2. Principle of the Method

The method was developed in accordance to the multi residue methods of the Deutsche Institut für Normung (DIN) (3) and of the European Committee for Standardization (CEN) (4) under special consideration of the liquid chromatographic standard method F12 (4) and F8 (5).

RH 2485 is concentrated from water samples by solid phase extraction (4, 6, 7), either off-line or on-line using an OSP-2A (On-line Sample Preparation Unit). Identification and quantitative determination is done by HPLC with UV detection (4) (see Figure 5 and Figure 6).

3. Test System

For method validation surface water from the river Rhine sampled in Leverkusen-Hitdorf was used. Analytical data for the surface water are listed in Table 1.

Table 1 Analytical data of the surface water from river Rhine, sampled in Leverkusen-Hitdorf on August 3, 1999

Test	Result
Total organic carbon (TOC)	6 mg/L
Dissolved organic carbon (DOC)	3 mg/L
Conductivity at 25 °C	587 µS/cm
pH	8.0
Water hardness	10.8 °dH
Dry residue after filtration	111 mg/L

4. Instruments

4.1. Sample Injection and Concentration

LC pump for sample injection:	L-7100 gradient pump Merck Co., D-64293 Darmstadt
16-port valve:	Multiposition Electric Actuator, VICI AG Valco Europa, Untertannenberg 7, CH-6214 Schenkon
OSP-2A:	On-line Sample Preparator, Merck Co., D-62493 Darmstadt
Autosampler:	L-7250 programmable autosampler, Merck Co., D-64293 Darmstadt

4.2. HPLC

LC pump:	L-7100 gradient pump Merck Co., D-64293 Darmstadt
LC column oven:	L-7350 column thermostat, Merck Co., D-64293 Darmstadt
LC detector:	L-7400 UV-detector, Merck Co., D-64293 Darmstadt

Alternatively comparable instruments of other manufacturers can be used.

Volumetric flasks, pipettes and other common laboratory equipment.

5. Reagents

Water:	deionized and cleaned in a milli-Q unit
RP-18 cartridges for on-line concentration:	LiChrospher 60, RP-18 (10 µm), 70 mg, Merck Co., D-64293 Darmstadt, article No. 1.10444
RP-18 cartridges for off-line concentration:	Bakerbond spe (6 mL), Octadecyl (C_{18}) (40 µm), 1 g, J.T. Baker, Phillipsburg NJ 08865 USA, Art. 7020-07
Solvent 1 (LM 1):	acetonitrile LiChrosolv, Merck Co., D-64293 Darmstadt, article No. 1.00030.9030
Solvent 2 (LM 2):	water, deionized and cleaned in a milli-Q unit
Reference substance:	RH 2485, batch 960507ELB01, purity 99.4% (confirmed by HPLC and GLC-head-space), identity ensured by mass spectrum and ^{1}H-NMR-spectrum, expiry date March 2000

For method validation a certified reference substance of batch no. 960507ELB01 was used. With the reference substance a primary stock solution of approx. 100 mg/L was prepared in acetonitrile. From this stock solution standard solutions were prepared by dilution with drinking water.

6. Safety Measures

The German guidelines for laboratories issued by the Trade Co-operative Association (e.g. Bulletin M006) or comparable guidelines in other countries must be considered when working according to this method.

The following solvents and pesticides classified as toxic and/or less toxic according to the Hazardous Substances Regulations are used.

Acetonitrile:	toxic and easily flammable
RH 2485:	A classification is not yet available. Due to this fact the compound has to be treated as a very toxic substance.

This classification is based on the German guidelines and has to be adapted to the respective national guidelines in case the method is used outside Germany.

7. Performance of Analysis

7.1. On-line Concentration

The water samples are concentrated by the OSP-2A and analyzed by HPLC.
For the determination of RH 2485 in the range from 0.05 µg/L to 0.5 µg/L 50 mL are concentrated.
If required, the concentration volume can be adapted to the concentrations to be measured.

The solid phase concentration of the water samples is carried out automatically and is integrated into the analytical method of determination. The instrument used for this purpose is described in the appendices A1 and A2.

7.2. Off-line Concentration

C_{18}-cartridges are washed with 10 mL of acetonitrile. After this the C_{18}-cartridges are conditioned with 10 mL of milli-Q water. After the conditioning step 200 mL of the water samples are sucked through the cartridges with a flow rate of approx. 5 mL/min. After this the cartridges are dried by sucking ambient air through the cartridges for one hour. To prevent pollution of the cartridges during the drying process activated carbon cartridges are placed on top of the C_{18}-cartridges. The suction pressure is approx. 20 mbar. After the drying procedure the cartridges are eluted with 3 mL of acetonitrile. The sample solution is evaporated to dryness and reconstituted in 1 mL of acetonitrile / milli-Q water (20/80, v/v). From each solution a volume of 250 µL is injected into the HPLC. The HPLC parameters are listed in appendix A3.
If required, the concentration volume can be adapted to the concentrations to be measured.

7.3. Evaluation

The evaluation was done by comparison of the peak areas of the samples with the peak areas of external standard solutions. For this purpose standard solutions of RH 2485 in drinking water were used.
Evaluation is performed using a laboratory data system by comparing the peak areas of the samples to the peak areas of external standard solutions. The concentration of the samples can be calculated according to the given formula:

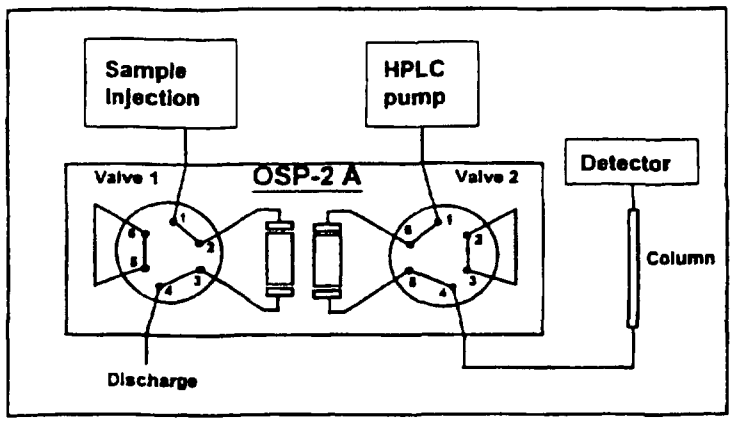
$$\text{Concentration}_{\text{Sample}}\,[\mu g/L] = \frac{\text{Peak Area}_{\text{Sample}} \cdot \text{Concentration}_{\text{Standard}}\,[\mu g/L]}{\text{Peak Area}_{\text{Standard}} \cdot \text{Concentration factor}}$$

Appendix

A 1 Principle of on-line Sample Preparation

The solid phase concentration of the water samples, is carried out automatically and is integrated into the analytical method of determination. The instrument used for this purpose is shown in Figure 1. The main module of the instrument is the OSP-2A (on-line sample preparation unit) of Merck Co.

Figure 1: OSP-2A loading position

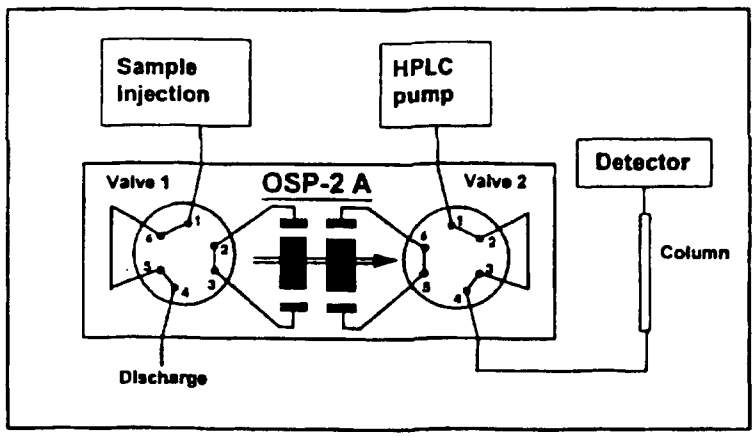


The OSP-2A allows the independent control of two switching valves as well as the automatic replacement of the extraction cartridges. Thereby the described instrument allows cleaning and conditioning of a C18 cartridge as well as the subsequent concentration of a water sample. Parallel to these steps the previously loaded cartridge is connected to the analytical separation system and analyzed there. The time events of the LC pump control the OSP-2A and the 16-port-valve are listed in Table A 1.

A 1.1 Sample Injection and Concentration

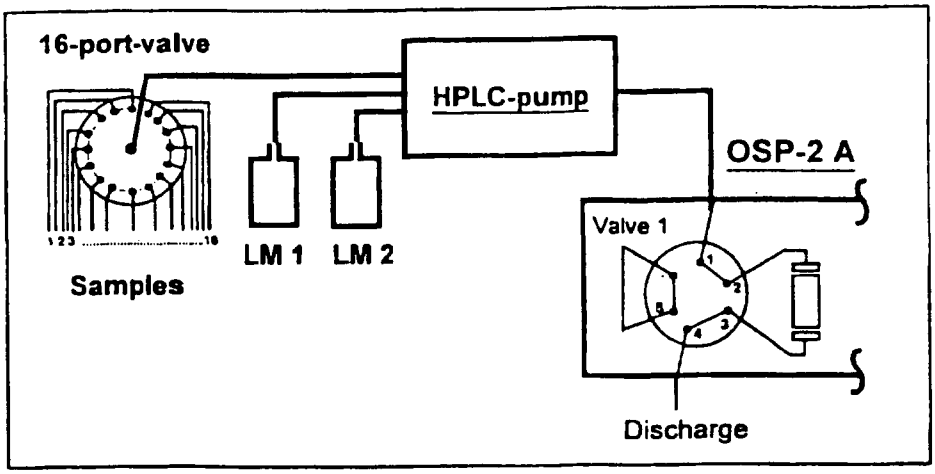
The individual steps of the concentration process are described in the following:
At the beginning of the concentration cycle the OSP-2A is in the "switching position" (see Figure 2). This means that the fixing clamp for the concentration cartridges is opened in order to allow a new cartridge to be positioned by turning the cartridge wheel. Valve 1 and valve 2 are in switching position 1, i.e. the flow is directed via the bypass to the discharge (valve 1) or to the column (valve 2).

Figure 2: OSP-2A switching position



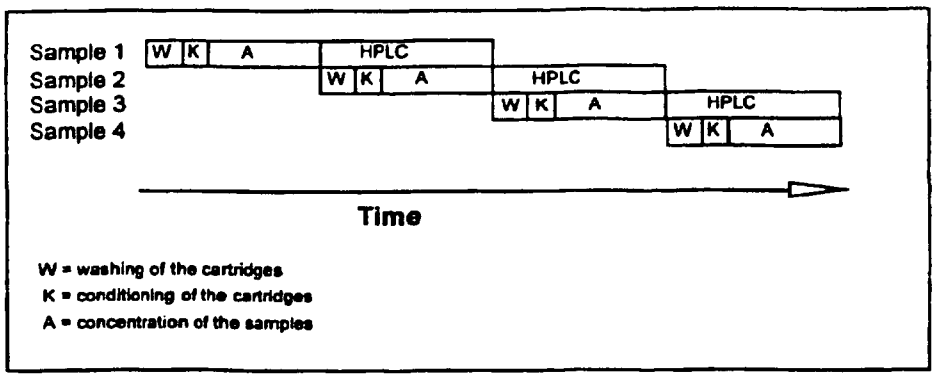
After delivery of the new cartridge, the OSP-2A is switched into "loading position" (see Figure 1). The fixing clamp is now closed and valve 1 and valve 2 are in position 2, i.e. the flow is directed via the cartridges. Now the right cartridge is connected to the analytical separation process, while the next sample is concentrated on the left cartridge. The sample injection and concentration is represented in Figure 3. For the concentration the cartridge is first washed with the solvent 1 (LM 1 = acetonitrile) and subsequently conditioned with solvent 2 (LM 2 = milli-Q water). After that the analytical sample is pumped through the cartridge and the active ingredient to be analyzed is adsorbed to the C_{18}-material. Washing, conditioning and sample injection are carried out by means of a suitable HPLC-pump (connection lines for at least 3 solvents/ternary gradient pump). The volumes needed for the above mentioned processes are adjusted via flow rate and duration of the pumping processes and are listed in Table A 2.

Figure 3: Sample injection



The use of a 16-port-valve allows to automatically concentrate up to 16 water samples in sequence in the described manner. The settings (volumes, times) used for the determination of RH 2485 are given in Table A 1 to Table A 3.
The interlacing of the sequent analytical processes is represented in Figure 4.

Figure 4: Sequence of the analyses



A 1.2 Chromatographic Determination

Parallel to the concentration process described in the previous section, which takes place on the left cartridge (see Figure 1), the chromatographic determination of the previously concentrated active ingredient is carried out on the right cartridge. The substance to be separated is rinsed by the eluent from the cartridge directly onto the chromatographic column. The chromatographic conditions for the determination of RH 2485 are described under item A 2.3 and A 2.4.

A 2 On-line Sample Preparation for RH 2485 Samples

The water samples were concentrated by the OSP-2A and analyzed by HPLC. For the determination of RH 2485 in the range from 0.05 µg/L to 0.5 µg/L 50 mL of water were concentrated.

A 2.1 Control of the OSP-2A for the Determination of RH 2485

The control of the OSP-2A is done by the time events of the sample injection pump. The used events are listed in Table A 1.

Table A 1: Description of the time events (sample injection pump)

Time events of the pump L-7100	Reaction at the OSP-2A
1 Off	Valve 1 in position 1 (bypass)
1 On	Valve 1 in position 2 (via cartridge)
2 Off	Valve 2 in position 1 (to column)
2 On	Valve 2 in position 2 (via cartridge to column)
3 Off	Open the fixing clamp
3 On	Close the fixing clamp
4 Pulse	Cartridge ring moved one position further

For the determination of RH 2485, the following volumes are chosen for conditioning of the cartridges and/or for the concentration:

Table A 2: Volumes for sample injection

Process	Valve 1 flow directed via	Solvent	Flow rate in mL/min	Duration in min	Volume in mL
Rinsing of the pipe	bypass	acetonitrile	2	1.7	3.4
Washing	cartridge	acetonitrile	2	2.5	5
Rinsing of the pipe	bypass	milli-Q water	2	1.4	2.8
Conditioning	cartridge	milli-Q water	2	2.5	5
Rinsing of the pipe	bypass	sample	2.5	4.4	11
Concentration	cartridge	sample	2.5	20	50

The program for the performance of the steps described in Table A 2 is listed in Table A 3.

Table A 3: Control program for sample injection

Time [min]	% A LM 1	% B LM 2	% C sample	Flow rate [mL/min]	Time event No. 1	No. 2	No. 3	No. 4
0.0	100	0	0	0			Off	
0.1	100	0	0	0				Pulse
0.2	100	0	0	0			On	
0.3	100	0	0	2	Off	On		
1.3	100	0	0	2		Off		
2.0	100	0	0	2	On			
4.5	100	0	0	2	Off			
4.6	0	100	0	2				
6.0	0	100	0	2	On			
8.5	0	100	0	2	Off			
8.6	0	0	100	2.5				
13.0	0	0	100	2.5	On			
33.0	0	0	100	2.5	Off			
33.1	100	0	0	0				

A 2.2 Chromatographic Conditions A (Enforcement Method)

C18-column:	LiChrospher 60 RP select B, length 250 mm; i.d. 4 mm, Merck Co., D-64293 Darmstadt
Particle size:	5 µm
Oven temperature:	40 °C
Concentr. volume:	50 mL
Wave length:	204 nm
Flow rate:	1 mL/min
Mobile phase:	Milli-Q water / acetonitrile = 55 / 45 (v/v)
Retention time:	approx. 15.6 min

The chromatographic determination is controlled via a time program proceeding on the HPLC pump. The time events used are described in Table A 4. The time program is listed in Table A 5 for OSP-2A analysis and in Table A 6 for direct injection.

Table A 4: Description of the time events (HPLC pump)

Time events of the pump L-7100	Reaction
2 Pulse	Starting signal for integrator
3 Pulse	16-port valve moves one position further

Table A 5: Control program of the HPLC pump (OSP-2A analysis)

Time [min]	% A	% B	Flow rate [mL/min]	Time event No. 1	No. 2	No. 3	No. 4
0.0	55	45	1				
0.3	55	45	1		Pulse		
33.1	55	45	1			Pulse *	

* Each sample can be injected repeatedly (e.g. for duplicate analysis) when the 16-port valve is not switched one position further after the first concentration cycle ("time event 3 Pulse").

Table A 6: Control program of the HPLC pump (direct injection)

Time [min]	% A	% B	Flow rate [mL/min]	Time event No. 1	No. 2	No. 3	No. 4
0.0	70	30	1		Pulse		
40.0	70	30	1				

A 3 Off-line Sample Preparation for RH 2485 Samples
Chromatographic Conditions B (Confirmatory Method)

CN-column:	LiChrospher 100 CN, length 250 mm; i.d. 4 mm, Merck Co., D-64293 Darmstadt
Particle size:	5 µm
Oven temperature:	40 °C
Injection volume:	250 µL
Wave length:	204 nm
Flow rate:	1 mL/min
Mobile phase:	Milli-Q water / acetonitrile = 70 / 30 (v/v)
Retention time:	approx. 14.1 min

Figure 5: Flow diagram of analysis procedure for on-line concentration

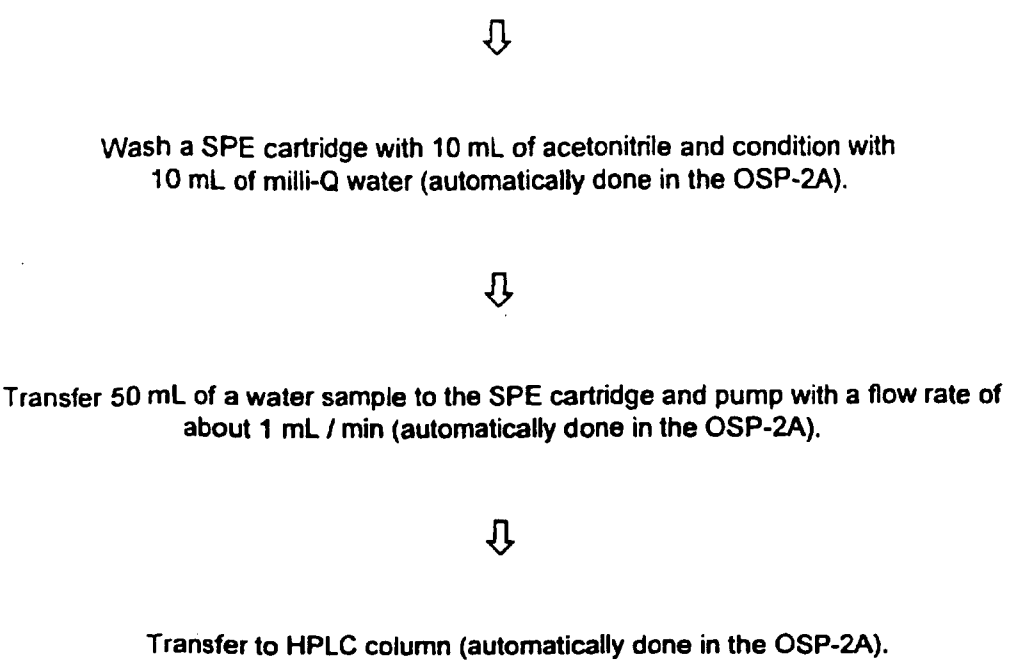


Figure 6: Flow diagram of analysis procedure for off-line concentration