Novel analytical methods and results of emerging contaminants in Finland

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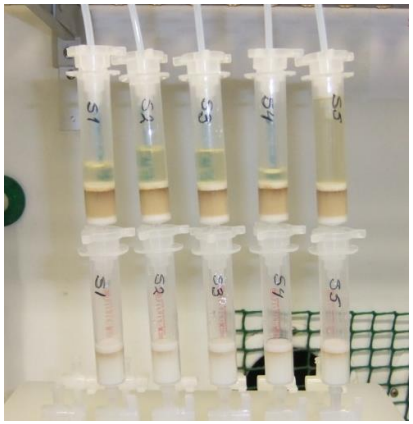
Content of the presentation

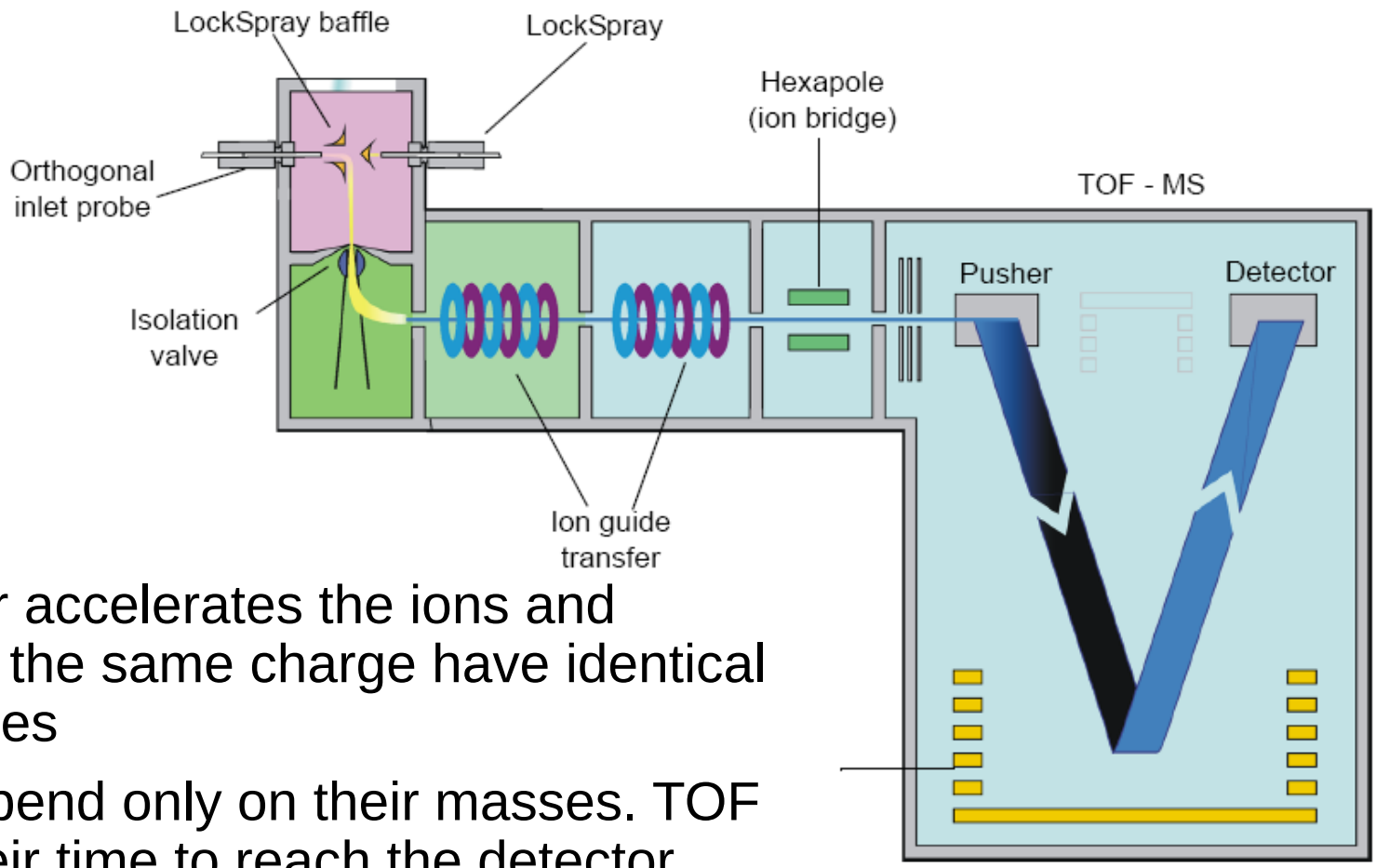
1. Sampling, sample treatment
2. Time-of-flight MS
3. GC-TOF-MS, nontarget
4. Passive samplers
5. LC-TOF-MS, posttarget
6. Conclusions

Materials and methods

- Samples: wastewater, surface waters, stormwater
- Sampling
  - Grab sampling
  - Passive samplers
- Sample pretreatment:
  - SPE
  - Liquid-liquid extraction, HEX, DCM
  - Solvent extraction to passive samplers
- Analytical techniques:
  - GC-TOF-MS, nontarget (and posttarget)
  - LC-TOF-MS, posttarget



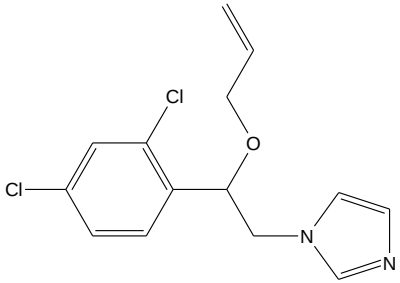
Time-of-flight mass spectrometry (TOF-MS)



- TOF analyzer accelerates the ions and particles with the same charge have identical kinetic energies
- Velocities depend only on their masses. TOF measures their time to reach the detector.
- Lighter ions will reach the detector first.
- Time of flight → mass of an ion

Waters LCT Premier XE Operator's guide, Waters Corp.

Time-of-flight mass spectrometry - accurate mass



Imazalil (pesticide)
$C_{14}H_{14}N_2OCl_2$

- TOF-MS is capable of measuring accurate mass (4 digits)
- Exact mass can be used to extract compound from raw data
- Measured accurate mass can be used to calculate possible elemental compositions → $C_AH_BN_CO_DCl_E$???
- Mass error can be used to reject elemental composition candidates

C: 14 x 12.0000 = 168.0000
H: 14 x 1.0078 = 14.1095
N: 2 x 14.0030 = 28.0060
O: 1 x 15.9949 = 15.9949
Cl: 2 x 34.9689 = 69.9378

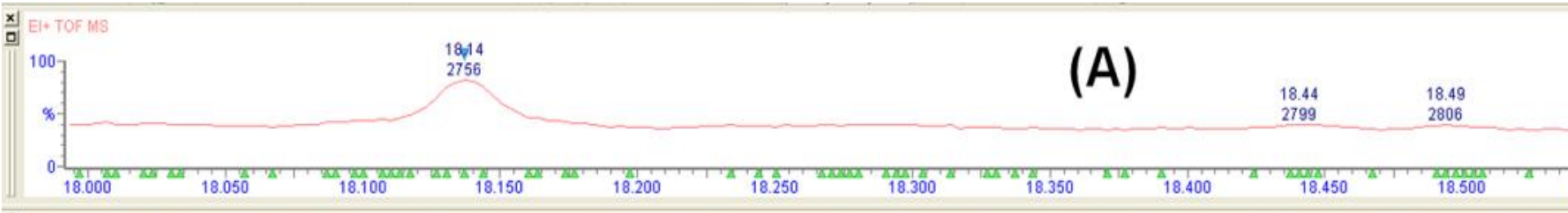
Exact mass: 296.0482

Mass	Calc. Mass	mDa	PPM	DBE	Formula
296.0482	296.0483	-0.1	-0.3	8.0	C14 H14 N2 O Cl2
	296.0478	0.4	1.4	12.5	C17 H11 N O2 Cl
	296.0488	-0.6	-2.0	3.5	C11 H17 N3 Cl3
	296.0473	0.9	3.0	17.0	C20 H8 O3
	296.0492	-1.0	-3.4	4.0	C8 H12 N2 O10
	296.0497	-1.5	-5.1	-0.5	C5 H15 N3 O9 Cl
	296.0500	-1.8	-6.1	21.5	C23 H6 N
	296.0501	-1.9	-6.4	3.0	C13 H19 O Cl3
	296.0461	2.1	7.1	-1.0	C8 H19 N2 O3 Cl3
	296.0460	2.2	7.4	17.5	C18 H6 N3 O2
	296.0506	-2.4	-8.1	-1.5	C10 H22 N Cl4

GC-TOF-MS:
How to accept a recognized compound?

Software for automated screening:

- peak detection and spectral deconvolution tool



	Ret. Time	Abundance	Rel. Abundance	Compound Name	Mass1	Mass2	Mass3	Mass4	Mass5	Mass6
1401	18.14	207	0.003	1H-Indole, 2,3-dihydro-1,2-dimethyl-	77.04	117.06	131.07	132.0808	146.10	147.10
1402	18.14	83	0.001	Benzene, 1-ethyl-3-isocyanato-	65.04	90.05	118.07	132.0807	147.10	148.11
1403	18.16	2	0.000	N-Methoxy-N-methylacetamide	104.03					
1404	18.16	5	0.000	S(IV)Thiirane-2-carboxylic acid, 2-amino-1-ethyl-	104.03	149.02				
1405	18.17	4	0.000	3-Nitro-1-phenyl-1-butanone	193.05					
1406	18.18	1	0.000	3-Nitro-1-phenyl-1-butanone	69.07					
1407	18.20	1	0.000	3-Nitro-1-phenyl-1-butanone	130.99					
1408	18.23	1	0.000	6-Methylbicyclo[2.2.1]hept-2-ene-5-carboxylic acid	79.05					

Library Match

Δmass (calculated – measured)

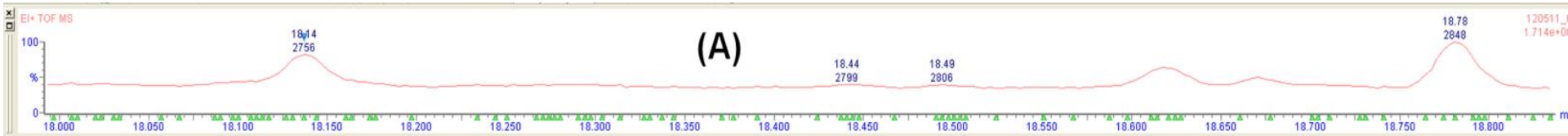
	Compound	Formula	Mol. Wt.	Forward Fit	EleComp Hits	132.0808 (mDa)	147.1046 (mDa)	117.0587 (mDa)	146.0970 (mDa)	131.0728 (mDa)	77.0402 (mDa)
1	1H-Indole, 2,3-dihydro-1,2-dimethyl-	C10H13N	147	725	6/6	-0.5, C9H10N	-0.2, C10H13N	0.9, C8H7N	0.0, C10H12N	-0.7, C9H9N	1.1, C6H5
2	Quinoline, 1,2,3,4-tetrahydro-2-methyl-	C10H13N	147	717	6/6	-0.5, C9H10N	-0.2, C10H13N	0.9, C8H7N	0.0, C10H12N	-0.7, C9H9N	1.1, C6H5
3	Benzenamine, 2-(1-methylcyclopropyl)-	C10H13N	147	715	6/6	-0.5, C9H10N	-0.2, C10H13N	0.9, C8H7N	0.0, C10H12N	-0.7, C9H9N	1.1, C6H5
4	Quinoline, 1,2,3,4-tetrahydro-3-methyl-	C10H13N	147	663	6/6	-0.5, C9H10N	-0.2, C10H13N	0.9, C8H7N	0.0, C10H12N	-0.7, C9H9N	1.1, C6H5
5	6-Methyl-1,2,3,4-tetrahydroquinoline	C10H13N	147	652	6/6	-0.5, C9H10N	-0.2, C10H13N	0.9, C8H7N	0.0, C10H12N	-0.7, C9H9N	1.1, C6H5
6	Pyridine, 4-methyl-2-(2-methyl-1-propenyl)-	C10H13N	147	649	6/6	-0.5, C9H10N	-0.2, C10H13N	0.9, C8H7N	0.0, C10H12N	-0.7, C9H9N	1.1, C6H5
7	2-Butanone, 4-(2,3-dihydro-1H-indol-1-yl)-	C12H15NO	189	645	6/6	-0.5, C9H10N	-0.2, C10H13N	0.9, C8H7N	0.0, C10H12N	-0.7, C9H9N	1.1, C6H5
8	Azetidine, 1-isopropyl-3,3-dimethyl-2-phenyl-	C14H21N	203	640	6/6	-0.5, C9H10N	-0.2, C10H13N	0.9, C8H7N	0.0, C10H12N	-0.7, C9H9N	1.1, C6H5

↑ elemental composition of compound

↑ elemental composition of fragment

Software for automated screening:

- peak detection and spectral deconvolution tool



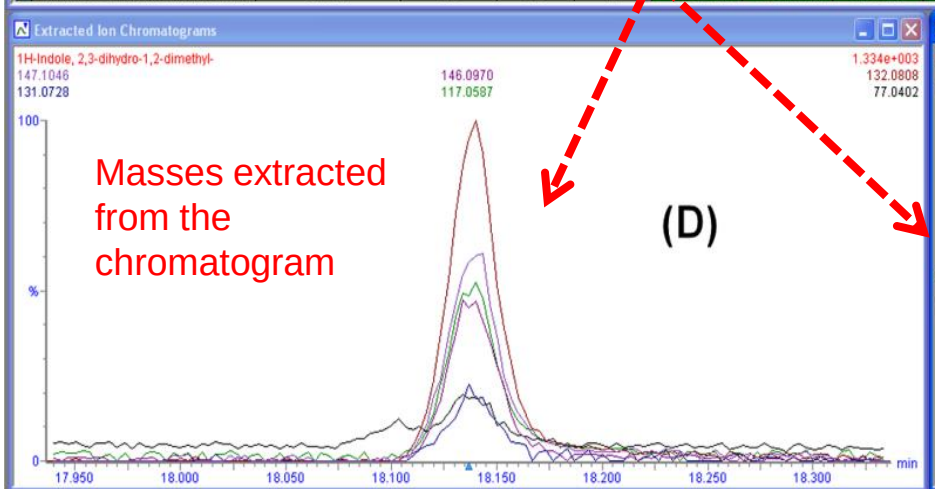
(B)

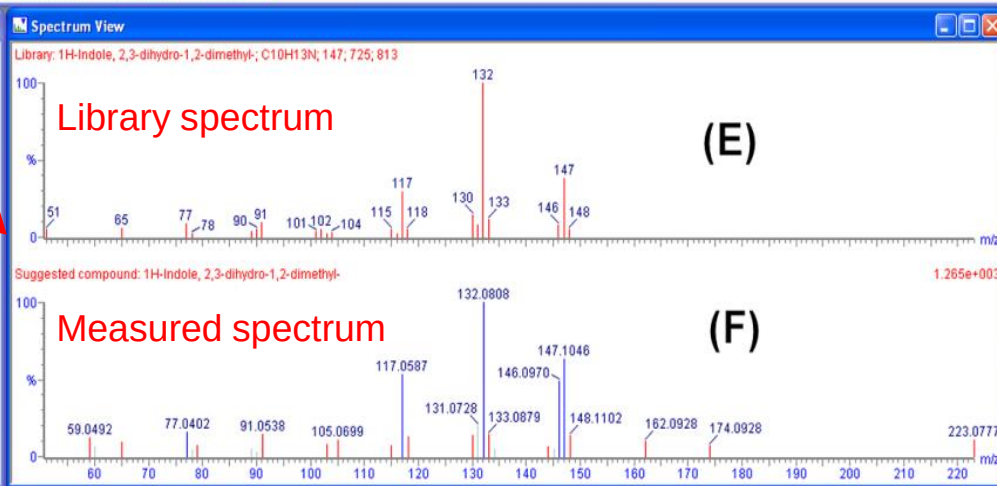
	Ret. Time	Abundance	Rel. Abundance	Compound Name	Mass1	Mass2	Mass3	Mass4	Mass5	Mass6	Match
1401	18.14	207	0.003	1H-Indole, 2,3-dihydro-1,2-dimethyl-	77.04	117.06	131.07	132.0808	146.10	147.10	430
1402	18.14	83	0.001	Benzene, 1-ethyl-3-isocyanato-	65.04	90.05	118.07	132.0807	147.10	148.11	
1403	18.16	2	0.000	N-Methoxy-N-methylacetamide	104.03						342
1404	18.16	5	0.000	S(IV)Thirane-2-carboxylic acid, 2-amino-1-ethyl-	104.03	149.02					282
1405	18.17	4	0.000	3-Nitro-1-phenyl-1-butanone	193.05						
1406	18.18	1	0.000	3-Nitro-1-phenyl-1-butanone	69.07						
1407	18.20	1	0.000	3-Nitro-1-phenyl-1-butanone	130.99						
1408	18.23	1	0.000	6-Methylbicyclo[2.2.1]hept-2-ene-5-carboxylic acid	79.05						

(C)

Library Match

	Compound	Formula	Mol. Wt.	Forward Fit	EleComp Hits	132.0808 (mDa)	147.1046 (mDa)	117.0587 (mDa)	146.0970 (mDa)	131.0728 (mDa)	77.0402 (mDa)
1	1H-Indole, 2,3-dihydro-1,2-dimethyl-	C10H13N	147	725	6/6	-0.5, C9H10N	-0.2, C10H13N	0.9, C8H7N	0.0, C10H12N	-0.7, C9H9N	1.1, C6H5
2	Quinoline, 1,2,3,4-tetrahydro-2-methyl-	C10H13N	147	71[illegible]	6/6	-0.5, [illegible]H10N	-0.2, C10H13N	0.9, C8H7N	0.0, C10H12N	-0.7, C9H9N	1.1, C6H5
3	Benzenamine, 2-(1-methylcyclopropyl)-	C10H13N	147	715	6/6	-0.5, [illegible]H10N	-0.2, C10H13N	0.9, C8H7N	0.0, C10H12N	-0.7, C9H9N	1.1, C6H5
4	Quinoline, 1,2,3,4-tetrahydro-3-methyl-	C10H13N	147	663	6/6	-0.[illegible], C9H10N	-0.2, C10H13N	0.9, C8H7N	0.0, C10H12N	-0.7, C9H9N	1.1, C6H5
5	6-Methyl-1,2,3,4-tetrahydroquinoline	C10H13N	147	652	6/6	-0.5, C9H10N	-0.2, C10H13N	0.9, C8H7N	0.0, C10H12N	-0.7, C9H9N	1.1, C6H5
6	Pyridine, 4-methyl-2-(2-methyl-1-propenyl)-	C10H13N	147	649	6/6	-0.5, C9H10N	-0.2, C10H13N	0.9, C8H7N	0.0, C10H12N	-0.7, C9H9N	1.1, C6H5
7	2-Butanone, 4-(2,3-dihydro-1H-indol-1-yl)-	C12H15NO	189	645	6/6	-0.5, C9H10N	-0.2, C10H13N	0.9, C8H7N	0.0, C10H12N	-0.7, C9H9N	1.1, C6H5
8	Azetidine, 1-isopropyl-3,3-dimethyl-2-phenyl-	C14H21N	203	640	6/6	-0.5, C9H10N	-0.2, C10H13N	0.9, C8H7N	0.0, C10H12N	-0.7, C9H9N	1.1, C6H5





Flow chart for recognition of unknowns with nontarget analysis

Stage 1.

- Components with forward fit value > 700 are qualified

Stage 2.

- Components containing three or more ions in deconvoluted component spectrum are qualified

Stage 3.

- Minimum component abundance of 10 counts is required

Stage 4.

- At least three ions must pass the accurate mass-scoring threshold
- At least four identification points are required for tentative identif

Stage 3.

- Minimum component abundance of 10 counts is required

Stage 4.

- At least three ions must pass the accurate mass-scoring threshold (5 mDa)
- At least four identification points are required for tentative identification
  - Accurate mass < 2 mDa → 2 IPs
  - Accurate mass 2–5 mDa → 1 IP

Stage 5a.

- Δ(forward fit) > 100 → tentative compound identification

Stage 5b.

- Δ(forward fit) < 100 → elemental composition proposed

Stage 6.

- Confirmation of identification result with a pure standard material

Tentatively identified compounds in aquatic samples

			Samples[b]			
Compound	Use of compound	ID[a]	WW	RW	ST1	ST2
Tetraacetyletlenediamine	Bleaching agent	-	x	x	-	-
Tris(2-chloroethyl)phosphate	Flame retardant	x	x	x	-	x
Tris(2-chloroisopropyl)phosphate	Flame retardant	x	x	x	-	x
Tris(1,3-dichloroisopropyl)phosphate	Flame retardant	x	x	x	-	-
Ethanol, 2-butoxy-, 1,1',1''-phosphate	Flame retardant	x	x	x	x	-
Triphenylphosphate	Flame retardant	x	x	x	x	-
4-Oxoisophorone	Personal care product	-	x	x	x	x
N,N-Diethyltoluamide	Personal care product	-	x	x	-	-
Galaxolide	Personal care product	x	x	x	-	-
Triethylphosphate	Plasticiser	x	x	x	x	x
Tri-isobutylphosphate	Plasticiser	x	x	x	x	x
Tri-n-butylphosphate	Plasticiser	x	x	x	x	x
Triethylcitrate	Plasticiser	-	x	x	x	-
N-butyl-benzenesulphoamide	Plasticiser	-	x	x	x	x
Bisphenol A	Plasticiser	x	x	x	x	x
Tributylacetylcitrate	Plasticiser	-	-	-	-	x
2-(Methylthio)benzothiazole	Rubber component	-	x	x	x	x
2(3H)-Benzothiazolone	Rubber component	-	x	-	x	x

[a] Confirmed with standard compound.
[b] WW: wastewater effluent, RW: Porvoonjoki-river, ST: stormwater

Nontarget analysis of passive samplers

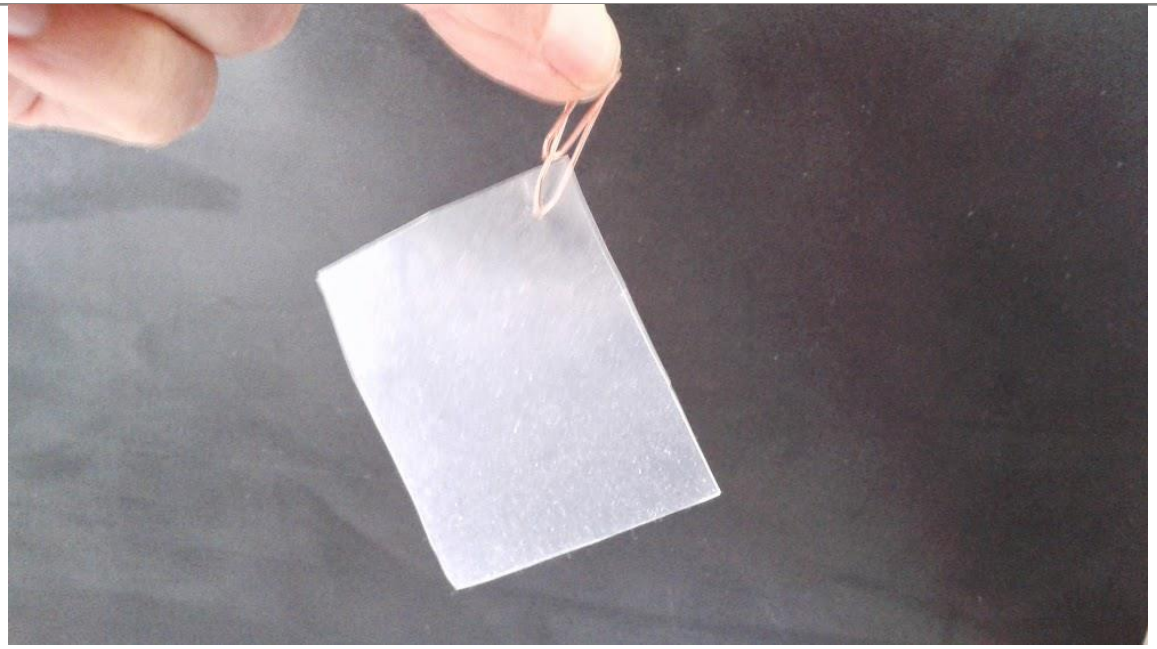
- Empore SPE disks were exposed to:
    1. River upstream WWTP
    2. River downstream WWTP
    3. Wastewater effluent

- Solvent extraction
- GC-TOF-MS nontarget

Findings in passive samplers with nontarget analysis

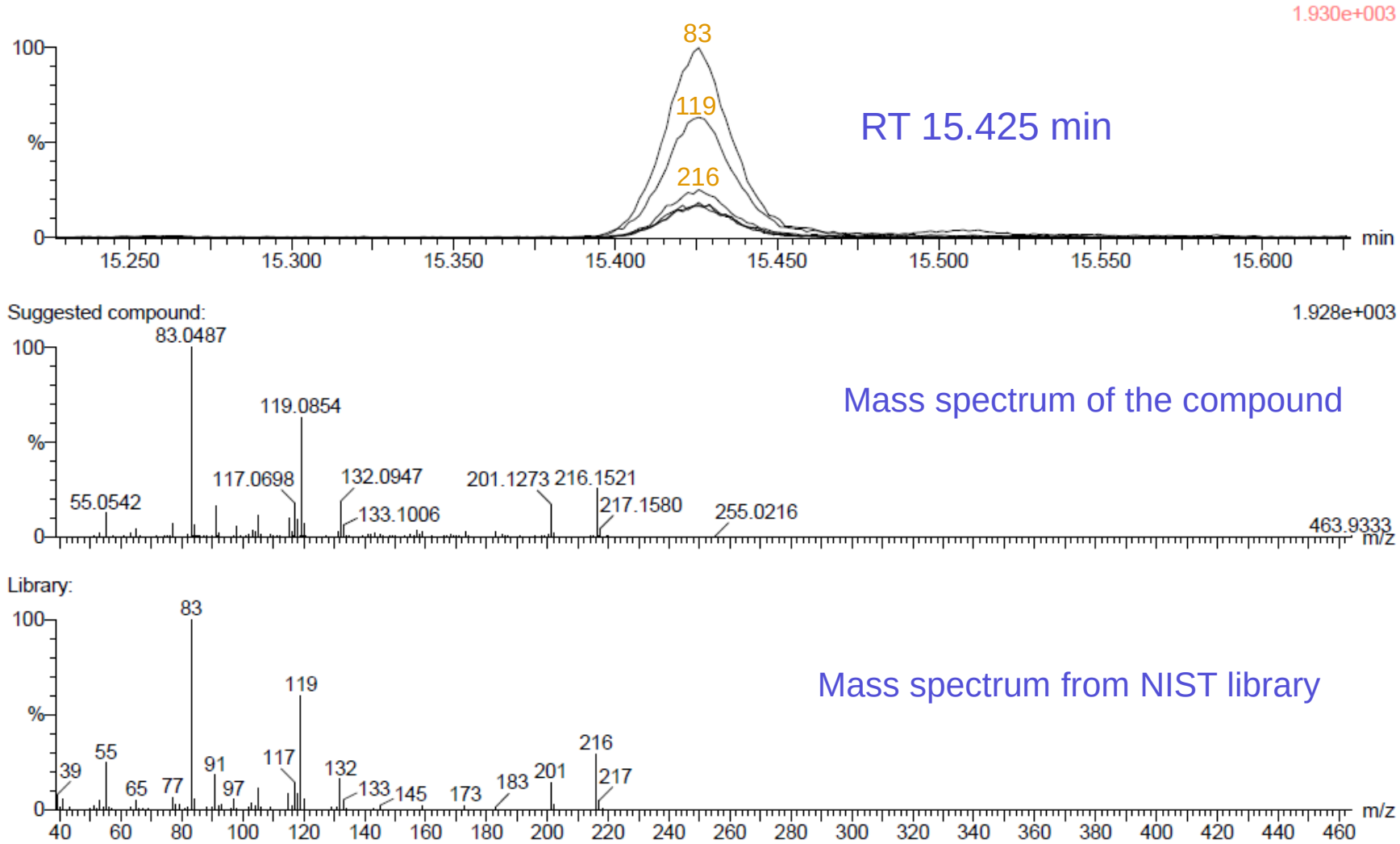
RT	Up WWTP	Down WWTP	Waste water	Elemental composition, name (usage)	Conf.
5.93		x		C_7H_6O Benzaldehyde (natural compound, food flavor, industry)	
14.96	x	x	x	$C_8H_7NS_2$ 2-(Methylthio)benzothiatzole (degradation of thiatzoles used in fungicides, rubber industry)	
15.28	x			$C_{10}H_{16}N_2O_4$ *N*,*N*,*N′*,*N′*-Tetraacetylethylenediamine, TAED (detergents, pulp industry)	
15.45	x	x	x	$C_{12}H_{20}O_7$ Triethyl citrate (food additive, plasticizer)	
16.63			x	$C_6H_{12}Cl_3O_4P$ Tris(2-chloroethyl)phosphate (fire retardant)	x
16.91	x	x	x	$C_{10}H_{15}NO_2S$ N-butylbenzenesulfonamide (plasticizer)	
17.05	x	x	x	$C_9H_{18}Cl_3O_4P$ Tris(1-chloroisopropyl)phosphate (fire retardant)	x
17.68	x	x	x	$C_{18}H_{26}O$ Galaxolide, HHCB (synthetic musk)	x
20.73			x	$C_{15}H_{16}O_2$ Bisphenol A (plasticizer)	x

Silicone rubber passive sampler

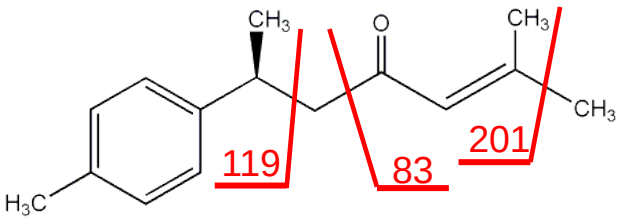


- 200 mL of waste water effluent
- One sampler / 200 mL
- Blank 200 mL deionized water
- Sampling time 3 weeks on a shaker

Unknown substance found by deconvolution software



Ar-tumerone MW 216
E100 food additive
color from turmeric plant



Compound	Formula	Mol. Wt.	Forward Fit	Reverse Fit	Hit Prob
Ar-tumerone	C15H20O	216	846	891	0.9651
Benzene, 1-(3-cyclopentylpropyl)-2,4-dimethyl-	C16H24	216	595	625	0.0096
Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	C15H22	202	572	699	0.0035
2,5,5-Trimethyl-3-phenyl-cyclohexanone	C15H20O	216	566	571	0.0027
Cyclobutanecarboxylic acid, 3-phenyl-2-propenyl ester	C14H16O2	216	563	634	0.0024
3-tert-Butyl-1-phenyl-2-pyrazolin-5-one	C13H16N2O	216	562	571	0.0023
Cyclohexanone, 3,3,5-trimethyl-5-phenyl-	C15H20O	216	558	573	0.0019
3-Methyl-2-butenoic acid, 3-phenyl-2-propenyl ester	C14H16O2	216	549	666	0.0014
Benzenebutanal, ç,4-dimethyl-	C12H16O	176	537	630	0.0009
Cyclobutane, tetrakis(1-methylethylidene)-	C16H24	216	531	532	0.0007

d	Library Na...	Cas No.	EleComp Hits	83.0487 (mDa)	119.0854 (mDa)	216.1521 (mDa)	132.0947 (mDa)	117.0698 (mDa)	201.1273 (mDa)
	C:\Nist08\...	Not found	6/6	-1.0, C5H7O	-0.7, C9H11	0.7, C15H20O	0.8, C10H12	-0.6, C9H9	-0.6, C14H17O
	C:\Nist08\...	54815-16-6	3/6		-0.7, C9H11		0.8, C10H12	-0.6, C9H9	
	C:\Nist08\...	644-30-4	3/6		-0.7, C9H11		0.8, C10H12	-0.6, C9H9	
	C:\Nist08\...	Not found	6/6	-1.0, C5H7O	-0.7, C9H11	0.7, C15H20O	0.8, C10H12	-0.6, C9H9	-0.6, C14H17O
	C:\Nist08\...	Not found	4/6	-1.0, C5H7O	-0.7, C9H11		0.8, C10H12	-0.6, C9H9	
	C:\Nist08\...	6631-89-6	5/6	(1) -1.0, C5H7O	(1) -0.7, C9H11		(1) 0.8, C10H12	(1) -0.6, C9H9	11.9, C13H15NO
	C:\Nist08\...	25109-54-0	6/6	-1.0, C5H7O	-0.7, C9H11	0.7, C15H20O	0.8, C10H12	-0.6, C9H9	-0.6, C14H17O
	C:\Nist08\...	56500-41-5	4/6	-1.0, C5H7O	-0.7, C9H11		0.8, C10H12	-0.6, C9H9	
	C:\Nist08\...	4895-19-6	4/6	-1.0, C5H7O	-0.7, C9H11		0.8, C10H12	-0.6, C9H9	

Contamination?

LC-TOF-MS
Waters LCT Premier XE TOF-MS

UHPLC-TOF-MS

Nontarget has challenges: low fractionation, spectral library?

Posttarget method was developed and applied to spiked and nonspiked wastewater

Posttarget is seaching compounds by using monoisotopic mass

Developed process for posttarget LC screening

Stage 1 - Candidates with mass error < 30 mDa are extracted from the total ion chromatogram

Stage 2 - Candidates with mass error < 5 mDa are qualified

Stage 3 - The regression of $LogK_{ow}$ and retention times of known compounds is used to predict the RT of screened compound (← Fig.)
- Candidates outside the calculated RT window are omitted

Stage 4 - Manual check of characteristic isotope peak pattern, if present
- Applied to all compounds containing chlorine or bromine atom(s)

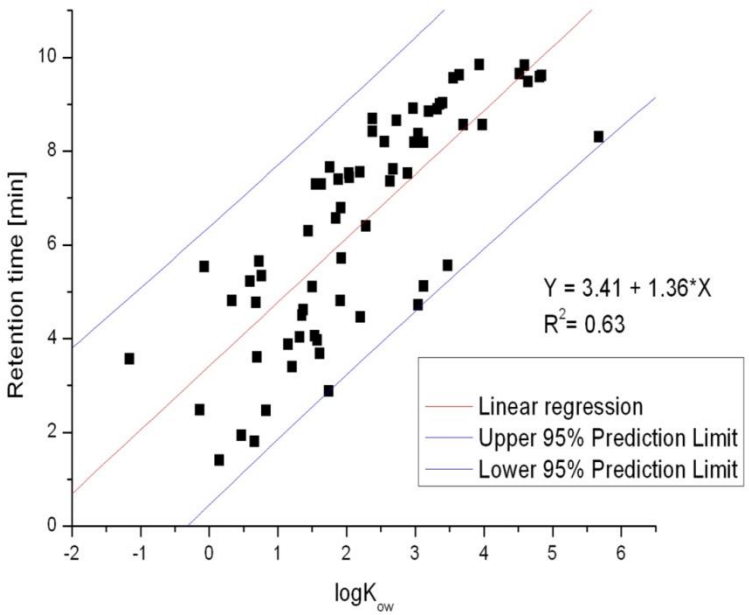


Fig. The linear regression line with 95% prediction intervals based on the $logK_{ow}$ and the retention time

Pharmaceuticals found in waste water effluent with posttarget analysis

Table 4. Identification results of posttarget analysis of unspiked wastewater effluent

Compound	Identification stage[a]	Confirmation with standard compound
Positive polarity		
Nordiazepam	Stage 4.	-
Oxazepam	Stage 4.	-
Temazepam	Stage 4.	-
Bisoprolol	Stage 3.	-
Candesartan	Stage 3.	-
Carbamazepine	Stage 3.	-
Enalapril	Stage 3.	-
Hydroxymetoprolol	Stage 3.	-
Lansoprazole	Stage 3.	-
Metoprolol	Stage 3.	-
Metronidazole	Stage 3.	Yes
Paroxetine	Stage 3.	Yes
Penicillin V	Stage 3.	-
Pentoxifylline	Stage 3.	-
Quetiapine	Stage 3.	-
Trimethoprim	Stage 3.	Yes
Venlafaxine	Stage 3.	-
Negative polarity		
Diclofenac	Stage 4.	Yes
Furosemide	Stage 4.	Yes
Acetylsalicylic acid	Stage 3.	-
Cholesterol	Stage 3.	-
Ibuprofen	Stage 3.	Yes
Mefenamic acid	Stage 3.	-
Naproxen glucuronide	Stage 3.	-
Salicylic acid	Stage 3.	-

Conclusions

- GC-TOF-MS is feasible for the nontarget and posttarget tentative identification
- LC-TOF-MS is feasible for posttarget identification
- The identification proposed should be treated as tentative, until it has been confirmed using a standard compound
- To avoid loosing a compound we should
  - Have right sampling method
  - Have right pretreatment, instrument, column….
  - Have high enough concentration ← LOI 100-2500 ng/L
  - Have not too concentrated/dirty sample
- Be careful with contamination

Thank you for your attention!

References

- Nurmi J. and Pellinen J. (2011). Multiresidue method for the analysis of emerging contaminants in wastewater by ultra performance liquid chromatography–time-of-flight mass spectrometry, *J. Chromatogr. A* 1218: 6712.

- Nurmi J., Pellinen J. and Rantalainen A.-L. (2012). Critical evaluation of screening techniques for emerging environmental contaminants based on accurate mass measurements with time-of-flight mass spectrometry, *J. Mass Spectrom.* 47: 303.

- Jernberg J., Pellinen J. and Rantalainen A.-L. (2013). Qualitative nontarget analysis of landfill leachate using gas chromatography time-of-flight mass spectrometry, *Talanta* 103: 384-391

- Jernberg J., Pellinen J. and Rantalainen A.-L. (2013). Identification of organic xenobiotics in urban aquatic environments using time-of-flight mass spectrometry, *Sci. Total Environ.* 450-451: 1-6