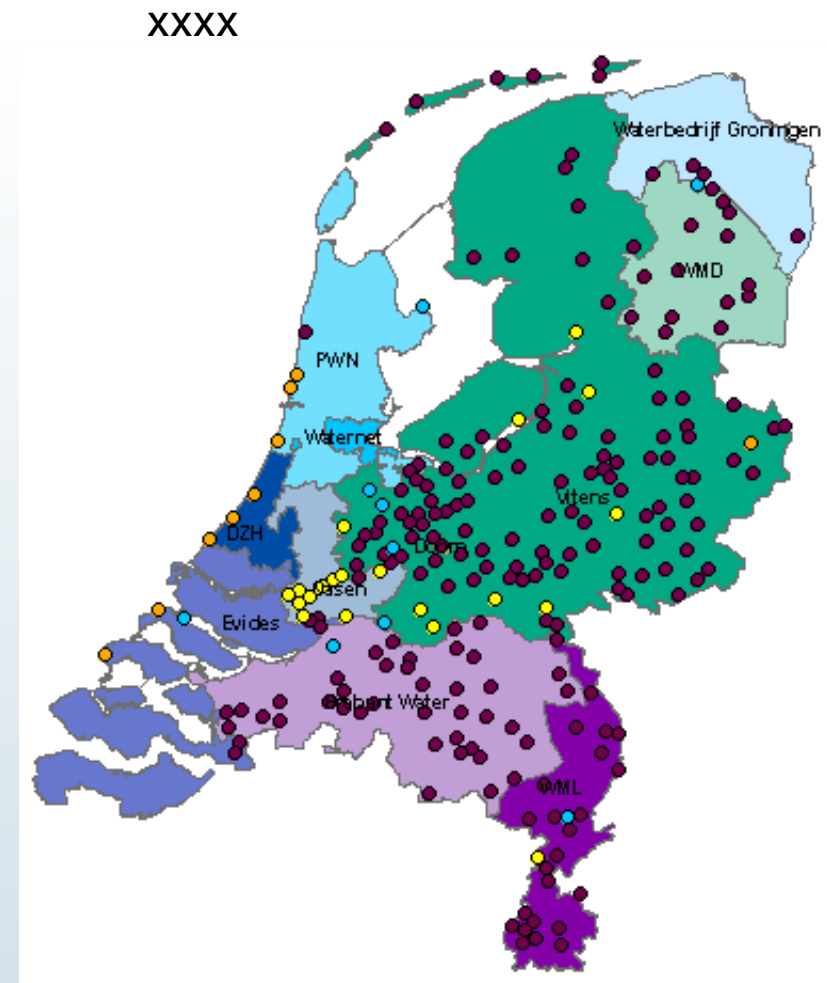
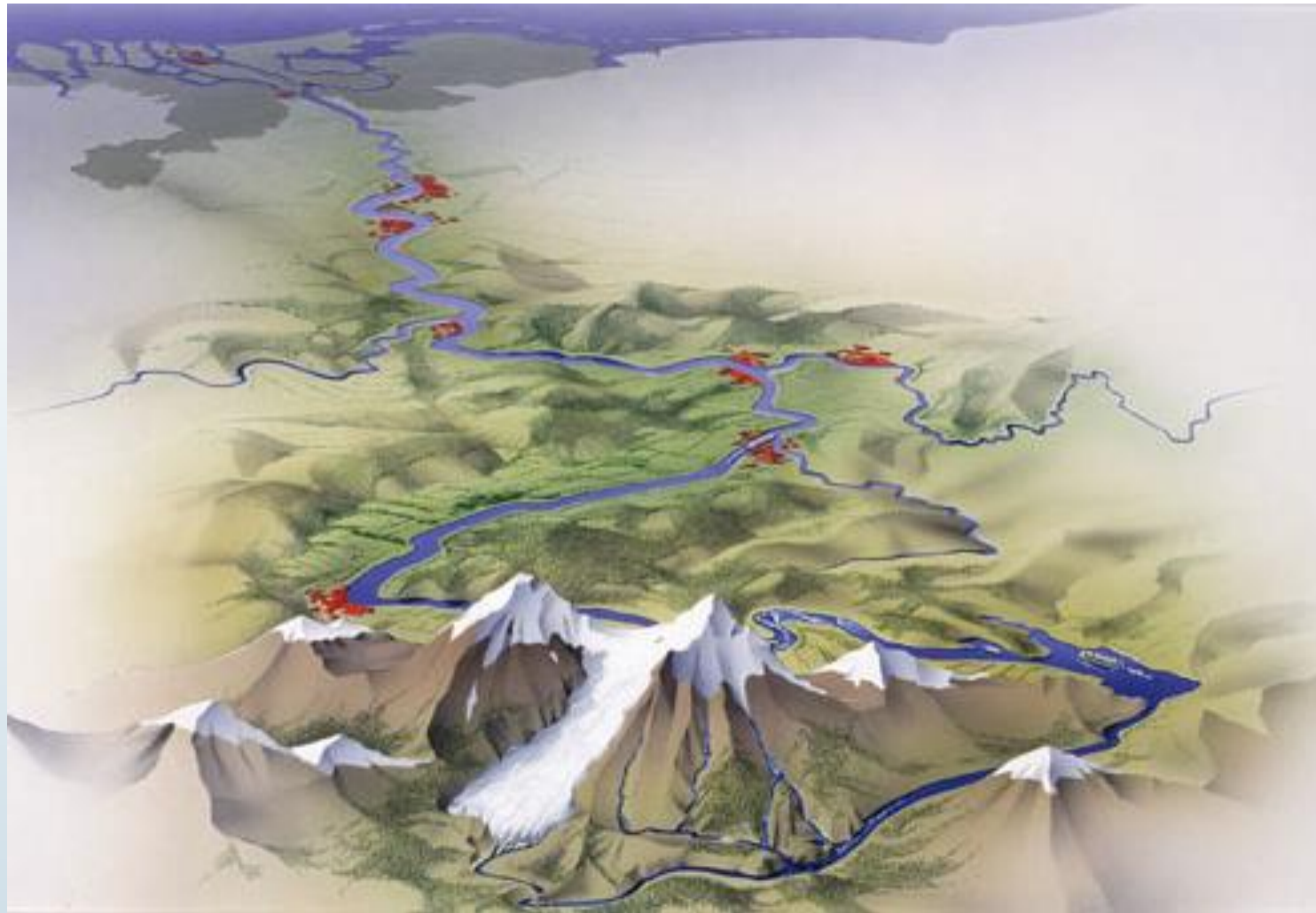


Elucidating the identity of a unknown contaminant in the river Meuse: Pyrazole, a new emerging polar industrial contaminant

Annemieke Kolkman, Erik Emke, Dennis Vughs, Kirsten Baken, Patrick Bäuerlein, Annemarie van Wezel and Pim de Voogt

Drinking water in the Netherlands

Drinking water sources



groundwater: ~66%
surface water: ~ 33%
(Rhine and Meuse)

Safeguarding the water quality

Monitoring: Early warning sensors

1. Basic parameters

- pH, Temperature, Oxygen, Electric Conductivity, Turbidity

2. Biological monitoring

- Waterflea (Daphnia Magna)
- Mussel monitor
- Algae monitor

3. Chemical monitoring

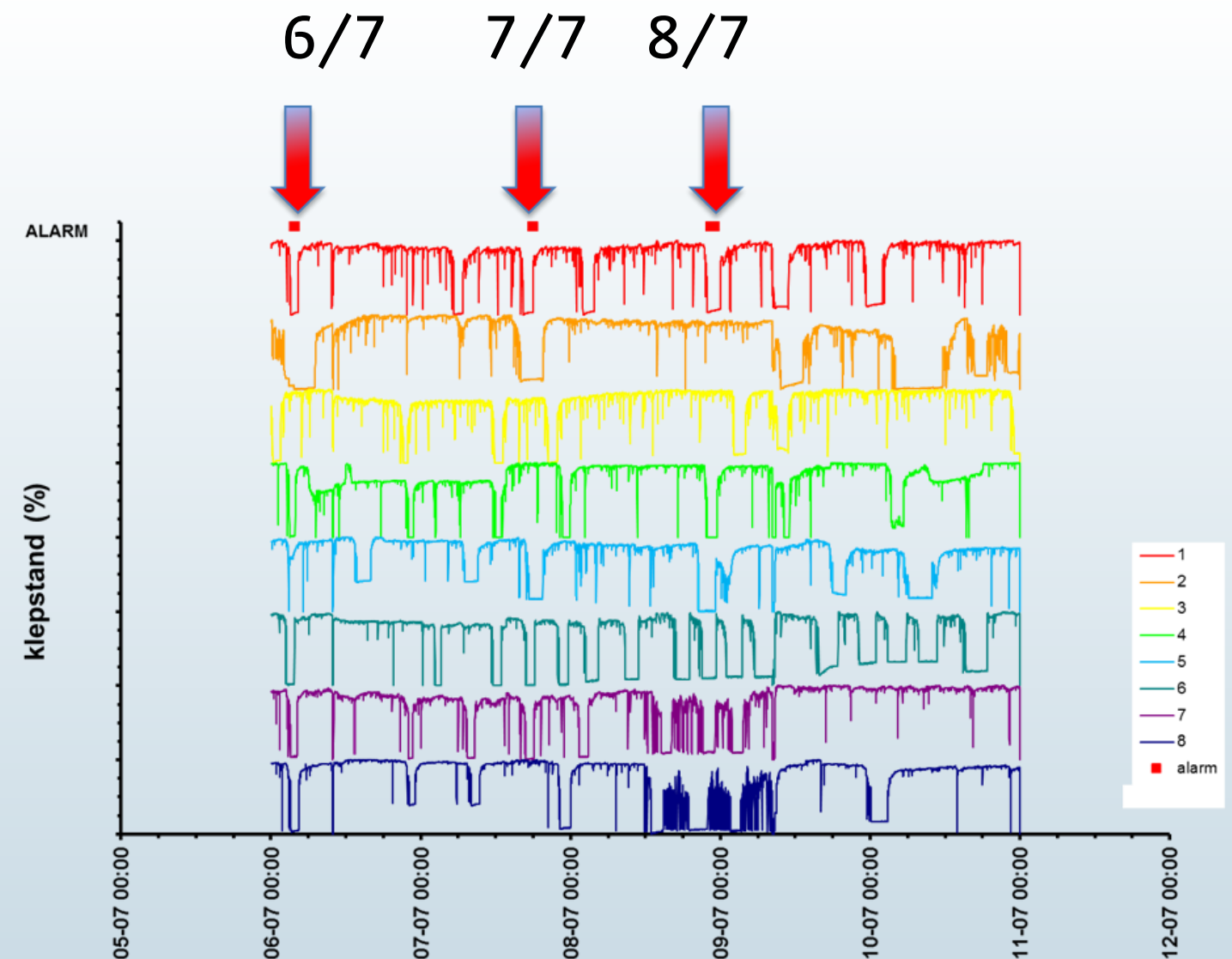
- HPLC-DAD so called HPLC-UV fingerprint
- GCMS screening



Biomonitor in River Meuse - mussle monitor

Alarm in July 2015 >> automatic stop DW intake

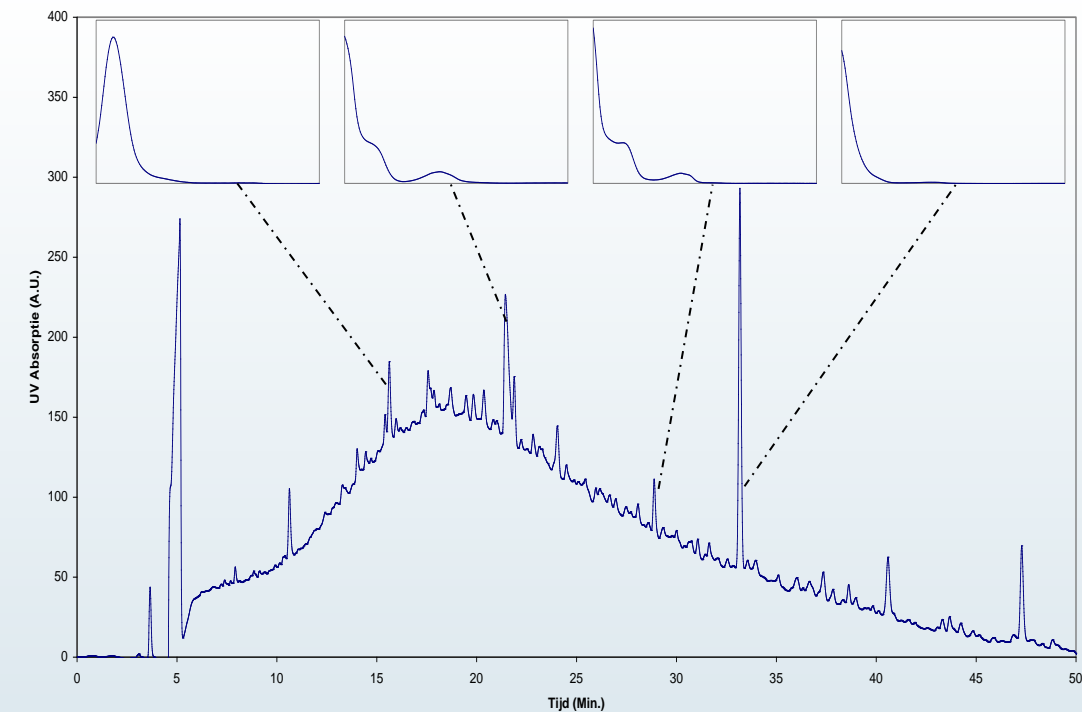
- Mussel monitor
 - Keeping the clams halve closed
 - Increased activity
 - Clamps longer closed than normal
 - Clamps are open (dead)



HPLC-UV screening

Harmonized method for efficient water quality monitoring

- Harmonized HPLC method
- SPE-On-Line-HPLC-DAD sensor
 - KWR, WML, Evides, Aqualab Zuid, RWS*
 - C18 column
 - Two internal standards (KRetI)
 - Gradient linear
 - Every peak has its characteristic UV spectrum
 - The use of the retention index KRetI gives additional information



HPLC-UV screening of River Meuse

Monitoring water quality – screening techniques



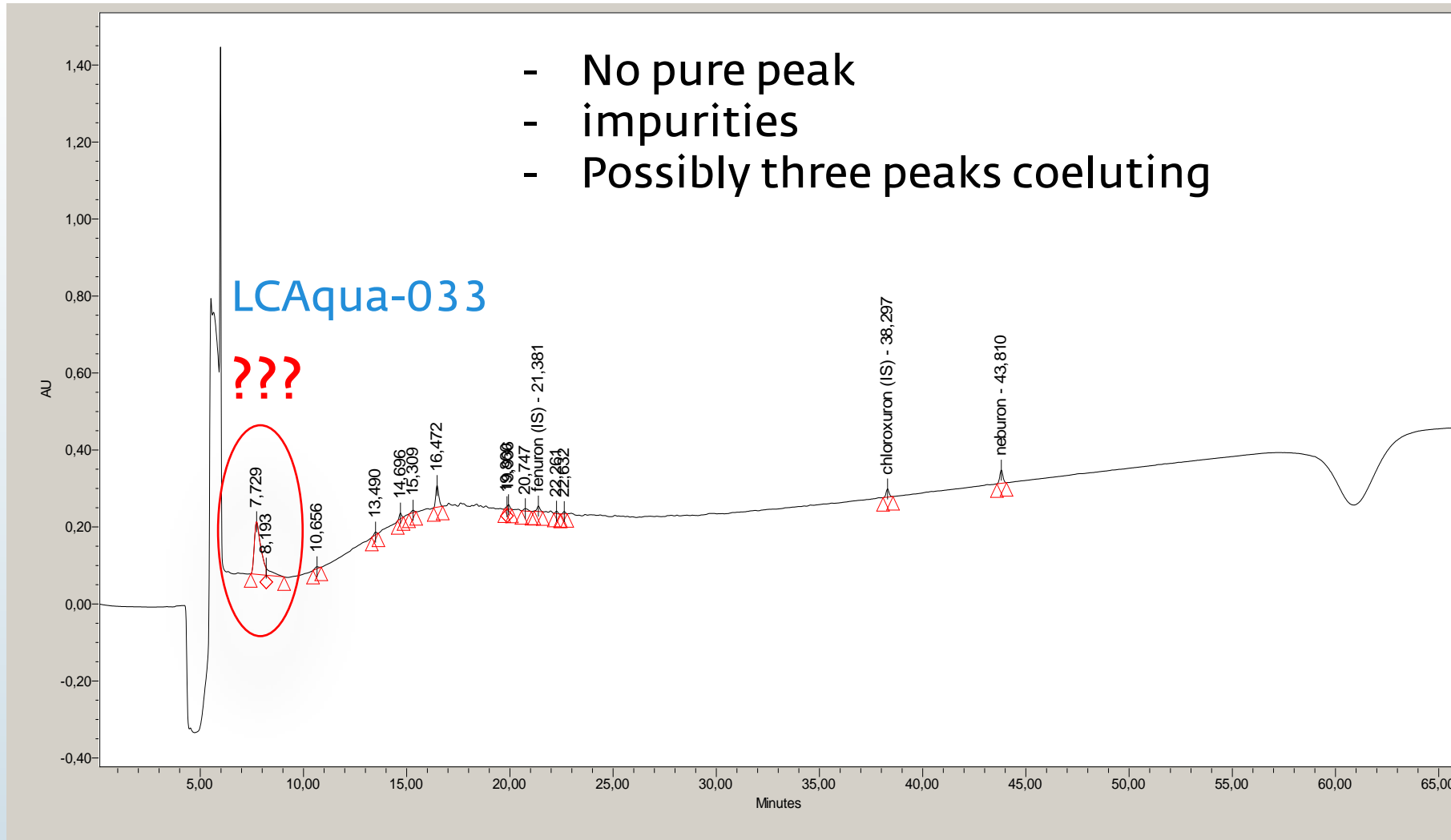
Eijsden - meetponton



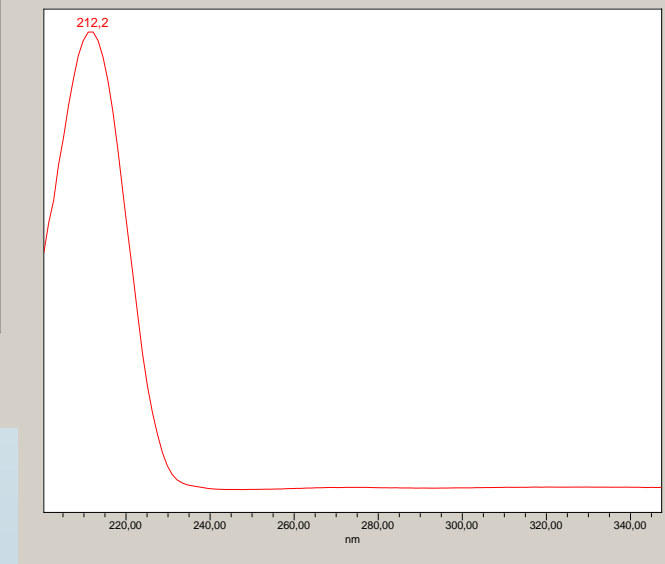
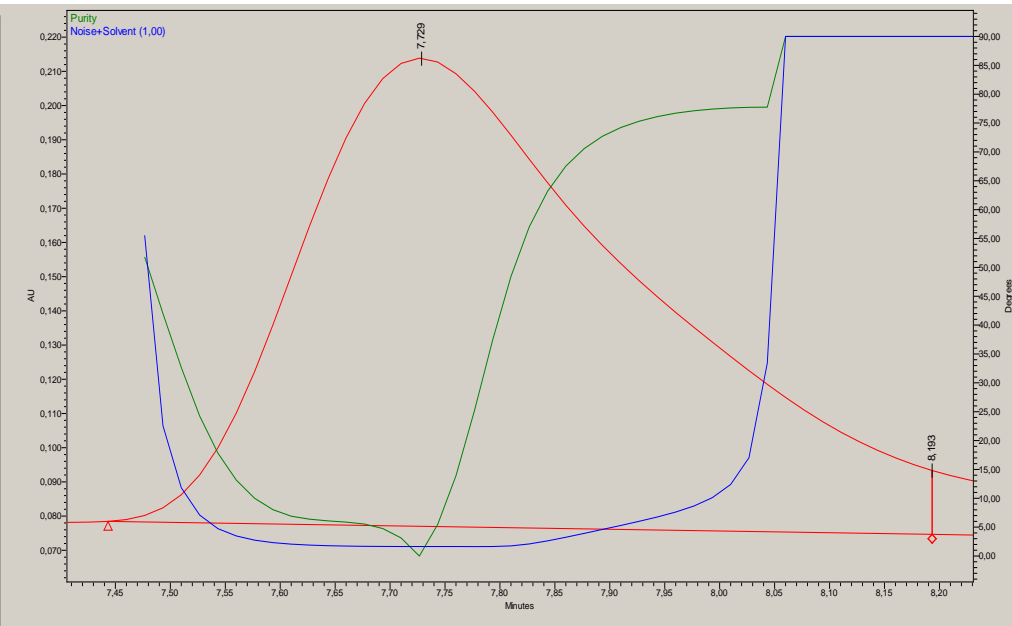
Monitoring waterquality of incoming rivers,
and at intake points for drinking water

Alarm sample - 9th of July 2015

LCAqua-033

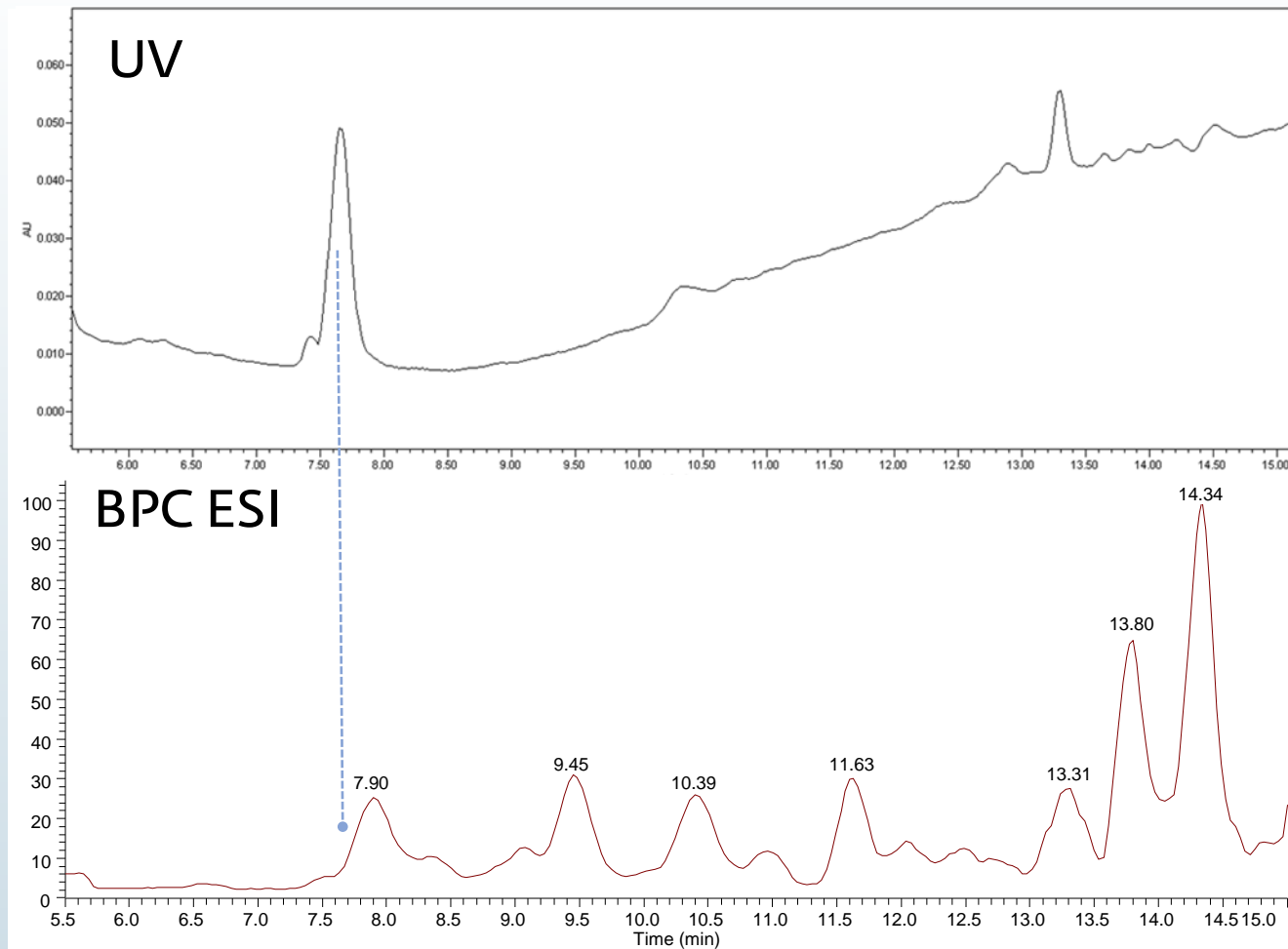


- No pure peak
- impurities
- Possibly three peaks coeluting



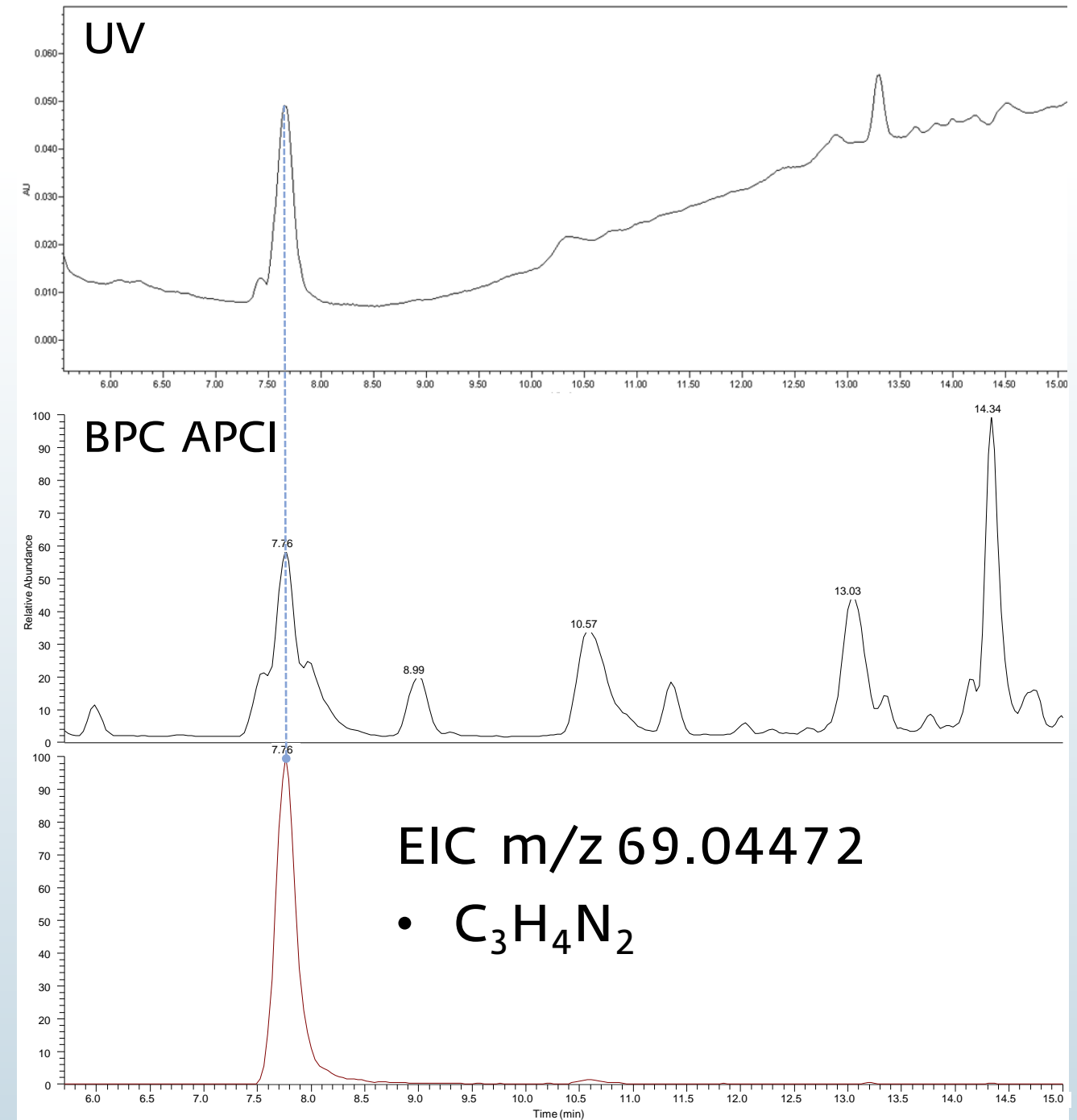
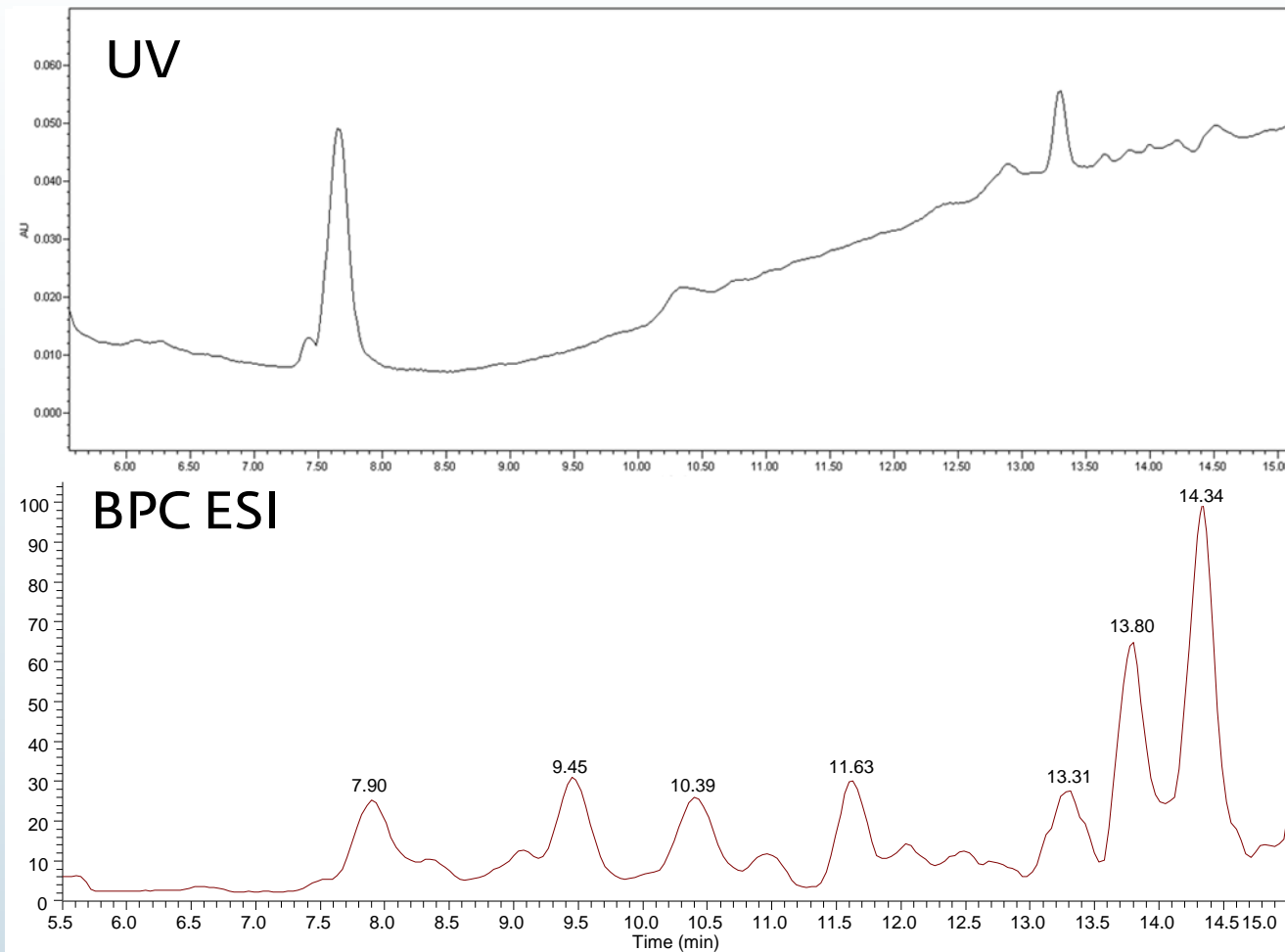
HPLC-UV-Orbitrap

ESI vs APCI



HPLC-UV-Orbitrap

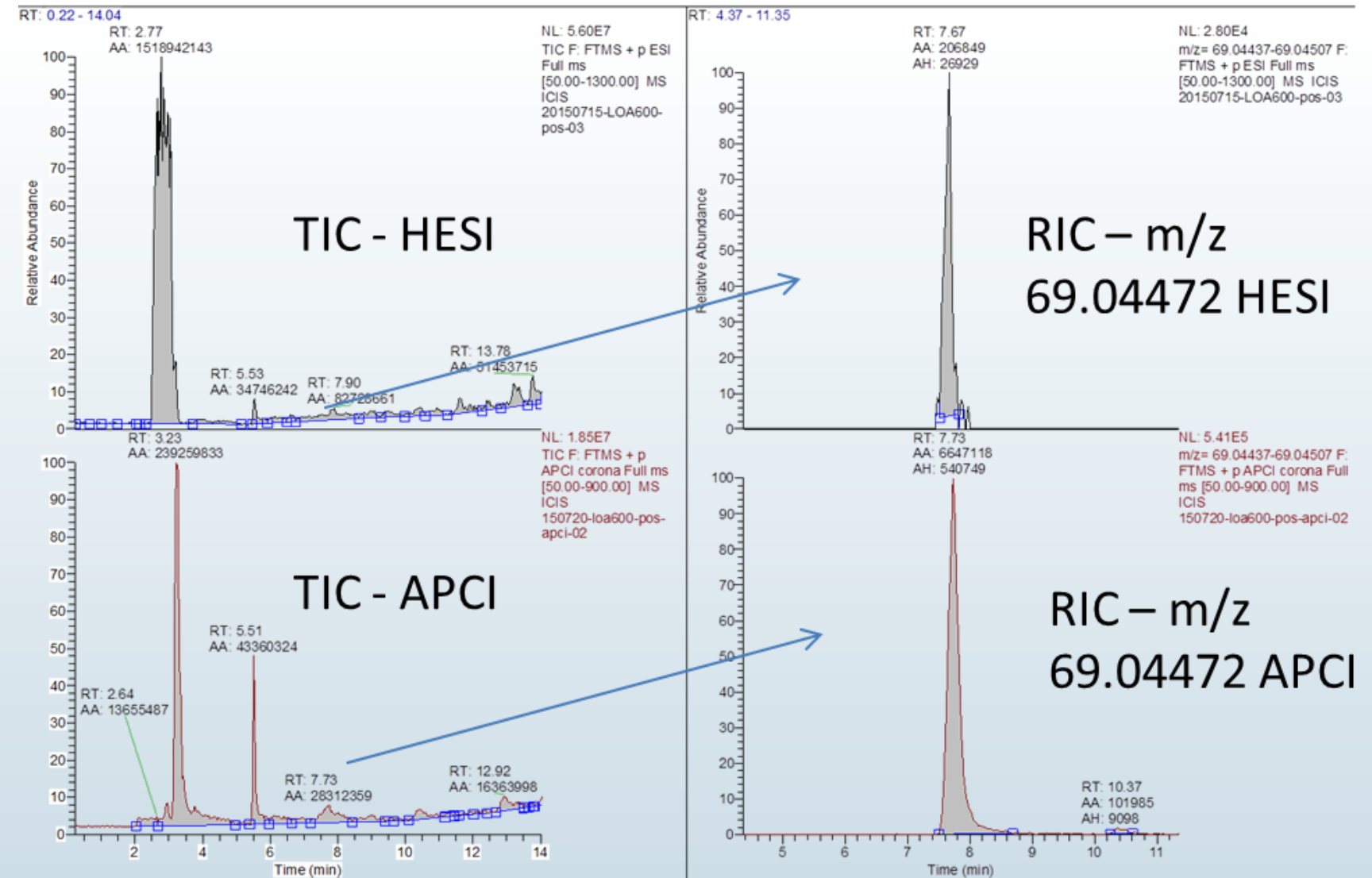
ESI vs APCI



On-line-SPE-HPLC-DAD-Orbitrap screening

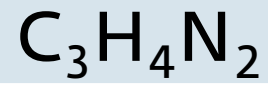
HESI vs APCI

- Switch to APCI
- ESI just above baseline signal
- APCI ~30 times higher
- m/z 69.04463 most probable formula $C_3H_4N_2$ (-1.2 ppm)





Found 21 results (by molecular formula)

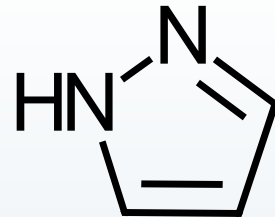


Final selection

- 1-H-Pyrazole

Properties:

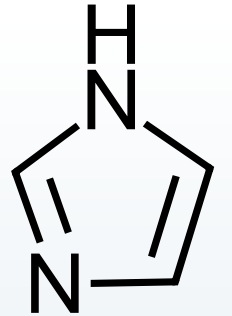
- ACD/LogD (pH 7.4): 0.43¹
- Water Solubility 8.5 g/l (25 deg C)²



- Imidazole

Properties:

- ACD/LogD (pH 7.4): -0.35¹
- Water Solubility 15.9 g/L (25 deg C)²



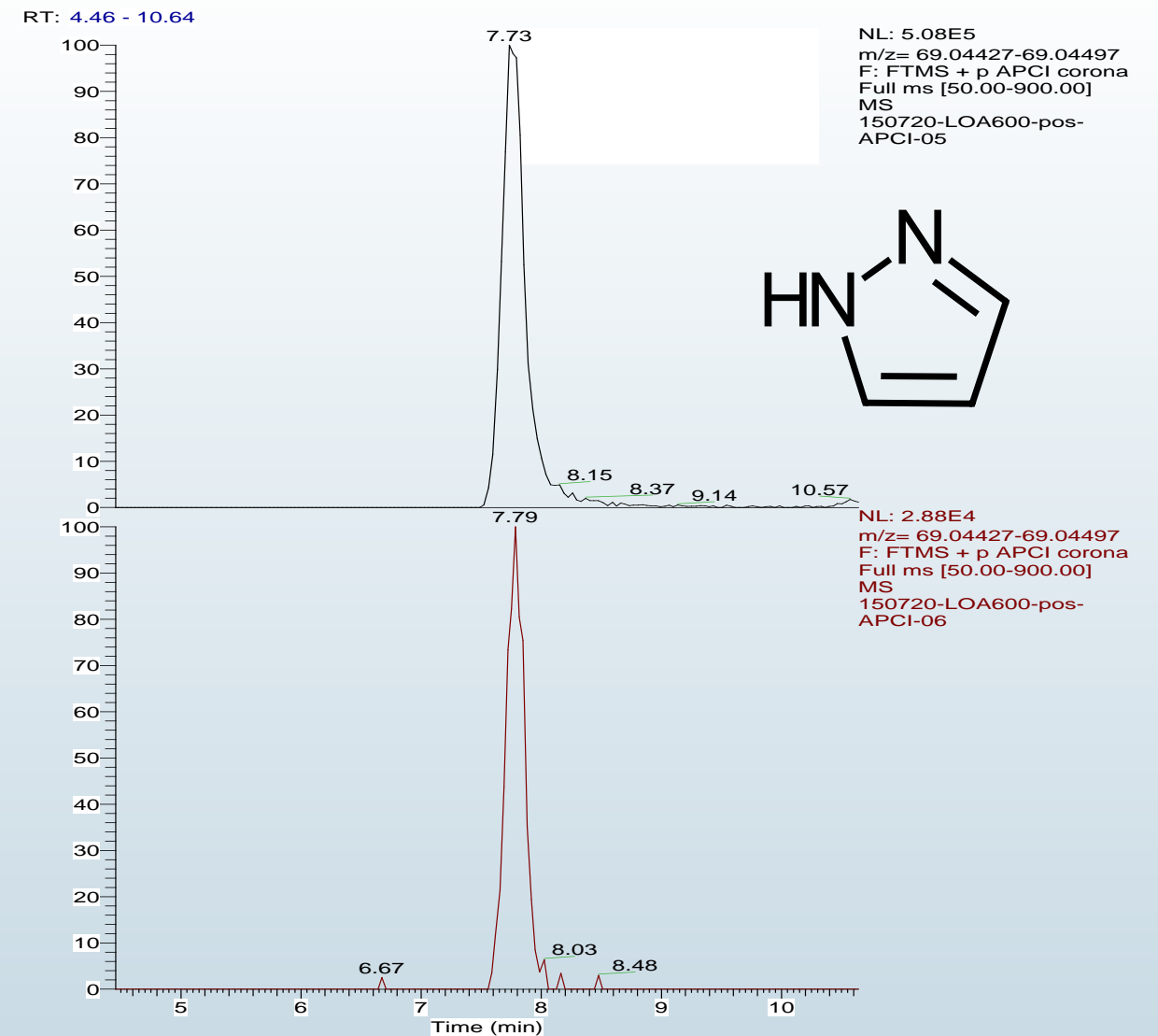
Rank	Name	# of Data sources	# of references	# of Pubmed	# of RSC
1	Imidazole	139	5985	13486	8954
2	Pyrazole	125	1556	5193	3750
3	(Methyleneamino)acetonitrile	57	70	0	1
4	2-Aziridinecarbonitrile	17	27	2	3
5	2-Aminoacrylonitrile	8	11	0	6

¹ACD/LABS

²calculated from Log Kow (EPI Suite)

Confirmation

- No observed fragmentation
 - (CID LTQ- Orbitrap Classic)
- Obtain reference material
 - Ordering takes a while
- Quick call ; one hour later we tried Imidazole kindly supplied by the Utrecht University synthesis department
- No luck. Imidazole (diffence rt 3 min)
- Pyrazole: confirmed by retention time and accurate mass
- conc. In River Meuse ~100 µg/L !!



Identifying the source

- LC-AQUA-033 now identified as pyrazole
 - Not found in Eijsden
- More samples were taken and small river Ur showed very high levels of pyrazole
- The STW plant of Chemelot, a large industrial site with > 50 chemical (pilot)plants discharges here.
 - Source ACRYLONITRILE plant
- Pyrazole-free surface water was difficult to obtain
 - Pyrazole also found in the river Rhine
 - (origin Cologne second largest acrylonitrile site)



NOS Teletekst 101 18:52:54

NOS

JOURNAL

Inname Maaswater gestaakt na lozing 111

Brugbouwer betaald

NOS Teletekst 111 18:52:42

Alphen wist van

Inname Maaswater gestaakt na lozing

Geen verdachten

Dodental migrant

Onderzoeksraad b

Vier eredivisie

Spies:situatie t

Hadi's troepen r

nieuwsverzicht

binnenland spo

Waterbedrijf WML heeft de inname van Maaswater in het noorden en midden van Limburg gestaakt, omdat de stof pyrazool in het water is geloosd. De stof werd op 9 juli gemeten en vrijwel onmiddellijk werd de inname gestaakt bij Heel, meldt Dagblad De Limburger.

De chemische verbinding Pyrazool wordt onder meer gebruikt in geneesmiddelen, kleurstoffen en bestrijdingsmiddelen. De stof zou door een fabriek zijn geloosd. Er wordt nog onderzocht welke fabriek dat kan zijn geweest.

Tienduizenden Limburgers krijgen nu grondwater uit een bekken in Beegden, bij Roermond.

Schultz: lozing Chemelot in Maas moet stoppen

REGIO | 04 september 2015 | 1 | Door onze verslaggever, Dichtbijredacteur



ROERMOND - Minister Melanie Schultz van Haegen van Infrastructuur en Milieu wil dat de lozing van pyrazolen in de Maas vanaf Chemelot stopt. Dat laat zij in een brief aan de drinkwaterbedrijven, waaronder de waterleidingmaatschappij Limburg (WML), weten. Zij wil op dit moment nog niet zelf ingrijpen, maar spreekt uitdrukkelijk de verwachting uit dat de betrokken partijen ervoor zorgen, dat er geen pyrazolen meer in de rivier terecht komen.



NEDERLANDSE WATERBEDRIJVEN AL SINDE 9 JULI GESTOPT MET GEBRUIK MA

Chemelot loost gif in Grensm

06/08/2015 om 06:02 door Guy THUWIS



Het was gisteren heerlijk peddelen op de Maas | geen kwaad voor de recreanten.

Foto: JEFFREY GAENS

nrc.nl

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NU IN HET NIEUWS: VLUCHTELINGENCRISIS

4 augustus 2015, 20:51

Maaswater vervuild door defect op industrieterrein Chemelot



Limburgs Dagblad



Achtergronden bij het nieuws

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[LEZERSERVICE](#)
[ABONNEREN](#)
[DIGIT](#)

[De Limburger Digitaal](#)
[Profclubs voetbal](#)
[Bijzond](#)

Drinkwatercrisis zorgde voor buikpijn

Door toedoen van een haperende afvalwaterzuiveringsinstallatie bij Chemelot hebben Zuid-Nederlandse waterbedrijven de afgelopen weken flink moeten interen op hun buffers. Hoe 'werkweigerende' bacteriën de chemiesite nieuwe imagoschade bezorgen.

Bram v/d Heijden en Roel Ophelders

Op 9 juli gaan alle seinen op rood bij de Waterleiding Maatschappij Limburg (WML). Er stroomt plotseling een chemische stof door de Maas die er niet thuishoort, geven de meters aan. Meteen wordt besloten de inname van Maaswater ten behoeve van de drinkwatervoorziening te stoppen en over te stappen op het water in de spaarbekkens. Op dat moment weet nog niemand welke chemische stof het nou exact is, die ineens in de Maas zit. De stof wordt alleen stroomafwaarts in de Maas gemeten vanaf het Chemelotterrein, dus al snel is duidelijk dat een van de fabrieken idelijkheid. Het zijn il-fabriek.

Pyrazole

Questions drinking water companies

- What is known about the toxicity of pyrazole
- What happens during drinking water treatment with pyrazole (removal?)
- There are also other peaks in chromatogram that are related to this incidence

Pyrazole

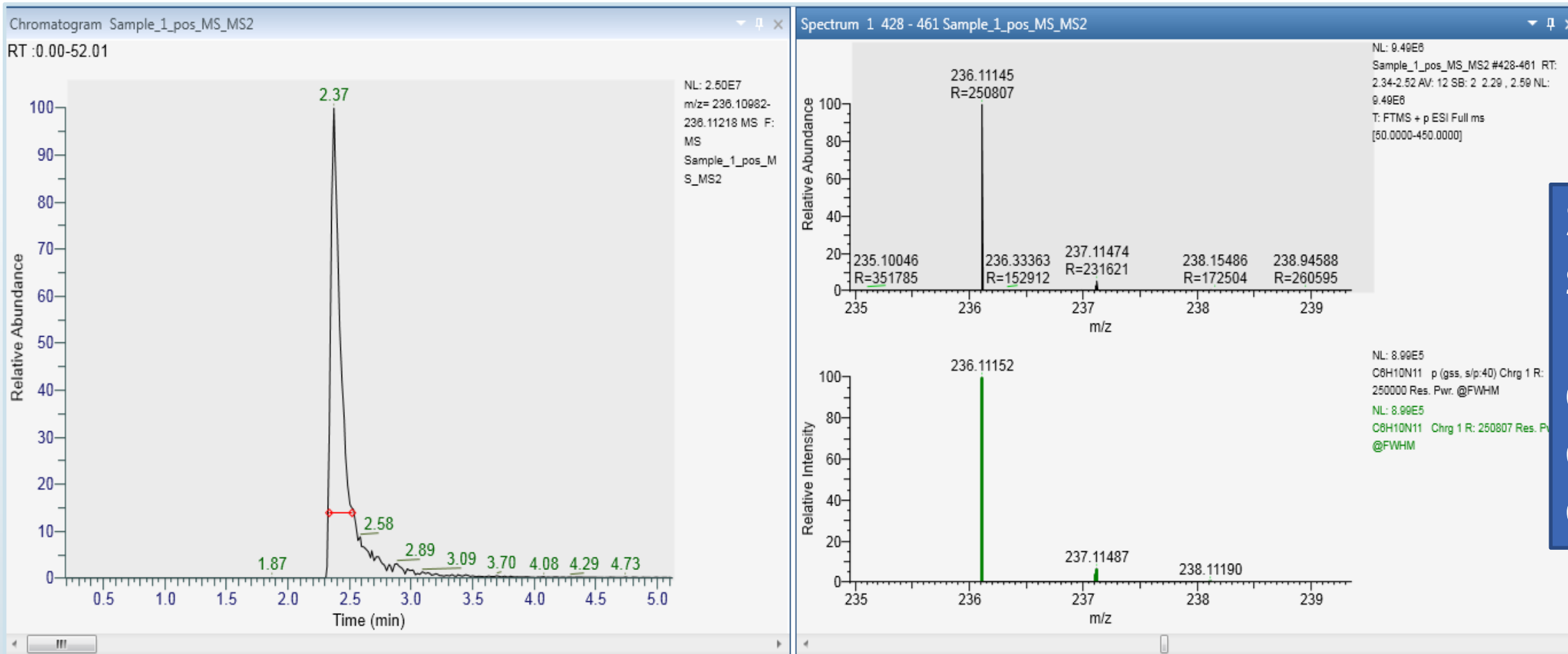
Questions drinking water companies

- What is known about the toxicity of pyrazole
 - Toxicity data on pyrazole is scarce; QSAR toolbox shows a structural alert for genotoxicity
- What happens during drinking water treatment with pyrazole (removal?)
 - No literature data available, small and polar compound >> probably poor removal
- There are also other peaks in chromatogram that are related to this incidence
 - What is the identity of these compounds?
 - Pyrazole derivatives?

Peak	RT (min)	m/z	Elemental composition	ID
1	7.56	136.05034	C6H5ON3	
2	7.97	205.13316	C12H16ON2	
3	10.57	191.09249	C9H10ON4	
4	11.35	211.18009	C12H22ON2	
5	12.27	205.13315	C12H16ON2	
6	13.03	167.07018	C9H10O3	
7	14.2	187.12271	C12H14N2	
8	14.31	207.14879	C12H18ON2	
9	16.17	164.07045	C9H9O2N	
10	18.71	205.1331	C12H16ON2	
11	19.49	150.06592	C7H7ON3	
12	19.97	150.06592	C7H7ON3	
13	20.02	178.08603	C10H11O2N	
14	21.19	178.08603	C10H11O2N	
LC-AQUA-033	7.73	69.04462	C3H4N2	Pyrazool
RWS-1**	RT RWS 2.48	219.08437	C6H6N10	Melem / casnr. 1502-47-2
RWS-4**	RT RWS 2.68	236.11090	C6H9N11	Melam / casnr. 3576-88-3

Orbitrap Fusion – demo – pyrazole samples

Direct water injection - m/z 236.11100 - C₆H₉N₁₁ - melam



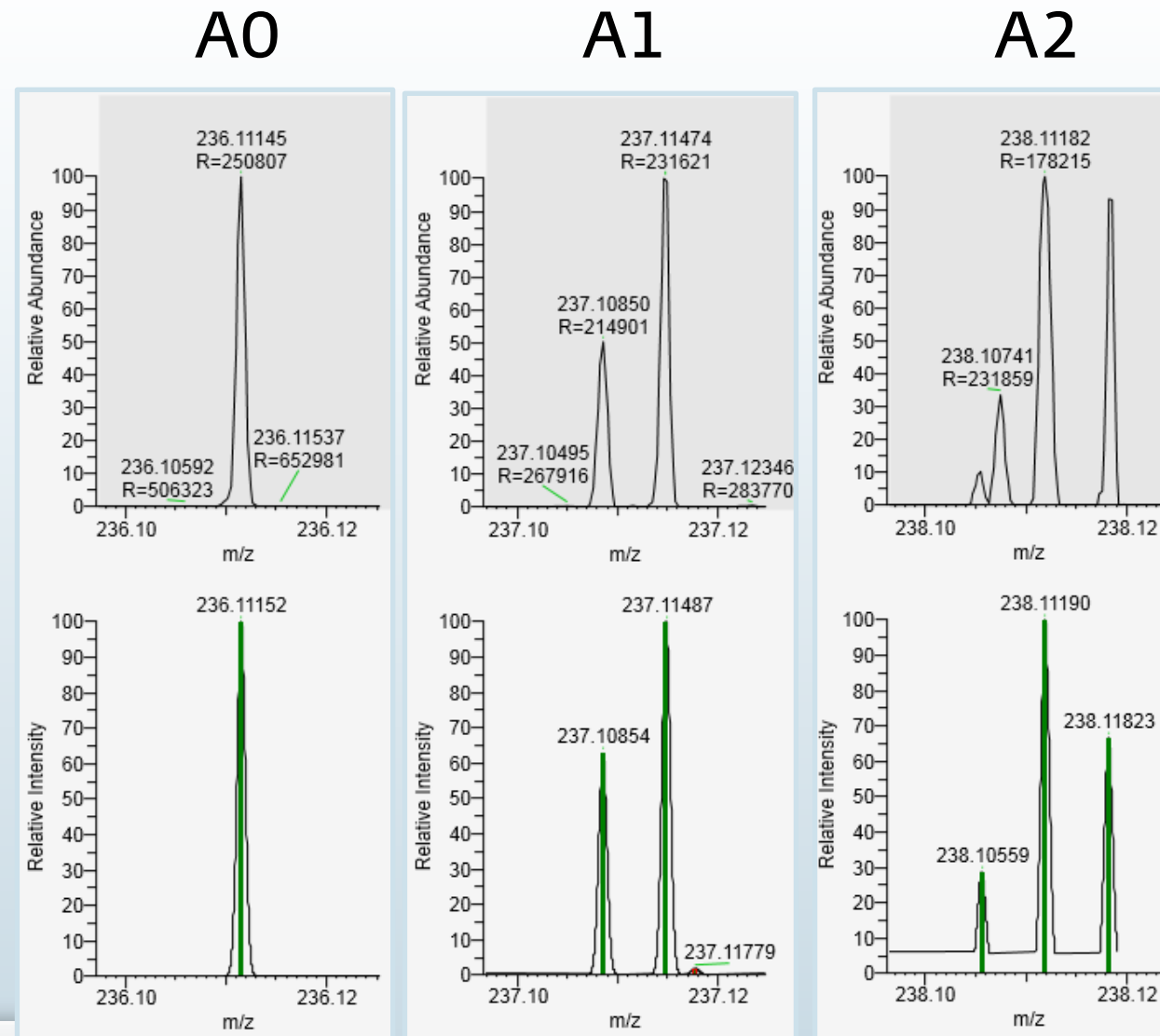
Software uses full strength of very high resolution for elemental composition determination

Elemental Composition Results

Index	m/z	Formula	Score	RDB	Delta ppm	OriginalFormula	Theo. Mass
1	236.11145	C ₆ H ₁₀ N ₁₁	100.00	7.50	-0.28	C6H10N11	236.11152
2		C ₈ H ₁₃ N ₅ F ₃	74.39	3.50	-1.29	C8H13N5F3	236.11176
3		C ₁₀ H ₁₉ O ₂ NF ³² S	55.41	1.50	-0.23	C10H19O2NF[32]S	236.11150
4		C ₉ H ₁₇ N ₃ F ₂ P	13.41	2.50	-3.46	C9H17N3F2P	236.11227
5		C ₁₂ H ₁₆ ON ³² S	0.00	5.50	4.61	C13H18ON[32]S	236.11036

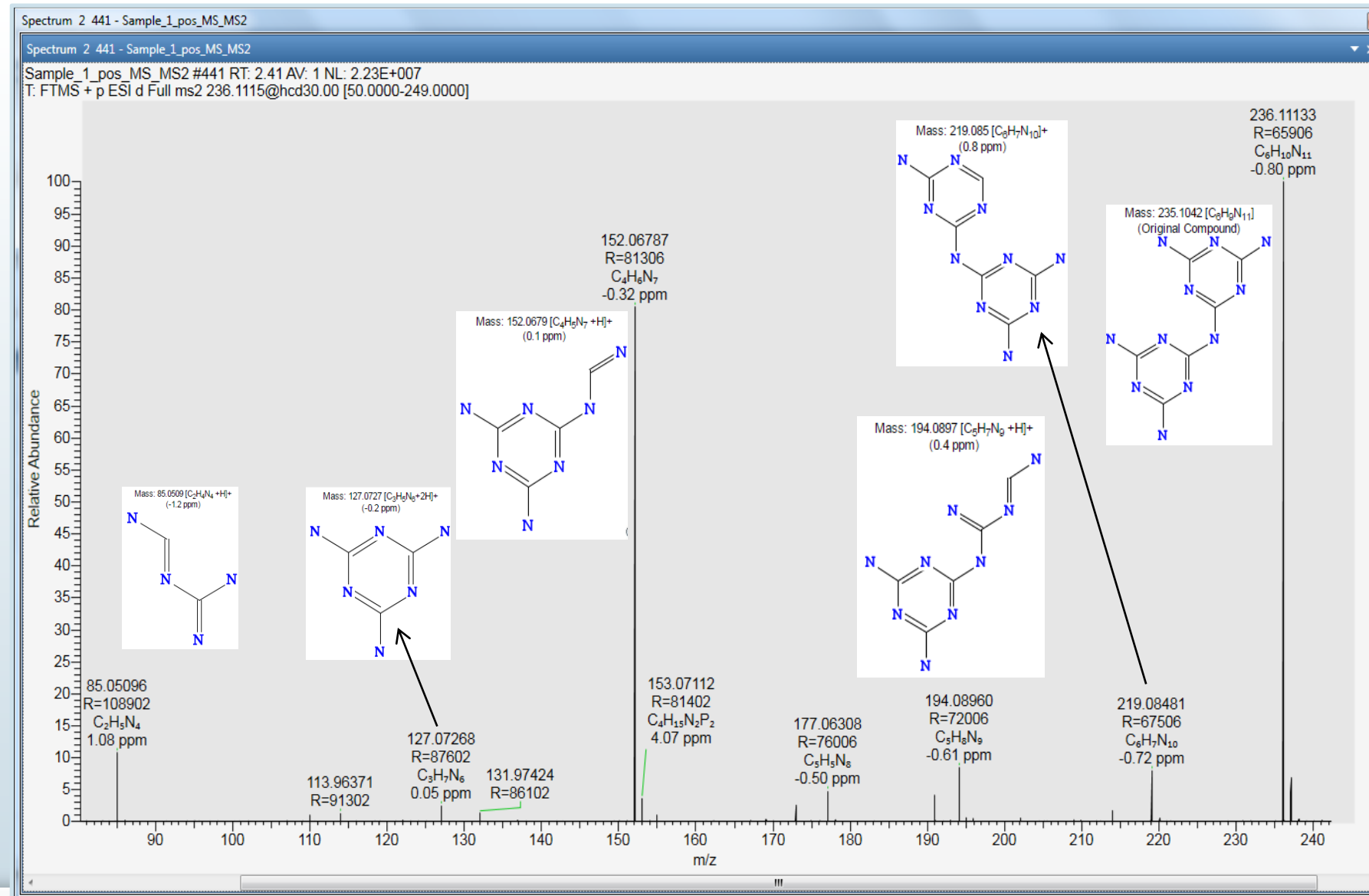
Orbitrap Fusion – demo – pyrazole samples

m/z 236.11100 - C₆H₉N₁₁ - melam



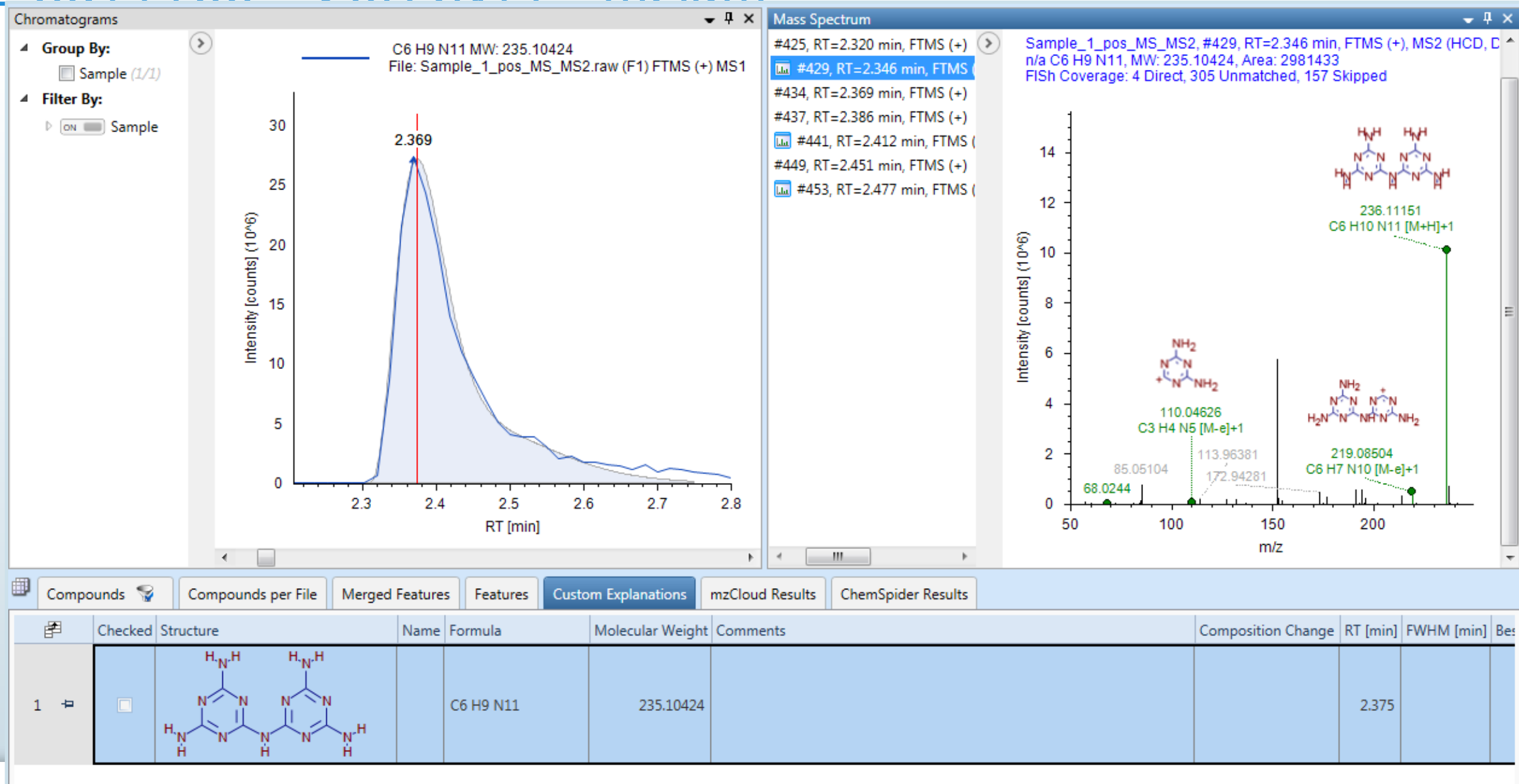
Orbitrap Fusion – demo – pyrazole samples

m/z 236.11100 - C₆H₉N₁₁ - melam



Orbitrap Fusion – demo – pyrazole samples

m/z 236.11100 - C₆H₉N₁₁ - melam



Conclusions

- HPLC UV screening is a valuable tool to safeguard water quality.
- Different ionisation techniques were required to elucidate identity of unknown compound;
- Identity of a unknown compound in the river Meuse was elucidated as pyrazole
- The consequences en implication for the Dutch drinking water companies were enormous
- Still a puzzle to solve >> 1 4 more unknowns
 - Orbitrap Fusion provides excellent mass accuracy and resolution in MS and MS2

Acknowledgements

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- Dennis Vughs
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WML

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Evides

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BRIDGING

science to practice