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Diphenyl ether – Determination of diphenyl ether in workplace air using high-performance liquid chromatography (HPLC-DAD)

Air Monitoring Method – Translation of the German version from 2022

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Keywords

diphenyl ether; air analyses; analytical method; workplace measurement; hazardous substance; high-performance liquid chromatography; quartz fibre filter; vapour particle mixture

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Abstract

This analytical method is a validated measurement procedure for the determination of diphenyl ether [101-84-8] in workplace air in a concentration range of one tenth up to twice the currently valid Occupational Exposure Limit Value (OELV) in Germany of 7.1 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 0.5 l/min and sampling duration is 2 hours (which corresponds to a sampling volume of 60 l). Diphenyl ether 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 0.008 mg/m^3 based on an air sample volume of 60 l. The mean recovery is 95.3% and the expanded uncertainty for the validation range of 0.71 to 14.2 mg/m^3 is 24 to 25%.

Method number	1
Application	Air analysis
Analytical principle	High-performance liquid chromatography with diode array detection (HPLC-DAD)

1 Characteristics of the method

Precision:	Standard deviation (rel.):	s = 2 to 9%
	Expanded uncertainty:	U = 24 to 25%
	in the concentration range from 0.71 to 14.2 mg/m³ and for n = 6 determinations	
Limit of quantification:	0.008 mg/m³ for an air sample volume of 60 l and a sampling period of 2 h	
Recovery:	η = 0.94 to 0.97 (94 to 96%)	
Sampling recommendations:	Sampling period:	2 h
	Air sample volume:	60 l
	Flow rate:	0.5 l/min

2 Description of the substance

Diphenyl ether (DPE) [101-84-8]

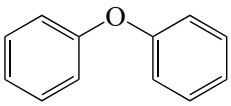

Fig. 1 Structural formula of DPE

DPE is a colourless, crystalline solid with a geranium-like odour (molecular mass 170.2 u, melting point 26.8 °C, boiling point 258 °C, density 1.07 g/cm³).

The OELV for DPE is 7.1 mg/m³ as the sum of the inhalable dust fraction and the vapour. For short-term exposures, the substance is classified in Peak Limitation Category I with an excursion factor of 1 (AGS 2022). The MAK value for DPE is the same as the OELV (DFG 2022). DPE is designated in the TRGS 900 (AGS 2022) and in the List of MAK and BAT Values (DFG 2022) as a vapour particle mixture, meaning that DPE can occur in particulate as well as in vaporous form. During sampling, a sampling device must be used that can simultaneously capture the inhalable dust fraction as well as the vapour.

3 General principles

The analytical method enables the determination of the content of diphenyl ether in the inhalable dust fraction of the workplace air in the range of a tenth to twice the currently valid OELV of 7.1 mg/m³.

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 the substances and the contents of the silica gel tube and, if necessary, the piece of connecting tube are transferred together into an amber glass vessel, covered with 2-propanol and shaken. The determination is carried out by means of HPLC-DAD. A linear calibration function is used for quantitative evaluation.

4 Equipment, chemicals and solutions

4.1 Equipment

For sampling:

- Sampling pump for personal or stationary sampling, suitable for a flow rate of 0.5 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 GmbH, 52068 Aachen, Germany)

For the sample preparation and the analytical determination:

- Ultrapure water unit (e.g. Millipore-Q-Gradient with Elix 3UV, from Merck KGaA, 64293 Darmstadt, Germany)
- Variable piston pipettes 10 to 100 µl and 100 to 1000 µl (e.g. Reference 2, from Eppendorf SE, 22366 Hamburg, Germany)
- Bottle attachment dispenser 1 to 10 ml (e.g. Dispensette S analog, from Brand GMBH + CO KG, 97877 Wertheim, Germany)
- Tube cutter (e.g. Supelco, from Merck KGaA, 64293 Darmstadt, Germany)
- 20 ml amber glass vessels with screw-caps (e.g. from CS-Chromatographie GmbH, 52379 Langerwehe, Germany)
- Laboratory compact shaker (e.g. KS 14 A Control Compact Shaker, from Edmund Bühler GmbH, 72411 Bodelshausen, Germany)
- Volumetric flasks (50 ml, glass) with glass stoppers (e.g. from Brand GmbH + CO KG, 97877 Wertheim, Germany)
- Chromafil syringe filter RC, pore width 0.45 µm, Ø 25 mm (e.g. from Carl Roth GmbH & Co. KG, 76185 Karlsruhe, Germany)
- Disposable syringes, 5 ml, made of polyethylene
- Analytical balance (e.g. XPE-20S Delta Range, from Mettler-Toledo GmbH, 35396 Gießen, Germany)
- Tweezers (e.g. from Plano W. Plannet GmbH, 35578 Wetzlar, Germany)
- High-performance liquid chromatograph with DAD (e.g. 20 Nexera XR HPLC, from Shimadzu GmbH, 47269 Duisburg, Germany)
- Autosampler (e.g. SIL-20AC XR, from 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 GmbH & Co. KG, 52335 Düren, Germany)
- Microlitre syringes, 50 µl and 200 µl (e.g. from Hamilton-Bonaduz AG, Bonaduz, Switzerland)

4.2 Chemicals

- Diphenyl ether (DPE), purity 99% (e.g. from Merck KGaA, 64293 Darmstadt, Germany)
- Biphenyl, purity 99% (e.g. from Merck KGaA, 64293 Darmstadt, Germany)
- *o*-Phenylphenol, purity 99% (e.g. from Merck KGaA, 64293 Darmstadt, Germany)
- 2-Propanol for HPLC, purity 99% (e.g. from Merck KGaA, 64293 Darmstadt, Germany)
- Acetonitrile (ACN), Rotisolv HPLC, ultra grade (e.g. from Carl Roth GmbH, 76185 Karlsruhe, Germany)
- Ultrapure water ($\rho \geq 18.2$ MΩ × cm at 25 °C) (e.g. Millipore, from Merck KGaA, 64293 Darmstadt, Germany)

4.3 Solutions

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

DPE Stock Solution 1: (14.2 mg DPE/ml in 2-propanol):
710 mg of DPE are 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 (1.42 mg DPE/ml in 2-propanol):
Approx. 30 ml of 2-propanol are placed into a 50-ml volumetric flask. Then, 5 ml of Stock Solution 1 are added. The volumetric flask is filled to the mark with 2-propanol and shaken.

Working Solution 2: 1:100 dilution of Stock Solution 1 (142 µg DPE/ml):
Approx. 30 ml of ACN are placed into a 50-ml volumetric flask. Then, 0.5 ml of Stock Solution 1 is added. The volumetric flask is filled to the mark with ACN and shaken.

Working Solution 3: 1:10 dilution of Working Solution 2 (14.2 µg DPE/ml):
Approx. 30 ml of ACN are placed into a 50-ml volumetric flask. Then, 5 ml of Working Solution 2 are added. The volumetric flask is filled to the mark with ACN and shaken.

Working Solution 4: 1:10 dilution of Working Solution 3 (1.42 µg DPE/ml):
Approx. 30 ml of ACN are placed into a 50-ml volumetric flask. Then, 5 ml of Working Solution 3 are added. The volumetric flask is filled to the mark with ACN and shaken.

4.4 Calibration standards

Calibration standards are prepared by dilution of the working solutions in acetonitrile according to the following specifications (see Table 1). 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 DPE/ml]	Volume of the working solution [µl]	Volume of the solvent (ACN) [µl]	Concentration of the calibration standard [µg DPE/ml]	Mass per 5 µl of injection [ng DPE]
4	1.42	300	700	0.426	2.13
4	1.42	500	500	0.71	3.55
4	1.42	750	250	1.065	5.325
3	14.2	150	850	2.13	10.65
3	14.2	300	700	4.26	21.3
3	14.2	500	500	7.1	35.5

Tab. 1 *(continued)*

Working solution	Concentration of the working solution [µg DPE/ml]	Volume of the working solution [µl]	Volume of the solvent (ACN) [µl]	Concentration of the calibration standard [µg DPE/ml]	Mass per 5 µl of injection [ng DPE]
3	14.2	750	250	10.65	53.25
3	14.2	1000	0	14.2	71
2	142	150	850	21.3	106.5
2	142	300	700	42.6	213

4.5 Control solutions

The reference standards are prepared as quality control samples from dilutions of Stock Solution 1 and Working Solution 1 and 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 were prepared as follows:

Control Solution 1 for one tenth of the OELV (42.6 µg of DPE in 20 ml of extraction solution or 10.65 ng per injection): 20 ml of 2-propanol are placed into a 20-ml vessel made of amber glass using a dispenser. Then, 30 µl of Working Solution 1 are added using a microlitre syringe and the vessel is shaken.

Control Solution 2 equivalent to the OELV (426 µg of DPE in 20 ml of extraction solution or 106.5 ng per injection): 20 ml of 2-propanol are placed into a 20-ml vessel made of amber glass using a dispenser. Then, 30 µl of Stock Solution 1 are added using a microlitre syringe and the vessel is shaken.

Control Solution 3 for twice the OELV (852 µg of DPE in 20 ml of extraction solution or 213 ng per injection): 20 ml of 2-propanol are placed into a 20-ml vessel made of amber glass using a dispenser. Then, 60 µl of Stock Solution 1 are added using a microlitre syringe and the vessel is shaken.

5 Sampling and sample preparation

5.1 Sampling

Sampling can be carried out as stationary or personal sampling. Samples 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 connected to the GSP sampling head without any gap. This is necessary to avoid losses from sorption onto the piece of tube. If a gap-free connection is not possible, 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 0.5 l/min. For 2 hours of sampling, this is equivalent to an air sample volume of 0.06 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 checked for constancy. If the deviation from the adjusted flow rate is ≥ ± 5%, it is advisable to repeat the measurement. The filters and the contents of the silica gel tubes are transferred into 20-ml amber glass vessels and covered with 20 ml of 2-propanol. The vessels are sealed air-tight and are transported to the laboratory at room temperature.

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 amber glass vessel. If necessary, the piece of tube between the GSP sampling head and the silica gel tube should also be transferred into a 20-ml vessel. Then, all vessels (containing filter and silica gel, piece of tube if applicable) are filled 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 by HPLC.

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

6 Operating conditions

Apparatus:	HPLC device with DAD and autosampler, e.g. 20 Nexera XR HPLC, 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):	0.55 ml/min	
Lamp:	D2	
Measured wavelength:	225 nm	
Mobile phase:	50% acetonitrile 50% ultrapure water	
Injection volume:	5 µl	

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 again.

8 Calibration

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

In each case, 5 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 (Figure 2) 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.

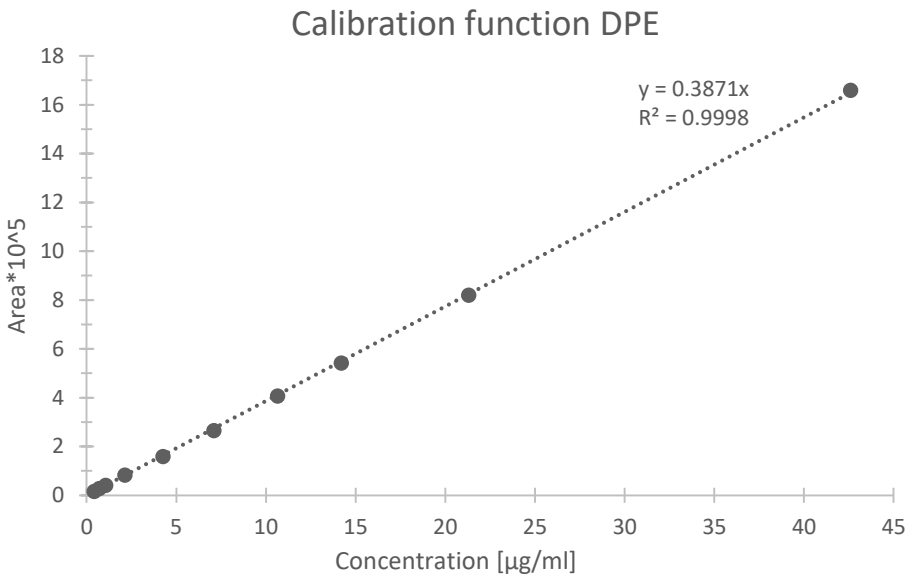

Fig. 2 Calibration function for DPE

9 Calculation of the analytical result

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

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

$$\rho = \frac{(c - c_{blank}) \times V}{V_{air}} \quad (1)$$

where:

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

c is the concentration of DPE in the measurement solution in µg/ml

c_{blank} is the concentration of the blank value in µg/ml

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), DIN EN 13936 (DIN 2014) and DIN 32645 (DIN 2008).

10.1 Recovery, precision and expanded uncertainty

In order to determine the precision and expanded uncertainty, six quartz fibre filters were each spiked with different masses of DPE (42.6 µg, 426 µg, 852 µg). Six filters were each spiked with 30 µl (equivalent to a content of 426 µg) of Stock Solution 1 (14.2 mg/ml) as well as six further filters with 60 µl each (equivalent to a content of 852 µg). Six filters were spiked with 30 µl each (equivalent to a content of 42.6 µg) of Working Solution 1 (1.42 mg/ml).

In each case, a silica gel tube was connected behind the loaded quartz fibre filter and then air was drawn through the combined sampling system for two hours at a flow rate of 0.5 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 in this manner from the spiked filters and collection tubes connected downstream were analysed after preparation. For an air sample volume of 60 l, these spiked masses are equivalent to concentrations of DPE in air of 0.71 mg/m^3, 7.1 mg/m^3 and 14.2 mg/m^3. The precision data were calculated from these results and are shown in Table 2. The precision and recovery data relate to the sum of DPE on the filters and silica gel tubes and, if applicable, the connection tube. The distribution of DPE between filter and collection tube (particle and vapour phase) varies depending on the investigated concentration.

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

Spiked mass [µg]	Concentration[a)] [mg/m^3]	Recovery [%]	Standard deviation (rel.) [%]	Expanded uncertainty *U* [%]
42.6	0.71	95.1	9	25
426	7.1	93.9	2	25
852	14.2	96.9	2	24

[a)] calculated on the basis of a 2-hour sampling period at a flow rate of 0.5 l/min

The expanded uncertainty is obtained by estimation of all the relevant influencing parameters. The uncertainty of the result consists of two important steps, 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 effectiveness for inhalable dusts were determined according to Appendix C in 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 by 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 an 11-point calibration in the lower concentration range of 14.2 to 88 ng/ml and is based on an injection volume of 5 µl.

The limit of quantification is 0.1155 ng of DPE absolute or 0.008 mg/m^3 for an air sample volume of 60 litres (0.5 l/min and a sampling period of 2 h).

10.3 Influence of the humidity

The influence of the humidity was examined at a concentration of 14.2 mg/m^3 (twice the OELV) at relative humidities of approx. 40, 60, 70 and 80%. In this manner, the relative humidity was shown to influence the breakthrough volume or the sum of DPE on the filter and collection tube. The breakthrough volume (or the breakthrough time) decreases with increasing humidity, therefore a sampling period of two hours can be recommended only for a relative humidity of less than 60%. A maximum sampling period of one hour is recommended at a relative humidity of between 60 and 70%. When the relative humidity is between 70 and 80% a maximum sampling period of 45 minutes is recommended.

10.4 Storage stability

The storage stability was determined by spiking six sample carriers each with one tenth, once and twice the OELV using standard solution. Subsequently air was drawn through them for two hours at a flow rate of 0.5 l/min. The filters and the contents of the collection tubes were transferred into 20-ml amber glass vessels, covered with 20 ml of 2-propanol and these were then sealed and stored at room temperature for 2 and 4 weeks. The extracts were then prepared and analysed as described in Sections 5, 6 and 7.

The mean recovery for the sum from filters and collection tubes was 96.5% after 2 weeks of storage. After 4 weeks of storage at room temperature, the mean recovery was 96.6%.

10.5 Selectivity

The analytical procedure by means of HPLC is specific and robust under the conditions stated here. No interference could be detected. Chromatographic differentiation between *o*-phenylphenol (OPP), DPE and biphenyl (BP) is ensured (Figure 3).

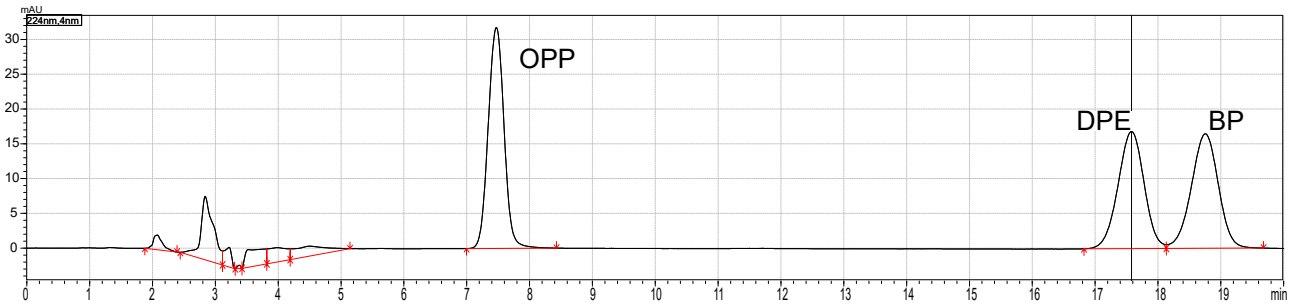

Fig. 3 Example of a chromatogram for the liquid chromatographic separation of OPP: *o*-phenylphenol, DPE: diphenyl ether, BP: biphenyl

11 Discussion

The analytical method described here permits the determination of DPE in workplace air in a concentration range from one tenth to twice the currently valid OELV of 7.1 mg/m³ as a sum of the inhalable and vaporous fraction. Furthermore, the analytical method is suitable for monitoring compliance with the short-term value.

A combined particulate and vaporous sampling is required for the measurement of diphenyl ether at workplaces.

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

Other sampling systems can be used as an alternative to the GSP sampling head, e.g. the GGP mini. In this case, a complete validation of the method is necessary.

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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