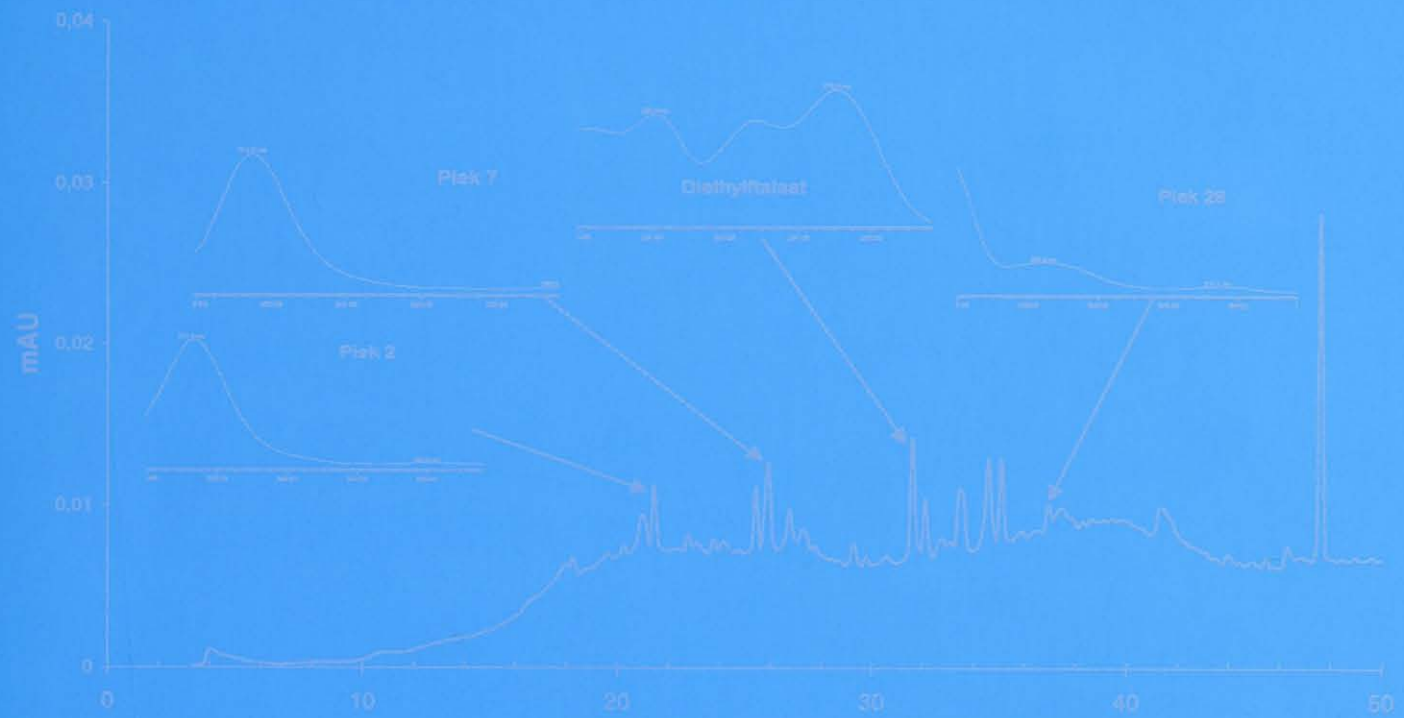
VERENIGING VAN RIVIERWATERBEDRIJVEN

RIWA

November 1999

Organic micropollutants in Rhine and Meuse

Monitoring with HPLC/UV-fingerprint



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Publisher

Vereniging van Rivierwaterbedrijven RIWA

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SUMMARY

The HPLC/UV indicates the presence of organic micropollutants in the form of peaks in a chromatogram. The micropollutants are characterized by their retention times and their UV spectra. The present report relates to micropollutants that exhibit UV absorption and are moderately polar (logKow values between 0 and 4). The mutagenicity of organic substances measured with the Ames test is also located in this polarity range.

In the period from March 1997 to February 1998, samples from the Rhine (Lobith) and the Meuse (Eijsden) were examined monthly with HPLC/UV fingerprinting. Both the overall water quality (as the sum of the individual peaks) and the presence of individual pollutants were monitored. The pollution of the Rhine, as measured with HPLC/UV fingerprinting, is approximately double that of the Meuse. The quality of the Meuse varies more than that of the Rhine, and the pollution in the Meuse is characterized primarily by a small number of compounds that occur sporadically in relatively high concentrations. The pollution of the Rhine is the most severe in autumn and winter, and that of the Meuse in summer and autumn. Several pesticides were identified in the samples. The concentrations measured generally agreed well with those reported in the framework of the regular RIWA research, with atrazine, diuron and isoproturon being present in the highest concentrations (diuron concentration in the Meuse up to 0.6 µg/l). A metabolite of diuron, 3,4-dichlorophenylmethyl urea, was encountered in concentrations of up to 0.08 µg/l. In addition, pesticides were encountered that are not covered by the regular RIWA investigation, with carbendazim, monuron and dimethachlor being the most important. Of the 67 compounds monitored in this study (of which the majority are still unidentified), 17 occur in both the Rhine and the Meuse. Apart from the pesticides referred to above, three of these substances occur frequently and - in the Rhine - in high concentrations; four substances occur frequently in generally low concentrations (sporadically in high concentrations), and the remaining six occur sporadically (three of these occasionally in high concentrations). Of the compounds that occur only in the Rhine, there are three that occur frequently and in high concentrations and four that occur sporadically and in high concentrations. Of those that were detected only in the Meuse, there is only one that occurs frequently and in high concentrations. Three occur frequently and in low concentrations, and six occur occasionally but in high concentrations.

It is recommended that further research be carried out to identify those substances that occur frequently and in high concentrations, and to assess their significance.

In the course of the study, HPLC/UV fingerprinting proved to be highly suitable not only for monitoring individual pollutants in time and place but also:
1. as an early warning system (dumping of chlortoluron in the Meuse in August),
2. for detection, in the old data files, of recently identified pollutants (the 4,4'-dihydroxydiphenyl sulphone identified in September 1998 was discovered already to have been present in 1997, in a concentration of approximately 4 µg/l), and
3. for selection of unidentified substances for further study, for the purpose of identification, with HPLC/MS (the xenoestrogenic substances diethylphthalate and dibutylphthalate in the Rhine in September).

1 INTRODUCTION

In the framework of the regular research done by RIWA into the quality of the Rhine and Meuse, these rivers are examined monthly for a large number of chemical compounds. These are identified pollutants whose presence in the surface water, which is used for drinking water production, is considered undesirable. In total, about 100 substances are involved, 50 of which are pesticides. In research done to ascertain the organic substances present in the Rhine and Meuse, it was established that hundreds or even thousands of substances can occur, of which several are highly undesirable from a health point of view (Van Genderen, Noij and Van Leerdam, 1994). The costs alone make it impossible to monitor all these substances with adequate frequency. In addition, many pollutants are not permanently present, and some are indeed present only infrequently (in the case of accidents). This means that if extensive monitoring programmes, in which hundreds of substances are tested for, were in place, the analysis results for the majority of the substances would be negative. For this reason, there is a need for a cost-effective method of obtaining non-specific information on the overall water quality, which allows immediate, targeted action to be taken (such as stoppage of intake, mixing with water of other qualities, or adaptation of the treatment conditions), should a limit value be exceeded. Biological monitoring systems which measure an overall effect of the water quality (fish monitoring, mussel monitoring, microtox monitoring) are currently receiving considerable attention.

In addition to biomonitoring, chemical monitoring is still very important. The challenge now is to obtain as complete as possible a picture of the water quality by the simplest and cheapest possible means. On the basis of laboratory methods for polar pesticides, an HPLC method has been developed which indicates the presence of a broad range of organic substances, without it being necessary to know exactly which substance is which ("fingerprint"). In a study commissioned by the Foundation for Applied Water Management Research (STOWA), the HPLC fingerprint was evaluated and applied to various surface water samples (STOWA, 1997). This fingerprint method is suitable for polar and weakly polar substances, can be used for monitoring of changes in concentration of identified and unidentified substances as a function of location (in the direction of flow), and proved to be capable of reliably measuring low concentrations of identified substances (pesticides). In addition, with this method, it was possible also to establish the content and type of natural organic material ("humus") present.

In the present study, which was commissioned by RIWA (reference 6/1:25138), the quality of the Rhine and the Meuse was examined by this "HPLC fingerprint" method. With this method, it is possible to obtain a picture of the overall water quality with reference to polar and weakly polar organic compounds. While the project was underway, a number of water companies (DZH, GWA, PWN, WBB/WBE, WRK and WZHO) decided to use the HPLC fingerprint for monitoring the effects of treatment processes on the quality of the Rhine and the Meuse. The research will be reported on separately to the companies concerned, use being made of the results of the RIWA study.

In the framework of the Water Companies Joint Research Programme, Kiwa is currently carrying out research into improvement of the HPLC fingerprint method. This improvement relates in particular to the isolation method and enables better

measurement of polar substances by use of a different adsorbent. It will have no further consequences for the research reported on in the present document.
In the framework of the same research, the possibility of coupling the HPLC directly to a genotoxicity test was also examined. On the basis of an exploratory study, it was concluded that such hyphenation is technically possible (Brandt and Van Genderen, 1998). The practical realization will be started at the end of 1998, the choice of genotoxicity test being made on the basis of the results of a joint RIWA-Kiwa study. When this combination is used, the individual pollutants, separated with HPLC, can each be awarded a genotoxicity score. This increases the value of the HPLC fingerprint considerably, because compounds that are thought to be harmful to health can immediately be traced (and if necessary identified with LC/MS).

2 MEANING AND INTERPRETATION OF THE HPLC/UV FINGERPRINT

2.1 Scope of the HPLC-UV fingerprint

In HPLC fingerprinting, a sample of a few mls is concentrated in an adsorption column in which the organic substances are adsorbed onto the adsorbent and the water passes through with the inorganic salts. The adsorbed material is subsequently desorbed and separated into its components by HPLC ("high performance liquid chromatography", also often referred to as LC or liquid chromatography). HPLC separates the components according to their polarity. After the separation, detection is performed, generally by UV absorption (hence "HPLC-UV" fingerprint).

This procedure determines the range of substances that can be examined by the method. Firstly, only substances that exhibit UV absorption are measured. This is, however, a very large fraction of the organic micropollutants. In general, UV detection functions for substances with double electron bonds such as aromatics and polyaromatics, nitro-compounds, aldehydes, ketones and sulphones. Compounds that cannot be detected by UV absorption are, for example, alkyl carboxylic acids (such as acetic acid), chloroalkanes, alkyl ethers and sugars.

The degree of UV absorption at a particular wavelength ("extinction coefficient") determines the sensitivity of the method and thus the minimum concentration of an organic substance for which detection is possible. Different substances have different extinction coefficients, which means that the detection limit (lowest concentration that will give a visible peak in the chromatogram) will vary from substance to substance. As an indication, under the HPLC/UV fingerprint conditions, pesticides such as atrazine and diuron can be detected from a concentration of 0.05 µg/l. The technique of direct coupling ("on-line") of solid-phase extraction with the HPLC separation makes it possible to analyse polar and moderately polar substances with logKow values between approximately 0 and 4. Recent research has shown that the mutagenicity measured with the Ames test is located primarily in this polarity range (Noordsij, 1998). Highly polar substances (with logKow < 0) are not quantitatively concentrated by the solid-phase extraction and will furthermore be subjected to severe interference from the humous matrix in the chromatogram. Unless special preventive measures are taken, highly non-polar substances (logKow > 4) will be adsorbed onto floating material, glass and metal, and will not be available for the analysis.

In summary:
HPLC/UV fingerprinting is suitable for polar and moderately polar substances that exhibit UV absorption and can be concentrated from water by solid-phase extraction; detection is possible from a concentration of approximately 0.1 µg/l.

2.2 Assessment of the HPLC/UV fingerprint

Figure 1 shows an example of an HPLC/UV fingerprint. The individual compounds can be seen as separate peaks. A UV absorption spectrum is established for each peak

(i.e. each compound). The UV absorption spectrum, together with the retention time, is characteristic of the compound. The size of the peak is a measure of the quantity present.

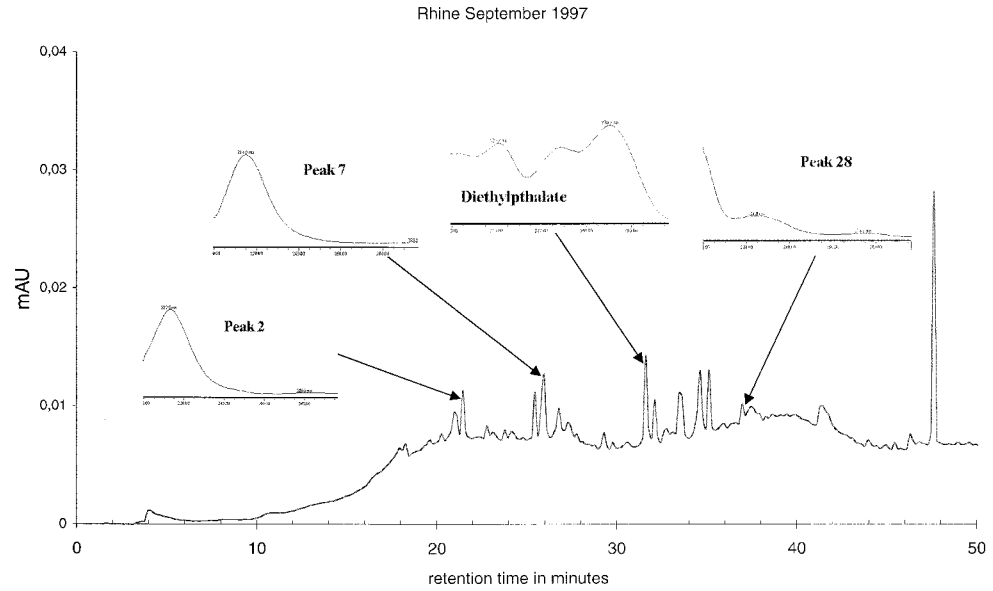

Figure 1 *Example of an HPLC/UV fingerprint with the UV absorption spectra of several components (Rhine, September 1997).*

When the fingerprints are being assessed, account must be taken of the different sensitivities of the different compounds. This means that, for example, even if the peaks of two different compounds are of equal height, the concentrations are not necessarily equal. What is true, however, is that for any one compound, if the concentration is increased by a factor of 10, then the peak will also be 10 times higher. In other words, the results must be considered relative to each other.

The HPLC/UV fingerprint gives information not only on the (relative) concentrations but also on the nature of substances present: the shorter the retention times, the more polar the substances.

In an HPLC fingerprint with a total length of 50 minutes, as used in this study, approximately 500 compounds can be distinguished on the basis of differences in their retention times (peaks can be distinguished for retention time differences of 6 seconds upwards). It is also the case that the more specific the UV absorption spectrum, the more effectively peaks that are close to each other can be distinguished. Only in cases in which different compounds have very similar retention times and similar UV absorption spectra is it not possible to distinguish the compounds by this method.

Earlier research has shown that surface water with pollution of industrial or other origin shows a large number of peaks, while surface water without industrial or agricultural pollution shows very few HPLC/UV peaks (Noij *et al*, (1989)). This research also indicated that the HPLC/UV fingerprint is suitable for following the effectiveness of treatment processes with regard to organic micropollutants even if the identity of all the peaks is not known. The same applies for the comparison of different surface waters and monitoring of the quality in time and location (STOWA,

1997). Not only can the peak pattern be assessed, dissolved organic carbon (DOC) can also be seen ("humous bulge"). The size of this bulge can also be taken into consideration in the assessment process.

Besides providing an overall picture of the water quality on the basis of the number of peaks and their sizes, HPLC/UV fingerprinting also permits monitoring of individual *known* pollutants. If, under the measuring conditions concerned, the retention time and the UV spectrum of a pollutant is determined, then this substance can be measured *quantitatively*. It is desirable to include these target compounds in a library. Several triazines and phenyl urea herbicides were included in this manner in this research.

Finally, it is possible to seek newly identified substances in existing HPLC/UV data files, by which means the occurrence of pollutants can also be traced back in time.

In summary:
The HPLC/UV fingerprint gives a picture of the degree of pollution of the water, with individual pollutants being distinguished on the basis of differences in retention time and UV absorption spectrum. The fingerprints can be compared with each other but do not give absolute concentrations for unidentified substances.

Identified pollutants, however, can be measured quantitatively in the same analysis.

New pollutants can be sought in old data files.

3 METHODOLOGY

3.1 HPLC/UV analysis

Samples are filtered in a 0.45 µm filter, after which a 15 ml sample is passed over an adsorption column with the aid of an autosampler (2 mm in diameter x 10 mm long) packed with PLRP-s adsorbent. This PLRP column is coupled on-line with the HPLC instrument. After charging, the PLRP column is flushed with 1 ml water containing 5% acetonitrile. Then the PLRP column is connected to the eluent stream from the HPLC, with the adsorbed material being desorbed in a counterflow process and subsequently separated in the analytical C-18 column. Detection is performed with a diode array detector (DAD), which not only measures the UV absorption signal at a reference wavelength of 215 nm but also records the entire UV absorption spectrum between 200 and 300 nm.

The arrangement used is shown schematically in Figure 2.

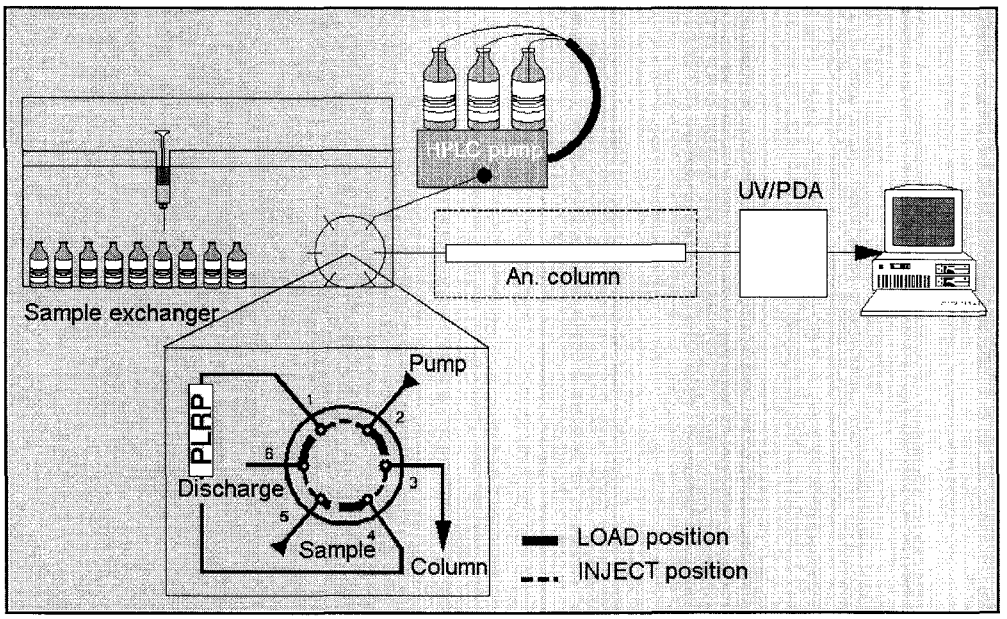

Figure 2 *Schematic representation of the measuring arrangement for the HPLC/UV fingerprint*

3.2 Water samples from the Rhine and Meuse

Every month from March 1997 until February 1998, samples from the Rhine at Lobith and the Meuse at Eijsden were submitted to the Kiwa laboratory by RIWA, and then analysed within a few days. The data from the samples are given in Appendix 1. When the results were being processed, the November sample from the Rhine was seen not to be reliable; it was subsequently disregarded.
The logistics, visual assessment and other results were unremarkable in terms of samples, preprocessing and analysis.

3.3 Processing of the data

Peaks from the HPLC/UV fingerprint were distinguished on the basis of retention time and UV absorption spectrum. The variations occurring in retention time (due to the 12 month duration of the project and the variations in the characteristics of the HPLC column and HPLC eluent in the course of this period) were corrected by means of two internal standards that were added to each sample (fenuron and chloroxuron, concentration approximately 0.6 µg/l water).
215 nm was taken as the reference wavelength for the presentation and processing of the data. Comparison of HPLC/UV fingerprints at 215 nm with the fingerprints at the (varying) wavelength at which the absorption is a maximum at any given moment of the analysis (as a result of which maximum sensitivity should be achieved) yields two very similar chromatograms (see Appendix 2). For the purpose of unambiguousness of the results, a fixed wavelength of 215 nm was chosen. Earlier research has also shown that this is the wavelength at which the greatest number of pollutants can be seen (STOWA, 1997).

For the overall water quality, the summed surface area of all discrete peaks visible above the humous bulge / base line was taken ("peak sum (PS)"; see STOWA, 1997). For the purpose of uniformity and comparability, the peaks were taken from the beginning of the chromatogram to the second internal standard (chloroxuron with a retention time of approximately 38 minutes). The two internal standards were excluded from the calculation, as was the frequently occurring interference peak ("peak 14"). A limited number of peaks (10 to 20 per chromatogram) was selected for monitoring of the behaviour of individual compounds. The selection was made on the basis of the size of the peak, occurrence in other samples and distinctiveness of the peak (separation, retention time and UV spectrum). The peaks in the different fingerprints were compared with each other on the basis of the heights of the peaks.

The peak size (surface area or height) is expressed relative to that of the chloroxuron used as the internal standard (represented as $1000 * (peak_x / peak_{chloroxuron})$). This provides a correction for variations in the sensitivity of the detector. For comparison, 1 µg/l atrazine at the reference wavelength of 215 nm corresponds to a relative peak height of 7400 units relative to chloroxuron and a relative peak surface area of 8300 units relative to chloroxuron (0.6 µg/l added to samples, peak size ≡ 1000).

The results for the Rhine and the Meuse are given in Appendices 3 and 4 (HPLC/UV fingerprints at 215 nm), in Appendices 5 and 6 (summed surface areas of the discrete peaks) and in appendices 7 and 8 (selected individual compounds and their peak heights).

4 QUALITY OF THE RHINE AND THE MEUSE

4.1 Overall quality

4.1.1 Sum of the peaks

The HPLC/UV fingerprints for the Rhine and the Meuse are given in Appendices 2 and 3. The overall development of the quality of the Rhine and the Meuse derived from the fingerprints is given in Figure 3. The discrete peaks from the fingerprints for the Rhine and the Meuse for the respective monthly samples are summed (see Section 3.3; for the numerical values, see Appendices 4 and 5).
Figure 3 indicates that the pollution of the Rhine with organic micropollutants varies over the course of the year, with the highest and lowest pollution levels differing by a factor of four. It is notable that the pollution is greater in the autumn and winter than in the spring and summer. Particularly noteworthy is the difference between the July-August period and September: this is the greatest difference, and it can be attributed more or less entirely to four peaks in the September fingerprint in the retention time range between 31 and 35 minutes, which include the diethylphthalate identified with LC/MS (see Section 4.2.3). In the Meuse, the levels of pollution can vary by a factor of 6, it being notable that the Meuse is least severely polluted with organic components in winter (the very period in which the Rhine is most severely polluted) and most severely in late summer and autumn. An abrupt decrease from November to December can also be seen.

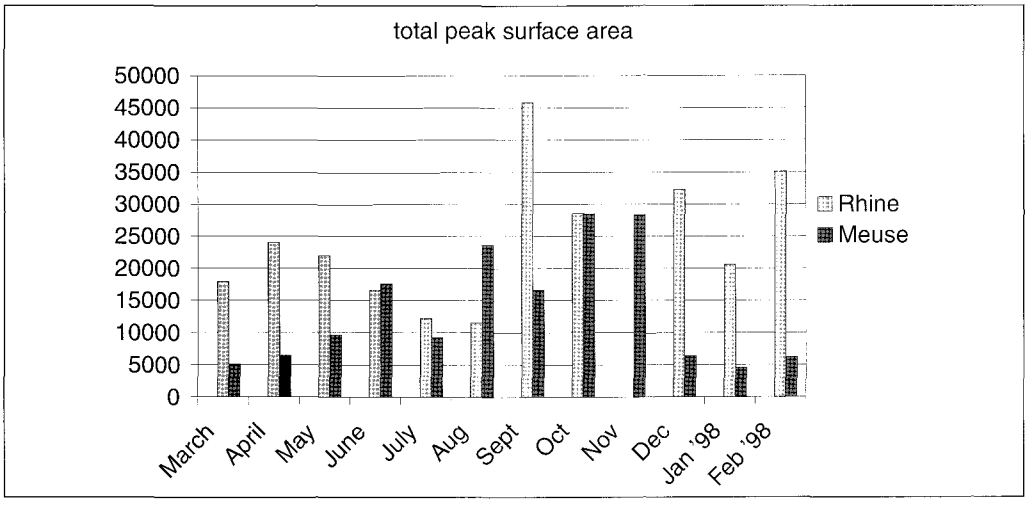

Figure 3 *Development of the overall quality of the Rhine and the Meuse, represented as summed peak sizes from the HPLC/UV fingerprints.*

In the mean, the pollution in the Rhine is more severe than that in the Meuse: summed over the 12 months, the total peak surface area for the Rhine is a factor of 1.8 higher than that of the Meuse. In the months when the pollution in the Rhine is not greater than that of the Meuse, this can be attributed to the (sporadic) occurrence of high concentrations of a small number of substances in the Meuse (June: atrazine and diuron; August: chlortoluron; October: "peak 5", which was later identified as 4,4'-dihydroxydiphenyl sulphone) (see 4.2).

What is noticeable about the fingerprints from August to December is that the humous bulge (a broad bulge at the start of the chromatograms with a maximum at a retention time of approximately 20 minutes) increases rapidly after the summer, reaches a maximum in November, and then decreases rapidly in December. This is the case for both the Rhine and the Meuse, although in the case of the Rhine, the bulge is still present in January. It is expected that this behaviour is related to the breakdown of biomass in the autumn.

4.1.2 Number of peaks

Figure 4 gives an overview of the number of pollutants that could be seen as discrete peaks in the fingerprints.

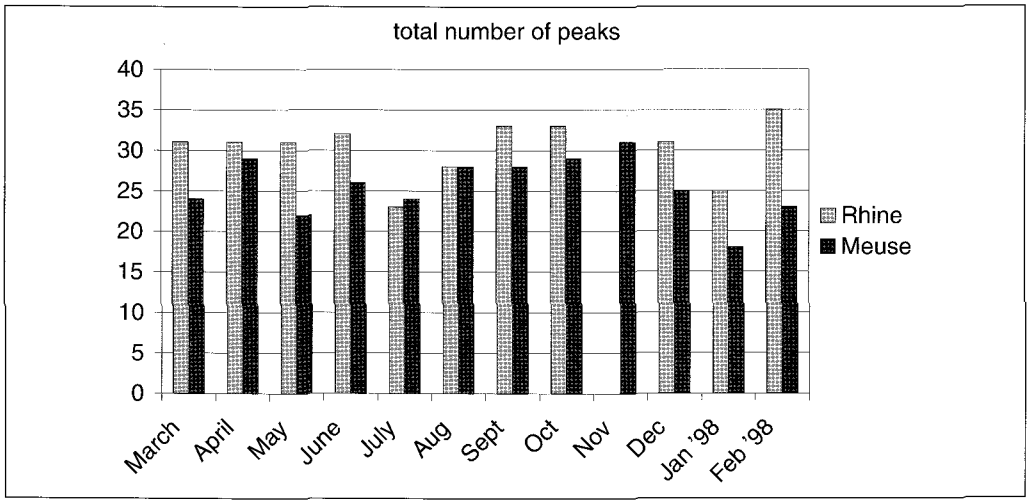

Figure 4 *Number of pollutants in the Rhine and the Meuse visible as discrete peaks in the fingerprints*

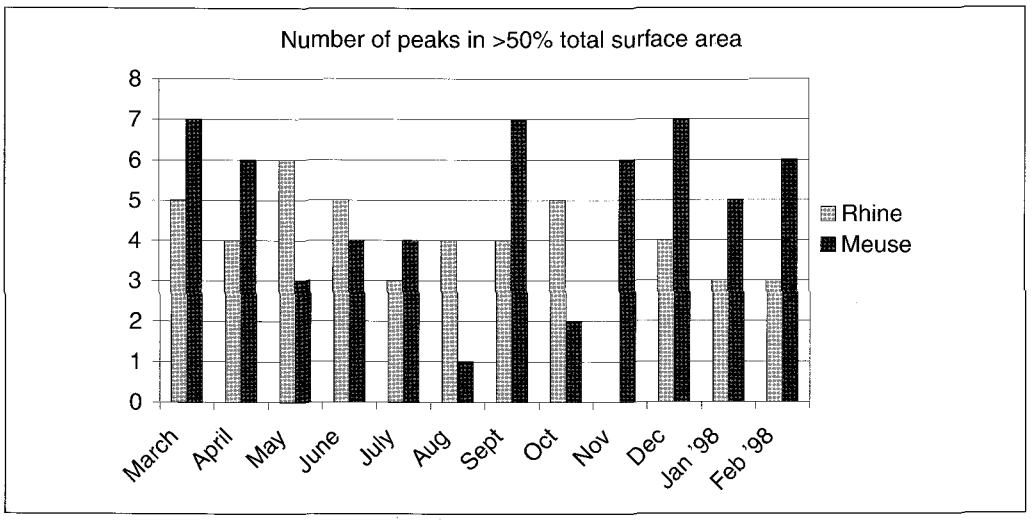

Figure 5 *Number of peaks that make up 50% of the total peak surface area of the fingerprint*

In the Rhine, the number remains fairly constant (variation within a factor of 1.3) and relates in many cases to compounds that are present for several months of the year (see 4.2.5 and 4.2.6). In the Meuse, there is a much greater variation (up to a factor of 1.8), which is caused by, inter alia, the sporadic occurrences of pollutants, often in high concentrations (see 4.2.7). This is to be seen in Figure 5, which shows the number of peaks that make up 50% of the total peak surface area in Figure 3. In the period from May to October (with September excluded), this number is four or less, which means that in the summer the pollution of the Meuse is determined by only a few substances that are present in relatively large concentrations. In the winter period, the number is approximately twice as high. In the case of the Rhine, the number of peaks that make up 50% of the total peak surface area is much more constant: over the whole year it varies between 3 and 6.

This picture is also given by Figure 6, which shows the contribution of the five largest peaks to the total peak surface area of the fingerprint. The five largest peaks (approximately 20% of the total number of peaks) make up 40% to 70% of the total peak surface area, with the Rhine showing a higher degree of constancy in this case too.

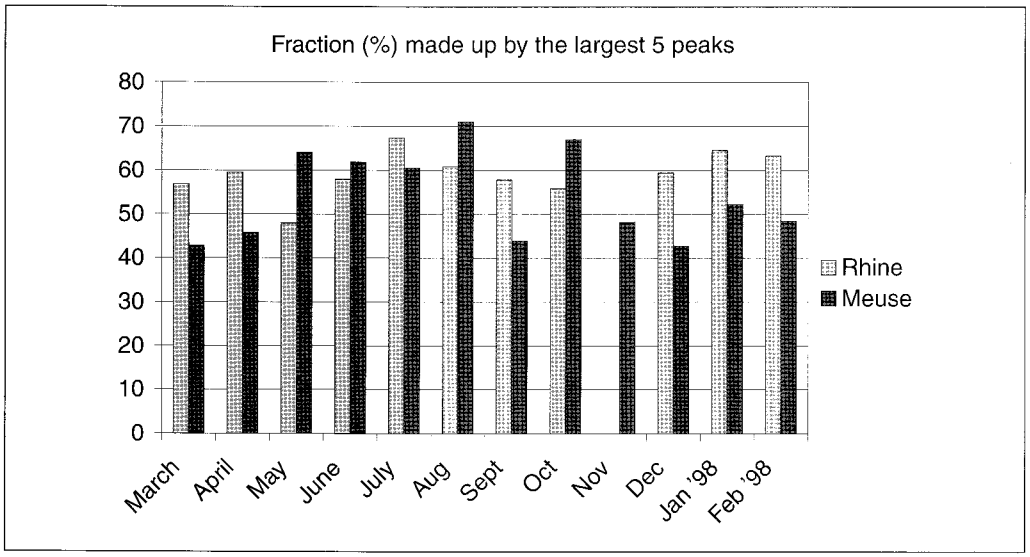

Figure 6 *Contribution of the five largest peaks of each fingerprint to the total peak surface area*

In summary:
The overall water quality of the Rhine varies within a factor of 4, the pollution in the autumn and winter being greater than that in the spring and summer. In the case of the Meuse, the overall water quality can vary within a factor of 6, with least pollution in the winter.
In the mean, the pollution in the Rhine is almost twice that in the Meuse. The quality of the Rhine fluctuates less than that of the Meuse. In the Meuse, the pollution is determined primarily by a small number of compounds that occur sporadically in relatively high concentrations.

4.2 Individual compounds

As stated in the introduction (Section 1), the HPLC/UV fingerprint can be used for monitoring individual compounds in place and time:

- comparison of different water types (for example raw water sources);
- monitoring, with respect to time, of the quality of the raw water used for drinking water;
- monitoring, with respect to time, of the quality of waste water streams and sewers;
- monitoring, with respect to place, of river water quality (monitoring of dilution, breakdown, and detection of discharges); and
- monitoring of the changes in the quality as a result of treatment processes.

The substances concerned here can be identified compounds (the retention time and UV absorption spectrum of which have been determined with the reference substance) or unidentified compounds, which are likewise characterized by retention time and UV spectrum. The HPLC peaks of these unidentified substances have been given arbitrary numbers ("peak #") in the reports. Depending on their occurrence and the behaviour, it may be decided, on the basis of fingerprints, to identify the respective compounds with LC/MS.

4.3 Pesticides

With existing determination methods for pesticides, it was possible to identify a number of peaks as pesticides on the basis of the retention times and UV absorption spectra. In the Rhine these were monuron, oxadixyl, isoproturon, atrazine, dimethachlor and possibly also anilazine and chlorobromuron. These compounds are encountered in the period from May to September, generally in low concentrations (lower than 0.1 µg/l). In the Meuse, the following are encountered: chloridazon, carbendazim, monuron, 3,4-dichlorophenylmethyl urea (metabolite of diuron), chlortoluron, isoproturon, atrazine, diuron, dimethachlor, metobromuron and possibly also desisopropyl atrazine (metabolite of atrazine). The period in which the compounds occur is considerably longer for the Meuse than for the Rhine: from March to November, and the concentrations are also substantially higher, in particular in the case of atrazine, diuron and chlortoluron (atrazine up to 0.3 µg/l, diuron up to 0.6 µg/l and chlortoluron up to 1.6 µg/l; see Section 4.2.2).
Although the HPLC/UV fingerprint is not intended primarily for the quantitative determination of individual compounds, it can be used for this purpose. The reliability is lower than that of the substance-specific, validated determination methods, but the HPLC/UV fingerprint does give an indication of the presence and concentrations of the various pollutants. Table 1 shows the above-mentioned pesticides for the Rhine and the Meuse together with their peak heights for the respective monthly samples.

A characteristic feature of the Meuse is the pollution with atrazine and diuron. Some of the pesticides given in Table 1 were also detected in an earlier study of the surface water quality, in which new analysis methods were used (Puijker and Janssen, 1998).

Table 1 *Detected pesticides and metabolites in the Rhine and the Meuse; peak heights relative to the internal standard (1 µg/l atrazine = 7,300 units) (blank cell indicates not detected)*

Pesticide	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov
Rhine									
monuron						*375*			
oxadixyl							*242*		
isoproturon		*193*	*201*						
atrazine		*69*	*201*	*731*	*428*	*382*			
dimethachlor		*166*	*200*	*258*					
Meuse									
chloridazon		*119*							
carbendazim							*315*	*665*	
monuron	*159*		*106*	*187*	*86*				
3,4-dichlorophenylmethyl urea			*250*	*442*	*140*	*102*	*187*	*267*	
chlortoluron						*11453*			
isoproturon	*386*	*304*							
atrazine	*138*	*304*	*1715*	*2438*	*1860*	*1125*	*1016*	*1351*	
diuron	*365*	*420*	*2144*	*3744*	*856*	*1259*	*1435*	*1103*	*1693*
dimethachlor	*106*	*108*	*127*	*207*	*121*				
metobromuron							*972*		

In Table 2, for atrazine, diuron and isoproturon, the peak heights are converted to concentrations and compared with the results of the specific determination methods used in the framework of the regular RIWA research (RIWA, 1998). In the HPLC/UV fingerprint of the Rhine, the indicated value for low diuron content was severely influenced by the presence of another peak. For this reason diuron was not included for the Rhine. With the exception of a single result, the table shows good to very good agreement between the methods, in particular where high contents are involved.

Table 2 *Concentrations of isoproturon, atrazine and diuron as determined with the HPLC/UV fingerprint and as measured within the framework of the regular RIWA research (blank cell indicates not detected).*

Pesticides		Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov
Rhine										
isoproturon	HPLC		0.1*	0.1*						
	RIWA	0.05		0.05						
atrazine	HPLC		0.01*	0.03*	0.10	0.06	0.05			
	RIWA	0.02	0.03	0.06	0.12	0.08	0.04		0.04	0.02
Meuse										
isoproturon	HPLC	0.2*	0.2*							
	RIWA	0.17	0.14	0.06			0.08			
atrazine	HPLC	0.02*	0.04*	0.23	0.33	0.25	0.15	0.14	0.18	
	RIWA	0.04	0.03	0.38	0.32	0.36	0.16	0.15	0.07	0.01
diuron	HPLC	0.06	0.07	0.35	0.61	0.14	0.21	0.24	0.18	0.28
	RIWA	0.06	0.12	0.44	0.74	0.21	0.21	0.22	0.21	0.17

* Accurate quantification not possible due to co-elution of atrazine and isoproturon peaks.

In this study, a metabolite of diuron, 3,4-dichlorophenylmethyl urea, was studied for an extended period for the first time. Whenever diuron is present (in the Meuse), the metabolite is also present, albeit in much lower concentrations (see Figure 7). The metabolite reaches its highest concentration in June: approximately 0.08 µg/l (diuron: approximately 0.6 µg/l). Other metabolites of phenyl urea herbicides, for which a method has recently been developed (Reimers, 1998), were not encountered in relevant concentrations.

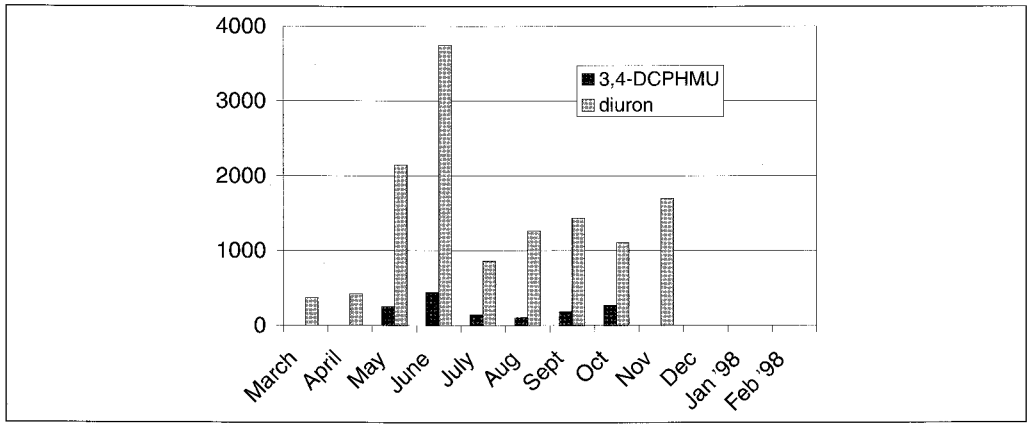

Figure 7 *Development of the peak heights of diuron and the metabolite 3,4-dichlorophenylmethyl urea in the Meuse*

4.4 HPLC/UV fingerprint as an early warning system: chlortoluron

The HPLC/UV fingerprint generates data on the overall water quality and on the presence of a broad range of individual (identified and unidentified) compounds. The method is thus highly suitable for use as an early warning system. Particularly since the fully automated method, which was applied in the laboratory in this study, can also be applied in an unmanned manner on location. This would only need a few adaptations of the instrument.
This method also has the advantage that in the case of a positive signal (for example a peak that exceeds a threshold value set beforehand), the peak might be identified by mass spectrometry (LC/MS). Then it would be possible to reach a conclusion, on the basis of literature or an expert opinion, on the significance (for example the health implications) of the pollution.

Chlortoluron incident
A very good example of the possibilities offered by the HPLC/UV fingerprint as an early warning system is the sudden occurrence of chlortoluron in the Meuse in August 1997. An extremely high peak in the August sample from the Meuse led to a more detailed investigation. Comparison with the method for phenyl urea showed that the retention time and the UV absorption spectrum agreed perfectly with those of chlortoluron. Inquiries with a small number of water companies that treat Meuse water, and with RIZA in Lelystad, did not at first yield any agreement. Finally, the RIZA measuring station in Eijsden confirmed that at the time of sampling for the RIWA study there had been an increased concentration of chlortoluron in the Meuse, which had lasted only a few days. Because, by coincidence, the RIWA samples had

been taken at precisely that time, chlortoluron was encountered in the Meuse sample with the HPLC/UV fingerprint. Comparison of the RIZA data with the estimated concentrations from the HPLC/UV fingerprint showed a very high level of agreement (see Table 3). Immediately after this was confirmed, this information was passed on to RIWA, WBB and DZH (Kiwa, 1997). If the HPLC/UV fingerprint had been used as a monitor with an analysis frequency of, for example, once every two hours, then this discharge incident would have been detected in good time.

Table 3 *Concentrations of chlortoluron (in µg/l) in the Meuse as measured by RIZA in Eijsden and by Kiwa with the HPLC/UV fingerprint*

date/time	RIZA	HPLC/UV fingerprint
11 August, 8:15	0.8	
16:00	1.6	
12 August, 8:30*	1.6 *	1.7
16:00	1.7	
13 August, 8:00	1.0	
14 August, 8:10	0.5	
16:00	0.4	

* Time corresponds to that of RIWA sampling

4.5 HPLC/UV databases: 4,4'-dihydroxydiphenyl sulphone in the Meuse

Another possibility offered by HPLC/UV fingerprinting is that of later identification of pollutants in data files of samples that were measured some time ago under similar conditions.
In September 1998, RIZA measured in the Meuse at Eijsden an increased concentration of a substance that was identified as 4,4'-dihydroxydiphenyl sulphone. In a project commissioned by the Brabantse Biesbosch Water Extraction Company (WBB), Kiwa examined the Meuse water for this compound with HPLC/MS. In the subsequent period, the compound was found to be present in the Meuse at Keizersveer in a concentration of approximately 0.1 µg/l. By analysis of a standard solution of this compound with the HPLC/UV fingerprint, the retention time and UV spectrum were established. Subsequently, the existing data files of analysed Rhine and Meuse samples from the research described in this report were searched in order to establish whether this compound had been encountered previously without it being known that it was 4,4'-dihydroxydiphenyl sulphone. Comparison of the data revealed that the substance in the September and October samples that had been referred to as "peak 5" was the sulphone in question. This "peak 5" was the most prominent pollutant in the October sample. Quantification *done subsequently in September 1998,* yielded a concentration of approximately 4 µg/l of 4,4'-dihydroxydiphenyl sulphone in the Meuse at Eijsden in October 1997 (peak height 10517), and approximately 0.1 µg/l in September and August 1997 (peak height 200 - 300).
The fingerprints of the October sample (1997) from the Meuse and a standard solution of 4,4'-dihydroxydiphenyl sulphone (4.5 µg/l) as measured in September 1998 agree well in terms of both (corrected) retention time and the (characteristic) UV absorption spectrum (see Figure 8).

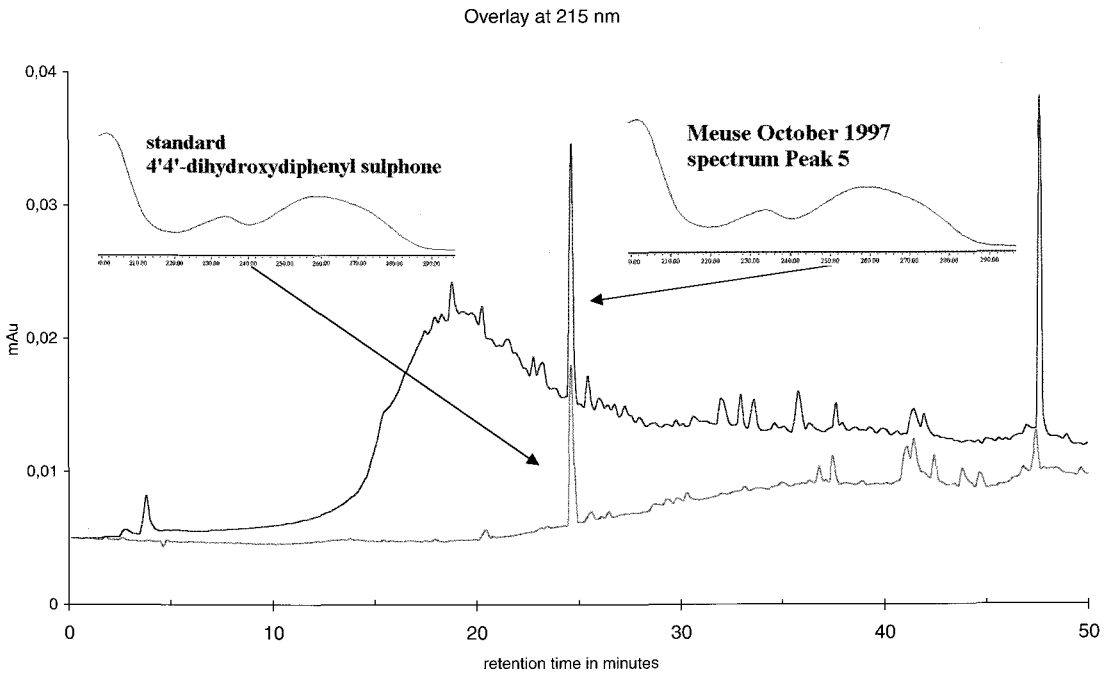

Figure 8 *HPLC/UV fingerprints of the October 1997 sample from the Meuse and of a standard solution of 4.5 µg/l 4,4'-dihydroxydiphenyl sulphone in September 1998. It has now been established that the October 1997 sample contained approximately 4 µg/l of this compound. At the time, the substance responsible for the peak had not been identified (and was referred to as unidentified pollutant "peak 5").*

4.6 Identification of unidentified peaks with HPLC/MS: diethylphthalate and dibutylphthalate in the Rhine

In the Rhine sample from September, a large peak that had not been encountered in earlier samples was measured at a retention time of approximately 32 minutes. Application of the methods that at the time were being developed for identification of unknown compounds with LC/MS (Bobeldijk, 1998) revealed that the peak was diethylphthalate. In the same sample, another peak at a retention time of approximately 47 minutes was identified as dibutylphthalate. The mass spectra are given in Appendix 9.
Although both compounds can also be measured with GC/MS, the result also indicates that the procedure followed, which comprises screening with HPLC/UV fingerprinting with subsequent LC/MS identification of important peaks, can be successful for monitoring of the water quality. This applies in this case all the more since the two phthalates are xenoestrogenic compounds (Denneman, 1997). Dibutylphthalate is a frequently occurring pollutant; diethylphthalate is encountered much less commonly.

In the framework of this project, no additional research for identification with LC/MS was done for any other marked peaks.

4.7 Behaviour and occurrence of individual compounds

In total, 38 characteristic peaks were monitored for the Rhine and 46 for the Meuse. Of the 38 peaks for the Rhine, six occur in all 11 samples, eight in at least eight samples, and 11 in at least six samples. 13 peaks occur in only one sample. Of the 46 peaks for the Meuse, none occurs in all 12 samples, five (including the pesticides atrazine and diron) occur in at least eight samples, and 12 in at least six samples. 13 peaks occur in one sample only. Overall, this means that only a quarter of the total number of compounds is present for at least half the year. Fewer than 10% occur all the year round, and about one third occurs very sporadically. In general it can be said that the situation in the Rhine is somewhat more constant than that in the Meuse but the type of pollution varies greatly in both rivers. Of the 38 (Rhine) and 46 (Meuse) selected characteristic peaks, 17, including the pesticides monuron, isoproturon, atrazine and dimethachlor mentioned previously, occur in both the Rhine and the Meuse. Over half the peaks encountered in both the Rhine and the Meuse occur frequently in both rivers.

There follows a description of the occurrence of a few very characteristic peaks (high concentrations or frequent occurrence). The relationship between the Rhine and the Meuse is also established. A number of these compounds will be discussed again in another study, related to the present research, which was done at six water companies in the field of treatment and/or pretreatment (Noij and Emke, 1998).

4.7.1 Compounds in both the Rhine and the Meuse

Of the 17 compounds from the selection of characteristic peaks that occur in both the Rhine and the Meuse, four are pesticides, namely monuron, isoproturon, atrazine and dimethachlor (see section 4.2.1). Of the remaining 13 compounds (see Table 3), "peak 2", "peak 7" and "peak 10" are very clearly present in the Rhine: they occur in all the samples as high peaks of mean height 2512, 5023 and 1441 respectively. In the Meuse, they occur in respectively five, 10 and seven of the 12 samples, in considerably lower concentrations than in the Rhine (mean heights respectively 300, 787 and 348); it should be noted that "peak 7" was present in a higher concentration in the November Meuse sample (height 3151). With the exception of the pesticide atrazine, discussed above, none of the common peaks is present to a high degree in the Meuse.

"Peak 6", "peak 15", "peak 17" and "peak 28" also occur frequently in the Rhine and the Meuse (in 40 to 75% of the samples), in some cases in relatively low concentrations (mean height between 200 and 800). It should be noted that "peak 6" and "peak 15" did occur in higher concentrations in the September sample from the Rhine (2055 and 2803 respectively).

The remaining 6 peaks occurring in both the Rhine and the Meuse occur only infrequently (in 10 to 40% of the samples). The individual concentrations are, however, in some cases quite high "peak 4" in the Rhine in May: 2108, "peak 16" in the Meuse in September and October: 2281 and 1808 respectively).

Table 3 *Frequency of occurrence and mean peak height in positive samples for compounds that were encountered in both the Rhine and the Meuse*

peak	n (Rhine; N=11)	mean peak height Rhine	n (Meuse; N=12)	mean peak height Meuse
a. prominent in the Rhine				
2	11	2512	4	300
7	11	5023	10	787 *
10	11	1441	7	348
b. peaks occurring frequently in the Rhine and Meuse				
6	9	774 *	5	487
15	8	686 *	7	339
17	6	171	6	286
28	7	552	9	370
c. peaks occurring occasionally in the Rhine and Meuse				
59	1	209	1	431
18	7	974 *	2	164
3	2	389	5	360
4	2	1218 *	4	802
44	2	742	4	635
16	1	88	4	1239 *

* increased concentration encountered occasionally

Table 4 *Frequency of occurrence and mean peak height in positive samples for compounds that were encountered in the Rhine only*

peak	n (Rhine; N=11)	mean peak height Rhine
peaks occurring frequently		
19	11	1505* #
43	11	2094* #
25	11	1042 *
large peaks occurring occasionally		
46	1	1730
60 + 61 ##	1	4719
22	5	1913 *
50	2	965 *

* increased concentration encountered occasionally
# The chromatography did not always give good separation of "peak 19" and "peak 43"; accurate quantification was thus not always possible. The means given here relate to samples for which sufficiently reliable quantification was possible for both peaks (August 1997 - February 1998); comparable peak sizes apply for the other months
"peak 60" and "peak 61" are scarcely separated by chromatography and are given here as one peak.

4.7.2 Compounds encountered only in the Rhine

Of the 18 remaining peaks in the Rhine (peaks not discussed in 4.2.1 (five pesticides), 4.2.4 (diethylphthalate and dibutylphthalate) or 4.2.6 (13 compounds also occurring in the Meuse)), three occur in all samples ("peak 19", "peak 43" and "peak 25"), often in high concentrations (see Table 4). The other 15 selected peaks for the Rhine occur fairly infrequently. It is unclear whether these substances are present every year with a low frequency or whether there is continuously a large number organic pollutants present, each occurring occasionally. Of the pollutants that occur occasionally, four are present in relatively high concentrations (peak heights between 1600 and 5000). These are pollutants that are encountered primarily in the winter period (October - February). The other 11 peaks appear to be less relevant in terms of both peak height and frequency, and are not included in table 4.

4.7.3 Compounds encountered only in the Meuse

Of the 21 remaining peaks in the Meuse (not discussed in 4.2.1 (10 pesticides), 4.2.2 (chlortoluron incident), 4.2.3 (4,4'-dihydroxydiphenyl sulphone) or 4.2.6 (13 compounds also found in the Rhine)), four occur in at least half of the samples ("peak 1", "peak 9", "peak 11" and "peak 12"). Of these, only "peak 1" occurs occasionally in high concentrations, while the other 3 occur only in low concentrations (see Table 5). The other 17 selected peaks for the Meuse occur fairly infrequently. Of these, 6 are substances that are present in relatively high concentrations (peak heights between 1400 and 2600). These are pollutants that were detected only once, in each case in the autumn (October - November). In view of the fact that these substances occur only once, the question presents itself (here more so than in the case of the Rhine) as to whether these substances are present, with a low frequency, every year or whether there is continuously a number of organic pollutants present, each occurring occasionally in a high concentration. The other 11 peaks appear to be less relevant in terms of both peak height and frequency.

Table 5 *Frequency of occurrence and mean peak height in positive samples for substances that were encountered only in the Meuse*

peak	n (Meuse; N=12)	mean peak height Meuse
peaks occurring frequently		
1	8	1498 *
9	6	329
11	6	309
12	8	221
large peaks occurring occasionally		
56	2	1750 *
57	1	1872
36	1	2374
31	1	1470
37	1	2567
49	1	1517

* increased concentration encountered occasionally

4.7.4 Notable monthly samples with reference to individual peaks

Although the study was not intended to investigate seasonal effects, it is notable that the substances that are present only occasionally, but in high concentrations, are detected primarily in the autumn and winter in the Rhine and the Meuse. With the exception of the pesticides atrazine and diuron, which are present primarily in the summer period, and the chlortoluron discharge in August, it was primarily the November sample from the Meuse that contained some compounds in relatively high concentrations. This applies to a lesser degree for the October and September samples from the Meuse. In the Rhine too, the compounds that occur occasionally are present in higher concentrations primarily in the winter (September - February), although this is less evident due to the presence of high concentrations of substances that are present virtually the whole year round.

5 MEANING FOR THE WATER QUALITY

The HPLC/UV fingerprint gives an indication of the presence of organic micropollutants in the form of a chromatogram. The micropollutants are characterized by their retention times and UV spectra. Determination of the fingerprints under fixed conditions yields data that can be used in the assessment and monitoring of water quality. Various practical examples, such as occurred during the execution of this project and the elaboration of the results, are given in Section 4.

In summary, the HPLC/UV fingerprint can be used for:

Monitoring of substances in time and place
With the HPLC/UV fingerprint, it is possible to monitor identified and unidentified substances. In this way, the dynamics of the water quality can be established (variations in type and quantity of the pollutants as a function of time). The changes can also be established as a function of place, which is useful in particular in the localization of point discharges and diffuse discharges. On the basis of the results, the most characteristic substances can, if required, be identified with HPLC/MS (see below).

Comparison of raw water sources
The HPLC/UV fingerprint can be used for comparing various sources of raw water for drinking water production. It is possible to assess the quality of the raw materials on the basis of the presence of peaks in the fingerprint, which is an indicator of the degree of pollution.

Assessment of treatment
Application of the HPLC/UV fingerprint in treatment processes makes it possible to assess the effects of processes and process steps on the presence of organic micropollutants. Combinations of processes can be assessed without it being necessary to use very extensive and expensive water quality measurements. If required, the HPLC/UV fingerprint can be used as a monitor for continuous monitoring of the process.

Early warning system
Application of HPLC/UV at the intake of raw water permits adequate monitoring for a broad range of organic micropollutants. Monitoring can be done for specific, identified compounds (for example pesticides), which are detectable from a concentration of approximately 0.1 µg/l. Monitoring for unidentified substances is also possible, however, with action being taken if a given peak height limit is exceeded.

Consultation of old data
Analysis results of the HPLC/UV fingerprint can always be consulted as an aid to establishing whether, where and when a certain pollutant was encountered in samples. Data collected in the past can be searched for pollutants that have since become important or been identified. It would be wise to include identified and unidentified substances with their retention times and UV spectra in a library that is preferably accessible to several users.

Selection of unidentified substances for identification

Organic micropollutants that, on the basis of the Ames mutagenicity test, are relevant from a toxicological point of view, have a polarity that corresponds to the area covered by the HPLC/UV fingerprint. It is therefore important that unidentified substances in this area in particular are identified. Identification with GC/MS is often not possible, since the compounds concerned are often too polar to be measured with GC/MS. Identification of unidentified substances by HPLC/MS is a much more expensive method than identification with GC/MS, because, per compound, much more time is required for structure elucidation. The HPLC/UV fingerprint can be used as a selection criterion: identification with HPLC/MS is appropriate above all for compounds that occur frequently and in high frequencies, and either are not removed by treatment, or are introduced by treatment.

6 CONCLUSIONS

- The HPLC/UV fingerprint is a good method for monitoring individual, identified and unidentified, organic micropollutants and for obtaining an overall picture of the water quality;
- The HPLC/UV peaks indicate that the pollution of the Rhine with organic micropollutants is somewhat more severe than that of the Meuse;
- The pollution of the Rhine is of a more constant nature than that of the Meuse;
- The pollution of the Meuse is characterized primarily by occasional, short-term pollution with substances which then occur in relatively high concentrations;
- Of the pesticides measured, it is still atrazine and diuron that are present for the longest periods in concentrations up to approximately 0.4 and 0.6 µg/l; dimethachlor is also frequently present (concentrations not quantified); carbendazim and monuron were encountered several times);
- If diuron is present, the metabolite 3,4-dichlorophenylmethyl urea is almost always present too, although in much lower concentrations (up to approximately 0.08 µg/l)
- In the Meuse, short-term, severe pollution with, inter alia, the pesticide chlortoluron (August 1997, up to 1.7 µg/l) and 4,4'-dihydroxydiphenyl sulphone (October 1997, 4 µg/l) was detected; chlortoluron was already included in the HPLC/UV library and 4,4'-dihydroxydiphenyl sulphone was added in September 1998;
- In the Rhine, increased concentrations of diethylphthalate and dibutylphthalate were measured in September; these compounds were identified with HPLC/MS;
- Of the selected 67 different pollutants in the Rhine and the Meuse, 17 occur in both rivers; these include four pesticides; three of the other pollutants occurring in both rivers are present to a prominent degree in the Rhine ("peak 2", "peak 7" and "peak 10"), both in terms of frequency of occurrence and concentration; four of the pollutants occurring in both rivers occur frequently in relatively low concentrations in the Rhine and the Meuse (of these, "peak 6" and "peak 15" are occasionally present in higher concentrations in the Rhine); the other 6 pollutants occurring in both rivers occur only occasionally, "peak 4" and "peak 18" occurring in relatively high concentrations in the Rhine and "peak 16" occurring in a relatively high concentration in the Meuse.
- Of the compounds that occur only in the Rhine (and have not already been mentioned above), there are three that occur frequently and in relatively high concentrations ("peak 19", "peak 43" and "peak 25"); four compounds occur occasionally and in relatively high concentrations. The remaining 11 appear to be less relevant in terms of both frequency and concentration;
- Of the compounds that occur only in the Meuse, there are four that are detected frequently but generally in low concentrations (with the exception of "peak 1", which occurs occasionally in high concentrations); six compounds occur occasionally and in relatively high concentrations. The remaining 11 appear to be less relevant in terms of both frequency and concentration;
- The occasional high concentrations of pollutants occur primarily in the winter period.

Recommendations

- In view of the occurrence of 4,4'-dihydroxydiphenyl sulphone (on which no toxicity information could be found) in the Meuse, diethylphthalate and dibutylphthalate (xenoestrogenic substances) in the Rhine and carbendazim, monuron, dimethachlor and 3,4-dichlorophenylmethyl urea (pesticides and

metabolites) in the Rhine and the Meuse, it is recommended that these substances be included in RIWA's regular measuring programme for 1999;

- In particular the following points (in order of urgency) merit further study for identification of unknown substances;
  - peaks frequently present in the Rhine and the Meuse, often in high concentrations: "peak 2", "peak 7", "peak 10", "peak 6" and "peak 15";
  - peaks frequently present in the Rhine, often in high concentrations: "peak 19", "peak 43" and "peak 25";
  - peaks frequently present in the Meuse, often in high concentrations: "peak 1";
  - peaks occasionally present in the Rhine and/or the Meuse, sometimes in high concentrations: "peak 18", "peak 4", "peak 16", "peak 46", "peaks 60+61", "peak 22", "peak 50", "peak 56", "peak 57", "peak 36", "peak 31", "peak 37" and "peak 49";
  - peaks frequently present in (apparently) low concentrations in the Meuse: "peak 9", "peak 11", "peak 12"
- It is recommended that it be established whether the cases of pollution occurring sporadically were events unique to 1997-1998 or annually recurring phenomena; it should also be established whether the low sampling frequency in 1997-1998 could have resulted in occasional cases of pollution (possibly with other substances) being missed in the periods between sampling.

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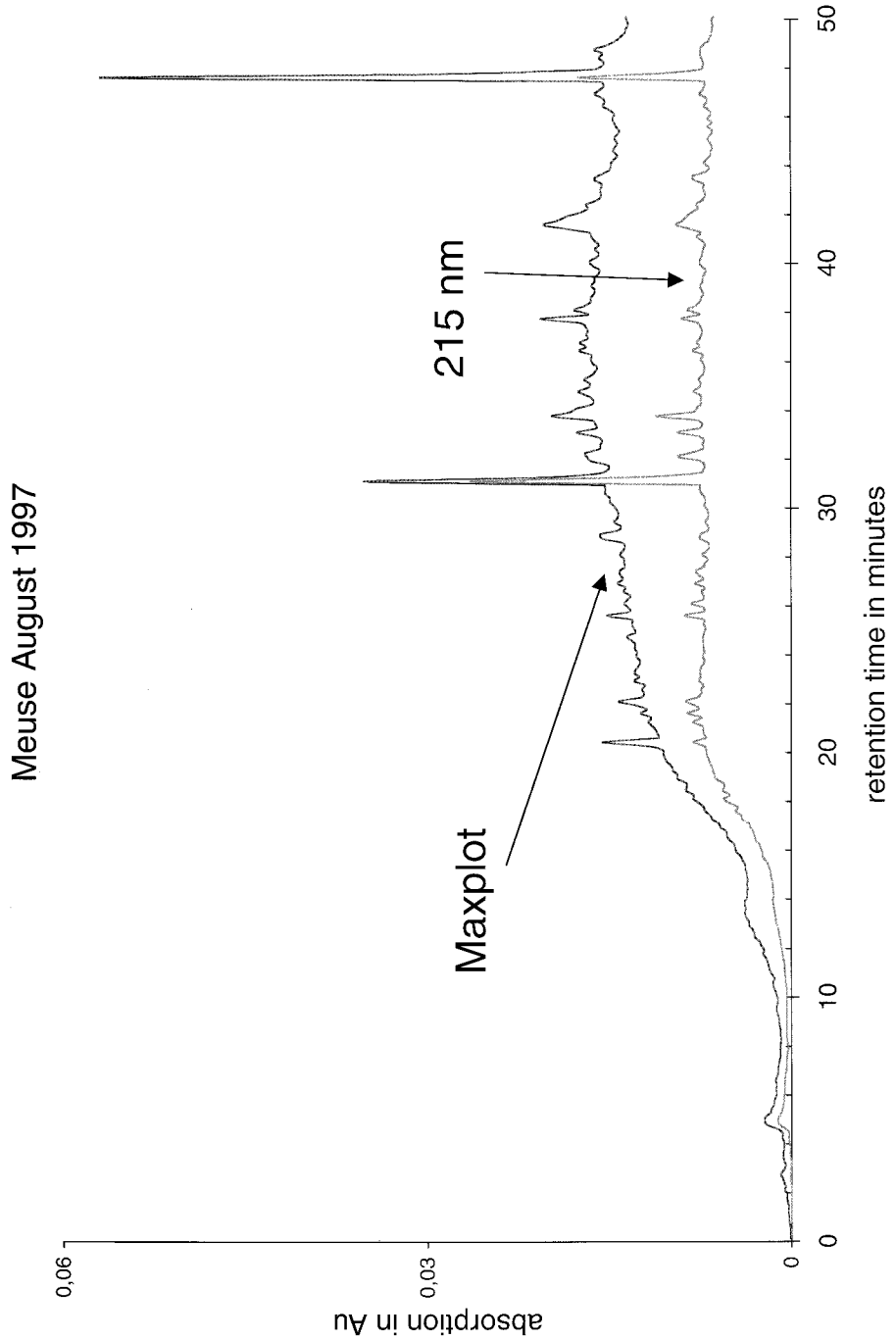
APPENDIX 1

Sampling data Rhine (Lobith) and Meuse (Eijsden)

Sample name	date Rhine	Date Meuse
March 1997	26-03	25-03
April 1997	22-04	22-04
May 1997	20-05	20-05
June 1997	18-06	17-06
July 1997	16-07	15-07
August 1997	13-08	12-08
September 1997	10-09	09-09
October 1997	08-10	07-10
November 1997	05-11	04-11
December 1997	03-12	02-12
January 1998	30-12-1997	29-12-1997
February 1998	28-01-1998	27-01-1998

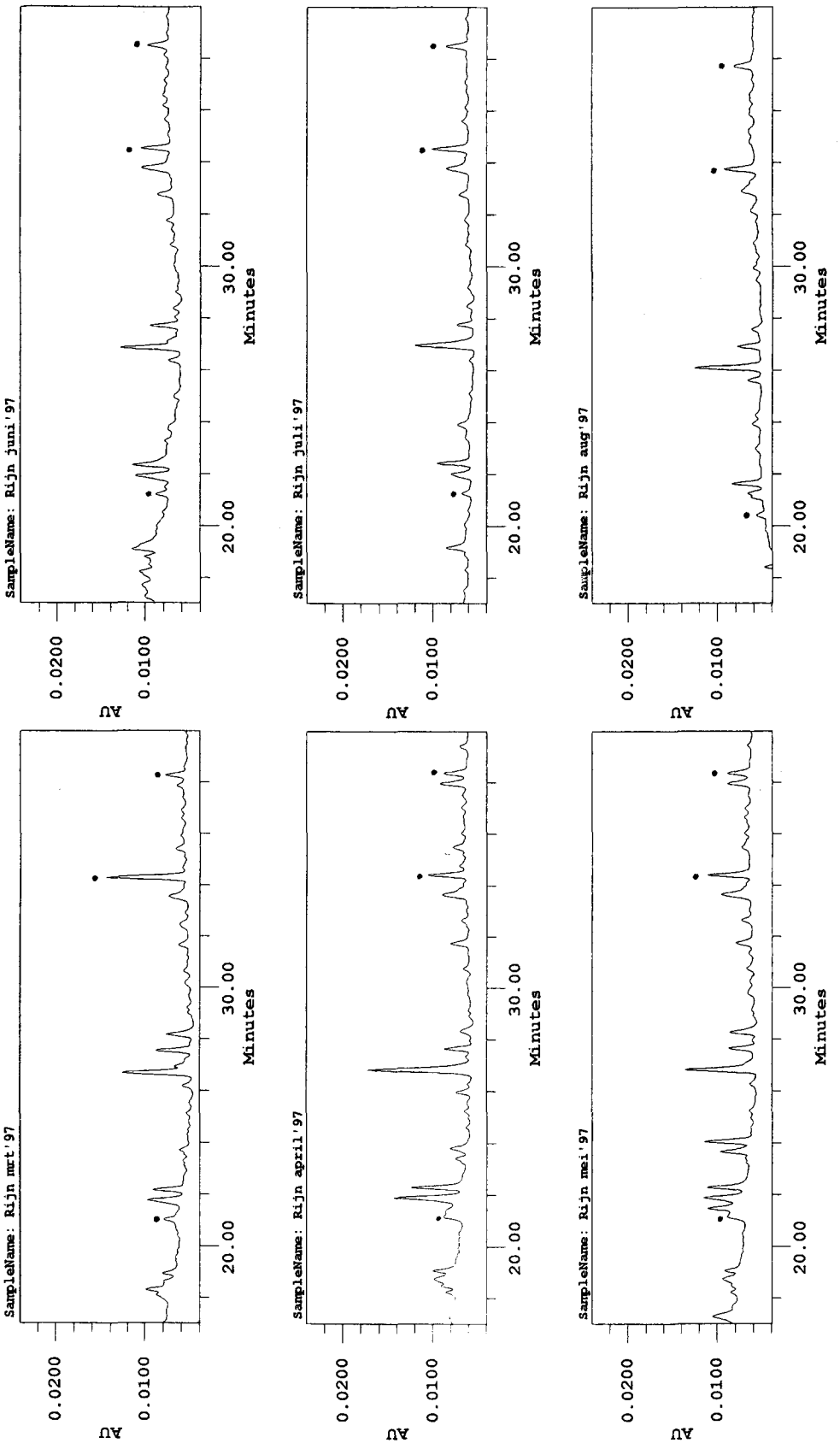
APPENDIX 2

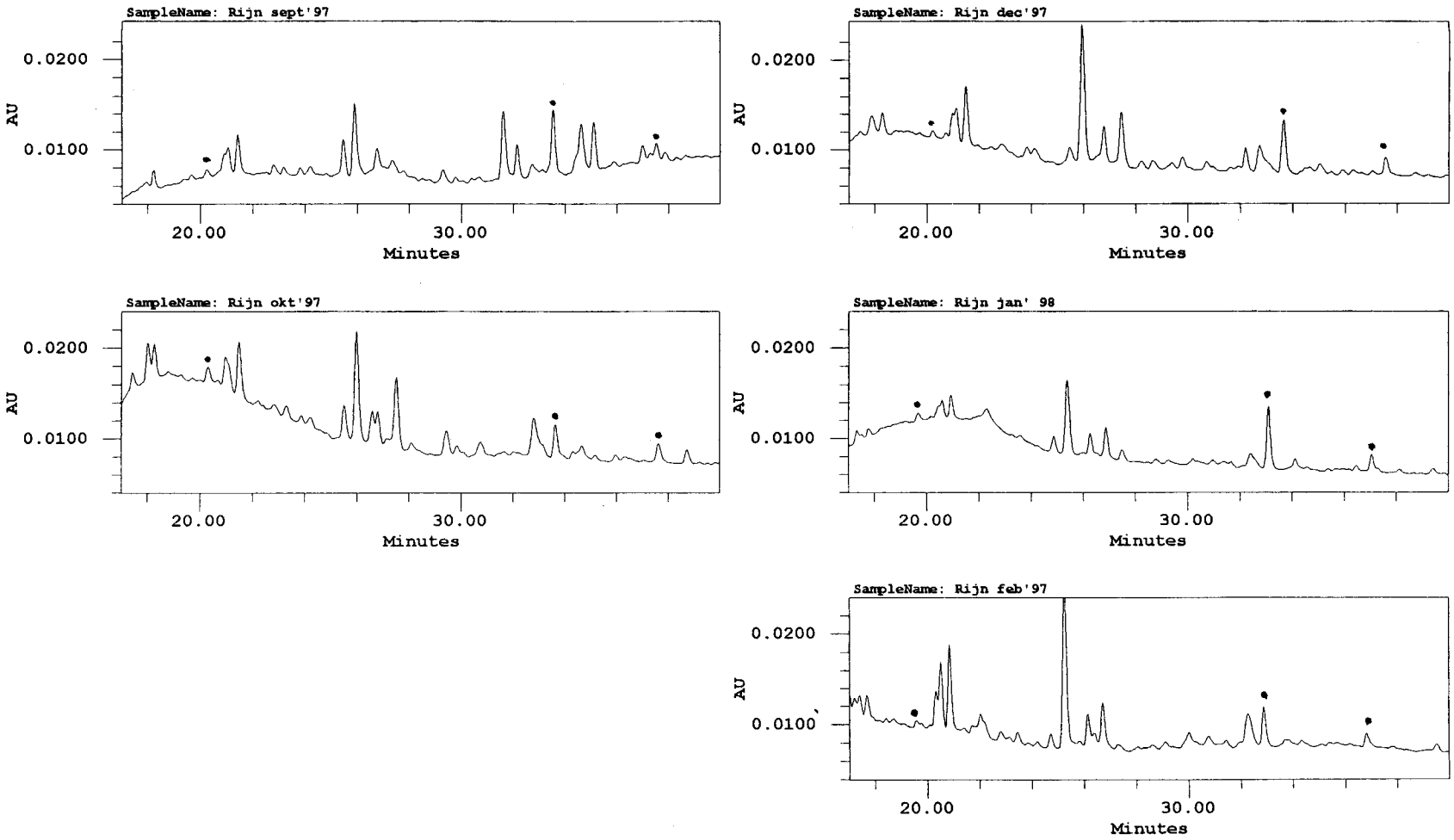
Comparison of the HPLC/UV fingerprint at an absorption wavelength of 215 nm and at the wavelength with maximum absorption.



APPENDIX 3

HPLC/UV fingerprint at 215 nm of the monthly Rhine samples at Lobith

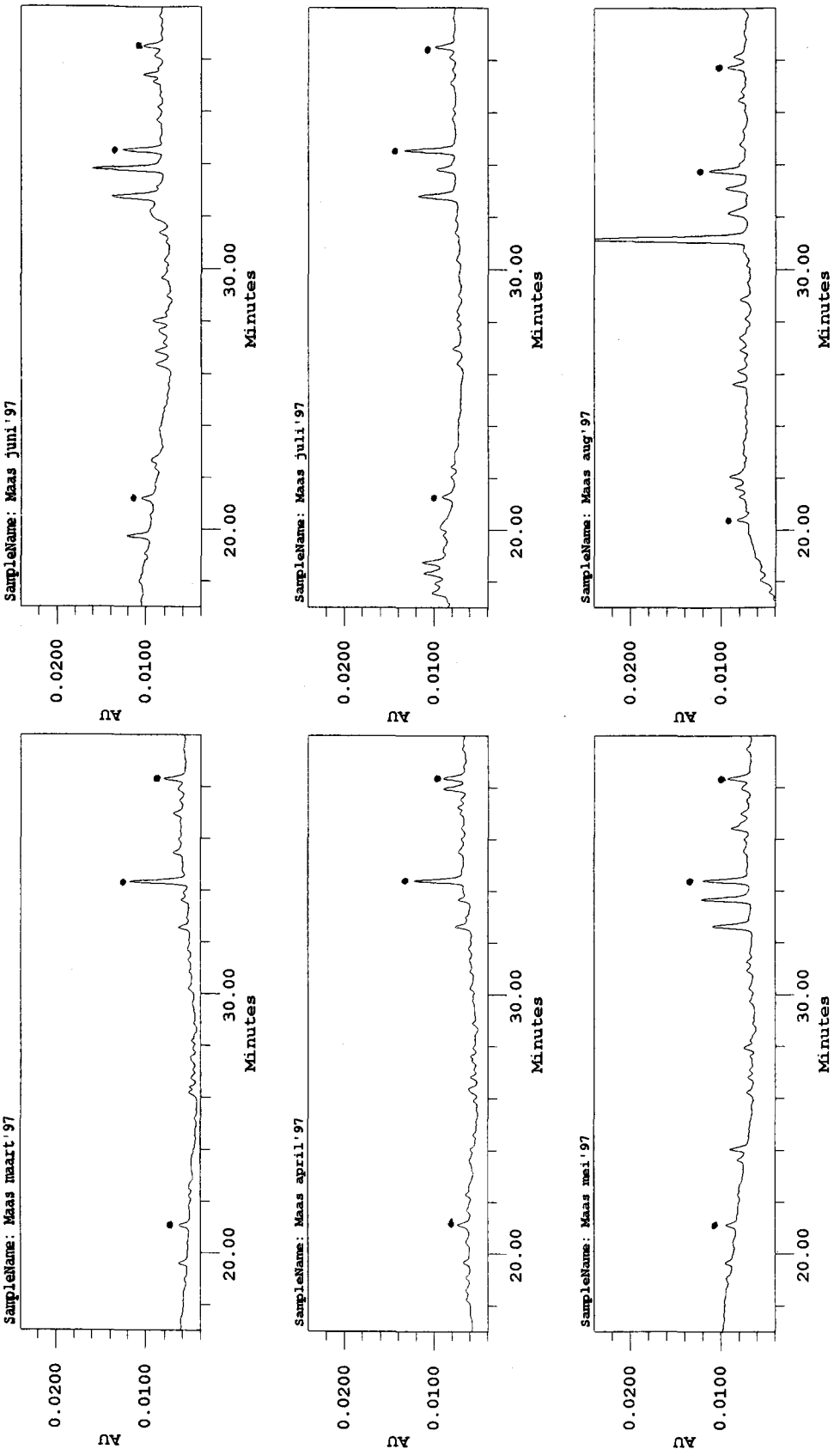


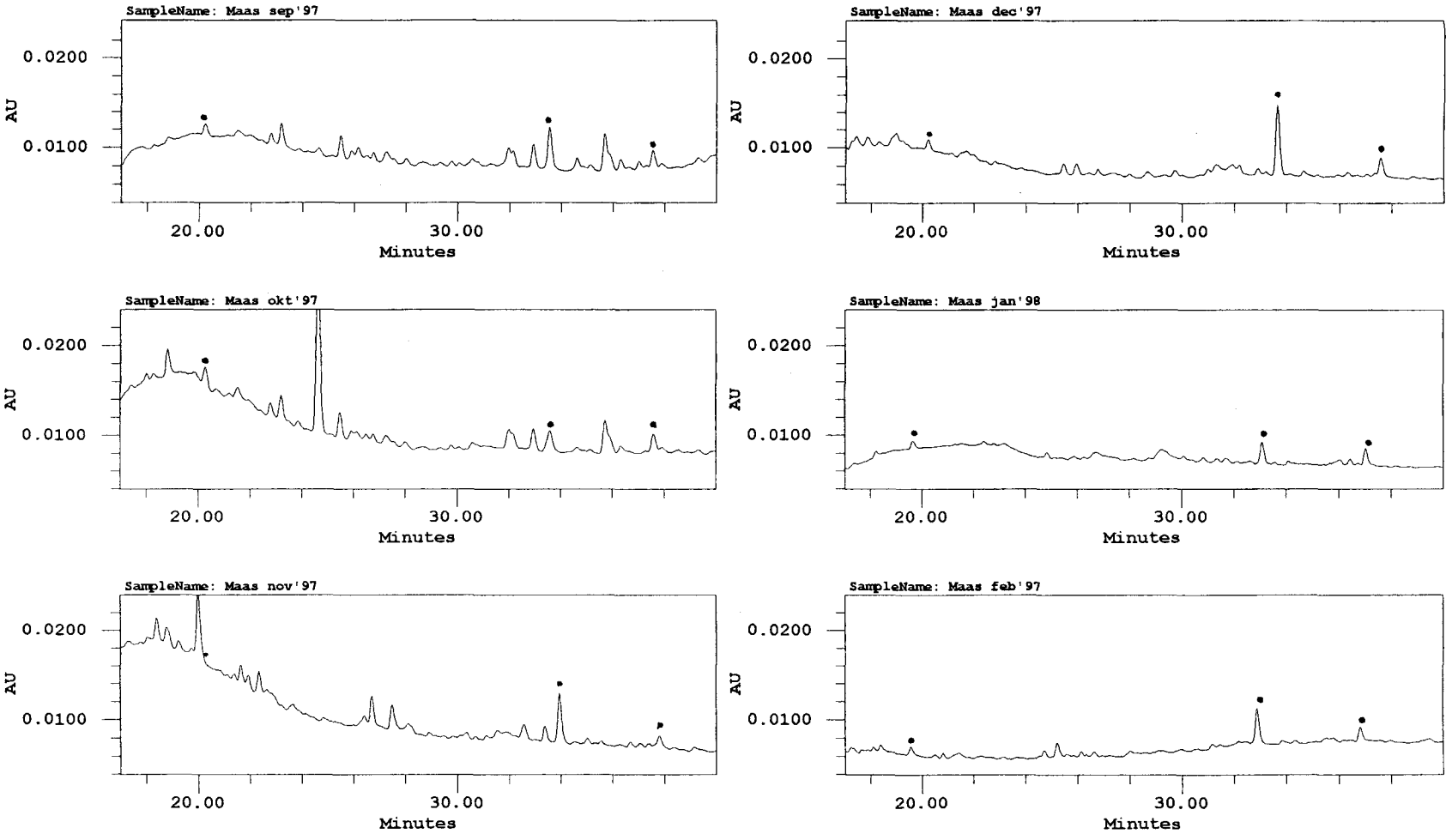


The peaks marked with* are the two internal standard components or disturbance peaks from the procedure and are disregarded in the processing of the results.

APPENDIX 4

HPLC/UV fingerprint at 215 nm of the monthly Meuse samples at Eijsden





The peaks marked with* are the two internal standard components or disturbance peaks from the procedure and are disregarded in the processing of the results.

APPENDIX 5

Summed peak surface areas of the discrete peaks from the HPLC/UV fingerprints for the Rhine

Sample	total peak surface area
March 1997	17862
April 1997	23968
May 1997	21989
June 1997	16547
July 1997	12182
August 1997	11511
September 1997	45844
October 1997	28556
November 1997	-
December 1997	32301
January 1998	20540
February 1998	35075

APPENDIX 6

Summed peak surface areas of the discrete peaks from the HPLC/UV fingerprints for the Meuse

Sample	total peak surface area
March 1997	5014
April 1997	6370
May 1997	9605
June 1997	17625
July 1997	9226
August 1997	23653
September 1997	16623
October 1997	28471
November 1997	28364
December 1997	6390
January 1998	4535
February 1998	6165

Corrected retention time	component	Rhine March	April	May	June	July	Aug	Sept	Oct	Dec	Jan '98	Feb '98	Frequency
18,79	peak 59	0	0	0	0	0	209	0	0	0	0	0	1
19,06	peak 18	603	977	632	1162	890	690	0	1863	0	0	0	7
19,21	peak 42	0	0	0	0	0	0	0	0	1189	0	0	1
19,24	peak 46	0	0	0	0	0	0	0	0	0	0	1730	1
21,12	fenuron	~~584~~	~~624~~	~~788~~	~~581~~	~~400~~	~~464~~	~~376~~	~~1003~~	~~386~~	~~473~~	~~397~~	11
21,79	peak 19	1709	2721	1871	1646	916	644	1025	1916	1676	715	3056	11
21,89	peak 43	+	+	+	+	+	822	1420	1636	2059	1089	5539	6
22,25	peak 2	1497	2113	1765	1736	1562	1616	2205	3027	3588	1407	7116	11
23,39	peak 20	189	475	0	0	0	0	0	0	0	588	0	3
23,64	peak 3	0	0	0	238	0	0	539	0	0	0	0	2
24,02	peak 4	0	0	2108	0	0	0	328	0	0	0	0	2
25,22	peak 21	197	226	0	0	0	0	0	0	0	0	0	2
25,94	peak 51	0	638	0	0	0	0	0	0	0	0	0	1
26,24	peak 6	487	169	253	548	154	723	2055	1667	0	0	0	8
26,68	peak 7	3568	4510	3093	2897	2718	3659	4207	6544	8185	1879	13991	11
26,80	peak 60 + 61	0	0	0	0	0	0	0	0	0	4719	0	1
27,56	peak 10	1715	1239	1083	1342	720	1247	1156	1352	2009	1324	2668	11
28,23	peak 22	1328	0	1152	356	0	0	0	0	3202	0	3526	5
28,26	monuron	0	0	0	0	0	375	0	0	0	0	0	1
30,17	peak 50	0	0	0	0	0	0	0	1656	0	274	0	2
30,61	oxadixyl	0	0	0	0	0	0	242	0	0	0	0	1
30,69	peak 23	235	252	263	260	152	0	0	0	0	0	0	5
31,71	peak 24	481	763	714	0	0	0	0	0	0	0	0	3
32,45	diethylphthalate	0	0	0	0	0	0	3938	0	0	0	0	1
32,61	isoproturon	0	193	201	0	0	0	0	0	0	0	0	2
32,61	atrazine	0	69	201	731	428	382	0	0	0	0	0	5
33,00	peak 44	0	0	0	0	0	0	0	0	1293	191	0	2
33,52	peak 25	908	1079	1136	1281	954	619	436	2011	1177	822	2445	11
34,29	peak 14	~~4161~~	~~1609~~	~~1846~~	~~1367~~	~~1633~~	~~1715~~	~~3636~~	~~1805~~	~~3249~~	~~3995~~	~~3228~~	11
34,75	peak 26	133	193	0	0	0	0	0	0	0	0	0	2
35,40	peak 15	454	498	199	0	237	140	2803	673	0	484	0	8
35,83	peak 45	0	0	0	0	0	0	0	0	443	0	0	1
36,00	dimethachloor	0	166	200	258	0	0	0	0	0	0	0	3
36,44	peak 16	0	0	88	0	0	0	0	0	0	0	0	1
36,68	peak 27	154	0	0	0	0	0	0	266	0	0	0	2
36,98	peak 17	192	183	193	149	104	0	0	204	0	0	0	6
37,76	peak 28	0	1164	982	199	0	101	991	102	0	327	0	7

Corrected retention time	component	Rhine March	April	May	June	July	Aug	Sept	Oct	Dec	Jan '98	Feb '98	Frequency
38,11	anilazin	0	0	0	0	0	0	478	0	0	0	0	1
38,35	chloroxuron	~~1000~~	~~1000~~	~~1000~~	~~1000~~	~~1000~~	~~1000~~	~~1000~~	~~1000~~	~~1000~~	~~1000~~	~~1000~~	11
38,70	chlorobromuron	0	0	0	0	0	0	410	0	0	0	0	1
47,15	dibutylphthalate	0	0	0	0	0	0	1267	0	0	0	0	1

+ Peak 19 coelutes with peak 43; both peaks present from August 1997 enough separated

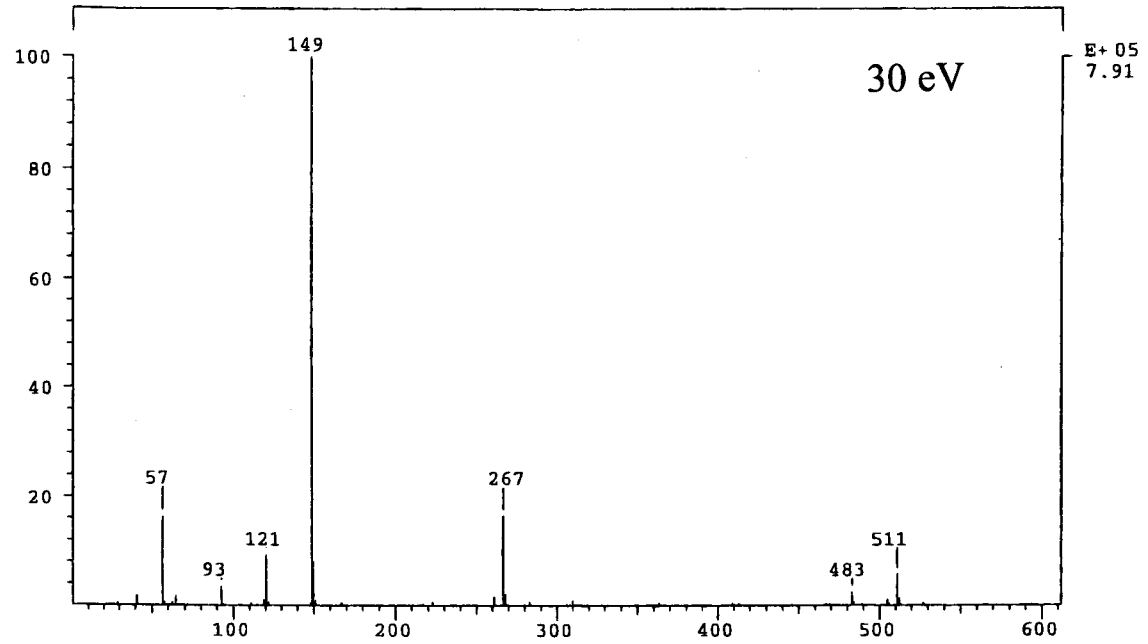
Corrected retention time	component	Meuse March	April	May	June	July	Aug	Sept	Oct	Nov	Dec	Jan '98	Feb '98	frequentie
16,44	peak 52	0	0	0	0	165	0	0	0	0	0	0	0	1
16,81	peak 53	0	0	0	0	200	0	0	0	0	0	0	0	1
17,41	peak 54	0	0	0	0	692	0	0	0	0	0	0	0	1
18,18	peak 56	0	0	0	0	938	0	0	0	2561	0	0	0	2
18,32	peak 55	0	0	0	0	0	253	0	0	0	0	0	0	1
18,37	peak 57	0	0	0	0	0	0	0	0	1872	0	0	0	1
18,42	desisopropylatrazine	0	0	0	0	980	0	0	0	0	0	0	295	2
18,77	peak 58	0	0	0	0	0	0	0	0	0	479	135	347	3
18,79	peak 59	0	0	0	0	0	431	0	0	0	0	0	0	1
18,84	peak 47	0	0	0	0	0	0	0	0	1042	0	0	0	1
19,06	peak 18	0	0	0	0	133	0	0	0	0	195	0	0	2
19,63	peak 1	444	277	308	1193	151	0	0	1735	7557	0	321	0	8
21,12	fenuron	~~502~~	~~496~~	~~456~~	~~628~~	~~511~~	~~717~~	~~731~~	~~1327~~	~~778~~	~~545~~	~~437~~	~~530~~	12
21,69	chloradizon	0	119	0	0	0	0	0	0	0	0	0	0	1
22,12	peak 36	0	0	0	0	0	0	0	0	2374	0	0	0	1
22,28	peak 2	121	0	0	233	253	592	0	0	0	0	0	349	5
22,36	carbendazim	0	0	0	0	0	0	315	665	0	0	0	0	2
23,64	peak 3	0	94	320	0	0	155	725	504	0	0	0	0	5
24,04	peak 4	0	150	758	0	0	0	1484	816	0	0	0	0	4
25,45	4,4'-dihydroxydiphenylsulfone	0	0	0	0	0	200	310	10517	0	0	0	0	3
26,24	peak 6	356	0	240	746	185	908	0	0	0	484	0	0	6
26,31	peak 31	0	0	0	0	0	0	0	1470	0	0	0	0	1
26,68	peak 7	213	291	226	774	0	541	494	547	3151	572	0	1061	10
26,96	peak 9	104	119	141	328	0	0	654	626	0	0	0	0	6
27,50	peak 37	0	0	0	0	0	0	0	0	2567	0	0	0	1
27,56	peak 10	218	257	0	550	131	442	447	394	0	0	0	0	7
27,90	peak 11	105	124	423	847	171	181	0	0	0	0	0	0	6
28,20	monuron	159	0	106	187	86	0	0	0	0	0	0	0	4
28,83	peak 12	88	195	119	246	124	0	308	328	360	0	0	0	8
29,52	peak 13	0	0	0	281	0	540	0	0	0	0	0	0	2
31,25	3,4-DCPHMU	0	0	250	442	140	102	187	267	0	0	0	0	6
31,80	chloortoluron	0	0	0	0	0	11453	0	0	0	194	0	0	2
32,63	isoproturon	386	304	0	0	0	0	0	0	0	0	0	0	2
32,63	atrazine	138	304	1715	2438	1860	1125	1016	1351	0	0	200	0	9
32,84	peak 49	0	0	0	0	0	0	0	0	1517	0	0	0	1
33,00	peak 44	0	0	0	0	0	0	923	1055	0	399	0	163	4
33,66	diuron	365	420	2144	3744	856	1259	1435	1103	1693	0	0	0	9
34,29	peak 14	~~2873~~	~~2198~~	~~2014~~	~~2092~~	~~2498~~	~~2280~~	~~2570~~	~~1235~~	~~5481~~	~~0~~	~~0~~	~~2677~~	10
35,40	peak 15	0	0	0	0	0	282	609	252	530	270	201	228	7
36,00	dimethachloor	106	108	127	207	121	0	0	0	0	0	0	0	5
36,42	peak 16	0	132	736	0	0	0	2281	1808	0	0	0	0	4
36,66	metobromuron	0	0	0	0	0	0	972	0	0	0	0	0	1
36,98	peak 17	427	184	305	358	213	0	0	0	0	227	0	0	6
37,09	peak 32	0	0	0	0	0	0	0	444	502	0	0	0	2

Corrected retention time	component	Meuse March	April	May	June	July	Aug	Sept	Oct	Nov	Dec	Jan '98	Feb '98	frequentie
37,76	peak 28	147	986	376	409	280	0	397	0	297	135	303	0	9
38,35	chloroxuron	~~1000~~	~~1000~~	~~1000~~	~~1000~~	~~1000~~	~~1000~~	~~1000~~	~~1000~~	~~1000~~	~~1000~~	~~1000~~	~~1000~~	12

APPENDIX 9

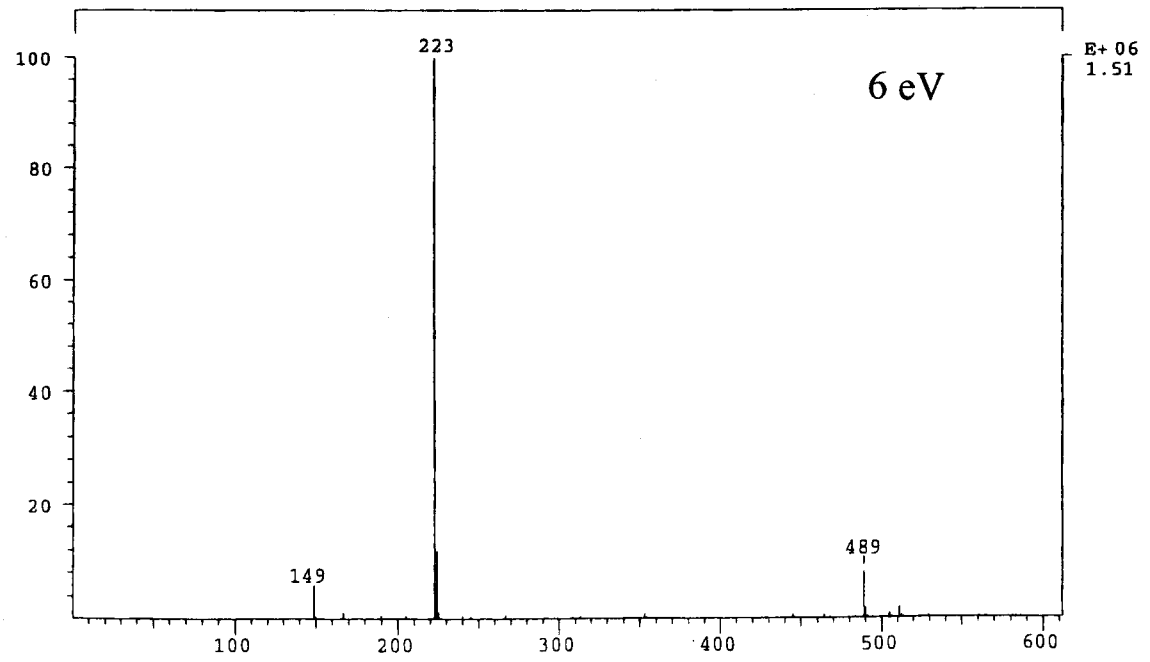
LC/MS mass spectra of diethylphthalate and dibutylphthalate

SPEC: lobith 23-SEP-97 DERIVED SPECTRUM #9
Samp: Rijn Lobith 10-9-97, 9 ml autosampler Start : 13:26:01 2315
Comm: Univ.150x3.0mm, 0.4 ml/min, H2O+NH4OAc/ACN 95->10
Mode: ESI +DAU 147.0 @ -30eV LMR GAS PROG RF UP LR
Oper: Piet Sp Client: grad. 3 metmieren Inlet :
Base: 148.9 Inten : 790986 Masses: 10 > 600
Norm: 148.9 RIC : 1578059 #peaks: 483
Peak: 1000.00 mmu
Data: +/2112>2142 - /2030>2064,2228>2262

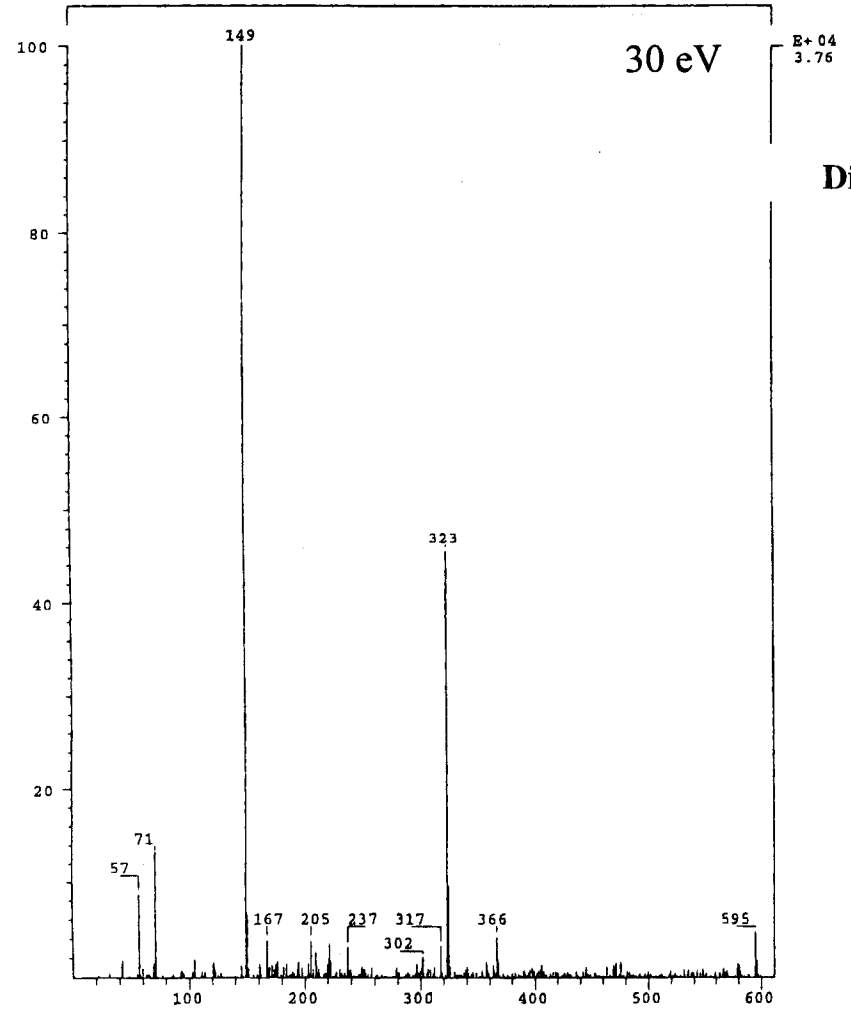


Diethylphtalate

SPEC: lobith 23-SEP-97 DERIVED SPECTRUM #9
Samp: Rijn Lobith 10-9-97, 9 ml autosampler Start : 13:26:01 2289
Comm: Univ.150x3.0mm, 0.4 ml/min, H2O+NH4OAc/ACN 95->10
Mode: ESI +DAU 145.0 @ -6eV LMR GAS PROG RF UP LR
Oper: Piet Sp Client: grad. 3 metmieren Inlet :
Base: 223.0 Inten : 1509074 Masses: 10 > 600
Norm: 223.0 RIC : 2213599 #peaks: 313
Peak: 1000.00 mmu
Data: +/2115>2138 - /2049>2086,2229>2259

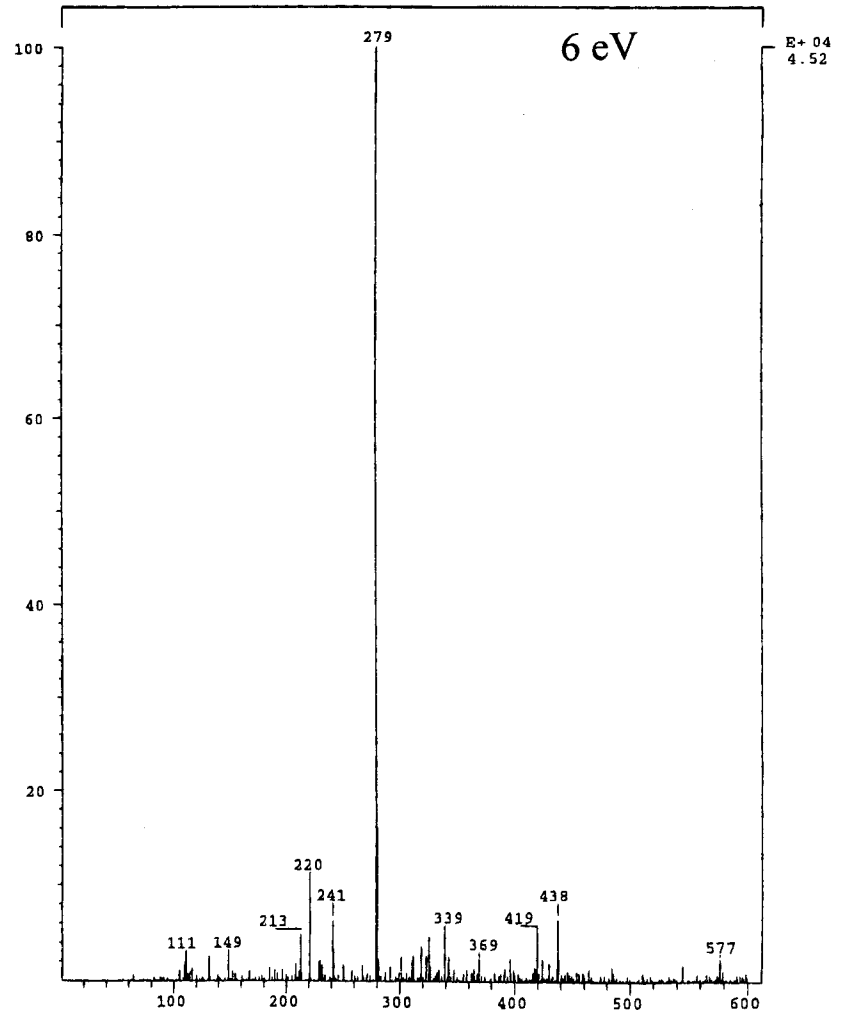


SPEC: lobith 23-SEP-97 DERIVED SPECTRUM #9
Samp: Rijn Lobith 10-9-97, 9 ml autosampler Start : 13:26:01 3392
Comm: Univ.150x3.0mm, 0.4 ml/min, H2O+NH4OAc/ACN 95->10
Mode: ESI +DAU 147.0 @ -30eV LMR GAS PROG RF UP LR
Oper: Piet Sp Client: grad. 3 metmieren Inlet :
Base: 148.9 Inten : 37557 Masses: 10 > 600
Norm: 148.9 RIC : 132237 #peaks: 330
Peak: 1000.00 mmu
Data: +/2762>2770 - /2728>2740,2797>2811



Dibutylphtalate

SPEC: lobith 23-SEP-97 DERIVED SPECTRUM #9
Samp: Rijn Lobith 10-9-97, 9 ml autosampler Start : 13:26:01 3392
Comm: Univ.150x3.0mm, 0.4 ml/min, H2O+NH4OAc/ACN 95->10
Mode: ESI +DAU 145.0 @ -6eV LMR GAS PROG RF UP LR
Oper: Piet Sp Client: grad. 3 metmieren Inlet :
Base: 279.2 Inten : 45179 Masses: 10 > 600
Norm: 279.2 RIC : 149150 #peaks: 344
Peak: 1000.00 mmu
Data: +/2758>2771 - /2723>2740,2792>2804



Colophon

Publisher	Vereniging van Rivierwaterbedrijven - RIWA
Cover and printing	B.V. Drukkerij De Eendracht, Schiedam

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