NUCLEODUR® C_{18} PAH
NUCLEOSIL® C_{18} PAH

Note: All HPLC columns from MACHEREY-NAGEL are supplied with a certificate, which contains specifications and test results of the column. NUCLEODUR® C_{18} PAH columns are quality products based on the high purity and very pressure stable silica NUCLEODUR®; NUCLEOSIL® C_{18} PAH is based on the robust silica NUCLEOSIL®. They are specifically developed for HPLC analysis. If carefully and properly used excellent chromatographic results and long column lifetime can be achieved. These HPLC columns can be used for the separation of mixtures of polycyclic aromatic hydrocarbons (PAH) and their quantitative determination. They must exclusively be used in accordance with universally accepted laboratory regulations and HPLC working methods. Before running the column the entire analytical system (column and equipment) must be carefully checked by the operator. Chromatographic conditions (mobile phase, flow, temperature etc.) has to be adapted to the analytical task. MACHEREY-NAGEL does not give any warranty and is not liable for the success of a separation or application. If you have any questions after reading this manual, please call our service / technical support.

Table of contents

Safety indication

Follow the general safety instructions for handling of HPLC solvents used as mobile phases (e.g., acetonitrile, methanol) and take precautions against any kind of injuries or damage to health (e.g., skin and eye protection in case of broken capillaries). Disposal of used HPLC columns must follow international, national and local environmental protection regulations. The use of HPLC columns is only permitted to staff members, who are qualified in their field. Keep HPLC columns away from children. MACHEREY-NAGEL disclaims and excludes all warranties of any kind or nature whatsoever and MN shall not be liable for any damages (whether direct, indirect, foreseeable, incidental, compensatory, consequential or special), whether based upon warranty, contract, tort or strict liability, if damages and / or losses occur caused by improper use, maintenance, neglect or improper treatment (especially opening of the column and exposure of the column bed).

Description of the column

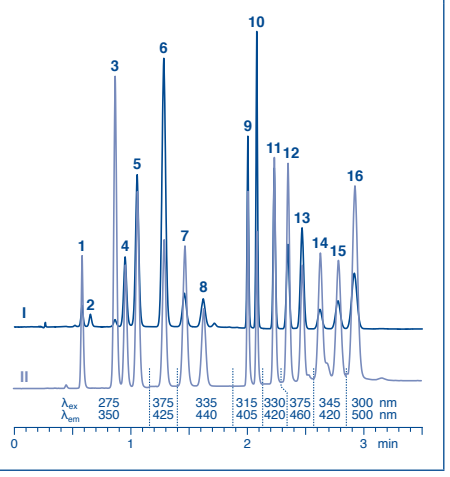
As stationary phase NUCLEODUR® C_{18} PAH columns contain a special octadecyl phase based on fully synthetical spherical silica (type B); NUCLEOSIL® C_{18} PAH columns are based on spherical silica (type A). The columns are recommended for a gradient separation of 16 PAHs in accordance with EPA. Moreover, separations of further PAH compounds such as PAHs according to EFSA can also be realized. A large assortment of PAH application notes can be found in the MN application database ***(www.mn-net.com/apps)***.

Analysis of 16 PAHs in accordance with EPA

Column: EC 100/4 NUCLEODUR® C_{18} PAH, 3 µm
Eluent: A) methanol – water (80:20, v/v)
B) acetonitrile
Gradient: 2–20 % B in 1.2 min, 20–100 % B in 0.5 min, 100 % B for 2.5 min, 100–2 % B in 0.4 min
Flow rate: 2.5 mL/min
Temp.: 35 °C
Detection: UV, 254 nm (I), Fluorescence (II)
Peaks:

1. Naphthalene
2. Acenaphthylene*
3. Acenaphthene
4. Fluorene
5. Phenantrene
6. Anthracene
7. Fluoranthene
8. Pyrene
9. Benz[a]anthracene
10. Chrysene
11. Benzo[b]fluoranthene
12. Benzo[k]fluoranthene
13. Benzo[a]pyrene
14. Dibenz[ah]anthracene
15. Benzo[ghi]perylene
16. Indeno[1,2,3-cd]pyrene

* not detectable by fluorescence



MN Appl. No. 123820

Installation

The column should be installed in the flow direction indicated on the column label. It is connected with 1/16" capillaries and fittings, typical for HPLC instruments.

Guard columns

For protection and an extension of column lifetime, particularly for samples with difficult matrices (e.g., soil, oil, food), the column should always be used with a guard column. The filter elements and the adsorbent in the guard column retain contaminants from the sample or the eluent. Connection of the guard column with the separation column is made by a suitable guard column holder (see ***www.mn-net.com*** or the MN chromatography catalog). Cartridge replacement is required when increased column pressure and / or loss of performance is observed.

Sample

Polycyclic aromatic hydrocarbons have to be determined in diverse matrices (e.g., water, soil, oil). Thus, an effective sample preparation is necessary. Therefore solid phase extraction (SPE) has been established in the last years (for SPE applications see ***www.mn-net.com/apps***). The prepared sample is reconstituted in acetonitrile or methanol. Residues of nonpolar solvents in the sample are detrimental to the reproducibility of retention times. Sample solutions should be passed through a syringe filter (e.g., CHROMAFIL® Xtra PET, 0.45 µm, 25 mm, REF 729220) before entering the column. If injected sample solutions are still turbid even after filtration, the lifetime of the column may be significantly reduced. The sample volume should be as small as possible to achieve an optimal resolution.

Eluent

PAH columns are supplied with the eluent acetonitrile – water (70:30, v/v). As eluents acetonitrile or methanol with pure water are typically used. Tetrahydrofuran can also be applied as eluent additive. Buffers can be used – but they are generally not added. The pH stability of the column from 2–8 must be considered. An eluent containing buffer must always be removed by rinsing the column with at least 10 column volumes acetonitrile – water or methanol – water (10:90, v/v) directly after finishing a measurement. Eluents should be filtered through a 0.2–0.45 µm membrane filter and degassed.

Flow rate and pressure

Flow rate (recommended for analytical columns with 2–4.6 mm ID: 0.2–2.5 mL/min) influences the time required, the resolution and the column lifetime. It is limited by the back pressure, which should not exceed the maximum of 600 bar (NUCLEODUR®) / 400 bar (NUCLEOSIL®). In mixtures of methanol and water viscosity reaches a maximum at about 40 % methanol. For this reason a reduced flow rate is recommended, when changing the eluent composition. We recommend controlling back pressure regularly. If a high pressure results from the use of the column at nominal flow rates, this usually indicates that some contaminants have become deposited on the packing material, which must be removed (see troubleshooting).

Temperature

Column temperatures from 20 °C up to 35 °C are common usage for PAH separations. 60 °C are possible as maximum temperature for the columns. But higher temperatures reduce the lifetime. However, the temperature should be at least 30 °C below the boiling temperature of the eluent, in order to ensure proper detection. Variation of the temperature influences retention times and especially the peak shape.

Detection

Spectrophotometers, mass spectrometers, refractometers and electrochemical detectors can be used with the columns. UV (250–280 nm), diode array or fluorescence detection at different wavelengths for excitation and emission are used for the detection of PAHs. (Acenaphthylene cannot be analyzed with fluorescence detection.)

Equilibration

Prior to measurement of samples the column must be rinsed with the eluent at the same flow rate and temperature as the method to be applied. Column equilibration is finished, when the baseline of the detector no longer shows a drift (generally after 10 column volumes).

Column storage

The original eluent acetonitrile – water (70:30, v/v) is recommended for storage. For long-term storage mobile phases containing inorganic salts are not recommended (see regeneration). Methanol is also not recommended for a longer storage, because of a possible impurity with metal ions (e.g., iron(III)). For column storage be sure the end fittings are tightly sealed using column end plugs, because storage without these seals can result in drying of the packing material. Under these circumstances rinse the column with approx. 10 column volumes of the eluent of storage at a flow rate of max. 0.2 mL/min.

Troubleshooting

The following outline describes the symptoms of performance loss and their cause. All columns are subject to the strict regulation and control of our quality assurance system. Columns based on silica are robust and hold their separation efficiency for long periods by correct maintenance and treatment. According to experience, column failures are mostly a result of injection of contaminants to the sorbent bed. The usage of a guard column, as well as an appropriate sample pretreatment will help to minimize these risks.
Use the outline below to help determine the cause of a possible performance loss:

Symptom / Error / Cause	Prevention / Remedy
Baseline drift · insufficient period for equillibration with the eluent · contaminated eluent · temperature	 longer or better equilibration use freshly prepared solvents and reagents column temperature control
Broad peaks · mixing and / or diffusion before / behind the column · too large sample volume	 keep length and ID of capillaries at a minimum smaller injection volume
Peak interference; too fast elution too fast elution and / or insufficient separation by: · improper column temperature or flow rate · elution power of eluent is too high	 optimize concerned parameter optimize eluent system
Increasing back pressure; degradation of the separation performance contamination of sorbent by: · particulate accumulation on frit or sorbent bed from sample, eluent or system · precipitation of buffer salts	 prepare fresh eluent; prefilter samples and eluent, use in-line filter / rinse LC system, clean the sorbent beforehand check solubility of buffer / remove them by rinsing (see column regeneration)
Insufficient separation; degradation of the separation with regular column pressure contamination with: · fats, oils, lipids from sample (coating of sorbent surface) and other organic substances from improperly prepared eluent or matrices	 remove organic substances by sample preparation / clean the sorbent (see column regeneration)
Double peaks (dead volume) · faulty fittings (capillaries, ferrules, nuts) · dissolution of silica by too high pH value of eluent	 use “PEEK Fingertight Fittings”, REF 718770 / replace fittings consider pH range 2–8 of column / replace column

Column regeneration

In some cases the perfomance of the column can be restored by removing contaminants from the sorbent bed or by regeneration of the phase. It is important, however, to locate the source of contamination before again using the column for the analysis of samples.

1. Prepare fresh eluent: Sometimes the performance loss is traced to eluent contamination. Therefore, prepare fresh eluent and flush all liquid lines before using the column again. The eluent should be filtered through a 0.2–0.45 µm membrane and degassed prior to use.
2. Cleaning of sorbent: To remove contamination rinse the column with a minimum of 10 column volumes (see table below) at the original flow rate and temperature as follows:
   - after usage of buffer first with acetonitrile – water or methanol – water (10:90, v/v)
   - 100 % methanol to remove polar organic compounds
   - 100 % acetonitrile to remove medium polar organic compounds (possibly T= 40 °C)
   - 100 % tetrahydrofuran to remove nonpolar organic compounds
   - if necessary, 100 % tetrahydrofuran with inverse flow direction at 1/5 of original flow rate
   - convert column to storage condition using acetonitrile – water (70:30, v/v) at original flow rate

   An adequate indicator for a clean column is a constant baseline. At constant temperature you should observe less than 2–3 mAU drift during a running time of 5 minutes with an isocratic run.
3. Regeneration: After the usage of buffer, directly after finishing a measurement and always before storage of the column rinse with a minimum of 10 column volumes at the original flow rate and temperature as follows:
   - acetonitrile – water or methanol – water (10:90, v/v) for removal of the buffer
   - increase the organic part in steps of 20 % to the conditions of a new measurement run
   - or gradually increase the part of acetonitrile in steps of 20 % to the storage conditions
4. Column replacement: The above procedures will restore performance only in certain cases. Some organic contaminants are particularly refractory and may not respond to treatment. Also dead volume, due to column compression can generally not be repaired. Under these circumstances, column replacement is necessary. It is highly advisable to locate the cause of the problem before installing a new column.

		Column volume [mL]			
Length [mm]	**ID [mm]:**	2	3	4	4.6
100		0.30	0.70	1.25	1.65
150		0.45	1.05	1.90	2.50
250		0.80	1.75	3.15	4.15

Abstract

To extend column lifetime, please keep in mind the following:

1. As eluents organic-aqueous eluent systems (e.g., acetonitrile or methanol – water) are recommendable. Please consider regeneration after usage of buffers. Eluents should be filtered through a 0.2–0.45 µm membrane and degassed.
2. Filter samples through a 0.2–0.45 µm CHROMAFIL® Xtra PET syringe filter before injection.
3. The usage of a guard column for samples with difficult matrices (e.g., soil, oil, food) is advisable.
4. The recommended flow rate for analytical columns (ID 2–4.6 mm) is 0.2–2.5 mL/min.
5. Adjust flow rate to keep column pressure below the maximum value of your column.
6. Store the column in acetonitrile – water (70:30, v/v) (after removal of buffer salts).
7. Use analytical grade reagents and HPLC grade solvents for all work. Discard any solutions that show evidence of bacterial growth.

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