An Unknown Screening Approach to Analyze Micro Pollutants Degradation by Disinfection Processes

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ABSTRACT

In this study an unknown approach (suspect screening) in combination with a non-target screening was used to analyze micro pollutants degradation by disinfection processes. In addition 60 identified organic micro pollutants were quantified. The samples analyzed were collected at the wastewater treatment plant Ruhleben and include samples of treated wastewater (TW) before and after disinfection by UV radiation (UV) or performic acid (PA) treatment. The workflow from discovering a feature to the proposed structure using statistical tools is shown for one feature which was proposed to be triphenylphosphine oxide.

INTRODUCTION

Six wastewater treatment plants clean the wastewater from Berlin (Germany) and the surrounding regions. Treated wastewater flows into the rivers Spree and Havel. Ruhleben, the biggest wastewater treatment plant, is directly located in the city. For disinfection the last treatment step in Ruhleben is an UV radiation or performic acid treatment. These techniques can also be used in parallel if the waste water flow is too high. In August 2015 samples of the treated wastewater (TW) were taken before and after disinfection by UV radiation (UV) or performic acid (PA) treatment. This study shows a suitable way to identify “unknowns” or transformation products.

MATERIALS AND METHODS

Sample Preparation

Samples were directly injected onto the online-SPE system without dilution.

Liquid Chromatography

A 1 mL sample was injected onto the online-SPE system. For pre-concentration a C18 column, 2.1 × 20 mm with 12 µm particle size was used. For compound separation, a C18 column, 2.1 × 50 mm with 1.8 µm particle size was used. A gradient formed from water and methanol (B), both spiked with 0.1% formic acid, was ramped up from 2% B to 95% B in 6.7 minutes. The total chromatographic cycle time including online pre-concentration was 15 minutes.[1]

Mass Spectrometry for Full Scan with variable Data Independent Acquisition

Mass spectrometric analysis was performed on a Thermo Scientific™ Q Exactive™ Focus hybrid quadrupole-Orbitrap™ mass spectrometer.

MS-parameter for screening and quantitative analysis in Full Scan Mode with variable Data Independent Acquisition (vDIA) or data dependent MS² (ddMS²):

<u>for screening</u>
Full scan (FS)
- ESI+ and ESI– separately
- R = 70,000; *m/z* 100–1000

variable Data Independent Acquisition (vDIA):
- R = 17,500
- CE = 20, 40, 60 eV
- vDIA segments: 5
(*m/z* 100–205, 195–305, 295–405, 395–505, 495–1000)

data dependent MS² confirmation (ddMS²):
- R = 17,500
- CE = 20, 40, 60 eV
- Inclusion list with prioritized features after a statistical selection with Compound Discoverer

<u>for quantitative analysis</u>
Full Scan
- polarity-switching (ESI+ / ESI–)
- R = 35,000; *m/z* 100-1000
- internal standards

All resolution settings are provided in FWHM at m/z 200

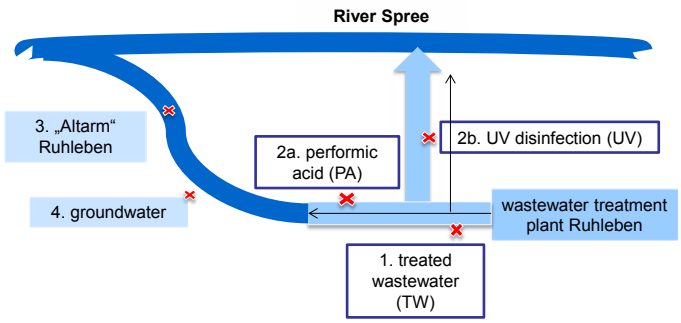
Note: vDIA method is not available in the United States of America

Data Analysis

Qualitative and quantitative analysis were performed with Thermo Scientific™ TraceFinder™ 3.3 software and Thermo Scientific™ Compound Discoverer™ 2.0 was used for the non-target screening approach.

SAMPLING

Figure 1. Schematic overview of the investigation area Berlin Ruhleben.



Investigation area

Figure 1 shows different sampling points in the area around the wastewater treatment plant Ruhleben. The following sampling points were in focus of the present study:

1. **treated wastewater** (**TW**) as control sample

The wastewater treatment plant has a throughput of around 247500 m³/day at dry weather. The wastewater treatment involves mechanical and biological purification steps. At first the water passes through a mechanically raked bar screen and six sand filters. This is followed by biological purification steps which include the removal of phosphate, nitrification and denitrification. [2]

2. **disinfection treatment steps (UV and PA)** to evaluate any possible microbiological pollution degradation

2a. **Performic acid** (**PA**): For chemical disinfection performic acid is used in a concentration of 15-20ppm and the reaction time is about 8 minutes. The throughput amounts to 500 L/s.[4]

2b. **UV radiation** (**UV**): The UV radiation unit consists of a total of 648 radiation sources. The throughput is variable and ranges between 500 L/s and 1000 L/s.[3]

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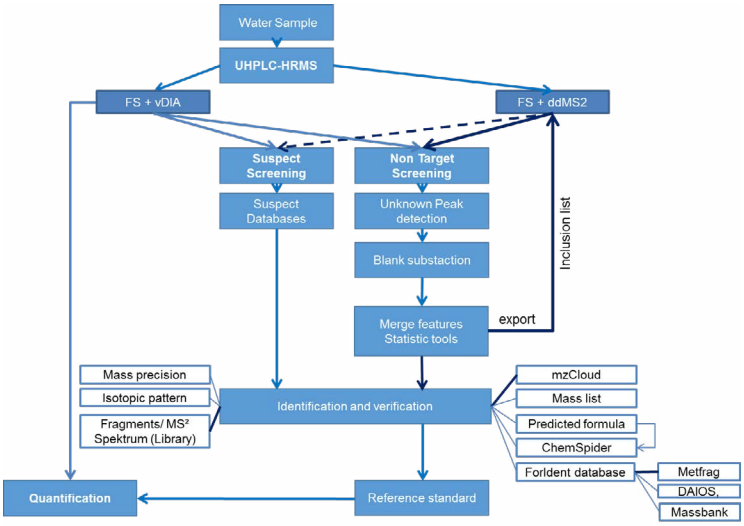
Workflow

Figure 2. Schematic overview of the workflow.



RESULTS - Suspect screening and quantification

Figure 2 shows a schematic diagram of the entire workflow carried out in this study. In the first step a suspect screening analysis was carried out with each sample (see fig.1). The database which was used contains a large number of compounds, e.g. pesticides, pharmaceuticals, industrial and household chemicals .

The suspect screening analysis resulted in a total of 381 suspects. By using confirmation criteria like mass precision and isotopic pattern match 192, 119 and 133 suspects could be confirmed in the control sample (TW) and the samples taken after UV or PA treatment, respectively. In addition 60 suspects could be quantified by reference standards. Table 1 shows the results of 17 selected compounds.

Table 1. Results of the suspect screening analysis and quantification.

Compound	Formula	used for/as	TW	UV	PA
			concentration [µg/L]		
Candesartan	$C_{24}H_{20}N_6O_3$	treatment of hypertension	1.70	1.70 -	1.60 -
1H-Benzotriazole	$C_6H_5N_3$	Corrosion inhibitor	3.90	4.00 -	3.40 ↓
2-Methylthiobenzothiazole	$C_8H_7NS_2$	vulcanization accelerator (metabolite)	0.47	0.55 -	x ↓
Bezafibrate	$C_{19}H_{20}ClNO_4$	lipid-lowering agent	0.15	0.14 -	0.14 -
Bisoprolol	$C_{18}H_{31}NO_4$	beta-blocker	0.43	0.51 -	0.25 ↓
Carbamazepine	$C_{15}H_{12}N_2O$	anticonvulsant	1.50	1.50 -	1.50 -
Diclofenac	$C_{14}H_{11}Cl_2NO_2$	NSAID	1.40	0.86 ↓	1.1 ↓
10,11-Dihydro-10,11-dihydroxy carbamazepine	$C_{15}H_{14}N_2O_3$	metabolite of carbamazepine	1.70	1.6 -	1.7 -
Carbendazim	$C_9H_9N_3O_2$	fungicide	✓	✓	✓
Amisulpride	$C_{17}H_{27}N_3O_4S$	antipsychotic	✓	✓	✓
Irbesartan	$C_{25}H_{28}N_6O$	treatment of hypertension	✓	✓	✓
Clindamycin	$C_{18}H_{33}ClN_2O_5S$	antibiotic	✓	✓	x ↓
Sotalol	$C_{12}H_{20}N_2O_3S$	beta-blocker	0.18	0.17 -	0.09 ↓
Diethyltoluamide (DEET)	$C_{12}H_{17}NO$	insect repellents	0.21	0.28 -	0.12 ↓
Phenazone	$C_{11}H_{12}N_2O$	analgesic	✓	✓	✓
Oxazepam	$C_{15}H_{11}ClN_2O_2$	benzodiazepine	✓	✓	✓
Primidone	$C_{12}H_{14}N_2O_2$	anticonvulsant	0.29	0.32 -	0.35 -

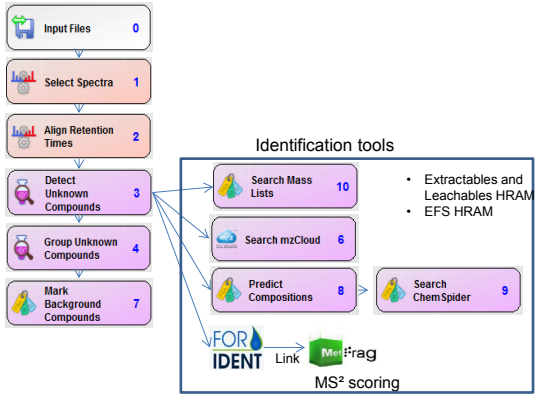
✓ identified suspect but not quantified, x not detected
↓ concentration decrease relating to TW, - no concentration change relating to TW

The concentration of candesartan, carbamazepine, bezafibrate, 10,11-Dihydro-10,11-dihydroxy carbamazepine and primidone did not change after UV or PA disinfection. Whereas the concentration of 1H-benzotriazole, 2-methythiobenzothiazole, bisoprolol, sotalol, diclofenac and diethyltoluamide decreased after treatment with PA. In addition the Diclofenac concentration also decreased significantly after UV treatment. That is well-known for diclofenac.

RESULTS - Non-Target Screening

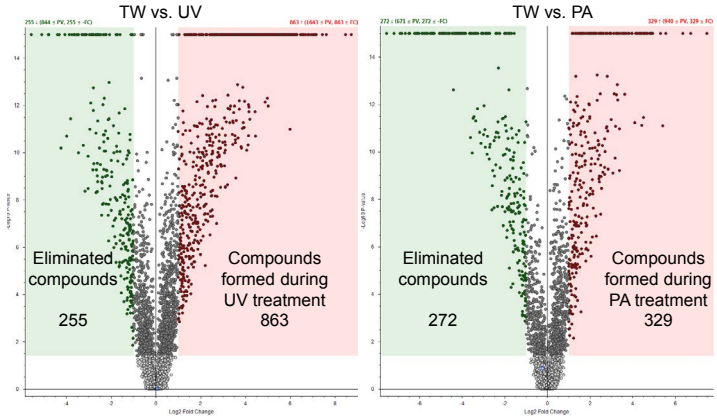
To identify further unknown compounds an unknown appraoch with Compound Discoverer 2.0 was conducted.

Figure 3. Unknown approach with Compound Discoverer 2.0 (workflow).



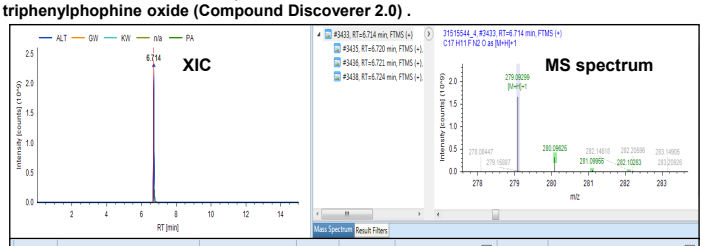
Compound Discoverer 2.0 (figure 3) is used to detect unknown compounds via elemental composition predictions. The software automatically subtracts the background (blanks), performs mzCloud and ChemSpider library searchings and scores the compounds based on defined patterns. In addition the database ForIdent which includes only water relevant data was used in this approach.

Figure 4. Uknown approach with Compound Discoverer 2.0 (workflow).



Statistic tools are useful to identify interesting features. In figure 4 the compounds formed after treatment with UV or PA are shown in red. At first glance it seems that more „new“ features were formed after UV treatment. A total of 15154 compounds were detected in all samples. After filtering the data by UV/TW >10 or PA/TW >10 the number of unique features decreased to 863 and 329 in the UV and PA samples, respectively. Only those features were in focus of this study.

FIGURE 5. Screenshot of compound m/z 278.08569 with masslist search hit triphenylphophine oxide (Compound Discoverer 2.0) .



Checked	Predicted Formula	Molecular Weight	RT [min]	Area (Max.)	Mass List Matches	# Adducts	Group Areas
	C18 H31 N O	277.24037	7.781	4485485		1	2.96e6 3.95e6 2.42e6 4.27e6 1.15e4
		278.07665	2.705	35608		2	2.88e4 2.55e4 3.18e4 1.58e4 7.32e3
		278.07899	5.471	95376		2	7.25e4 7.65e4 5.64e4 3.96e4 7.53e4
		278.07914	4.902	51911		2	4.22e4 4.86e4 4.65e4 2.00e4 1.43e4
	C14 H15 Cl N2 O2	278.08220	4.801	304698		1	2.97e5 2.70e5 2.35e5 1.72e5 3.26e3
	C17 H11 F N2 O	278.08569	6.713	127486929		4	3.62e6 1.11e6 1.17e7 1.26e6 7.06e5

Identification results

Predicted Compositions | Compounds per File | mzCloud Results | Mass List Search Results

	Checked	ΔMass [Da]	ΔMass [ppm]	Formula	Molecular Weight	RT [min]	Structure	Name
1		0.00036	1.31	C18 H15 O P	278.08605	0.000		Triphenylphosphine oxide

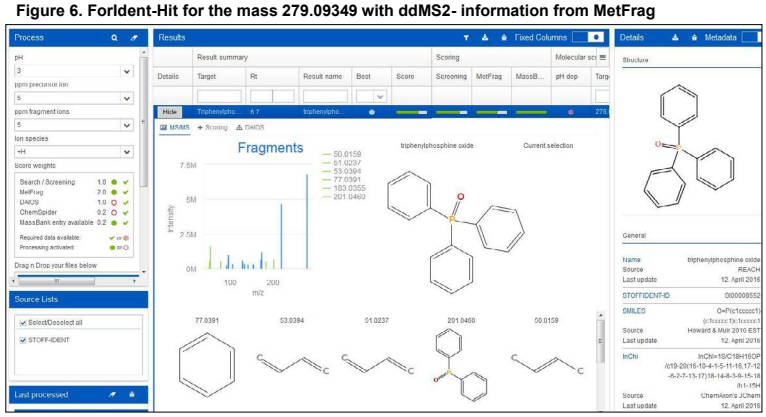
Compounds per File | mzCloud Results | Mass List Search Results

	Checked	ΔMass [Da]	ΔMass [ppm]	Scan Number	mzCloud ID	Formula	Structure	Molecular Weight	Match	Best Match	Name
1		0.00027	0.98	1497	932	C18 H15 O P		278.08605	71.5	71.5	Triphenylphosphine oxide

Figure 5 shows a selected compound with the monoisotopic mass 278.08569. Besides $[M+H]^+$ the adducts $[M+Na]^+$,$[2M+H]^+$ and $[2M+Na]^+$ were also detected. This compound was only found in the UV sample. The masslist search resulted in triphenylphosphine oxid (TPPO) which is a waste product in various chemical reactions e.g. Wittig reaction. A library score of 71.5% was given by mzcloud.

FOR-IDENT is an online database especially for compounds relevant in water analysis. 9500 compounds are currently registered and the database is continously updated and extended.[4]
A feature list with approximately 5000 features including their exact masses and retention times can be uploaded. The main criterias used for compound identification include exact masses (max. tolerance set at Δ 5 ppm), fragment ions (Δ 5 ppm), the ion species (+H) and the retention time index (RTI). If MS2-data are available the linked in MetFrag tool can be used additionally to further prioritize suggested compounds via *in silico* fragmentation.
In case of the compound with m/z 279.09349 the structure with the highest score is triphenylphosphine oxide (see figure 6). For final confirmation a reference standard needs to be checked.

Figure 6. Forldent-Hit for the mass 279.09349 with ddMS2- information from MetFrag



CONCLUSION

The present study is a combination of quantification and screening. With the suspect screening a high number of suspect could be identified and verified by reference standards. Whereby the behaviour of the different compounds could be observed. The quantified compounds showed no significant decrease of concentration by the disinfection step UV with the exeption of diclofenac. With PA treatment often the concentration decreases. With statistical tools in the non-target appraoch the data set can be reduced significantly to figure out relevant features. For identification beside isotopic pattern and formula prediction a pattern with databases and librarys are feasible. The producure of the non-target workflow is showed from Pekfinding to identifing with an example. To identify „new“ unknowns results it is a long and difficult way to discover.

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