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Ambient air — Determination of particle-phase polycyclic aromatic hydrocarbons by high performance liquid chromatography

Air ambiant — Détermination des particules d'hydrocarbures aromatiques polycycliques par chromatographie liquide à haute performance

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Foreword

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International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 16362 was prepared by Technical Committee ISO/TC 146, *Air quality*, Subcommittee SC 3, *Ambient atmospheres*.

Introduction

Several polycyclic aromatic hydrocarbons (PAHs) are considered to be potential human carcinogens. PAHs are emitted into the atmosphere primarily through combustion of fossil fuel and wood. Two- and three-ring PAHs are typically present in urban air at concentrations ranging from ten to several hundred nanograms per cubic metre (ng/m^3); those with four or more rings are usually found at concentrations of a few nanograms per cubic metre or lower. PAHs possess saturation vapour pressures at 25 °C that range from 10^{-2} kPa to less than 10^{-13} kPa. Those with vapour pressures above 10^{-8} kPa may be substantially distributed between the gas phase and particle-associated (particulate) phase in the atmosphere. The distribution between phases depends on ambient temperature, humidity, types and concentrations of PAHs and particulate matter, and residence time in the air. PAHs, especially those having vapour pressures above 10^{-8} kPa, tend to vaporize from particle filters during sampling.

This International Standard allows the determination of low volatility, particle-bound PAHs, in contrast to ISO 12884[1] which allows the measurement of PAHs in the gas phase. This International Standard allows the use of a range of sampler flowrates, and requires the use of high performance liquid chromatography (HPLC) with the detection carried out by either fluorescence detection or UV absorption.

Ambient air — Determination of particle-phase polycyclic aromatic hydrocarbons by high performance liquid chromatography

1 Scope

This International Standard specifies sampling, clean-up and analysis procedures for the quantitative determination of low volatility (particle-bound) polycyclic aromatic hydrocarbons (PAHs) in ambient air. For sampling, a low-volume or a medium/high-volume sampling device may be used. Sampling times between 1 h and 24 h are possible. The sampling volume flowrates can range from 1 m^3/h to 4 m^3/h ("low volume sampler") or from 10 m^3/h to about 90 m^3/h ("medium/high-volume sampler"). In any case, the linear face velocity at the collection filter should range between about 0,5 m/s and 0,9 m/s.

The method has been validated for sampling periods up to 24 h. The detection limits for single PAHs and the standard deviations resulting from duplicate measurements are listed in 9.2 and Annex D respectively.

This International Standard describes a sampling and analysis procedure for PAH that involves collection from air onto a filter followed by analysis using high performance liquid chromatography usually with fluorescence detector (FLD). The use of a diode array detector (DAD) is possible. The combination of both detector types is also possible (see Annex B). Total suspended particulate matter is sampled.

Generally, compounds having a boiling point above 430 °C (vapour pressure less than 10^{-9} kPa at 25 °C, e.g. chrysene, benz[*a*]anthracene) can be collected efficiently on the filter at low ambient temperatures (e.g. below 10 °C). In contrast, at higher temperatures (above 30 °C, see also ISO 12884[1]), only PAHs having boiling points above 475 °C (vapour pressure less than 10^{-10} kPa at 25 °C) are determined quantitatively (see Annex F).

2 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

2.1
blank value solution
solution which contains the chemicals used in making up the sample solution batch and the constituents influencing the measurement in the same or similar concentration as the sample to be analysed, but to which the compound to be determined has expressly not been added

2.2
low-volume sampling device
sampling device with a volume flowrate of 1 m^3/h to 4 m^3/h

2.3
medium/high-volume sampling device
sampling device with a volume flowrate of 10 m^3/h to about 90 m^3/h

3 Symbols and abbreviated terms

3.1 Symbols

A_i peak area of component i

A_{IS} peak area of internal standard

ρ mass concentration

f response factors, slope of straight line

m_i mass of component i

m_{IS} mass of internal standard

M_r relative molecular mass (molecular weight)

V volume

3.2 Abbreviated terms

ASE accelerated solvent extraction

b.p. boiling point

DAD diode array detector (UV absorption)

FLD fluorescence detector

HPLC high performance liquid chromatography

PAH polycyclic aromatic hydrocarbon

SOP standard operating procedure

UV ultraviolet

WHO World Health Organization

4 Principle of the procedure

For sampling, sampling devices with volume flowrates from 1 m^3/h to about 90 m^3/h may be used. The particulate matter, onto which the PAHs are adsorbed, is collected on glass or quartz fibre filters.

The PAHs are extracted and the extract concentrated. If necessary, the extracts may be cleaned by column chromatography using silica gel.

The PAHs are determined by HPLC using DAD or FLD. For quality assurance, internal standards are added.

5 Reagents, apparatus and materials

5.1 Reagents

5.1.1 Solvents for analysis: water, acetonitrile, toluene (all solvents of chromatographic grade).

5.1.2 Solvents for sample preparation: chromatographic grade toluene, cyclohexane and acetonitrile.

The chromatograms of the solvents obtained under the conditions of the illustrative example shall not exhibit any interfering peaks.

5.1.3 Helium, purity 99,999 %; for degasification of solvents.

To avoid interferences, no plastic hoses shall be employed, preferably metal hoses are recommended.

5.1.4 Internal standard

If using DAD: indeno[1,2,3-*cd*]fluoranthene dissolved in toluene, mass concentration e.g. 3 µg/ml (see 6.2). If using FLD: 6-methylchrysene.

5.1.5 Calibration standards

Cyclopenta[*c,d*]pyrene	CPP
Benz[*a*]anthracene	BaA
Chrysene	CHR
Benzo[*b*]fluoranthene	BbF
Benzo[*j*]fluoranthene	BjF
Benzo[*k*]fluoranthene	BkF
Benzo[*a*]pyrene	BaP
Benzo[*e*]pyrene	BeP
Indeno[1,2,3-*cd*]pyrene	INP
Dibenz[*a,h*]anthracene	DBahA
Dibenz[*a,c*]anthracene	DBacA
Benzo[*g,h,i*]perylene	BghiP
Anthanthrene	ANT
Coronene	COR
Dibenzo[*a,l*]pyrene	DBalP
Dibenzo[*a,i*]pyrene	DBaiP
Dibenzo[*a,e*]pyrene	DBaeP
Dibenzo[*a,h*]pyrene	DBahP
Benzo[*a*]chrysene (= picene)	BaC

5.2 Apparatus

5.2.1 Sampling device, consisting of the following parts (commercially available).

5.2.1.1 Sampling head, usually containing the filter.

5.2.1.2 Pumping system, e.g. sliding vane-pump or turbine.

5.2.1.3 Volume meter, for measuring the sample volume or a flowrate-measuring device.

5.2.1.4 Electronic or mechanical device, to establish a constant flow.

5.2.1.5 Timer, for selecting the time and duration of the sampling.

5.2.1.6 Blunt tweezers (optional), for handling the filters.

5.2.2 Sample preparation equipment

The PAH extraction (see 7.2) is carried out using ordinary laboratory equipment. This may include:

5.2.2.1 Flasks/reflux condenser, round-bottomed flask (e.g. 250 ml, or 100 ml if the small filter device is used) with matched reflux condenser and heating bath, or

5.2.2.2 Ultrasonic bath, beaker, capacity e.g. 50 ml or 100 ml, or

5.2.2.3 Soxhlet extractor, capacity e.g. 30 ml to 50 ml, cellulose extraction thimble, round-bottomed flask (100 ml) with reflux condenser and heating bath, or

5.2.2.4 ASE apparatus, device for extracting samples at elevated temperatures and under high pressure.

5.2.2.5 Vacuum pump, e.g. a membrane or water-jet pump**.**

5.2.2.6 Centrifuge, with inserts; e.g. of volume 20 ml each.

5.2.2.7 Chromatography column, internal diameter e.g. 10 mm, length 230 mm (silica gel column).

5.2.3 Analytical apparatus

5.2.3.1 High performance liquid chromatograph, fitted with an isothermal column device, solvent purge system, gradient pump system and a FLD or DAD.

5.2.3.2 Separation columns, reverse phase-sorbent columns optimized for PAH analysis (see Annex G).

5.2.3.3 Recording equipment, work station with screen and printer/plotter for acquiring, processing, storing and interpreting the data and the possibility of a later baseline correction.

5.2.3.4 GC microliter syringes, suitable for metering aliquots.

5.3 Materials

5.3.1 Collection filter, glass or quartz fibre filters, collection efficiency better than 99,9 % for particles $< 0{,}5$ µm in diameter, without organic binder, appropriate for the sampling device (circular or square).

NOTE Filters coated or impregnated with polytetrafluoroethene (PTFE) have been used for collection of particle-associated PAHs [2]. Use of these filters, in lieu of those specified, requires validation of their performance by the user.

5.3.2 Sorbent for column chromatography

Silica gel, high purity grade, type 60, particle diameter 70 µm to 200 µm; 15 % mass fraction of water is added 24 h before use. To pack the column, a slurry is formed of 10 g of moistened silica gel in 40 ml of cyclohexane. The slurry, freed from air bubbles by shaking, is packed into the chromatography column. Prior to use, the cyclohexane is drawn off until the level of liquid drops to the surface of the silica gel layer.

6 Measurement procedure

6.1 Sampling

Choose a sampling device appropriate to the measurement task.

Label each collection filter in the laboratory, and, by means of tweezers, place it into the appropriate filter holder. Ensure that the labelling material is not extracted. Fix the filter with a supporting ring. Put the filter holder, with the filter inserted, into a Petri dish and place it in an airtight shipping container (transport box) for transport to the measuring site. At the measuring site, insert the filter holder containing the filter into the sampling head, which is connected to the suction tube, and fix it.

Set the sampling time from 1 h to 24 h, depending on the sampling task. Set the pump and the timer in operation synchronously.

If the flow-controlling device is used in combination with a total volume meter, the sample volume is derived from the volume meter readings at the beginning and the end of the sampling period.

If the flow-controlling device is combined with a flowrate-measuring device, the sample volume is derived from the average flowrate (calculated from the flowrates at the least at the beginning and end of the measurement period) and the elapsed time.

The flowrate-measuring device should also be used to check proper operation of the flow-controlling device at the beginning and end of the sampling period.

Turn the pump off after sampling. Remove the filter holder with the exposed filter from the sampling head. Put the filter holder containing the filter again into a Petri dish and place it in an airtight shipping container (transport box) with the exposed filter side facing upwards for transport. Transport the shipping container (transport box) horizontally. In the laboratory, remove the exposed filter from the filter holder with the aid of tweezers.

Prior to extraction, store the filter in the dark at ambient temperature or below.

At least 10 % of the samples, or a minimum of one per sampling site if fewer than 10 samples are taken at the site, shall be field blanks.

6.2 Sample preparation

Check the purity of the filters and glassware and the purity of the solvents and reagents. For this purpose, add the internal standard solution to an unexposed filter and subject it to the entire analytical procedure (blank value).

The blank values are not taken into account in the calculation, but shall not exceed 10 % of the value of the sample or 10 % of a limit/guide value to be monitored.

Protect the samples and sample solutions against direct light during preparation.

For the extraction, place the filter in a 250 ml round-bottomed flask, cover it with 150 ml of toluene (or 70 ml of toluene in a 100 ml round-bottomed flask if small filters are used) and add then 50 µl of the internal standard solution (see 5.2.2.3). Toluene is especially suitable for the extraction of PAHs[3]. If other solvents (e.g. dichloromethane, acetonitrile) are used, the procedure shall have been validated using NIST standards. Insert the reflux condenser and heat the contents of the flask to boiling for about 20 min. Separate the extract from the filter material and dust particles by filtration.

The extraction may also be performed quantitatively by various other methods:

— in a Soxhlet apparatus (toluene: 8 h, at least 10 cycles per hour);

— in the ultrasonic bath in a beaker (toluene: at least 15 min);

— in an ultrasonic bath followed by centrifugation. The filter is cut into pieces and placed in centrifuge tube, followed by the addition of 15 ml toluene. The tubes are placed in the bath and extracted for 15 min. They are then centrifuged (10 min, 3 000 r/min) and the solvent is decanted. The whole extraction procedure is repeated. Both extraction solvents are combined;

— by accelerated solvent extraction (ASE). The filter is placed in the extraction vessel and extracted at a temperature of 150 °C with toluene.

All methods shall be validated.

Concentrate the toluene solution to few microlitres under reduced pressure (e.g. 13 kPa). Keep this evaporation step under observation at all times.

NOTE If evaporation is continued to dryness and the residue allowed to remain for a time in a vacuum, some PAHs could be lost.

If further clean-up is necessary, add 2 ml of cyclohexane.

Add the cyclohexane solution to the pre-prepared silica gel column (see 5.2.2.7) using a syringe. Rinse the flask then with 2 ml of cyclohexane which is also added with a syringe to the silica gel column. Carry out the elution using 100 ml of cyclohexane. To remove the cyclohexane, concentrate the eluate to a volume of a few milliliters and evaporate it then under a nitrogen stream almost to dryness. Dissolve the residue then in the correct solvent for injection into the HPLC column, e.g. 100 µl of acetonitrile.

NOTE If the origin of the air sample is known and interferents are low, the clean-up using a silica gel column may be unnecessary.

If the extract is not analysed immediately, it shall be stored in a refrigerator. Before analysis, it is allowed to warm to room temperature.

6.3 HPLC analysis

Inject an aliquot of the sample (e.g. 20 µl) into the HPLC apparatus.

An example of operation conditions for HPLC analysis with FLD and DAD in series is given in Annex B.

7 Establishment of the calibration function and verification of the measurement values

7.1 Identification

The separation conditions are optimized with aid of multi-component calibration standard solutions (see 5.1.5), which gives adequate separation of the compounds of interest.

A component in the sample is primarily identified by comparison of its retention time to that of the same substance in the calibration solution analysed under identical conditions. The level of identity shall be reported.

The concentrations of the calibration standards should be in the range of (depending on the measurement task):

— 2 ng/ml to 200 ng/ml, when a FLD is used;

— 20 ng/ml to 1 000 ng/ml, when a DAD is used.

7.2 Instrument calibration

Prepare calibration standard solutions of different concentrations for each new substance i to be determined using acetonitrile. The calibration does not include the overall measurement procedure. Handle the calibration standard solutions identically to the sample solutions.

Starting with the lowest concentration, inject the standard solution three times into the HPLC. Keep the injection volume as well as the other parameters of the calibration and the sample measurement constant.

The functions shall be presented as a graph in a diagram. To achieve this, plot the measurement values A_i (peak areas in the units of the integration systems) for each substance i on the axis as ordinates and the corresponding mass concentrations m_i of substance i on the abscissa. When using an internal standard, plot the ratios of the measurement values of A_i of substance i to A_{IS} of the internal standard as ordinate and the ratios of the corresponding mass concentrations of m_i of substance i to m_{IS} of the internal standard on the abscissa.

The basic function equation and the correlation coefficient are calculated and are part of the analytical result.

If an electronic integration system is used, the determined peak areas shall be checked for plausibility by means of the plotted chromatogram. The measurement values shall be stored as complete raw data to enable re-integration.

7.3 Determination of response factors and quantification

Prior to analysis, determine the response factors for the PAHs by injecting a calibration solution containing known masses of the components of interest and the internal standard (e.g. 6-methylchrysene for FLD or indeno[1,2,3-*cd*]fluoranthene for DAD) at least three times. Calculate the response factors f_i by comparing the peak areas in the chromatogram and the corresponding masses of the substances according to Equation (1).

$$f_i = \frac{A_{IS} \quad m_{ic}}{A_{ic} \quad m_{IS}} \tag{1}$$

where

f_i is the response factor of the ith PAH compound;

A_{IS} is the area of the internal standard in the chromatogram of the calibration solution;

A_{ic} is the area of the ith PAH compound in the chromatogram of the calibration solution;

m_{ic} is the mass of the ith PAH compound in the calibration solution;

m_{IS} is the mass of the internal standard in the calibration solution.

The average values of the response factors for the three injections can be used for further analysis.

Quantitative determination of the PAH compounds in the sample extracts is performed by the internal standard method. Before sample preparation, add a known mass of the internal standard (e.g. 6-methylcrysene in the case of FLD or indeno[1,2,3-*cd*]fluoranthene in the case of DAD) to the sample. This should be equivalent to three to five times the mass of benzo[*a*]pyrene. A guide value which may be used as a basis is 1 ng/m^3 to 10 ng/m^3 of benzo[*a*]pyrene in the sample.

Calculate the masses of the PAH compounds in the sample extracts according to Equation (2).

$$m_{iE} = \frac{f_i \quad A_{iE} \quad m_{ISE}}{A_{ISE}} \tag{2}$$

where

A_{ISE} is the area of the internal standard in the chromatogram of the sample extract;

m_{ISE} is the mass of the internal standard in the sample extract;

$A_{i\,E}$ is the area of the ith PAH compound in the chromatogram of the sample extract;

$m_{i\,E}$ is the mass of the ith PAH compound in the sample extract.

For verification of the analytical procedure, at the beginning of an analytical series and at an interval of 10 to 15 samples, carry out a control analysis using calibration solutions.

To calibrate the overall determination procedure, place at least one aliquot of one calibration solution into a 250 ml round-bottomed flask (when the medium/high-volume sampler is used) or into a 100 ml round-bottom flask (when the low-volume sampler is used); add thereafter 150 ml or 70 ml of toluene, respectively, and shake the mixture. Then add the internal standard and a blank filter and prepare the mixture in accordance with 6.2. All apparatus, solvents and operating steps which are normally employed in the analysis of the samples shall be included. The deviations of the response factors f_i, that are obtained on calibrating the overall measurement procedure, shall not differ from each other by more than 10 %. If the differences are greater, the system shall be checked. After the reason has been found carry out a complete recalibration.

The measurement procedure shall be calibrated with a multi-component calibration standard solution (see 5.1.5) in which the analyte is included. The calibration function has to be linear for the range of the complete measurement procedure.

In the context of this International Standard, the complete measurement procedure comprises all steps beginning with the filter preparation. It is not possible to include sampling into the calibration step.

The linear range shall be determined over a range of at least 5 different concentrations. The calibration function determined for a substance is only valid for the calibrated concentration range. Furthermore, the calibration function depends on the operating conditions of the chromatographic systems and shall be checked regularly. For routine measurements it is sufficient to adjust the calibration function by a one-point-check.

The calibration with standard solutions of different concentrations within the linear range of the detector gives information about performance characteristics. Generally, the signal of the compound to be determined in the sample has to be in the linear range of the detector used. In case where this requirement is not met, a statement must be included in the quality manual.

The mass concentration of an identified substance in a sample is calculated by comparing the signal intensity (peak area) in the chromatogram to the intensity of the reference compound.

The concentration determination is independent of the sample preparation steps, from losses during the preparation, from inaccuracies in injection and in certain cases from matrix effects.

In some cases the use of an internal standard may be limited to the detection with the DAD, due to the fact that not all PAHs are fluorescence-active.

The substance used as an internal standard shall have physical and chemical characteristics (extraction behaviour, vapour pressure, retention time, detection response) similar to the substance to be determined. This or another substance with the same retention time shall not be present in the sample. The mass of the internal standard added before the analysis shall be adapted to the measurement task, the concentration is chosen such that the signal of the internal standard is in the linear range of the detector and is greater than the signal of the substance to be determined, but is smaller than ten times the signal.

Often, the choice of a suitable substance is difficult. However, 6-methylchrysene is recommended if FLD is used and indeno[1,2,3-*cd*]fluoranthene is recommended if DAD is used.

7.4 Determination of the extraction efficiency

To check the sample preparation, determine the extraction efficiency of the most common and important PAHs by spiking blank filters with standard solutions. The compounds are extracted and analysed by the method described. The efficiencies should fall between 50 % and 150 %. Samples for which the extraction efficiencies are less than 50 % or more than 150 % shall be discarded. Some results from laboratory tests are summarized in Table C.1 in Annex C.

8 Calculation of the result

The analytical result is calculated as follows:

$$\rho_i = \frac{m_i}{V} \tag{3}$$

where

ρ_i is the concentration of component i, in micrograms per cubic metre;

m_i is the mass of component i, in micrograms [see Equation (1)];

V is the sample volume, in cubic metres.

If the sample volume shall be reported on the basis of standard conditions (101,325 kPa; 273,15 K) or other reference conditions, pressure, temperature, humidity or other parameters shall be measured during sampling as necessary.

9 Performance characteristics

9.1 Standard deviations of the overall measurement procedure

The extracts resulting from the analytical procedures are chromatographed by means of HPLC in combination with an FLD or alternatively a DAD. The linear range is set depending on the application and the expected PAH concentrations. The working range differs for both detectors. Using a DAD, the lower detection limit is considerably higher compared to that of an FLD. The DAD has the advantage of a greater linear working range.

The analytical performance characteristics depend on the injection quantities and the concentration of the extracts, as well as on the modes of application of the detectors and the detector-specific configurations. In the case of DAD these are: the choice and the bandwidth of the wavelengths as well as the resolutions of the detectors. For the FLD these are: the choice of the wavelengths, the excitation light intensity and the detector geometry as well. According to the measurement tasks, a suitable working range has to be fixed by the choice of the parameters available. The lower limit of the working range is defined by the lower detection limit. The upper limit of the working range may be adjusted by dilution of the solutions.

In Table D.1, as an example, the results of collocated duplicate measurements for each PAH quantified by DAD are listed, together with the standard deviation and the mean values.

9.2 Detection limits

For a routine method in field studies, the following detection limits (signal/noise = 3/1) should be achievable: the injection volume is 20 µl:

Table 1 — Detection limits

PAH	FLD ng/ml	DAD ng/ml
Benz[*a*]anthracene	4	50
Chrysene	4	50
Benzo[*b*]fluoranthene	4	100
Benzo[*k*]fluoranthene	4	50
Benzo[*a*]pyrene	4	100
Dibenz[*a,h*]anthracene	4	25
Benzo[*ghi*]perylene	4	50
Indeno[1,2,3-*cd*]pyrene	4	50
Coronene	10	20

The following equipment and operating parameters were used for the determination of the above-mentioned detection limits:

a) Parameters

HPLC (binary system) with DAD and FLD

Column: Vydac 201 TP 52 (250 mm long by 4,6 mm internal diameter)

Isothermal temperature: 20 °C

Flowrate: 1,5 ml/min

Injection volume: 20 µl

b) Gradient: Acetonitrile/Water

Time (min)	**Acetonitrile** (% volume fraction)	**Water** (% volume fraction)
3	50	50
10	100	0
18	100	0
20	50	50

c) Parameters for fluorescence detection:

Compound	Excitation wavelength (nm)	Emission wavelength (nm)
Benz[*a*]anthracene	260	420
Chrysene	260	420
6-methylchrysene	260	420
Benzo[*e*]pyrene	260	420
Benzo[*k*]fluoranthene	290	430
Dibenz[*a,h*]anthracene	290	430
Benzo[*ghi*]perylene	290	430
Indeno[1,2,3-*cd*]pyrene	250	500
Coronene	290	430

10 Interferences

Individual reactive PAHs may degrade on the filter during the collection period and subsequent storage. This applies in particular to the following substances dealt with in this International Standard: cyclopenta[*c,d*]pyrene, anthanthrene, benzo[*a*]pyrene, dibenz[*a,h*]anthracene. The filters should be analysed as soon as possible in order to prevent further degradation of the PAHs.

At higher ambient air temperatures, reduced recoveries of components having boiling points < 475 °C (vapour pressure above 10^{-9} kPa at 25 °C) may be expected.

Alkyl PAH, if present, may co-elute with analytes of interest, but should rarely present problems.

Exposure to heat, ozone, nitrogen dioxide (NO_2) and ultraviolet (UV) light may cause PAH degradation during sampling, sample storage and processing. These problems shall be addressed as part of a standard operating procedure prepared by the user. Where possible, incandescent or UV-filtered (excluding wavelengths below 365 nm) fluorescent illumination shall be used in the laboratory to avoid photodegradation during analysis.

NOTE Reactive gases, such as ozone and nitrogen oxides, may lead to degradation of PAH on the filter during sampling [4, 5, 6, 7, 8]. For benzo[*a*]pyrene, significant losses by ozone degradation were observed.

Smoking of tobaccco during sample preparation or in the analytical laboratory or in adjoining areas can result in contamination of samples with PAH.

11 Quality assurance

Users shall generate standard operating procedures (SOPs) describing the following activities in their laboratory: assembly, calibration and operation of the sampling system (stating also the manufacturer and model of equipment used); preparation, purification, storage and handling of sampling reagent and samples; assembly, calibration and operation of the HPLC system (stating also the manufacturer and model of equipment used); furthermore all aspects of data recording and processing (including lists of computer hardware and software used).

The SOPs shall provide specific stepwise instructions and shall be readily available to and understood by the laboratory personnel conducting the work. The SOPs shall be consistent with this International Standard.

Calibration standards shall be freshly prepared at least every two months and checked for accuracy against certified PAH standard mixtures[1)].

Calibration standards shall be analysed before and after each set of samples.

To monitor instrument/operator variability, 6-methylchrysene for FLD and indeno[1,2,3-*cd*]fluoranthene for DAD may be added to the pretreated sample extract prior to analysis. The results shall be plotted on control charts.

Recovery efficiencies of the surrogates added to the samples prior to extraction and analysis shall be closely monitored to assure the effectiveness of sample work-up and analytical procedures. The surrogate recoveries should fall between 75 % and 125 %. Samples for which surrogate recoveries are less than 50 % or more than 150 % shall be discarded.

Approximately 10 % of the sample extracts shall be subjected to duplicate HPLC analysis to assure acceptable analytical precision.

To assure acceptable analytical accuracy, periodic analyses shall be made of a known standard reference material[2)].

12 Test report

The test report shall contain at least the following information:

a) complete identification of the sample;

b) reference to this International Standard or any supplementary standards;

c) the sampling location, sampling time period and volume of air pumped;

d) the barometric pressure and temperature if required (Clause 8);

e) the test result;

f) any unusual features noted during the determination;

g) any operation not included in this International Standard, or in the International Standard to which reference is made, or regarded as optional.

1) NIST 1647d is an example of a suitable product available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

2) NIST SRM 1649a “Urban Dust” is an example of a suitable product available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

Annex A
(informative)

General information

PAHs are formed from organic material on heating in the absence of oxygen (pyrolysis) or in the case of incomplete combustion (lack of oxygen in micro-regions of the fuel/air mixture) and in the conversion of vegetable matter into coal (mineral oil and coal formation).

The state of knowledge on the occurrence and chemistry of PAHs is outlined in several reports [3, 4, 9, 10].

The PAH measurement procedure described in this International Standard has been studied with regard to the sampling time in research and development projects [11, 12, 13, 14, 16] (see also Annex E).

The following information may be of assistance in practical application of the analytical procedure described in this International Standard:

Different PAHs may be important, depending on the objective for the PAH air pollution measurement (measurements for monitoring the exceeding of guide values or measurements with the objective of analysing causes, or measurements in connection with epidemiological surveys and the like).

In the case of benzo[*a*]pyrene, the substance most frequently measured in previous air pollution measurements, attention should be paid to the significance of the concentration values, as it can be used as an indicator for the carcinogenic activity of PAH mixtures which regularly occur in ambient air. According to the report in the WHO Air Quality Guidelines[5], benzo[*a*]pyrene is a useful indicator for the carcinogenic potential of the total PAH emission in connection with lung cancer, in the case of coking plant emissions.

Annex B
(informative)

Examples of operation parameters for HPLC analysis with FLD and DAD in series

Sampling: 360 m^3/24 h ambient air (medium-volume sampler)

Filter: diameter 120 mm, glass fibre; extraction in toluene, 1 h ultrasonic bath

Clean-up: silica gel 60 (0,063 mm to 0,200 mm), cyclohexane

Column chromatography

- Column: MZ-PAH C18, 250 long × 3 mm internal diameter, particle size 5 µm
- Oven temp.: 30 °C
- Solvent: water/acetonitrile
- Flowrate: 0,5 ml/min
- Gradient: 60 % acetonitrile — 2 min
  60 % → 100 % — 26 min
  100 % — 17 min
- Injection volume: 20 µl

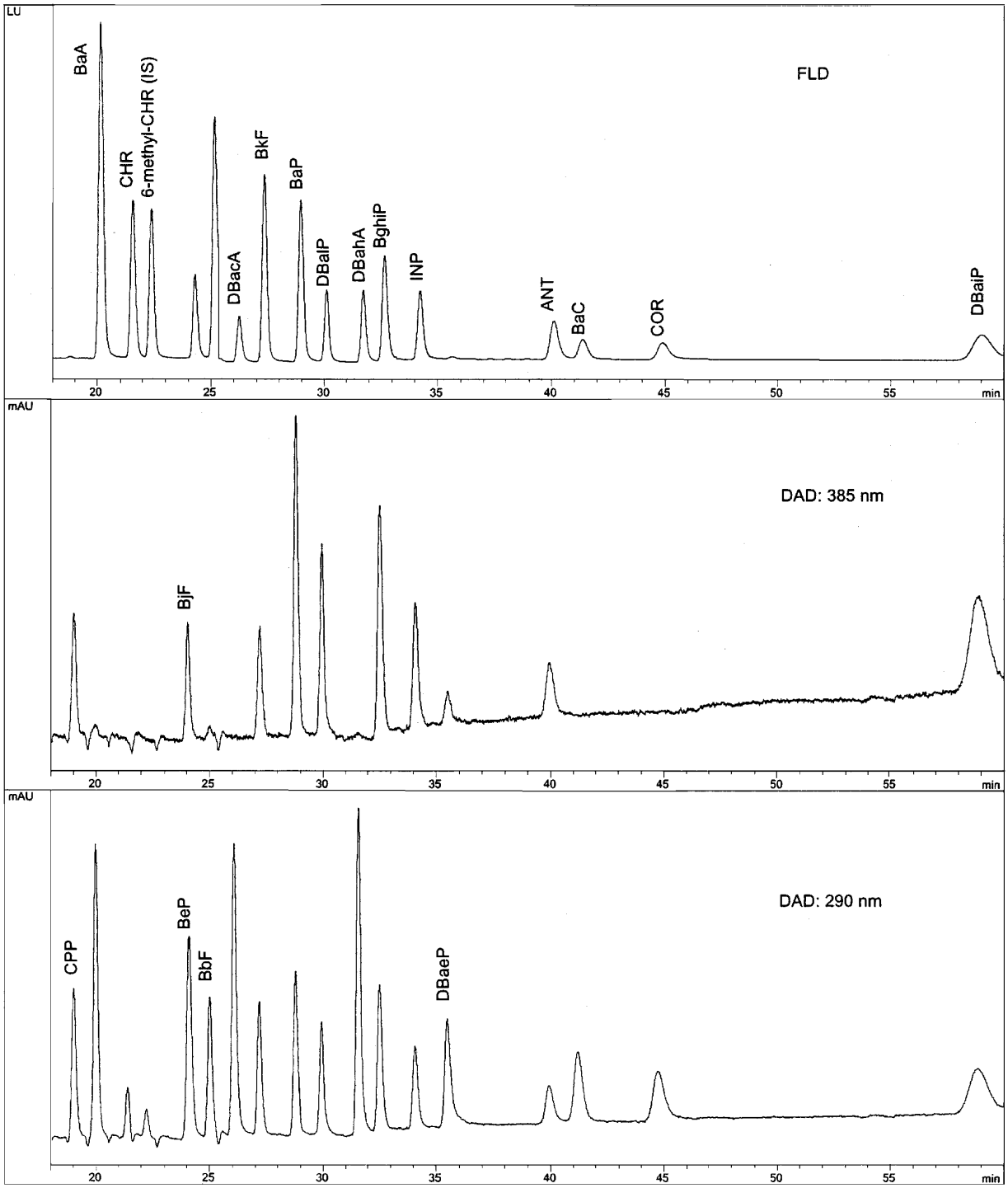
UV-Detector DAD:

Sample wavelength nm	**Reference wavelength** nm
385	500
290	500

Fluorescence detector FLD, Type HP 1100:

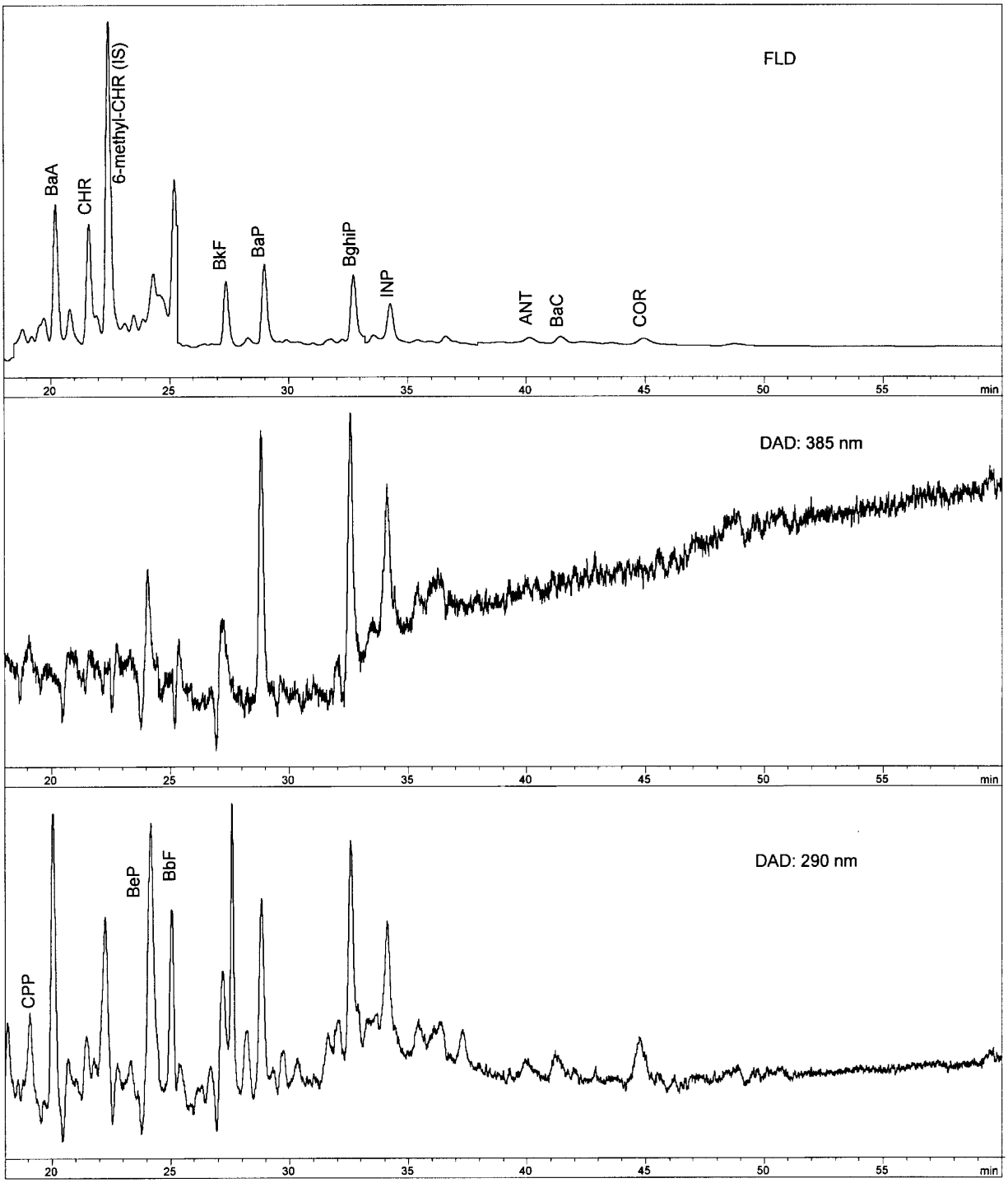
Time min	**Excitation wavelength** nm	**Emission wavelength** nm
13,50	270	440
18,50	260	420
25,00	290	430
33,00	250	500
38,00	290	430

For chromatograms of a calibration mixture and an air sample detected with these parameters, see Figures B.1 and B.2. For abbreviations, see Annex F.



CPP	10,80 ng	DBalP	8,00 ng
BaA	11,11 ng	DBahA	10,91 ng
CHR	10,61 ng	BghiP	10,94 ng
6-methyl-CHR (IS)	7,00 ng	INP	10,20 ng
BbF	13,20 ng	DBaeP	10,24 ng
BeP	10,84 ng	ANT	10,20 ng
BjF	10,19 ng	BaC	10,00 ng
DBacA	10,70 ng	COR	7,20 ng
BkF	8,60 ng	DBaiP	10,24 ng
BaP	11,32 ng		

Figure B.1 — Chromatogram of a PAH calibration mixture



CPP	0,060 ng/m^3	DBalP	n.d.
BaA	0,101 ng/m^3	DBahA	n.d.
CHR	0,183 ng/m^3	BghiP	0,011 ng/m^3
6-methyl-CHR (IS)	0,426 ng/m^3	INP	0,133 ng/m^3
BbF	0,150 ng/m^3	DBaeP	n.d.
BeP	0,185 ng/m^3	ANT	0,038 ng/m^3
BjF	n.d.	BaC	0,106 ng/m^3
DBacA	n.d.	COR	0,271 ng/m^3
BkF	0,081 ng/m^3	DBaiP	n.d.
BaP	0,132 ng/m^3		

n.d.: not detectable

Figure B.2 — Chromatogram of an air sample

Annex C
(informative)

Extraction efficiencies for some PAH compounds

Table C.1 provides extraction efficiencies for some PAH compounds.

Table C.1 — Extraction efficiency

Compound	%	Compound	%	Compound	%
Dibenzo[*a,l*]pyrene [a]	36	Cyclopenta[*cd*]pyrene	80	Benzo[*ghi*]perylene	83
Anthanthrene	68	Dibenz[*a,h*]anthracene	80	Chrysene	84
Benzo[*b*]naphtho [2.1-*d*]thiophene	70	Benzo[*e*]pyrene 1,2-Benzopyrene	81	Benzo[*a*]pyrene	86
Indeno[1,2,3-*cd*]pyrene	73	Dibenz[*a,c*]anthracene[a]	81	Benz[*a*]anthracene Tetraphene	90
Perylene [a]	76	Coronene	81	Benzo[*k*]fluoranthene	90
Dibenzo[*a,e*]pyrene [a]	79	Triphenylene	82	Benzo[*b*]fluoranthene	92
Number of measurements = 4					
[a] Number of measurements = 2					

Annex D
(informative)

Results of duplicate measurements

Collocated duplicate measurements of PAHs were carried out at the station “Neumarkt” in the city of Cologne (Germany) in February 1992. The measurement results as well as the standard deviations are listed in Table D.1, and evaluation of the data in Table D.2. The sampling time was 24 h each at a volume flow rate of 16 m^3/h. The analysis was performed as described.

Table D.1 — Concentration values and standard deviation of PAHs resulting from double measurements

Sample Number	Compound														
	TRI	CPP	BaA	CHR	BHT	BjF	BeP	BbF	BkF	BaP	DBahA	BghiP	INP	ANT	COR
	Ambient air concentrations ng/m^3														
N1a*)	1,2	2,5	1,6	2,6	0,8	1,4	1,3	2,0	1,1	0,1	0,3	3,3	1,8	0,1	1,2
N1b*)	2,0	4,0	2,5	4,4	1,1	2,8	2,1	3,6	1,6	1,0	0,2	5,0	3,1	1,0	2,0
N2a	0,3	2,6	6,4	6,28	2,0	2,8	2,7	5,9	2,6	2,3	0,1	6,9	2,6	0,7	3,1
N2b	2,6	2,7	6,2	6,23	2,2	2,4	2,7	5,9	2,8	2,1	0,1	6,7	2,6	0,7	2,9
N3a	2,8	16,3	7,4	7,7	2,2	4,8	3,3	6,5	2,8	2,7	1,1	8,9	3,8	2,7	3,9
N3b	2,9	16,0	7,2	7,8	2,2	4,7	3,2	6,2	2,7	2,5	1,1	8,5	3,7	2,4	3,4
N4a	0,9	5,3	2,3	2,6	1,0	1,1	1,2	2,2	0,9	0,8	0,1	3,0	1,2	0,3	2,0
N4b	3,7	4,9	5,9	6,4	1,9	3,9	3,5	6,0	2,3	2,1	0,2	8,1	3,4	1,7	3,1
N5a	2,4	2,8	5,0	6,2	2,0	1,7	2,9	5,0	1,7	1,4	0,2	6,1	2,9	0,4	2,3
N5b	2,4	3,2	4,3	6,3	2,1	3,3	2,9	4,9	1,7	1,3	0,1	6,1	3,1	0,5	2,4
N6a	2,9	2,7	2,9	4,2	1,8	2,4	1,4	3,0	1,4	0,8	0,3	2,5	1,4	0,4	1,1
N6b	2,6	2,4	2,6	3,7	1,7	2,1	1,4	2,8	1,2	0,7	0,2	2,3	1,3	0,4	0,7
N7a	3,7	6,5	6,2	8,2	3,3	7,2	2,8	5,7	2,3	1,8	0,4	5,1	2,8	1,0	2,1
N7b	3,2	5,1	4,9	6,7	2,8	3,7	2,2	4,4	1,9	1,3	0,4	3,8	1,91	0,7	1,3
N8a	2,8	9,7	5,0	7,1	2,5	3,1	2,5	4,9	2,0	1,5	0,4	5,2	2,9	0,9	1,8
N8b	2,7	9,3	4,9	7,0	2,5	3,2	2,5	4,7	1,9	1,6	0,1	5,1	2,9	0,8	2,0
N9a	2,8	12,8	5,0	7,2	2,2	3,3	2,8	4,9	1,8	1,7	0,8	6,9	3,2	0,9	2,5
N9b	3,1	9,8	5,3	7,7	2,4	3,3	3,0	5,2	1,9	1,7	0,1	7,0	3,4	1,0	2,6
N10a	3,5	14,9	5,8	8,1	2,7	4,2	3,2	5,8	2,2	2,0	0,1	7,8	3,9	1,4	3,7
N10b	3,1	14,6	5,2	7,2	2,4	3,6	2,9	5,3	1,9	1,8	0,9	6,9	3,5	1,3	2,8
N11a	4,6	19,7	8,3	11,9	3,9	5,6	4,9	8,5	3,3	2,9	1,4	10,7	5,3	2,0	3,8
N11b	4,4	19,7	7,8	11,3	3,7	4,9	4,5	8,0	3,1	2,7	1,3	10,0	4,9	1,9	4,1
N12a	3,4	14,0	5,6	8,0	2,5	3,8	3,3	5,8	2,1	1,9	0,1	7,5	3,7	1,4	2,8
N12b	3,3	13,3	5,4	7,7	2,4	3,7	3,1	5,5	2,0	1,8	0,1	6,9	3,4	1,2	2,5
$\bar{\rho}$	2,8	8,9	5,2	6,8	2,3	3,5	2,8	5,1	2,0	1,7	0,4	6,3	3,0	1,1	2,5
σ	0,8	0,8	0,9	1,0	0,3	1,1	0,5	0,9	0,3	0,3	0,2	1,2	0,6	0,3	0,4
σ_{rel}	27,5	8,9	16,5	14,1	11,4	30,4	18,9	17,9	15,9	20,3	54,1	18,7	18,5	30,1	16,0

*) Adjacent numbers represent paired values

Abbreviations N1a and b relate to instruments 1 and 2; $\bar{\rho}$: arithmetic mean value; σ: standard deviation; σ_{rel}: relative standard deviation (related to $\bar{\rho}$).

Table D.2 — t-statistic of the differences between paired values from Table D.1 for calculation of the probability P (%) of conformity

Compound	$\lvert t \rvert$	Critical value	Significantly different?	P %
TRI	1,268	2,201	Not sig	23,1
CPP	1,298	2,201	Not sig	22,1
BaA	0,163	2,201	Not sig	87,3
CHR	0,488	2,201	Not sig	63,5
BHT	0,409	2,201	Not sig	69,0
BjF	0,038	2,201	Not sig	97,1
BeP	0,644	2,201	Not sig	53,3
BbF	0,504	2,201	Not sig	62,4
BkF	0,478	2,201	Not sig	64,2
BaP	0,163	2,201	Not sig	87,3
DBahA	0,428	2,201	Not sig	67,7
BghiP	0,423	2,201	Not sig	68,0
INP	0,594	2,201	Not sig	56,4
ANT	0,790	2,201	Not sig	44,6
COR	0,240	2,201	Not sig	81,5
NOTE If $\lvert t \rvert <$ critical value, the values are not significantly different.				

Annex E
(informative)

Comparison measurements and invariance test of the PAH profiles

E.1 General

The measurement procedure described has been validated for sampling time [11] and for the choice of filters, the influences of oxidants and the determination of the maximum sampling volumes. The results were confirmed by the studies [12, 13, 14]. The constancy of the PAH profile (constant PAH composition) for up to 24 h was also confirmed.

Due to the different chemical and photochemical reactivities of the PAHs and their different vapour pressures, with increasing sampling time there is the risk of chemical degradation (oxidation, nitration) and also of evaporative losses (blow off) of PAHs already collected on the filter [5]. The partial decomposition of individual PAHs (e.g. benzo[*b*]naphtho[2,1-*d*]thiophene, cyclopenta[*cd*]pyrene, anthanthrene) can be recognized by checking whether the PAH profile [9] is stable or variable during the chosen sampling time. This can be done by comparing the PAH profile of a sample taken over the total period with the PAH profiles of samples taken sequentially during shorter time intervals within the same sampling period at the same site (e.g. $1 \times$ 24-h sampling in comparison with $24 \times$ 1-h sampling).

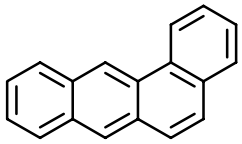
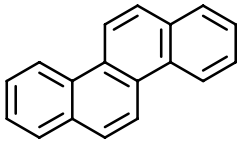
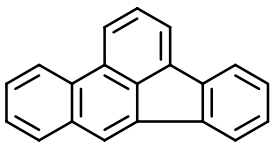
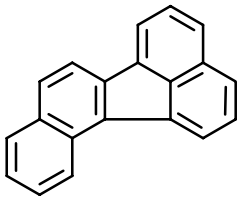
E.2 Similarity of the PAH air pollution profiles

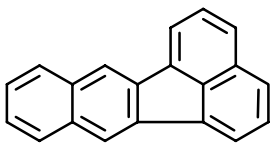
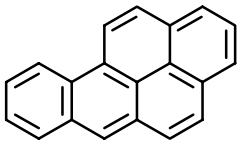
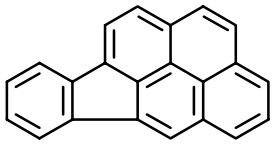
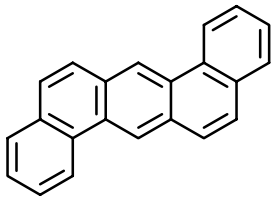
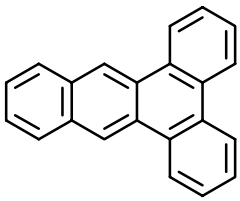
The apparent similarity of the PAH profiles (that is the relations between the concentrations of the individual PAHs) has been repeatedly noted in various publications [5, 9], especially in the case of the annual mean values. These earlier observations have been confirmed by a detailed study [15], using various statistical analyses of the results of extensive PAH air pollution measurements.

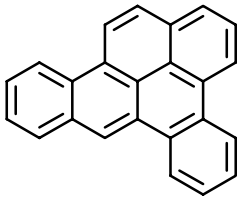
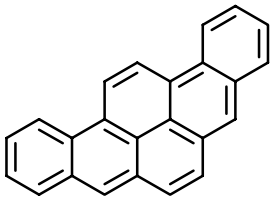
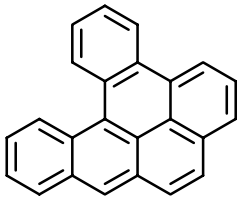
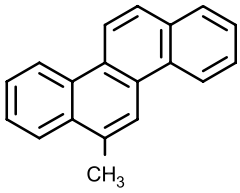
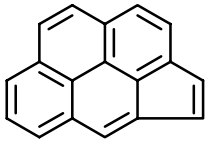
Various PAH emission sources, such as coking plants, household wood burning or motor vehicle traffic, give off PAH emissions with different profiles. This has been illustrated by the scattering of the 24-h individual values occurring at the many individual measuring stations and by the profiles calculated there over a longer period of a year. This has also been shown with changing meteorological conditions. Apparently an “averaging out” of the profile differences occurs over the long term. In studies [15], with the aid of the regression calculation, each of the correlations between the mass concentration of benzo[*a*]pyrene and those of five other frequently occurring PAHs (benzo[*e*]pyrene, benz[*a*]anthracene, bibenz[*a,h*]anthracene, benzo[*ghi*]perylene, coronene) were calculated, as were those between the mass concentration of benzo[*a*]pyrene and the sum of the mass concentrations of the five other PAHs at all 85 measurement stations. The scatter of the individual station-related annual mean values about the regression line benzo[*a*]pyrene/$\sum$PAH, which served as a measure of quality, was small and was of the order of at most 20 %. The differences between the station-related profiles thus are of an order of magnitude where differences in the effects of PAH air pollution, indicated by benzo[*a*]pyrene, are not yet discernible according to medical opinion.

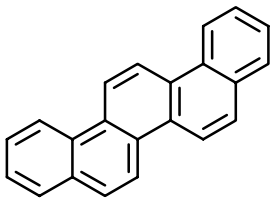
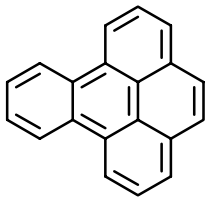
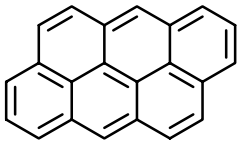
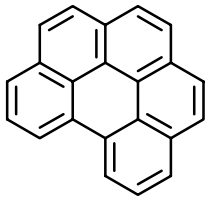
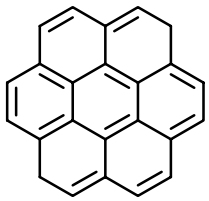
Annex F
(informative)

Physical constants of PAHs

Benz[*a*]anthracene		**BaA**
Molecular formula:	$C_{18}H_{12}$	
CAS Registry No.:	56-55-3	
Boiling point °C:	400	
Melting point °C:	160,7	
Vapour pressure (kPa at 25 °C):	$1{,}5 \times 10^{-8}$	
Chrysene		**CHR**
Molecular formula:	$C_{18}H_{12}$	
CAS Registry No.:	218-01-9	
Boiling point °C:	448	
Melting point °C:	253,8	
Vapour pressure (kPa at 25 °C):	$5{,}7 \times 10^{-10}$	
Benzo[*b*]fluoranthene		**BbF**
Molecular formula:	$C_{20}H_{12}$	
CAS Registry No.:	205-99-2	
Boiling point °C:	481	
Melting point °C:	168,3	
Vapour pressure (kPa at 25 °C):	$6{,}7 \times 10^{-8}$	
Benzo[*j*]fluoranthene		**BjF**
Molecular formula:	$C_{20}H_{12}$	
CAS Registry No.:	205-82-3	
Boiling point °C:	480	
Melting point °C:	165,4	
Vapour pressure (kPa at 25 °C):	$2{,}0 \times 10^{-9}$	

Benzo[*k*]fluoranthene	**BkF**
Molecular formula: $C_{20}H_{12}$	
CAS Registry No.: 207-08-9	
Boiling point °C: 480	
Melting point °C: 215,7	
Vapour pressure (kPa at 25 °C): $2{,}1 \times 10^{-8}$	
Benzo[*a*]pyrene	**BaP**
Molecular formula: $C_{20}H_{12}$	
CAS Registry No.: 50-32-8	
Boiling point °C: 496	
Melting point °C: 178,1	
Vapour pressure (kPa at 25 °C): $7{,}3 \times 10^{-10}$	
Indeno[1,2,3-*cd*]pyrene	**INP**
Molecular formula: $C_{22}H_{12}$	
CAS Registry No.: 193-39-5	
Boiling point °C: 536	
Melting point °C: 163,6	
Vapour pressure (kPa at 20 °C): $1{,}3 \times 10^{-11}$	
Dibenz[*a,h*]anthracene	**DbahA**
Molecular formula: $C_{24}H_{14}$	
CAS Registry No.: 53-70-3	
Boiling point °C: 524	
Melting point °C: 266,6	
Vapour pressure (kPa at 25 °C): $1{,}3 \times 10^{-11}$	
Dibenz[*a,c*]anthracene	**DBacA**
Molecular formula: $C_{24}H_{14}$	
CAS Registry No.: 215-58-7	
Boiling point °C: 518	
Melting point °C: 205 to 207	
Vapour pressure (kPa at 25 °C): $1{,}3 \times 10^{-12}$	

Dibenzo[*a,e*]pyrene	**DBaeP**
Molecular formula: $C_{24}H_{14}$ **CAS Registry No.:** 192-65-4 **Boiling point °C:** 592 **Melting point °C:** 244,4 **Vapour pressure (kPa at 25 °C):** ca. 10^{-10}	
Dibenzo[*a,i*]pyrene	**DBaiP**
Molecular formula: $C_{24}H_{14}$ **CAS Registry No.:** 189-55-9 **Boiling point °C:** 594 **Melting point °C:** 282 **Vapour pressure (kPa at 25 °C):** $3{,}2 \times 10^{-10}$	
Dibenzo[*a,l*]pyrene	**DBalP**
Molecular formula: $C_{24}H_{14}$ **CAS Registry No.:** 191-30-0 **Boiling point °C:** 95 **Melting point °C:** 162,4 **Vapour pressure (kPa at 25 °C):** ca. 10^{-10}	
6-methylchrysene	**IS**
Molecular formula: $C_{19}H_{14}$ **CAS Registry No.:** 1705-85-7 **Boiling point °C:** 458 **Melting point °C:** 160,3 **Vapour pressure (kPa at 25 °C):** ca. 10^{-9} (estimated)	
Cyclopenta[*cd*]pyrene	**CPP**
Molecular formula: $C_{18}H_{10}$ **CAS Registry No.:** 27208-37-3 **Boiling point °C:** 439 **Melting point °C:** 170 **Vapour pressure (kPa at 25 °C):** ca. 10^{-7}	

Benzo[*a*]chrysene	**BaC**
Molecular formula: $C_{22}H_{14}$	
CAS Registry No.: 213-46-7	
Boiling point °C: 518 – 520	
Melting point °C: 367 – 369	
Vapour pressure (kPa at 25 °C): ca. 10^{-11} (estimated)	
Benzo[*e*]pyrene	**BeP**
Molecular formula: $C_{20}H_{12}$	
CAS Registry No.: 192-97-2	
Boiling point °C: 492,9	
Melting point °C: 179	
Vapour pressure (kPa at 25 °C): $7{,}3 \times 10^{-10}$	
Anthanthrene	**ANT**
Molecular formula: $C_{22}H_{12}$	
CAS Registry No.: 191-26-4	
Boiling point °C: 547	
Melting point °C: 246	
Vapour pressure (kPa at 25 °C): ca. 10^{-10} (estimated)	
Benzo[*ghi*]perylene	**BghiP**
Molecular formula: $C_{22}H_{12}$	
CAS Registry No.: 192-97-2	
Boiling point °C: 550	
Melting point °C: 278	
Vapour pressure (kPa at 25 °C): $1{,}3 \times 10^{-11}$	
Coronene	**COR**
Molecular formula: $C_{24}H_{12}$	
CAS Registry No.: 191-07-1	
Boiling point °C: 525	
Melting point °C: 438 to 440	
Vapour pressure (kPa at 25 °C): $2{,}0 \times 10^{-13}$	

Other data: see References [17] and [18].

Annex G
(informative)

List of HPLC columns

All listed PAHs should be separated. The separation depends on operative conditions.

Columns characteristics, temperature

RP columns (Type C-18) capable of separating the PAH according to 5.2.3.2; column length varies from 250 mm to 50 mm and the particle size from 10 µm to 3 µm, depending on the commercially available system, a selection of which is given below[3)].

Type LiChroCART 250-4 LiChro-spher PAH;

Type MZ-PAH;

Type Supelcosil LC PAH;

Type UltraSep ES PAH;

Type Vydac 201 TP 52.

3) The columns listed here and elsewhere in this International Standard are those known to perform as specified under ISO 16362. Each column that is identified by a trademarked name is unique and has a sole manufacturer; however, they are widely available from many different suppliers. This information is given for the convenience of users of ISO 16362 and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to give the same results.

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