Cover Sheet for

ENVIRONMENTAL CHEMISTRY METHOD

Pesticide Name:	Propoxycarbazone-sodium
MRID # :	450127-32
Matrix:	Water
Analysis:	HPLC/UV

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450127-32

109114

Study Title

Method for the determination of MKH 6561 in drinking water by HPLC using direct injection as well as on-line solid phase extraction.

Data Requirement

Author

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Completion Date

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Submitted by:

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Performing Laboratory ID

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109114

Page 1a plus pages 1b, 1c, and 1d for a total of 22 pages

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Company: Bayer Corporation
Agriculture Division
Research and Development Department
Environmental Research Section

Company Agent: R. L. Graney, Vice President Date: 6/29/99

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Good Laboratory Practice Certification

Although scientifically valid, the study described in this document was not conducted under Good Laboratory Practices (GLP).

Submitted by: Bayer Corporation
Agriculture Division

R. L. Graney, Ph.D. Date: 6/29/99
Vice President, Environmental Research

Sponsor Representative: Date: 6/21/99
V. E. Clay, Ph.D.
Director, Environmental Fate

Study Director: See Page 13 Date:
B. Brumhard
Bayer AG

Certification of Availability of Raw Data

It is hereby certified that the registrant possesses or has access to the raw data for this study. A permanent record of all raw data and final report are filed at Bayer AG, Crop Protection Research, Institute for Environmental Biology, Leverkusen, Germany. A copy of the final report will be retained in the Environmental Fate Group files at the Bayer Research Park, 17745 South Metcalf Avenue, Stilwell, Kansas.

Company Agent: Date: 6/29/99
P.A. Toll
Supervisor, Quality Assurance

Certification of Authenticity

With the exception of addition of a Bayer Report Number, subsequent pages of this report are presented as received from the performing laboratory.

Sponsor: Bayer Corporation
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Vice President, Environmental Research
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Bayer AG
Crop Protection-Development
Institute for Metabolism Research
and Residue Analysis

Monheim, October 01, 1998
Dr. B. Brumhard/ SA
MR-741/98
Method 00549

Method for the determination of MKH 6561 in drinking water by HPLC using direct injection as well as on-line solid phase extraction

Björn Brumhard

Summary

The method describes the determination of MKH 6561 in drinking water by means of HPLC with UV-detection. The water samples with concentrations ranging from 0.05 µg/L to 10 µg/L are concentrated by on-line solid phase extraction (OSP-2A). Samples ranging from 10 µg/L to 10000 µg/L are measured by direct injection. The limit of quantification is 0.05 µg/L. The linearity of the detector was checked for MKH 6561 in the range from 0.05 µg/L to 10 µg/L (concentration volume 50 mL) and in the range from 10 µg/L to 10000 µg/L (injection volume 250 µL). The correlation coefficients were 0.99964 (0.05 - 10 µg/L) and 0.99997 (10 - 10000 µg/L). The relative standard deviations were between 0.002 and 0.03 for the peak areas and between < 0.001 and 0.001 for the retention times.

Zusammenfassung

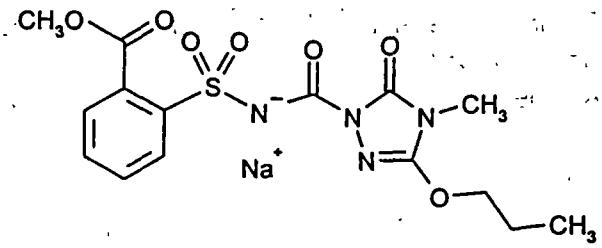
Die Methode beschreibt die Bestimmung von MKH 6561 in Trinkwasser durch HPLC mit UV-Detektion. Die Messung der Wasserproben mit Konzentrationen von 0,05 µg/L bis 10 µg/L erfolgt nach vorheriger on-line Festphasenextraktion (OSP-2A). Proben mit 10 µg/L bis 10000 µg/L werden mit HPLC-Direktinjektion gemessen. Die Bestimmungsgrenze der Methode liegt bei 0,05 µg/L. Die Linearität des Detektors für MKH 6561 wurde im Bereich von 0,05 µg/L bis 10 µg/L (Anreicherungsvolumen 50 mL) und im Bereich von 10 µg/L bis 10000 µg/L (Injektionsvolumen 250 µL) geprüft. Die Korrelationskoeffizienten betrugen 0,99964 (0,05 -10 µg/L) und 0,99997 (10 - 10000 µg/L). Die relative Standardabweichung der Methode lag für die Peakflächen zwischen 0,002 und 0,03 und für die Retentionszeiten zwischen < 0,001 und 0,001.

Table of contents

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

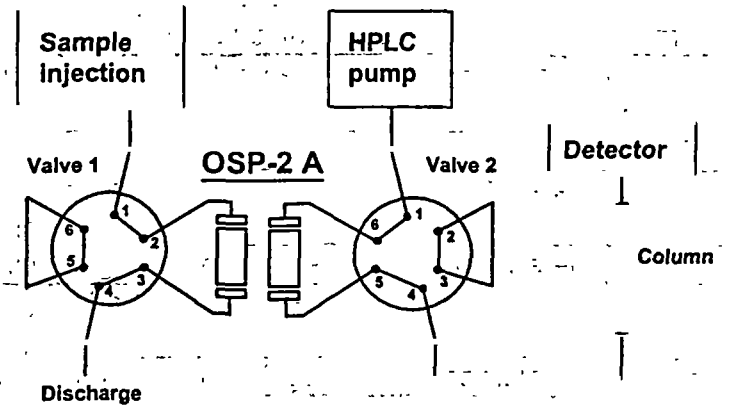
Water	:	deionized and cleaned in a milli-Q-unit
Methanol	:	methanol HPLC-grade, Promochem Co., D-46469 Wesel, article No. 3041
o-phosphoric acid	:	o-phosphoric acid suprapur, Merck, D-64293 Darmstadt, article no. 552.0250
RP-18 cartridges	:	LiChrospher 60, RP-18 (10 µm), 70 mg, Merck Co., D-64293 Darmstadt, 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

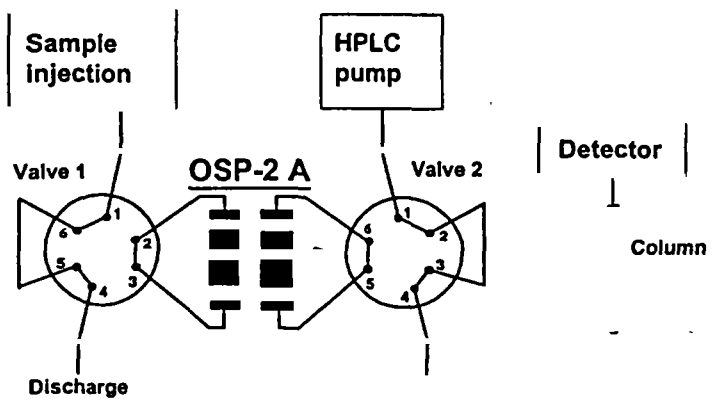


The OSP-2 A allows the independent control of two switching valves as well as the automatic replacement of the extraction cartridges. Thereby the described apparatus allows cleaning and conditioning of a C18 cartridge as well as the subsequent concentration of a water sample. Parallel to these steps 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

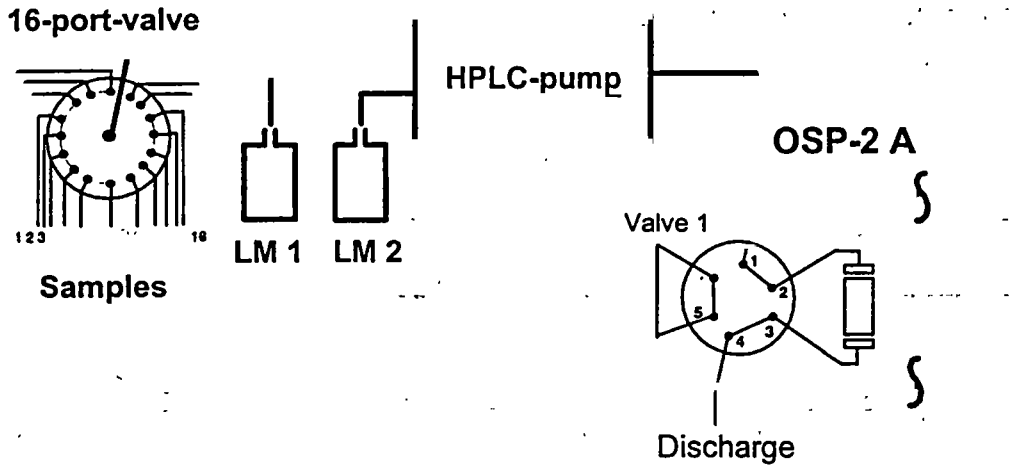
The individual steps of the concentration process are described in the following:
At the beginning of the concentration cycle the OSP-2 A is in the "switching position" (see Fig. 2). This means that the fixing clamp for the concentration cartridges is opened in order to allow a new cartridge to be positioned 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



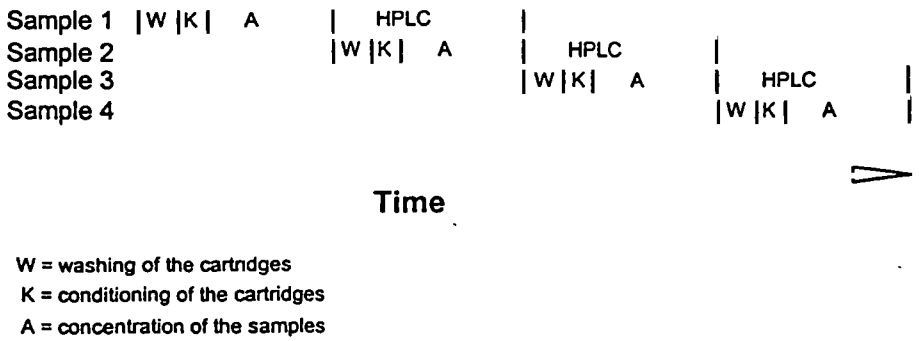
After delivery of the new cartridge, the OSP-2 A is switched into "loading position" (see Fig. 1). The fixing clamp is now closed and the valves V1 and V2 are in position 2, i.e. the flow is directed via the cartridges. Now the right cartridge is connected to the analytical separation process, while 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]

8. Validation

8.1. HPLC system

Injection of the external standard solutions is performed in the same way as described for the samples. The peak areas of the external standards can therefore directly be compared to the peak areas of the samples. Therefore, determination of recovery rates is not required. For the peak areas and the retention times the following relative standard deviations were determined:

Table 7: OSP-2A analysis

Sample material	Conc. [µg/L]	n	Peak area Average [area counts]	RSD*	Retention time Average [min]	RSD*
Drinking water	0.052	9	2247	0.03	14.34	0.001
Drinking water	10.48	10	395436	0.01	14.39	< 0.001

RSD* = Relative Standard Deviation

Attention: To guarantee similar conditions it is necessary to prepare the standard solutions in water with the same matrix load like the test water samples.

Table 8: Direct injection

Sample material	Conc. [µg/L]	n	Peak area Average [area counts]	Peak area RSD*	Retention time Average [min]	Retention time RSD*
Drinking water	10.48	10	2167	0.03	3.93	< 0.001
Drinking water	10482	10	2299445	0.002	3.96	< 0.001

8.2. *Comparison of response*

A response comparison for MKH 6561 was performed to determine the recovery rate during the automatic on-line extraction.
For the response comparison the absolute amount of MKH 6561 which is concentrated during the OSP-2A analysis from 50 mL test water sample, was directly injected (without concentration) in 250 µL test water via an autosampler.

Table 9: Comparison of response

	n	Solution concen-tration [µg/L]	Injected amount of MKH 6561 [µg]	Peak area (average)	Response factor [μg^{-1}]	%
direct	10	10.48	0.0026	2167	833462	100
OSP-2A	9	0.052	0.0026	2247	864231	104
direct	2	2096	0.524	458755	875487	100
OSP-2A	10	10.48	0.524	395436	754649	86

$$\text{Response factor} = \frac{\text{Peak area}}{\text{Amount MKH 6561}}$$

Analysis of the external standard solutions was performed in the same way as described for the samples, i.e. the standard was also subjected to concentration on the C18-cartridges (see item 5 of the method). Therefore the peak areas of the external standard can directly be compared to the peak areas of the samples. Correction of sample measurements by recovery rates determined off-line are not necessary.

8.3. Blank values

In the blank samples MKH 6561 was not detected (Appendices 1 and 2).

8.4. Linearity

The linearity of the method was checked for MKH 6561 in the range from 0.05 µg/L to 10 µg/L with a concentration volume of 50 mL and in the range from 10 µg/L to 10000 µg/L with an injection volume of 250 µL. The resulting curves are represented in the Appendices 7 and 8. The correlation coefficients for the OSP-2A analysis was 0.99964 and for direct injection 0.99997.

9. Limit of quantification

The limit of quantification for MKH 6561 is 0.05 µg/L.

10. Safety instructions

The German guidelines for laboratories issued by the Trade Cooperative Association (e.g. Bulletin M006) or comparable guidelines in other countries must be observed 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.

Methanol	:	Toxic and highly inflammable
o-phosphoric acid	:	Corrosive
MKH 6561	:	Classification not yet available. Due to this the compound has to be handled like a very toxic substance.

This classification is based on German guidelines and must be adapted to the respective national guidelines if the method is used outside of Germany.

11. Appendices

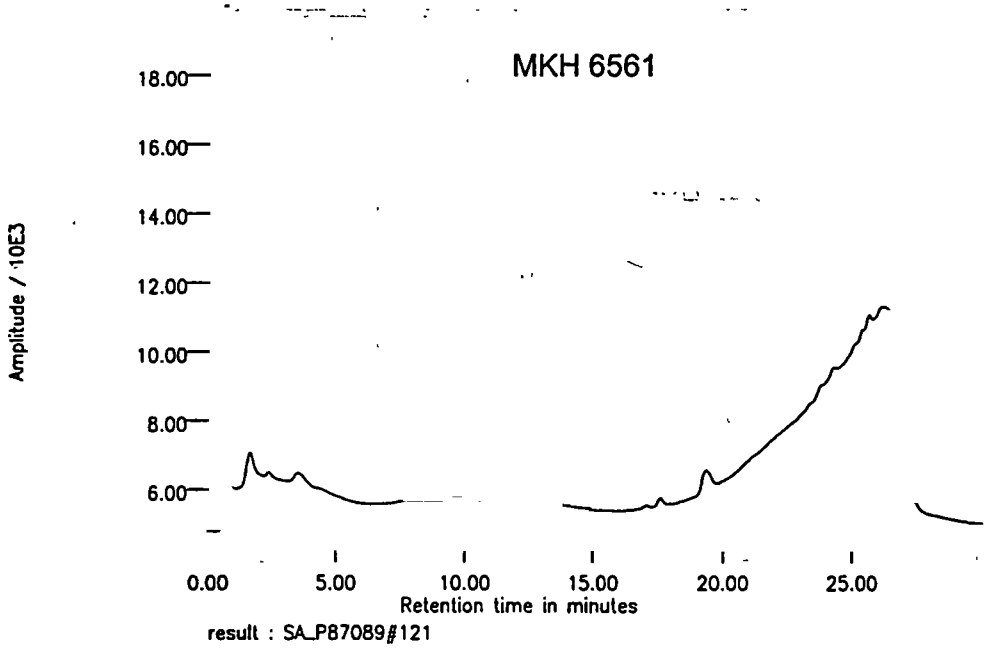
Appendix 1 : Chromatogram standard MKH 6561 10.48 µg/L in test water (concentration volume 50 mL)
Appendix 2 : Chromatogram standard MKH 6561 0.052 µg/L in test water (concentration volume 50 mL)
Appendix 3 : Chromatogram blank sample test water (concentration volume 50 mL)
Appendix 4 : Chromatogram standard MKH 6561 10482 µg/L in test water (injection volume 250 µL)
Appendix 5 : Chromatogram standard MKH 6561 10.48 µg/L in test water (injection volume 250 µL)
Appendix 6 : Chromatogram blank sample test water (injection volume 250 µL)
Appendix 7 : Linearity of detection for MKH 6561 from 0.05 µg/L to 10 µg/L in test water (concentration volume 50 mL)
Appendix 8 : Linearity of detection for MKH 6561 from 10 µg/L to 10000 µg/L in test water (injection volume 250 µL)

The experiments were carried out by Mr. Stefan Schwedhelm.

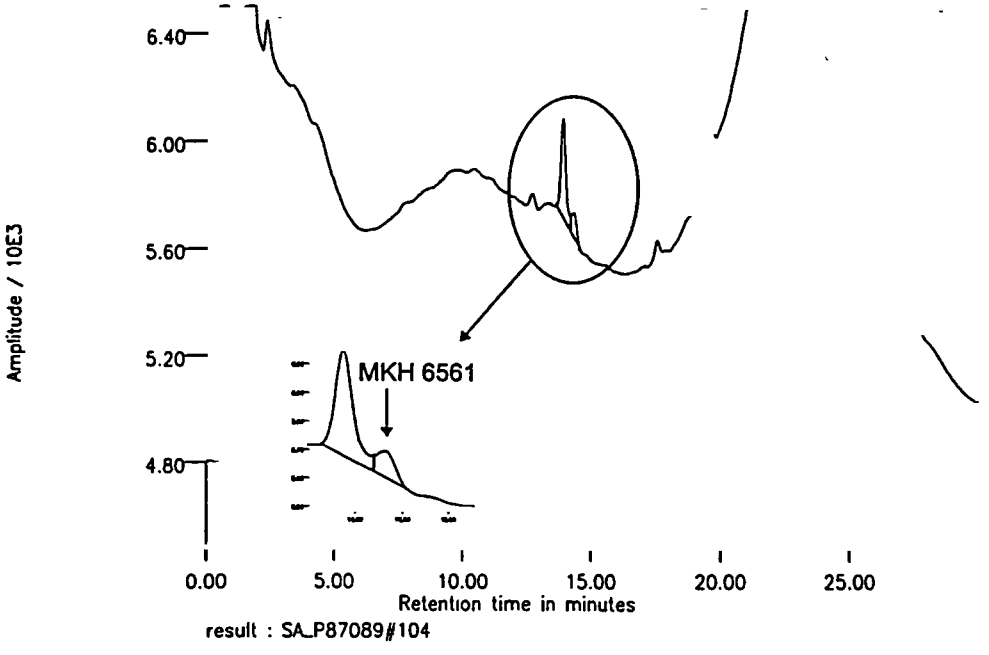
B. Brumhard Oct. 1, 1998
Dr. B. Brumhard
(study director)

J. Köhler 10/2/98
Dr. J. Köhler
(head of testing facility)

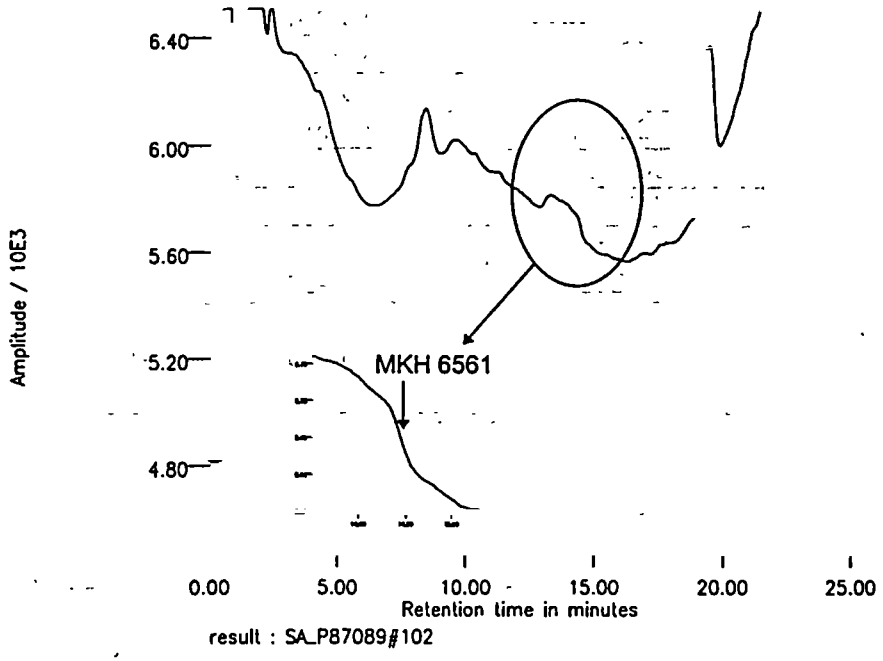
Appendix 1 : Chromatogram standard MKH 6561 10.48 µg/L in test water (concentration volume 50 mL)



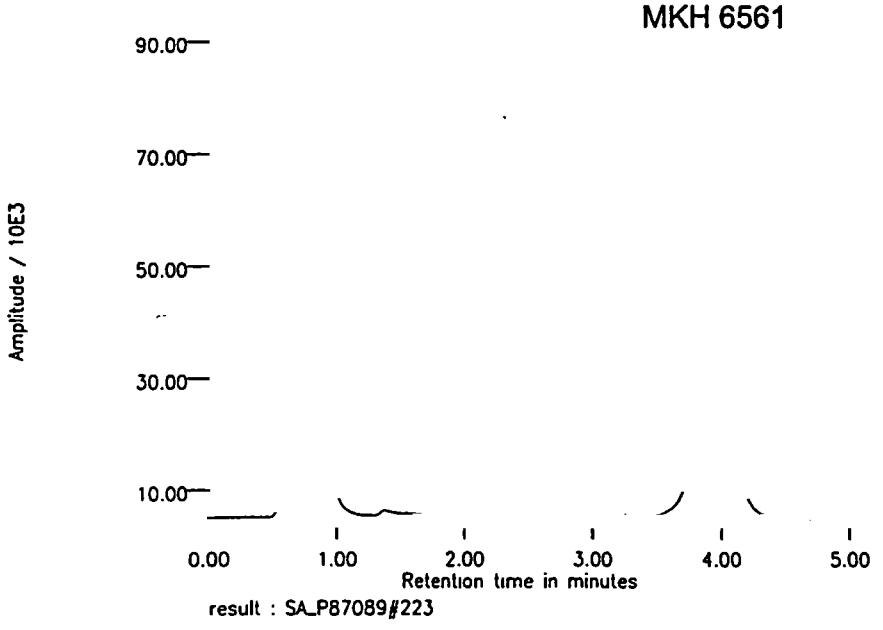
Appendix 2 : Chromatogram standard MKH 6561 0.052 µg/L in test water (concentration volume 50 mL)



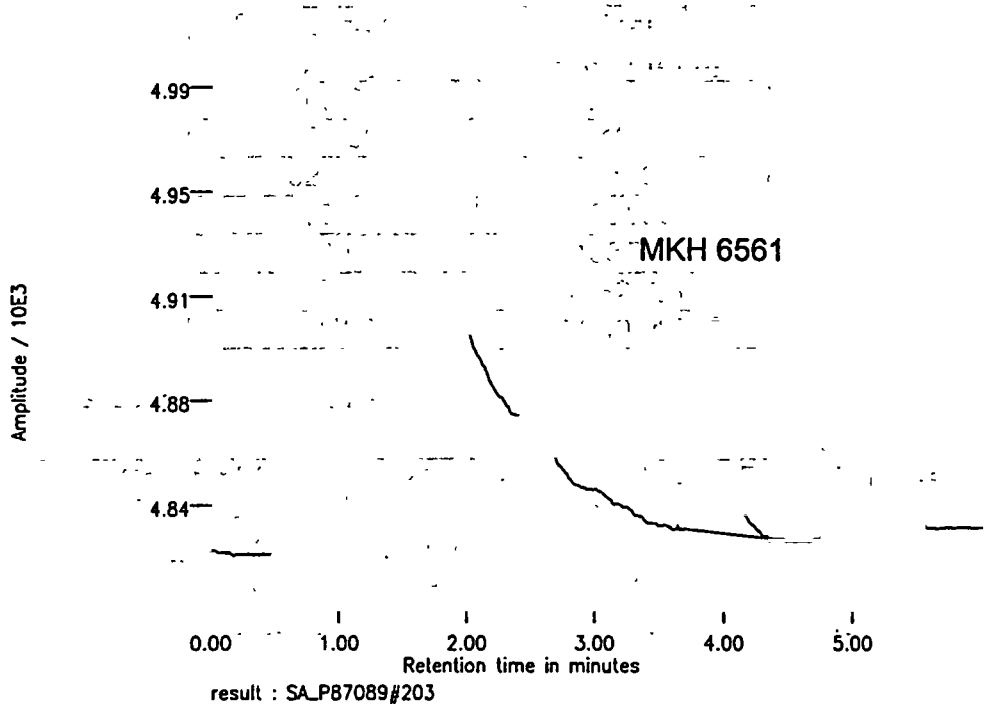
Appendix 3 : Chromatogram blank sample test water (concentration volume 50 mL)



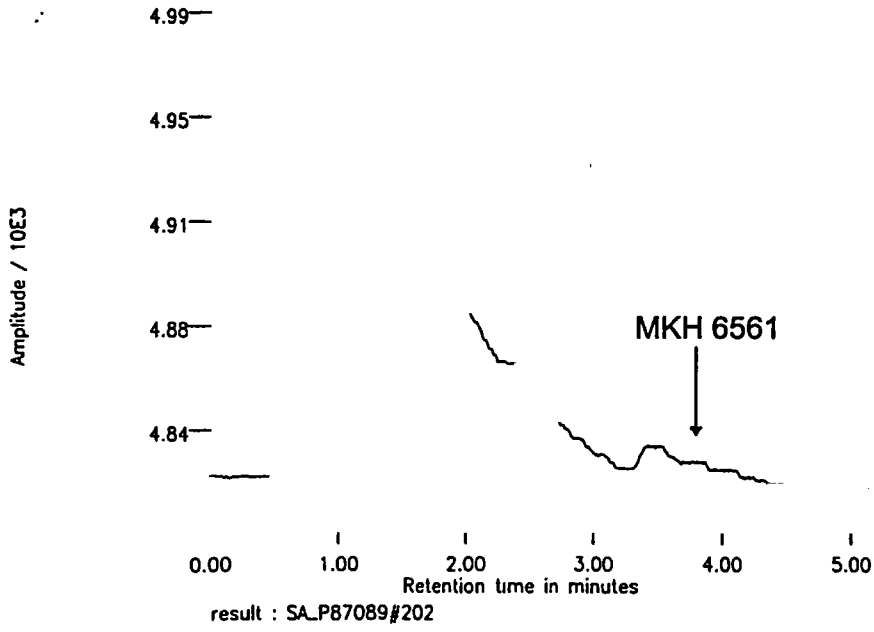
Appendix 4 : Chromatogram standard MKH 6561 10482 µg/L in test water (injection volume 250 µL)



Appendix 5 : Chromatogram standard MKH 6561 10.48 µg/L in test water (injection volume 250 µL)



Appendix 6 : Chromatogram blank sample test water (injection volume 250 µL)



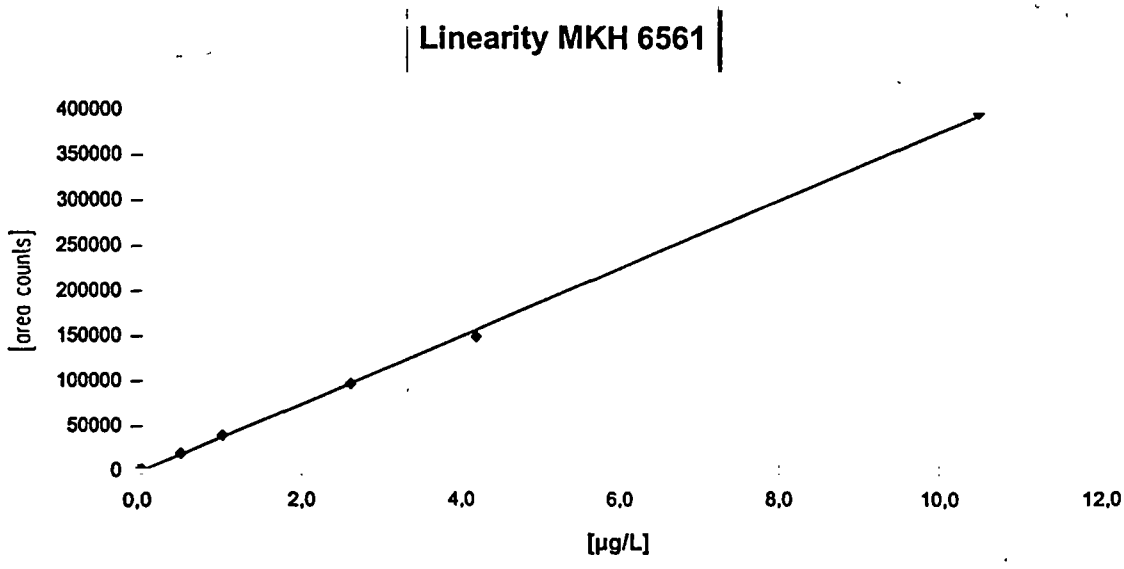
Appendix 7 : Linearity of detection for MKH 6561 from 0.05 µg/L to 10 µg/L in test water (concentration volume 50 mL)

Concentration [µg/L]	Mean [area counts]
0.052	2247
0.524	19454
1.048	39476
2.621	96670
4.193	148333
10.482	395436

statistical evaluation

Constant (a)	37606,946	-1651,4	Point of intersection (b)
Std. deviation	501,2420	2383,75	Std. deviation. for b
Certainty (r^2)	0,99929	4370,81	Std. deviation. for y
F-Statistic	5629,13	4	Degrees of Freedom
Quadratic regression	1,08E+11	7,64E+07	Quadr. Resid.

correlation coefficient : 0.99964



Appendix 8 : Linearity of detection for MKH 6561 from 10 µg/L to 10000 µg/L in test water (injection volume 250 µL)

Concentration [µg/L]	Mean [area counts]
10.482	2167
104.82	24201
524.11	114931
1048	228165
2096	458755
5241	1132728
10482	2299445

statistical evaluation

Constant (a)	218.912	-1399.3	Point of intersection (b)
Std. deviation	0.7128	3223.17	Std. deviation. for b
Certainty (r^2)	0.99995	6715.76	Std. deviation. for y
F-Statistic	94312.42	5	Degrees of Freedom
Quadratic regression	4.25E+12	2.26E+08	Quadr. Resid.

correlation coefficient : 0.99997