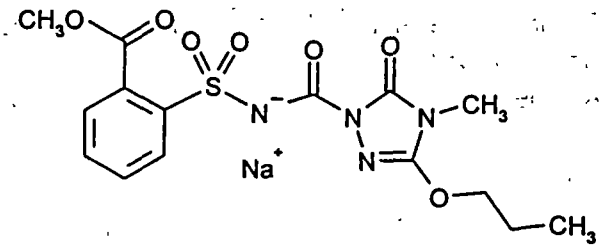
1. Introduction

The method has been developed for the determination of MKH 6561 in drinking water, but may also be used for determination of MKH 6561 in test water from aquatic toxicity tests. The method has to be validated for test water from aquatic toxicity tests if necessary.

1.1. Chemical and physical properties of MKH 6561

Structural formula	:	
Chemical designation (CAS)	:	Benzoic acid, 2-[[[(4,5-dihydro-4-methyl-5-oxo-3-propoxy-1H-1,2,4-triazol-1-yl)carbonyl]amino]sulfonyl]-, methyl ester, sodium salt
Empirical formula	:	$C_{15}H_{17}N_4NaO_7S$
Molecular weight	:	420.4 g/mole
Solubility in water (20 °C)	:	pH 4 = 2.9 g/L pH 7 = 42 g/L pH 9 = 42 g/L unbuffered = 42 g/l

2. Principle of the method

The determination is done by means of HPLC with UV-detection. Water samples of < 10 µg/L are concentrated on a RP-18 cartridge using the OSP-2 A (On-line Sample Preparation Unit) of Merck Co., while water samples > 10 µg/L are directly injected into the HPLC.

3. Instruments

3.1. 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

3.2. 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.

4. Reagents

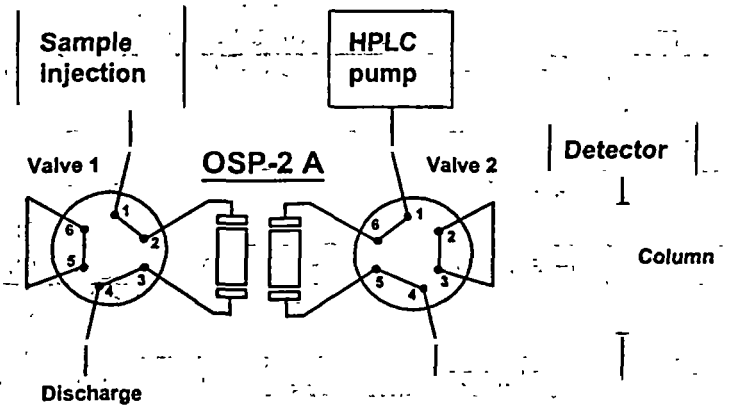
Water	:	deionized and cleaned in a milli-Q-unit
Methanol	:	methanol HPLC-grade, Promochem Co., D-46469 Wesel, article No. 3041
o-phosphoric acid	:	o-phosphoric acid suprapur, Merck, D-64293 Darmstadt, article no. 552.0250
RP-18 cartridges	:	LiChrospher 60, RP-18 (10 µm), 70 mg, Merck Co., D-64293 Darmstadt, article No. 1.10444
Solvent 1 (LM 1)	:	methanol HPLC-grade, Promochem Co., D-46469 Wesel, article No. 3041
Solvent 2 (LM 2)	:	water, deionized and cleaned in a milli-Q-unit
Reference substance	:	MKH 6561, batch 960229ELB02, purity 97.6%, expiry date March 2000

For method development certified reference substance of batch 960229ELB02 (MKH 6561) was used. With the reference substance primarily stock solutions of approx. 500 mg/L were prepared in acetonitrile. From the stock solutions common standard solutions are prepared by dilution with drinking water (adjusted with o-phosphoric acid to pH=3). It is necessary to prepare the standard solutions in water with a comparable matrix load like the water samples to be analyzed.

5. Performance of analyses

The analysis of MKH 6561 in the low ppb as well as high ppb range is described in the present method. The solid phase concentration of the water samples, which is necessary for the measurements of concentrations ranging from 0.05 µg/L to 10 µg/L, is carried out automatically and is integrated into the analytical method of determination. The apparatus used for this purpose is shown in Fig. 1. The main module of the apparatus is the OSP-2 A (on-line sample preparation unit) of Merck Co.
Water samples with concentrations higher than 10 µg/L can be directly injected into the HPLC via an autosampler.

Fig. 1: OSP-2 A loading position

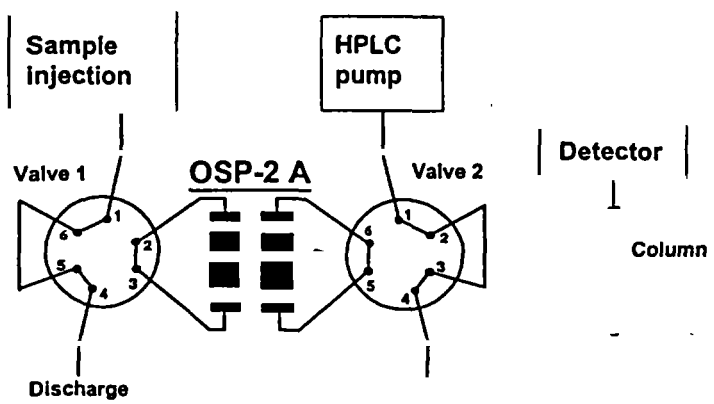


The OSP-2 A allows the independent control of two switching valves as well as the automatic replacement of the extraction cartridges. Thereby the described apparatus allows cleaning and conditioning of a C18 cartridge as well as the subsequent concentration of a water sample. Parallel to these steps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

5.1. Sample injection and concentration

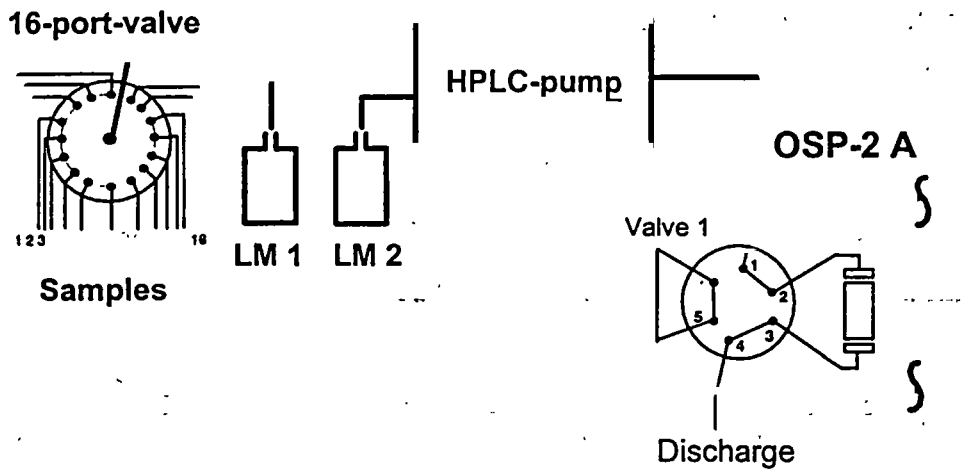
The individual steps of the concentration process are described in the following:
At the beginning of the concentration cycle the OSP-2 A is in the "switching position" (see Fig. 2). This means that the fixing clamp for the concentration cartridges is opened in order to allow a new cartridge to be positioned by turning the cartridge wheel. The valves V1 and V2 are in switching position 1, i.e. the flow is directed via the bypass to the discharge (V1) or to the column (V2).

Fig. 2: OSP-2 A switching position



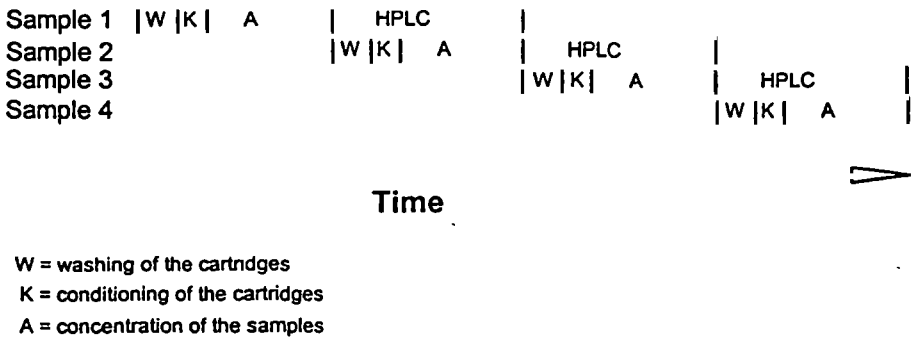
After delivery of the new cartridge, the OSP-2 A is switched into "loading position" (see Fig. 1). The fixing clamp is now closed and the valves V1 and V2 are in position 2, i.e. the flow is directed via the cartridges. Now the right cartridge is connected to the analytical separation process, while the next sample is concentrated on the left cartridge. The sample injection and concentration is represented in Fig. 3. For the concentration the cartridge is first washed with the solvent 1 (LM 1 = methanol) 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.

Fig. 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 MKH 6561 are given under item 6.2. The interlacing of the sequent analytical processes is represented in Fig. 4.

Fig. 4: Sequence of the analyses



W = washing of the cartridges
K = conditioning of the cartridges
A = concentration of the samples

5.2. Chromatographic determination

Parallel to the concentration process described under item 5.1, which takes place on the left cartridge (see Fig. 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 MKH 6561 are described under item 6.3.

6. Determination of MKH 6561

6.1. Sample preparation

The water samples (adjusted with o-phosphoric acid to pH=3) are directly injected into the HPLC or concentrated by the OSP-2A and determined by HPLC.
For the determination of MKH 6561 in the range from 0.05 µg/L to 10 µg/L 50 mL are concentrated.

6.2. Control of the OSP-2A for the determination of MKH 6561

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

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

Time events of the pump L-7100	Reaction at the OSP-2 A
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 MKH 6561, the following volumes are chosen for conditioning of the cartridges and/or for the concentration:

Table 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	methanol	2	1.6	3.4
Washing	cartridge	methanol	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 2 is listed in Table 3.

Table 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		
2.0	100	0	0	2	On			
4.5	100	0	0	2	Off	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			

6.3. Chromatographic conditions

Column	:	LiChrospher 60, RP-select B, 125 mm, 4 mm i.d., Merck Co., D-64293 Darmstadt, article No. 50829
Particle size	:	5 µm
Oven temperature	:	40 °C
Injection volume	:	250 µL *
Flow rate	:	2 mL/min
Solvent A	:	milli-Q-water (adjusted with o-phosphoric acid to pH=3)
Solvent B	:	methanol
Wavelength	:	235 nm
Stop time	:	6 min (direct injection) 33 min (OSP-2A analysis)
Retention time	:	MKH 6561 approx. 3.9 min (direct injection) approx. 14.4 min (OSP-2A analysis)

* If required, the injection volume can be adapted to the concentrations to be measured.

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

Table 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 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	80	20	2				
0.3	80	20	2		Pulse		
4.5	80	20	2				
25.0	30	70	2				
25.5	30	70	2				
26.0	80	20	2				
33.0	80	20	2			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 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	60	40	2		Pulse		
6.0	60	40	2				

7. Evaluation

The evaluation is made by means of a laboratory data system via comparison of the peak areas of the sample with the peak areas of the external standard solutions. The active ingredient content of the sample can be calculated according to the following formula:

$$C = \frac{As \times Cs}{A}$$

A = peak area of the standard solution [area counts]
As = peak area of the sample solution [area counts]
C = active ingredient content of the sample [µg/L]
Cs = concentration of the standard solution [µg/L]