Rhine Monitoring Programme

Chemistry

HPLC MS/MS Special Assay 2013

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1. Introduction

Within the Rhine Monitoring Programme Chemistry 2013, a special monitoring programme was implemented, in order to detect new substances in the Rhine water by applying the Swiss developed high performance liquid chromatography/mass spectrometry (HPLC MS/MS) analysis method. During the monitoring year 2013, samples were taken four times at the following monitoring stations:

- Weil am Rhein (see photo 1)
- Karlsruhe/Lauterbourg
- Koblenz/Rhine (see photo 2)
- Koblenz-Moselle
- Bad Honnef
- Bimmen-Lobith (see photo 3)
- Bischofsheim-mouth R. Main
- Mannheim-Neckar
- Maassluis

Photo 1: Monitoring station Weil am Rhein (photographic rights AUE-BS)

Photo 2: Monitoring station Koblenz Rhine (photo: Schwandt, rights BfG)

Photo 3: Monitoring station Bimmen (rights LANUV-NRW)

2. Present State of HPLC-MS / MS Analytics

Environmental analytics represent an important means of detecting unwanted trace substances such as household chemicals, agrochemicals, industrial chemicals, pesticides or pharmaceutical substances in wastewater, surface waters and groundwater. Deep concentrations and the variety of substances to be determined by sensitive analysis represent the greatest challenges. Therefore, it is most important to continue to develop specific and sensitive methods of detection.

In environmental analytics, the desire to analyse as many substances as possible in multi-methods and to detect unknown compounds at the same time has resulted in the use of new high-resolution mass spectrometry devices, such as Orbitrap. Such a system was used for this special monitoring programme.

During the last three years, this new method of analysis has been used daily at the monitoring station Weil am Rhein. Every day, water samples are measured, so as to be able to identify longstanding trends in water quality and to rapidly detect discharges and accidents. To this end, the EAWAG (Institute for Water Research of the ETH) and the Amt für Umwelt und Energie Basel-Stadt have developed a multi-stage analysis strategy:

Target Area

Within one day, the samples are analysed for known substances. At present (state September 2014) the list of known substances and their transformation products consists of 300 chemical substances belonging to the group of pesticides, biocides, pharmaceuticals, anaesthetic agents, industrial chemicals (ICPR report 202), anti-corrosive agents (ICPR report 281) and sweeteners. This list is continuously being updated (monitoring results of national and international monitoring programmes).

This multi-component method has also been applied for the special assay at hand.

Non-Target Area

In spite of this vast range of substances of the target screening it remains unclear, which share of substances existing in the Rhine is actually being identified. In order to also identify unknown substances, the EAWAG has developed a programme extracting all existing signals of substances from the mass spectrometry data. About 8,000 unknown substances remain, once background signals and blank value signals have been eliminated. In a first step, and based on the exact mass and isotopic pattern, the presumed empirical formula can be assigned to a substance. Next, specialized data bases are searched for appropriate substance structures. So far, it is not possible to automate these steps, which are thus rather time consuming. However, new substances are successfully recognized and identified. Methadone is one example. The occurrence of a certain mass during three days in a row leads to further results. After determining the empirical formula and comparing it with data banks in public spectrum libraries it was possible to assign a structure to the unknown substance. The final identification resulted from matching with a reference material. The substance turned out to be methadone and after its identification it was also possible to determine the discharger.

Presently, a new software tool is being implemented for the non-target sector. This tool is supposed to lead to a better prioritisation of data and thus improved recognition of "interesting" masses.

New substances for the extended Rhine Monitoring Programme Chemistry 2015-2020 exclusively belong to the "target list". The non-target analytics will gain in importance once the method will have been implemented in several laboratories along the Rhine.

3. Special assay of samples of the longitudinal profile of the Rhine and its tributaries applying HPLC-MS/MS

Chapter 3 summarizes the results of the four samplings.

3.1 Evaluation based on the frequency of occurrence

The detailed validated monitoring data of the special assay including a statistical evaluation are compiled in an Excel table available upon request.

Table 1 holds the evaluation of the special assay for substances identified above the limit of quantification at all monitoring stations, pharmaceuticals being the group of substances with most identified individual substances.

Table 1: Substances occurring at all monitoring stations (above the limit of quantification)

Pharmaceuticals	**Individual substances**
Candesartan	5-Methylbenzotriazole
Metoprolol	Benzotriazole
Sitagliptin	Naphthalene-2-sulfonic acid
Carbamazepine	Tetraglyme
Lamotrigin	
Sulfamethoxazole	**Metabolites**
Levetiracetam	Valsartan acid
Telmisartan	4-Formylaminoantipyrin
Oxazepam	N-acetyl-4-aminoantipyrine
Amisulpride	Atenolol acid
Venlafaxin	10,11 - Dihydro-10,11-Dihydroxy Carbamazepine
Phenazone	Metolachlor-ESA
Clarithromycin	Clopidogrel acid
Lidocain	2-Hydroxyatrazine
Metformin	
Gabapentin	**Sweeteners**
Valsartan	Acesulfam
O-Desvenlafaxine[1]	Cyclamic acid
Tramadol[1]	Sucralose
	Saccharin
Plant protection agents	
DEET	**Radiopaque contrast agents**
Carbendazime	Iomeprol[2]
Metolachlor	Iopamidol[2]

3.2. Evaluation based on concentration

For the evaluation based on concentration, a value of 0.3 µg/l was chosen, corresponding to the guide value of the International Warning and Alarm Plan Rhine for biocides (individual substances), plant protection agents (individual substances) and pharmaceuticals (individual substances).

The evaluation of the special assay based on concentrations is detailed in Table 2.

[1] was identified as sum of O-Desvenlafaxine and Tramadol

[2] was identified as sum of Iomeprol and Iopamidol

Table 2: Substances with a concentration > 0.3 µg/l

Pharmaceuticals	**Individual substances**
Furosemide (4.37 µg/l)	Toluene-4-sulfonic acid monohydrate (1.88 µg/l)
Metformin (1.87 µg/l)	5-Methylbenzotriazole (1.61 µg/l)
Hydrochlorothiazide (0.35 µg/l)	Benzotriazole (1.44 µg/l)
Gabapentin (0.87 µg/l)	2.7-Naphthalenedisulfonic acid (0.68 µg/l)
Valsartan (0.31 µg/l)	2-Naphthalenedisulfonic acid (0.44 µg/l)
Metabolites	**Radiopaque contrast agents**
Valsartan acid (0.55 µg/l)	Iopromid (0.54 µg/l)
Metazachlor-OXA (0.41 µg/l)	
Metazachlor-ESA (0.54 µg/l)	**Sweeteners**
4-Formylaminoantipyrine (0.40 µg/l)	Acesulfam (3.17 µg/l)
N-acetyl-4-aminoantipyrine (0.38 µg/l)	Cyclamic acid (1.29 µg/l)
Metoprolol Acid (0.32 µg/l)	Sucralose (0.83 µg/l)
	Saccharin (0.41 µg/l)

3.3 Evaluation based on concentration and frequency

It is planned to include those substances into the extended Rhine Monitoring Programme Chemistry 2015-2020 from the special monitoring programme 2013, which prove to be particularly conspicuous. To this end, the results of the special monitoring programme have been evaluated in two ways (Table 3):

1. Based on the maximum concentration of the substance.
2. Based on the frequency of occurrence at the monitoring stations investigated.

Table 3: Scoring system aimed at assessing the substance occurrence

Score for			
Concentration (µg/l)		Frequency of occurrence compared to the number of measurements	
Value	Score	Value	Score
> 1	100	100 %	100
> 0,75	75	> 75 %	75
> 0,5	50	> 50 %	50
> 0.3	30	> 25 %	25
> 0.1	10	> 0 %	20
> 0.03	4		
> 0.01	1		

Scores were given for the substances based on the result of this evaluation. The maximum achievable score for a substance was thus 200. The list of substances of the extended monitoring programme now includes substances with a score above 100. The list of new substances was supplemented with additional substances reported by the

states or German federal states, which could not be captured within the special assay, e.g. seasonally occurring plant protection agents.

Table 4: Substances from the special assay with a score > 100

Score 200	**Score > 100**
Metformin	Metazachlor-ESA
5-Methylbenzotriazole	O-Desvenlafaxine[1]
Benzotriazole	Candesartan
Iomeprol[2]	Metoprolol
Iopamidol[2]	Sitagliptin
Acesulfam	Carbamazepine
Cyclamic acid	Lamotrigin
	Sulfamethoxazole
Score > 175	Levetiracetam
Gabapentin	10,11 - Dihydro-10,11-Dihydroxy Carbamazepine
Toluene-4-sulfonic acid monohydrate	Metolachlor-ESA
Caffeine	Hydrochlorothiazide
Sucralose	Telmisartan
	Oxazepam
Score > 130	Amisulpride
Valsartan acid	Venlafaxin
Valsartan	Phenazone
Naphthalene-2-sulfonic acid	Clarithromycin
4-Formylaminoantipyrin	Clopidogrel acid
N-acetyl-4-aminoantipyrine	DEET
Atenolol acid	Carbendazime
Saccharin	Lidocain
	Tramadol[1]
	2-Hydroxyatrazine

4. Most important findings of the special assay

Until a few years ago, the scope of analysis of the Rhine monitoring station Weil am Rhein primarily concerned volatile and covalent substances (analysis by GC-MS technology). Since the introduction of the high-resolution HPLC-MS/MS method, analysis has been extended to polar trace substances, thus filling in this analytical "gap". However, there are limits to the HPLC-MS/MS method applied, set by the preparation of the method, the principles of separation and measurement. So this method only allows to identify those organic compounds, which are neither very volatile, nor very covalent nor extremely polar. Furthermore, only substances with a molar mass above 114 and which may be ionized are identified. This means that the applied multi-method is not capable of identifying a large number of solvents and the majority of substances belonging to the group of polycyclic aromatic hydrocarbons. The method is nevertheless a valuable completion which can however not yet completely replace the GC-MS technology which continues to be applied.

Within the special assay in the longitudinal profile of the Rhine, and along selected tributaries and due to applying the established HPLC-MS/MS analytics, numerous substances could be identified at all monitoring stations in concentrations above 0.1 µg/l. So far, most of these substances had not been part of the monitoring programme. The results show that the greatest permanent pollution is discharged by wastewater treatment plants. In particular, pharmaceuticals and their metabolites range among the

substances concerned (see ICPR report 182). Pesticides do not range among the compounds with the greatest permanent loads (exemption: pesticide metabolites metazachlor-ESA and -OXA). Seasonally and locally, they are however recurrent in higher concentrations.

With the results of the special assay we now dispose of comparable results in the longitudinal profile of the Rhine for about 300 substances. Based on the evaluation described, relevant substances may now be included into the extended water monitoring programme.

Recommendations

The results of this special monitoring programme should also serve as an extended basis for decision-taking by ICPR member states with respect to a complementation of the analytics by (high-resolution) liquid chromatography/mass spectrometry analytics (LC-MS/MS).

In this connection, the method/measurement technique to be included should fulfil the following criteria:

- The evaluation of the target substances must be possible within 6 hours after receipt of the samples.

- The identification and clarification of non-target substances requires a high-resolution LC-MS capable of generating a resolution of 60.000 (in routine check) in the relevant mass range of 150-400 m/z (Singer, H.W. et al., 2009, Multi-component screening for the Rhine at Basel - final report).