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o-Phenylphenol (OPP) – Determination of o-phenylphenol in workplace air by means of high performance liquid chromatography (HPLC-DAD)

Air Monitoring Method – Translation of the German version from 2021

Keywords

o-phenylphenol; hazardous substance; air analyses; workplace measurement; high-performance liquid chromatography; silica gel sorbent tube; quartz fibre filter; vapour particle mixture

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Abstract

This analytical method is a validated measurement procedure for the determination of o-phenylphenol [90-43-7] in workplace air in a concentration range of one tenth up to twice the currently valid occupational exposure limit value in Germany of 5 mg/m^3. For sampling a defined volume of air is drawn through a binder-free quartz fibre filter followed by a silica gel sorbent tube. The flow rate is set to 1 l/min and sampling duration is 2 hours (which correspond to a sampling volume of 120 l). o-Phenylphenol is extracted with 2-propanol and subsequently analysed using liquid chromatography with diode array detection. The quantitative determination is based on a calibration function. The limit of quantification is 0.0183 mg/m^3 based on an air sample volume of 120 l. The mean recovery is 94% and the expanded uncertainty for the validation range of 0.5 to 10 mg/m^3 is 27 to 30%.

Method number	1
Application	Air analysis
Analytical principle	High performance liquid chromatography (HPLC)

1 Characteristics of the method

Precision:	Standard deviation (rel.):	s = 3 to 11%
	Expanded uncertainty:	U = 27 to 30%
	in the concentration range of c = 0.5 to 10 mg/m^3 and for n = 6 determinations	
Limit of quantification:	0.02 mg/m^3 for an air sample volume of 120 l and a sampling period of 2 h	
Recovery:	η = 0.88–0.97 (88–97%)	
Sampling recommendations:	Sampling period:	2 h
	Air sample volume:	120 l
	Flow rate:	1 l/min
	Sampling period (short-term exposure limit):	15 min
	Flow rate:	1 l/min
	Air sample volume:	15 l

2 Description of the substances

o-Phenylphenol [90-43-7]

o-Phenylphenol (OPP) is a colourless, crystalline solid with a slight odour (molecular weight 170.2 u, melting point 57 °C, boiling point 286 °C, density 1.2 g/cm^3).

OPP is widely used as a fungicide and a germicide. OPP is used as a disinfectant in hospitals and households, as a preservative for water/oil emulsions, such as in cooling lubricants employed in the metal working industry, as a preservative for citrus fruit and vegetables after harvest as well as a preserving agent in the leather, paper, glue, textile, plastic and cosmetics industries.

The Occupational Exposure Limit Value (OELV) and the MAK value of OPP [90-43-7] is 5 mg/m^3 in the inhalable dust fraction (I dust). The short-term exposure limit is assigned to Peak Limitation Category I with an excursion factor of 1 (AGS 2021; DFG 2021). OPP is a vapour-particle mixture, i.e. it can be present in particulate as well as in vapour form in the workplace air. When sampling, a sampling device that can simultaneously capture the inhalable dust fraction as well as the vapour phase must be used.

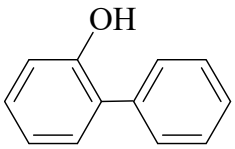

Fig. 1 Molecular structure of OPP

3 General principles

The method enables the determination of the content of OPP in the workplace air in the inhalable dust fraction in the range of a tenth to twice the currently valid Occupational Exposure Limit Value (OELV) and MAK value of 5 mg/m^3 (inhalable dust fraction) (AGS 2021).

Sampling is carried out using a suitable sampling pump to draw a defined volume of air through a combined sampling system consisting of a quartz fibre filter free from binding agents and an adsorbent tube (silica gel). The quartz fibre filter loaded with hazardous substances and the contents of the silica gel tube are each transferred into an amber glass vessel, covered with 2-propanol and shaken. The determination is carried out by means of high performance liquid chromatography with DAD (diode array detector) detection. Quantitative evaluation is based on a linear calibration graph.

4 Equipment, chemicals and solutions

4.1 Equipment

For sampling:

- Sampling pump for personal or stationary sampling, suitable for a flow rate of 1 l/min (e.g. GilAir Plus, from DEHA Haan & Wittmer GmbH, 71296 Heimsheim, Germany)
- Personal sampling head for the inhalable fraction (GSP) (e.g. from GSA Messgerätebau GmbH, 40880 Ratingen, Germany)
- PGP filter cassette made of plastic supplied with covers for the filters, 37 mm diameter (e.g. from GSA Messgerätebau GmbH, 40880 Ratingen, Germany)
- Quartz fibre filter (free from binding agents), Ø 37 mm (e.g. MN QF-10, from Macherey-Nagel GmbH, 52355 Düren, Germany (or of similar quality))
- Silica gel tubes ORBO™ 506 Activated Silica Gel (45/60) 300/150 mg, 8 × 75 mm (e.g. Supelco, Merck KGaA, 64293 Darmstadt, Germany)
- Flow meter (e.g. TSI Flowmeter 4146, from TSI, 52068 Aachen, Germany)

For the sample preparation and the analytical determination:

- Ultrapure water unit (e.g. Millipore-Q-Gradient® with Elix® 3UV, from Merck, 64293 Darmstadt, Germany)
- Variable piston pipettes, 10 to 100 µl and 100 to 1000 µl (e.g. Reference 2®, from Eppendorf, 22366 Hamburg, Germany)
- Bottle-top dispenser attachment, 1 to 10 ml (e.g. Dispensette S® analog, from Brand, 97877 Wertheim, Germany)
- Tube cutter (e.g. Supelco, Merck KGaA, 64293 Darmstadt, Germany)
- 20-ml amber glass vessels with screw-caps (e.g. Fa. CS-Chromatographie, 52379 Langerwehe, Germany)
- Laboratory compact shaker (e.g. Compact Shaker KS 14 A control, from Edmund Bühler GmbH, 72411 Bodelshausen, Germany)
- Volumetric flasks (50 ml, glass) with glass stoppers (e.g. from BRAND, 97877 Wertheim, Germany)
- Chromafil® syringe filter RC, pore width 0.45 µm, Ø 25 mm (e.g. from Carl Roth, 76185 Karlsruhe, Germany)
- Disposable syringes, 5 ml made of polyethylene

- Analytical balance (e.g. XPE-20S Delta Range®, from Mettler-Toledo, 35396 Gießen, Germany)
- Tweezers (e.g. from Plano W. Plannet GmbH, 35578 Wetzlar, Germany)
- High performance liquid chromatograph with DAD (e.g. HPLC 20 Nexera XR, from Shimadzu GmbH, 47269 Duisburg, Germany)
- Autosampler (e.g. SIL-20AC XR, Shimadzu GmbH, 47269 Duisburg, Germany)
- Detector (e.g. SPD-M20A prominence DAD, from Shimadzu GmbH, 47269 Duisburg, Germany)
- C18-column, length: 250 mm; inner diameter: 3 mm; particle size: 5 µm (e.g. CC 250/3 Nucleosil 100-5 C18, from Macherey-Nagel, 52355 Düren, Germany)
- Microlitre syringes, 50 µl, 200 µl (e.g. from Hamilton, 52379 Langerwehe, Germany)
- 2-ml vials (e.g. from Macherey-Nagel, 52355 Düren, Germany)

4.2 Chemicals

- o-Phenylphenol 99% (e.g. from Merck KGaA, 64293 Darmstadt, Germany)
- 4-tert-Amylphenol 99% (e.g. from Merck KGaA, 64293 Darmstadt, Germany)
- 2-Benzyl-4-chlorophenol 95% (e.g. from Merck KGaA, 64293 Darmstadt, Germany)
- 2-Propanol for the HPLC, 99.9% (e.g. from Merck KGaA, 64293 Darmstadt, Germany)
- Acetonitrile, Rotisolv HPLC ultra grade (e.g. from Carl Roth GmbH, 76185 Karlsruhe, Germany)
- Ultrapure water ($\rho \geq 18.2\ M\Omega \times cm$ at 25 °C) (e.g. Millipore, Merck KGaA, 64293 Darmstadt, Germany)

4.3 Solutions

The following solutions, which can be stored in the refrigerator at +4 °C for at least 3 months, are prepared from the chemicals listed in Section 4.2:

OPP Stock Solution 1 (20 mg of OPP per ml of 2-propanol):
1 g of OPP is transferred into a 50 ml volumetric flask and dissolved in 50 ml of 2-propanol.

The following working solutions are obtained by dilution of Stock Solution 1:

Working Solution 1: 1:10 dilution of Stock Solution 1 (2 mg/ml)
Approx. 30 ml of 2-propanol are placed into a 50 ml volumetric flask. Then 5 ml of Stock Solution 1 are added and the volumetric flask is filled to 50 ml with 2-propanol.

Working Solution 2: 1:100 dilution of Stock Solution 1 (200 µg/ml)
Approx. 30 ml of acetonitrile (ACN) are placed into a 50 ml volumetric flask. Then 0.5 ml of Stock Solution 1 is added and the volumetric flask is filled to 50 ml with ACN.

Working solution 3: 1:10 dilution of Working Solution 2 (20 µg/ml)
Approx. 30 ml of ACN are placed into a 50 ml volumetric flask. Then 5 ml of Working Solution 2 are added and the volumetric flask is filled to 50 ml with ACN.

Working Solution 4: 1:10 dilution of Working Solution 3 (2 µg/ml)
Approx. 30 ml of ACN are placed into a 50 ml volumetric flask. Then 5 ml of Working Solution 3 are added and the volumetric flask is filled to 50 ml with ACN.

4.4 Calibration standards

Calibration standards are prepared by dilution of the working solutions in acetonitrile according to the following specifications. 2 ml vials are used for this purpose.

Tab. 1 Preparation and concentrations of the calibration solutions

Working solution	Concentration of the working solution [µg OPP/ml]	Volume of the working solution [µl]	Volume of the solvent (ACN) [µl]	Concentration Calibration standard [µg OPP/ml]	Mass per 10 µl of injection [µg OPP]
4	2	500	500	1	0.01
4	2	750	250	1.5	0.015
4	2	1000	0	2	0.02
3	20	125	875	2.5	0.025
3	20	175	825	3.5	0.035
3	20	250	750	5	0.05
3	20	375	625	7.5	0.075
3	20	500	500	10	0.10
3	20	750	250	15	0.15
3	20	1000	0	20	0.2
2	200	150	850	30	0.3

4.5 Control solutions

The reference standards are prepared as quality control samples by dilution of Stock Solution 1 and Working Solution 1 and they are regularly checked in the analytical run. 5 µl of the prepared sample are injected into the liquid chromatograph by means of the autosampler and analysed under the conditions described in Section 6. The quality control samples are prepared as follows:

Control Solution 1 for one tenth of the OELV (60 µg of OPP or 0.015 µg per injection):
20 ml of 2-propanol are placed into a 20 ml vessel made of amber glass using a dispenser and then 30 µl of Working Solution 1 are added using a microlitre syringe and shaken.

Control Solution 2 equivalent to the OELV (600 µg of OPP or 0.15 µg per injection):
20 ml of 2-propanol are placed into a 20 ml vessel made of amber glass using a dispenser and then 30 µl of Stock Solution 1 are added using a microlitre syringe and shaken.

Control solution 3 for twice the OELV (1200 µg of OPP or 0.30 µg per injection):
20 ml of 2-propanol are placed into a 20 ml vessel made of amber glass using a dispenser and then 60 µl of Stock Solution 1 are added using a microlitre syringe and shaken.

5 Sampling and sample preparation

5.1 Sampling

Sampling can be carried out as stationary or personal sampling. Measurements are taken in the breathing zone in the case of personal sampling. It is important to ensure that the inlet of the sampling head is freely accessible.

The quartz fibre filter free from binding agents is inserted into the GSP sampling head, an adsorption tube (silica gel) is connected on one side of the GSP sampling head by means of a piece of tube and connected to the pump on the other

side. The adsorption tube must be directly connected to the GSP sampling head without any gap. This is necessary to avoid sorption onto the piece of tube. If a connection without gaps proves impossible, then the connection tube must also be analysed. The air sample is drawn through the combined sampling system (GSP, silica gel tube) by means of a flow-regulated pump at a flow rate of 1 l/min. After 2 hours of sampling, this is equivalent to an air sample volume of 0.12 m^3. The important parameters for the determination of the concentration in air (sample volume, temperature, air pressure and relative humidity) are documented in the sampling record.

After sampling, the flow rate must be tested for constancy. If the deviation from the adjusted flow rate is ≥ ± 5%, it is advisable to repeat the measurement. The sample carriers are sealed air-tight and are transported to the laboratory at room temperature and kept sealed there until preparation. After sampling, the filters are transferred into vessels made of amber glass, in order to avoid sample losses through evaporation.

5.2 Sample preparation

The loaded quartz fibre filter is transferred into a 20 ml vessel made of amber glass using tweezers. The silica gel tubes are opened and the contents of the tubes (including the control layer) are transferred into a 20 ml vessel made of amber glass. If necessary, the piece of tube between the GSP sampling head and the silica gel tube can also be transferred into a 20 ml vessel. Then all the vessels (filter and silica gel, piece of tube if applicable) are spiked with 20 ml of 2-propanol. Subsequently, the vessels are shaken for at least one hour at 200 rpm. The extracts are filtered and an aliquot is transferred into 2-ml vials. The vials are placed into the autosampler; the extracts are analysed in the HPLC.

A blank value determination is performed with each sample series. For this purpose a filter, an adsorption tube and, if necessary, a piece of tube are subjected to the entire sample preparation and analysed.

6 Operating conditions for HPLC-DAD

Apparatus:	HPLC device with DAD and autosampler, e.g. HPLC 20 Nexera XR, from Shimadzu GmbH	
Separation Column:	Material:	NUCLEOSIL 100-5 C18
	Length:	250 mm
	Inner diameter:	3 mm
	Particle size:	5 µm
	Column temperature:	25 °C
Flow rate (sample):	0.5 ml/min	
Lamp:	D_2	
Measured wavelength:	285 nm	
Mobile phase:	55% acetonitrile 45% ultrapure water	
Injection volume:	5 / 10 µl	
Run time:	15 min	

7 Analytical determination

5 µl of the prepared sample are injected into the liquid chromatograph by means of the autosampler and analysed under the conditions described in Section 6.

If the measured concentrations are above the calibration range, then a suitable dilution of the sample to be measured must be prepared with 2-propanol and analysis must be carried out anew.

8 Calibration

The calibration solutions described in Section 4.4 are used to obtain the calibration functions.

In each case 10 µl of the calibration solutions are injected and analysed in the same manner as the sample solutions. The resulting peak areas are plotted versus the corresponding concentrations. The calibration function is typically linear in the investigated concentration range.

Control samples must be analysed each working day to check the calibration function. The calibration must be performed anew if the analytical conditions change or the quality control results indicate that this is necessary.

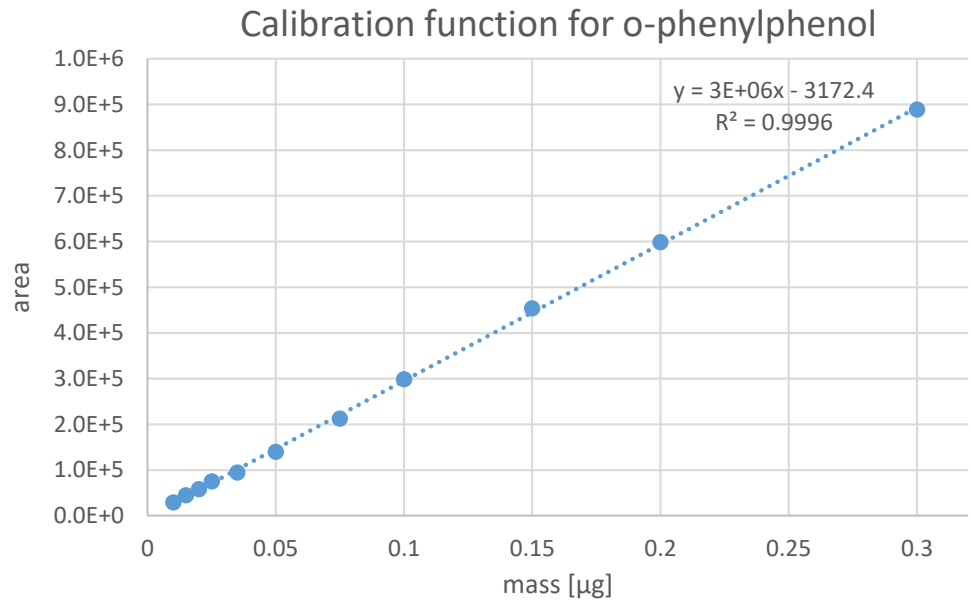

Fig. 2 Calibration function for OPP

9 Calculation of the analytical result

The concentration of OPP in the workplace air is calculated from the concentration of OPP in the measurement solution by the data evaluation unit. The data evaluation unit uses the calculated calibration functions for this purpose. The concentrations of OPP in the workplace air are obtained from the OPP concentrations, taking the corresponding dilutions and the air sample volume into account.

The concentration by mass of the analyte is calculated using Equation 1:

$$\rho = \frac{(C - C_{blank}) \times 0.001 \times f_d \times V}{V_{air}} \quad (1)$$

where:

ρ is the mass concentration of OPP in the air sample in mg/m³

C is the concentration of OPP in the measured solution in µg/l

C_{blank} is the concentration of the blank value in µg/l

0.001 is the conversion factor [µg → mg]

f_d is the dilution factor

V is the volume of the sample solution in l

V_{air} is the air sample volume in m³

10 Reliability of the method

The characteristics of the method were calculated as stipulated in DIN EN 482 (DIN 2021 a), DIN EN ISO 22065 (DIN 2021 b), DIN EN 13205-1 (DIN 2014 a), DIN EN 13936 (DIN 2014 b) and DIN 32645 (DIN 2008).

10.1 Precision, recovery and expanded uncertainty

In order to determine the precision and expanded uncertainty, six quartz fibre filters in each case were spiked with different masses of OPP (60 µg, 600 µg, 1200 µg). Six filters were each spiked with 30 µl (equivalent to a content of 600 µg) of Stock Solution 1 (20 mg/ml) and six further filters were spiked with 60 µl each (equivalent to a content of 1200 µg). Six filters were spiked with 30 µl (equivalent to a content of 60 µg) of Working Solution 1 (2 mg/ml).

In each case, a silica gel collection tube was connected downstream from the loaded quartz fibre filter and then air was drawn through the combined sampling system for two hours at a flow rate of 1 l/min. Then the filters and collection tubes were subjected to all the steps of the sample preparation and analysis, as described in Sections 5.2, 6 and 7.

The sample solutions prepared from the spiked filters and collection tubes connected downstream were analysed after preparation. For an air sample volume of 120 l these spiked masses are equivalent to OPP concentrations of 0.5 mg/m³, 5 mg/m³ and 10 mg/m³ in air. The precision data were calculated from these results and are shown in Table 2. The precisions and recovery data are the sum of OPP on the filter and silica gel tubes and, if applicable, on the connection tube. The distribution of OPP between filter and collection tube (particle and vapour phase) varies according to the investigated concentration. At OPP concentrations of 0.5 mg/m³, 5 mg/m³ and 10 mg/m³ and after two hours of simulated sampling at room temperature and 40% relative humidity OPP masses of approx. 24%, 40% or 51% were recovered from the filter, the rest from the collection tube.

Tab. 2 Standard deviation (rel.) and expanded uncertainty U for n = 6 determinations

Spiked mass [µg]	Concentration [a] [mg/m³]	Recovery [%]	SD (rel.) [%]	Exp. uncertainty U [%]
60	0.5	88	3	30
600	5	97.1	11	28.2
1200	10	96.6	3.8	27

[a] The concentration is based on a sampling period of two hours at a flow rate of 1 l/min

The expanded uncertainty is obtained by estimation of all the relevant influencing parameters. The uncertainty of the result consists of two important contributions, the uncertainty components for sampling and for analysis.

In order to estimate the uncertainty components of sampling, the uncertainty associated with the air sample volume and the sampling efficiency for inhalable dusts were determined according to Appendix C of ISO 21832 (DIN 2020).

The combination of all uncertainty contributions results in the concentration-dependent combined uncertainties of the entire method. The values for the expanded uncertainty of the entire method listed in Table 2 are obtained by multiplying with the expansion factor k = 2.

10.2 Limit of quantification

The limit of quantification was determined on the basis of standard DIN 32645 (DIN 2008). The limit of quantification was calculated after carrying out a 13-point calibration in the lower concentration range of 20–260 ng/ml and for an injection volume of 10 µl.

The limit of quantification was 0.55 ng of OPP absolute (based on an injection of 5 µl) or 0.0183 mg/m^3 for an air sample volume of 120 litres (flow rate of 1 l/min and a sampling period of 2 h).

10.3 Influence of the relative humidity

The influence of the humidity was examined at concentrations of a tenth up to twice the OELV at relative humidities of approx. 40 and 80%. No influence from the relative humidity on the sum of OPP on the filter and collection tube could be detected. The deviations discovered in the recovery (the sum of particles and vapours) were significantly less than 5%.

10.4 Capacity of the sampling system

Breakthrough experiments by spiking OPP concentrations of twice the OELV were carried out to determine the capacity of the sample system used. Then air with a relative humidity of approx. 80% was drawn through the sampling system for three hours at room temperature. In this case the control layer of the silica gel tube was analysed separately.

After three hours no breakthrough was observed. The recovery (the sum from the filter and collection tube) was 95.3%. Less than 0.5% of the concentration was detected in the control layer.

10.5 Storage stability

The storage stability was determined by spiking six sample carriers each with standard solution as described in Section 10.1. Then air was drawn through them for two hours at a flow rate of 1 l/min. The filters were transferred into 20 ml amber glass vessels, which were sealed and stored for 14 days at room temperature. The collection tubes were sealed with air-tight plastic caps and stored for 14 days at room temperature. The filters and collection tubes were then prepared and analysed as described in Sections 5, 6 and 7.

The mean recovery for the sum from filters and collection tubes after two weeks storage was 90.3%. A further measurement series with two sample carriers each per concentration resulted in a mean recovery of 87.2% after four weeks of storage at room temperature. It is not advisable to store OPP samples for more than two weeks before they are extracted and analysed.

Comparative measurements were carried out to check whether the spiked filters must be eluted immediately after sampling. For this purpose six sample carriers were each loaded with 600 µg of OPP. Then laboratory air was drawn through them at a flow rate of 1 l/min for two hours (equivalent to 5 mg/m^3, i.e. the OELV). Two filters were stored in amber glass vessels, two filters in amber glass vessels additionally sealed with parafilm and two filters were immediately covered with 2-propanol after being loaded and also sealed with parafilm. The corresponding silica gel tubes were each sealed with airtight plastic caps. Analysis of the filters and silica gel tubes was performed after two weeks of storage. As Table 3 shows, no significant differences could be found for the sum from filters and silica gel tubes between simple storage of the filters, storage with additional sealing or filters that were eluted immediately.

Tab. 3 Comparative measurements for filter differently stored for two weeks

Storage (2 weeks)	Recovery [%]
Amber glass vessels with caps	92.9
Bottles additionally sealed with parafilm	92.1
Covered with 2-propanol, sealed with parafilm	94.0

10.6 Selectivity

The analytical procedure by means of HPLC is specific and robust under the conditions stated here. No interference was detectable. A chromatographic distinction between the three substances that most commonly occur in biocide products based on OPP is ensured (Figure 2).

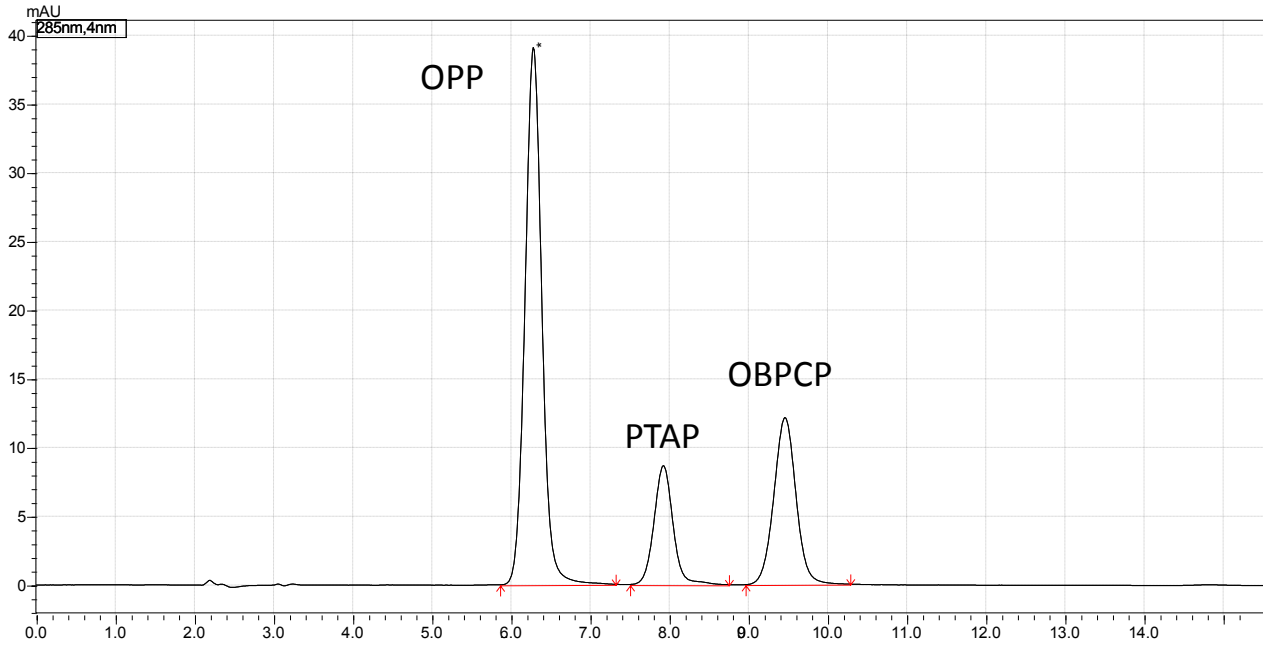

Fig. 3 Example of a chromatogram for the liquid chromatographic separation of three biocide products (OPP: o-phenylphenol; PTAP: p-tert-amylphenol; OBPCP: o-benzyl-p-chlorophenol)

11 Discussion

The analytical procedure described here permits the determination of OPP in the workplace air in a concentration range from a tenth to twice the currently valid OELV of 5 mg/m^3 in the inhalable fraction. Furthermore, the analytical method is suitable for monitoring compliance with the short-term value.

A combined vapour-particle sampling system is necessary for measurement of OPP at the workplace. The investigations carried out have shown that OPP can evaporate from filters. Approx. 50% of the OPP mass (at twice the OELV; 10 mg/m^3) was recovered from the silica gel tubes after two hours of sampling.

Principally all working conditions, in particular sample preparation und and analytical conditions must be adapted to the respective HPLC device used.

Notes

Competing interests

The established rules and measures of the Commission to avoid conflicts of interest (www.dfg.de/mak/conflicts_interest) ensure that the content and conclusions of the publication are strictly science-based.

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