Novel monitoring concepts to acquire new water quality knowledge

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Abstract Two novel water quality monitoring concepts were developed: the HPLC-fingerprint for the monitoring of yet unidentified pollutants and the HPLC-Toxprint® for the recognition of (unknown) toxic or genotoxic compounds. The paper describes applications of both concepts. The HPLC-fingerprint is used for the evaluation of the overall water quality in addition to the monitoring of individual pollutants. Based on their occurrence (frequency, concentration, location) a listing of unknown priority pollutants is set up. Participating waterworks monitor for these compounds using a dedicated HPLC-DAD library that contains the required compound data (UV-absorbance spectrum, retention time index). In five years of experience with this concept, the HPLC-fingerprint was also found very suitable for the retrieval of new priority pollutants in existing HPLC-fingerprint databases, providing historic data on these new compounds. The HPLC-fingerprint concept was also used as an Early Warning System for accidental spills or sabotage. The HPLC-Toxprint was successfully applied in identifying genotoxicity (in the umu-test) in various waste water samples. By the application of LC-MS/MS genotoxicity could be assigned to acridine-derivatives in one of these wastewaters. To enable the evaluation of drinking water resources, the sensitivity of the HPLC-Toxprint was improved, now allowing the detection of pollutants with a 10% genotoxic potential as compared to 2-aminoanthracene (the positive control compound) at concentrations as low as 0.1 µg/l.

Keywords Early warning systems; genotoxicity; HPLC; LC-MS/MS; monitoring strategy; organic micro-pollutants

Introduction

Every year hundreds of new chemical compounds are synthesized of which many finally may appear in the environment. These compounds pose a possible threat to water quality and to drinking water production. For only a few of these compounds specific sampling strategies are designed in order to monitor their occurrence and their behaviour in the environment and in water treatment processes. Recent examples include pesticides, endocrine disrupters, pharmaceuticals and single compounds like MTBE. For these known substances risk assessment is possible through their physico-chemical properties, their emission sources, occurrence and their toxicological properties.

However, many chemical substances are not readily known, i.e. man-made compounds that are present in the environment, but have not yet been recognized nor identified.

A recent study in The Netherlands (RIWA, 2000) presents the presence of 1,328 identified organic compounds in surface water intended for drinking water production. These results were largely obtained by GCMS-screening of the rivers Rhine and Meuse, the two largest rivers in The Netherlands. It appeared that of these 1,328 compounds, 388 compounds were found frequently. Toxicological data showed that 36 of them were relevant with respect to human health. Estimating the presence of e.g. 10,000 largely not yet identified organics in surface water, this implies that we have to consider several hundred of toxicologically relevant and possibly harmful compounds.

How to deal with harmful, but still unidentified chemicals?

Water quality control, following several national or international directives, is usually limited to the analysis of regulated compounds like pesticides, THMs, bromate and heavy
metals. Methods to analyse these compounds are harmonized through international standardization bodies (ISO, CEN). For the organic pollutants, specific analytical methods are generally based on GC or HPLC (Barceló, 1993). In addition to the target compounds, other peaks in the chromatograms frequently reveal the presence of non-target compounds in the water sample that are not readily known. When GCMS is the applied technique, “forward search” of the mass spectrum might lead to the identification of the unknown peak, using MS libraries or (joint) data systems (Bobeldijk et al., 2001a). However, in routine analysis there is often no time for this as the laboratory is only commissioned to present data on the requested target compounds. When MS detection is applied in HPLC analysis for the analysis of target compounds, identification of unknown peaks is not possible because the MS detection is done only at specific $m/z$ values.

A different approach than the traditional application of GC or HPLC in water quality monitoring is required if unknown harmful compounds need to be tracked. Experimental evaluation of the genotoxicity of organic compounds present in water shows that the genotoxicity (as determined by the Ames test) is mainly present in the fraction of intermediate polarity, i.e compounds with logKow values between 0 and 3 (Kiwa, 1996; Figure 1).

A critical evaluation of the application range of analytical techniques indicates that GCMS is capable of analyzing non-polar compounds down to about logKow = 2. This means that the largest part of the organics that contribute to the Ames genotoxicity cannot be measured by GCMS. The analytical technique most suitable for this is Reversed Phase HPLC, in combination with UV or MS detection.

In addition, because of the ease of automation, HPLC analysis (in combination with on-line preconcentration) is the best way to go for the on-site monitoring of organic micropollutants in water (Slobodnik et al., 1993; Noij and Brandt, 1995) and is also the best approach for biospecific detection (Emnéus and Marko-Varga, 1995). This is important for Early Warning Systems as a means to safeguard drinking water quality from accidental spills or terrorist attacks.

In this paper the novel concepts of HPLC-fingerprinting and HPLC analysis in combination with (geno)toxicity detection (HPLC-Toxprint®) are presented. These concepts allow the tracking of harmful unknown compounds and enable their monitoring in drinking water production.

**Methods**

**HPLC-fingerprint**

The HPLC-fingerprint is based on a routine method for the analysis of phenylurea herbicides (Noij and Brandt, 1995) and is very similar to internationally standardized methods (ISO, 1997).
**Instrumental set-up.** The equipment for the HPLC-fingerprint consists of a Perkin-Elmer model 250 HPLC pump (Perkin Elmer, Shelton, USA) and a Waters model 991 Photo Diode Array Detector (Waters, Milford, USA). The analytical column is a 250 mm × 4 mm Inertsil ODS-2 column (particle size 5 µm) from GL Sciences (Chrompack, Middelburg, NL). A Gilson 232-401 autosampler (Gilson International, Rijswijk, NL) is used with a 20 mm × 3 mm pre-concentration column replacing the sample loop of the injection valve. The sorbent material is 25–35 µm OASIS (Waters). The equipment is schematically shown in Figure 2.

**Procedure.** After filtering the water sample over a 0.2 µm regenerated cellulose filter and the addition of two retention time standards (the herbicides fenuron and chloroxuron), a volume of 4 ml is passed over the pre-concentration column where the organics are adsorbed onto the OASIS material. After rinsing with 1 ml ultrapure water, the valve is switched into the desorption position and the HPLC-eluent flushes the compounds onto the analytical column. Here the separation takes place under conditions of gradient elution from 90% H₂O/10% acetonitrile to 20% H₂O/80% acetonitrile in 40 minutes at a flowrate of 0.7 ml/min, followed by an additional cleaning step of 100% acetonitrile for 2 minutes. The Diode Array Detector (DAD) records continuously at a scan speed of 1 scan/s, a spectral resolution of 1.2 nm and a spectral range of 200–350 nm.

**Data management.** Peaks in the chromatograms are characterized by their retention time and UV absorption spectrum. These data are stored in a HPLC-DAD library. Peaks are assigned a (random) ranking number when their identity is still unknown. Apparent concentrations are estimated against atrazine under the pre-assumption of equal sensitivity at an absorbance wavelength of 215 nm.

**HPLC-Toxprint**

The HPLC equipment and procedure are very similar to those of the HPLC-fingerprint, except that for reasons of sensitivity a volume of 20 ml instead of 4 ml of the filtered sample is taken. At the outlet of the DAD a Model 202 fraction collector (Gilson) is installed, and from retention times 11 to 46 minutes, 1 minute fractions are collected in a 96-well polypropylene microtiter plate. A small volume of DMSO is added as a keeper solvent prior to evaporation of the collected fractions. In each well the umu-genotoxicity test is performed using the Salmonella TA98 stem with and without the liver homogenate S9, all according to the procedure described in Bobeldijk et al. (2001b).

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**Figure 2** Instrumental set-up of the HPLC-fingerprint and the HPLC-Toxprint methods
Results and discussion

HPLC-fingerprint for the overall evaluation of water quality

The HPLC-fingerprint visualizes the presence of organic compounds without necessarily knowing their identity. Based on their retention time and their UV absorbance spectrum, individual unknown compounds can be monitored (simultaneously with monitoring known targeted compounds!). Applications of the HPLC-fingerprint concept are presented below as they were developed in the period 1997–2001.

The HPLC-fingerprint can be used for monitoring the overall water quality: the number of peaks indicates the number of pollutants present, and the peak areas indicate their concentrations. Figure 3 gives an example for the river Meuse at two different moments in 1997: June and October. The insert shows the variation in total peak area as it was monitored over a 1 year period. In this way temporal and spatial differences in overall water quality can be monitored, but also e.g. the overall efficiency of water treatment processes.

HPLC-fingerprint as an early warning system for accidental spills of pollutants

In August 1997 a large peak showed up in the HPLC-fingerprint chromatogram of the river Meuse. Retrieval of existing chromatographic data on target compounds showed a good agreement between this peak and the retention time and UV absorbance spectrum of the herbicide chlorotoluron. This was confirmed by standard addition of chlorotoluron to the sample and the concentration was estimated as being 1.7 µg/l, i.e. 10 to 30 times more than usual. These results compare very well to those obtained by RIZA (Institute for Inland Water Management and Waste Water Treatment) at their monitoring station at the Belgian-Netherlands’ border (Eijsden) (RIZA, 1997).

Retrieval of “new” pollutants in existing HPLC-fingerprint databases

The HPLC-fingerprint concept to monitor water quality yields databases containing a list of unknown compounds (each characterised by its assigned peak number, retention time index and the peak’s UV absorbance spectrum) and an estimated concentration (relative to the internal standard compound). When a “new” compound is identified or needs regular monitoring, these old databases can be searched for the presence of this new compound in previously analysed water samples.

Table 1 Chlorotoluron in the river Meuse in August 1997 as observed by the HPLC-fingerprint and compared to the RIZA monitoring data (concentrations in µg/l); August 12, 08.30 is the corresponding sampling time

<table>
<thead>
<tr>
<th>Date/time</th>
<th>RIZA Eijsden</th>
<th>HPLC fingerprint</th>
</tr>
</thead>
<tbody>
<tr>
<td>August 11, 16.00 h</td>
<td>1.6</td>
<td></td>
</tr>
<tr>
<td>August 12, 08.30 h</td>
<td>1.6</td>
<td>1.7</td>
</tr>
<tr>
<td>August 13, 08.00 h</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>August 14, 08.10 h</td>
<td>0.5</td>
<td></td>
</tr>
</tbody>
</table>
An example of this application of the HPLC-fingerprint was the retrieval of the pollutant 4,4′-dihydroxy-diphenylsulphone that was first identified by RIZA in the river Meuse in September 1998. Analysing a standard solution of this compound and comparing both the retention times and the UV absorbance spectra with unknown peaks in the HPLC-DAD library revealed the presence of this compound in the river Meuse also earlier than 1998 (Figure 4), listed as “Peak 5”. Afterwards quantification (1) of this “Peak 5” in the 1997 samples showed 4 µg/l in the October sample and 0.1 µg/l in both the August and September samples.

Assessment and identification of unknown pollutants

The HPLC-fingerprint allows us to monitor for organic pollutants even without knowing their identity yet. In close cooperation with 7 water laboratories in The Netherlands an inventory of occurring pollutants was performed followed by an assessment of their relevance to drinking water. These 7 laboratories are associated with water supply companies that abstract water from the rivers Rhine or Meuse for the production of drinking water and they use more or less the same HPLC-DAD method for the routine analysis of pesticides like phenylurea herbicides.

In 1997 a HPLC-fingerprint monitoring programme was executed for 68 water samples. After a first harmonisation of the method amongst the 7 water laboratories a second inventory was carried out in 1999. Existing HPLC-DAD data of 80 water samples that were analysed by these laboratories as part of their regular quality control programme were reprocessed.

A total of 90 peaks were present at apparent concentrations >0.05 µg/l (calculated as equivalent to atrazine, see “Methods”). Of these 90 peaks, 17 were known compounds that are already regularly monitored (eg the herbicides atrazine, diuron, etc). During this research 7 peaks were identified that were not known beforehand: they could be assigned names by comparison of existing GCMS data, by exchange of water quality data among research laboratories or by LCMS identification (Bobeldijk et al., submitted). Their identity was confirmed by analysing standard solutions of the pure compounds. Of the 90 peaks, 14 compounds appear frequently at relatively high concentrations. Further details on the occurrence of the 90 peaks are given in Table 2.

In drinking water samples 15 different unknown compounds were occasionally found, though generally at very low concentrations (apparent concentration <0.1 µg/l).

Figure 4  The unknown “Peak 5” in the October sample of the river Meuse compared to the aqueous standard solution of 4,4′-dihydroxy-diphenylsulphone (4.5 µg/l) as it was measured in September 1998 after it was designated to be a priority pollutant. The inserts show the UV absorbance spectra.
Based on these findings a listing of unknown priority pollutants was made including those occurring frequently and at high concentrations in raw water and those appearing in drinking water. These priority pollutants are monitored by the water laboratories as part of their regular monitoring programme and research is being started to elucidate their identity by LC-MS/MS.

The monitoring of HPLC-fingerprint peaks by the participating laboratories is supported by a joint HPLC-DAD library. Apart from reading from the library, users can also add entries for newly found unknown peaks, allowing all participants to search for these new compounds. A screen dump of this library is shown in Figure 5.

**HPLC-Toxprint for the risk assessment of unknown pollutants**

Peaks appearing in HPLC-UV chromatograms do not give any information on toxicity or human health. To direct water quality attention to toxicologically relevant compounds, the HPLC-Toxprint was developed. Although the approach is still very new and not widely nor routinely applied, some initial results and applications are presented here.

The HPLC-fingerprint defines compounds as being of interest whenever a compound occurs frequently at high concentrations or when it passes the water treatment process. In order to allow the assessment of peaks from the point of view of consumer health or ecotoxicity the HPLC-Toxprint was developed (Bobeldijk et al., 2001b).

Because it failed sufficient sensitivity the original HPLC-Toxprint was only applied successfully to wastewater samples. Examples of this application are shown in Figure 6 for an industrial and a hospital wastewater before water treatment. The results show clear differences in the character of the samples: in the hospital wastewater genotoxicity is found in a broad polarity range, whereas in the industrial wastewater genotoxicity is found in distinct

### Table 2
The 90 peaks assessed in this study, divided by their frequency and concentration of occurrence (“frequently”: >20% positive samples, “high conc.”: >250 ng/l apparent concentration) and the contribution of already known compounds and compounds identified within this study

<table>
<thead>
<tr>
<th>Occurrence</th>
<th>Total number of peaks</th>
<th>Already known</th>
<th>Identified in this study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequently, high conc.</td>
<td>14</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Occasionally, high conc.</td>
<td>26</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Frequently, low conc.</td>
<td>13</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Occasionally, low conc.</td>
<td>37</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td><strong>90</strong></td>
<td><strong>17</strong></td>
<td><strong>7</strong></td>
</tr>
</tbody>
</table>

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**Figure 5** Screen dump of the HPLC-DAD library as an aid for laboratories to monitor unknown compounds appearing in the HPLC-fingerprint
fractions. The identification effort with LC-MS/MS (using a Q-TOF mass spectrometer) was focused on fraction 43. Two acridine compounds were tentatively identified: 9-amino-2-hydroxy-acridine and 9-hydroxy-acridine-N-oxide or its structural isomer (di-hydroxy-acridine) (Bobeldijk et al., submitted). Because of the lack of standard compounds the identification could not be confirmed. However from the industrial process the presence of acridine-like compounds is to be expected. Moreover it is known that acridine derivatives are genotoxic compounds which is in accordance with the genotoxicity of fraction 43.

In order to be able to evaluate drinking water resources, the original HPLC-Toxprint was adapted. The sensitivity was enhanced by a combination of an optimised solvent manipulation and umu-test procedure. The adapted procedure now allows the detection of pollutants with a genotoxic potential of only 10% of the positive control compounds 2-aminoanthracene (2-AA) and 4-nitroquinoline-N-oxide (4-NQO) at concentrations as low as 0.1 µg/l.

As an example the detection of a genotoxic compound in Lake IJssel (December 2001) is shown in Figure 7. This corresponds to a small peak in the HPLC-fingerprint at a retention
time of 22.5 minutes. This peak was not yet included in the HPLC-DAD library, meaning that it concerns a newly tracked genotoxic compound. It will be monitored from now on and if possible be identified by LC-MS/MS.

Conclusions
The two new concepts of HPLC-fingerprint and HPLC-Toxprint provides new water quality knowledge in addition to target compound analyses. The HPLC-fingerprint allows the monitoring of yet unidentified compounds (simultaneously with target compounds) and it provides:

- the temporal and spatial distribution of pollutants in river basins and in water systems;
- the assessment of water treatment processes;
- an early warning system for accidental spills or sabotage;
- long term water quality data that can be searched afterwards for new priority pollutants.

The HPLC-Toxprint pinpoints unidentified genotoxic or toxic compounds and directs both the regular monitoring for these compounds by HPLC-fingerprint as well as the identification of unknown compounds by LC-MS/MS.

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