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1.50156.0001

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LiChrospher® PAH Instruction Manual



Polycyclic aromatic hydrocarbons (PAH) are produced by pyrolysis or incomplete combustion of organic substances. Main sources of PAH are exhaust fumes of private or industrial furnaces, car exhausts and tobacco smoke. The determination of PAH is of great importance, because some PAH are carcinogenic.

For identification of PAH the use of an HPLC column with high selectivity and separation efficiency is of greatest importance. LiChrospher® PAH is a new stationary phase based on a special modified RP-18 silica gel, to achieve high resolved PAH separations.

According to the EPA method 610 (EPA = Environmental Protection Agency) 16 PAH must be separated. In almost all cases benzo(e)pyrene and perylene must be determined in addition to the 16 PAH. Using LiChroCART® 250-4 LiChrospher® PAH, 16 PAH of EPA 610 + benzo(e)pyrene + perylene can be separated by HPLC gradient and determined by UV or fluorescence detection (figure 1).

LiChrospher® PAH can be used for the determination of 7 PAH in tap water according to the method DIN 38 407, part 8, defined by german regulation (figure 2).

To protect the analytical column LiChroCART® 250-4 against contamination the use of the guard column LiChroCART® 4-4 is recommended.

LiChroCART® 4-4 cartridges can be mounted conveniently by using the manu-CART® endfittings (1.51486.0001).

Specifications:

Column:	LiChroCART® 4-4 (1.50148.0001) LiChroCART® 250-4 (1.50149.0001)
Sorbent:	LiChrospher® PAH, spherical RP-18 silica gel with high selectivity for PAH
pH-stability:	pH 2–7.5

Column equilibration:

LiChroCART® 250-4 LiChrospher® PAH is supplied in acetonitrile/water (75/25) (v/v) and should be stored in the same solvent mixture.

When unpacking your LiChroCART® 250-4 LiChrospher® PAH keep in mind that it may dry out during stocking and shipping period. This does affect neither efficiency nor life time of the column. To get the whole surface activated and to receive reproducible results it is highly recommended to start equilibration procedure with pure organic modifier such as acetonitrile or methanol. After this procedure you should start equilibrating with the mobile phase.

**Figure 1: Separation of 18 PAH
(16 PAH according to EPA 610 + Benzo(e)pyrene + Perylene)**

Column: LiChroCART® 250-4 LiChrospher® PAH
Flow rate: 1.0 ml/min

Temperature: 20 °C

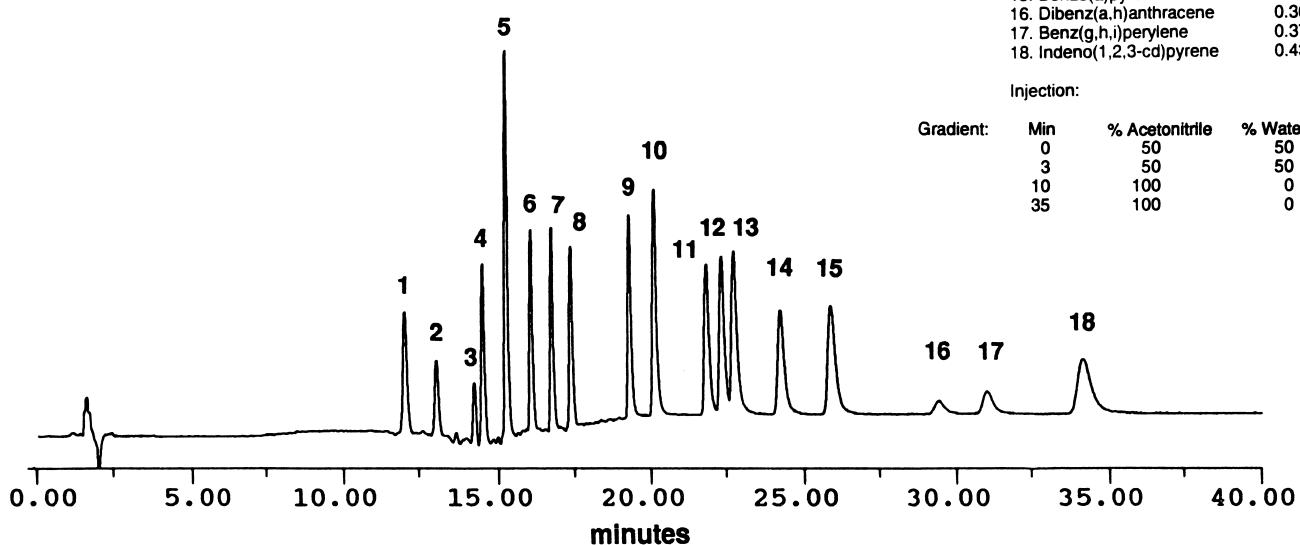
Detection: UV 254 nm

(Detector: LiChroGraph® L-4500)

Sample:	1. Naphthalene	2.00 µg/ml
	2. Acenaphthylene	1.54 µg/ml
	3. Acenaphthene	2.06 µg/ml
	4. Fluorene	0.48 µg/ml
	5. Phenanthrene	0.35 µg/ml
	6. Anthracene	0.08 µg/ml
	7. Fluoranthene	0.77 µg/ml
	8. Pyrene	0.85 µg/ml
	9. Benz(a)anthracene	0.41 µg/ml
	10. Chrysene	0.37 µg/ml
	11. Benzo(e)pyrene	0.74 µg/ml
	12. Benzo(b)fluoranthene	0.42 µg/ml
	13. Perylene	0.72 µg/ml
	14. Benzo(k)fluoranthene	0.47 µg/ml
	15. Benzo(a)pyrene	0.49 µg/ml
	16. Dibenz(a,h)anthracene	0.36 µg/ml
	17. Benz(g,h,i)perylene	0.37 µg/ml
	18. Indeno(1,2,3-cd)pyrene	0.43 µg/ml

Injection: 30 µl

Gradient:	Min	% Acetonitrile	% Water
	0	50	50
	3	50	50
	10	100	0
	35	100	0



Detection: Fluorescence
(Detector: LiChroGraph® F-1080)

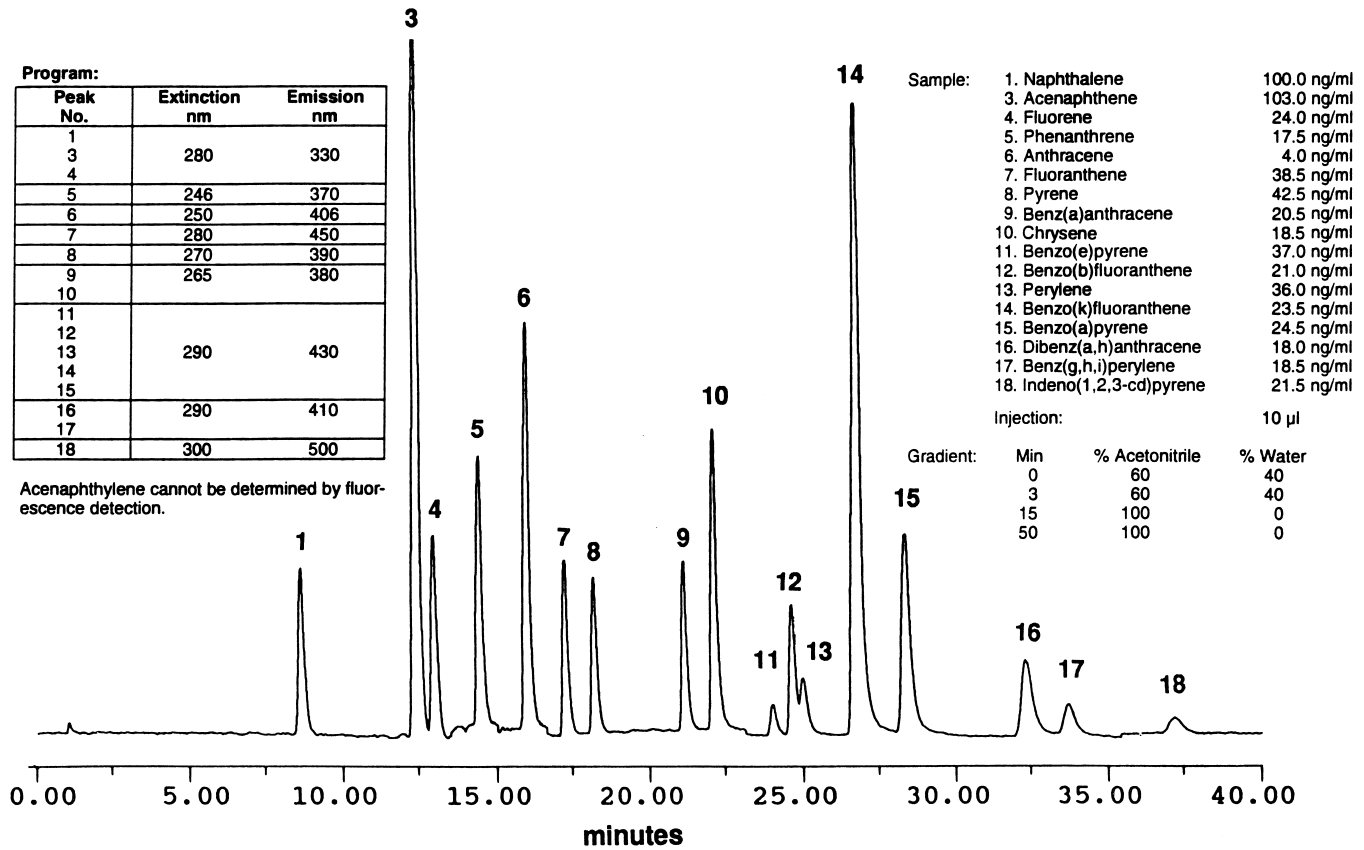
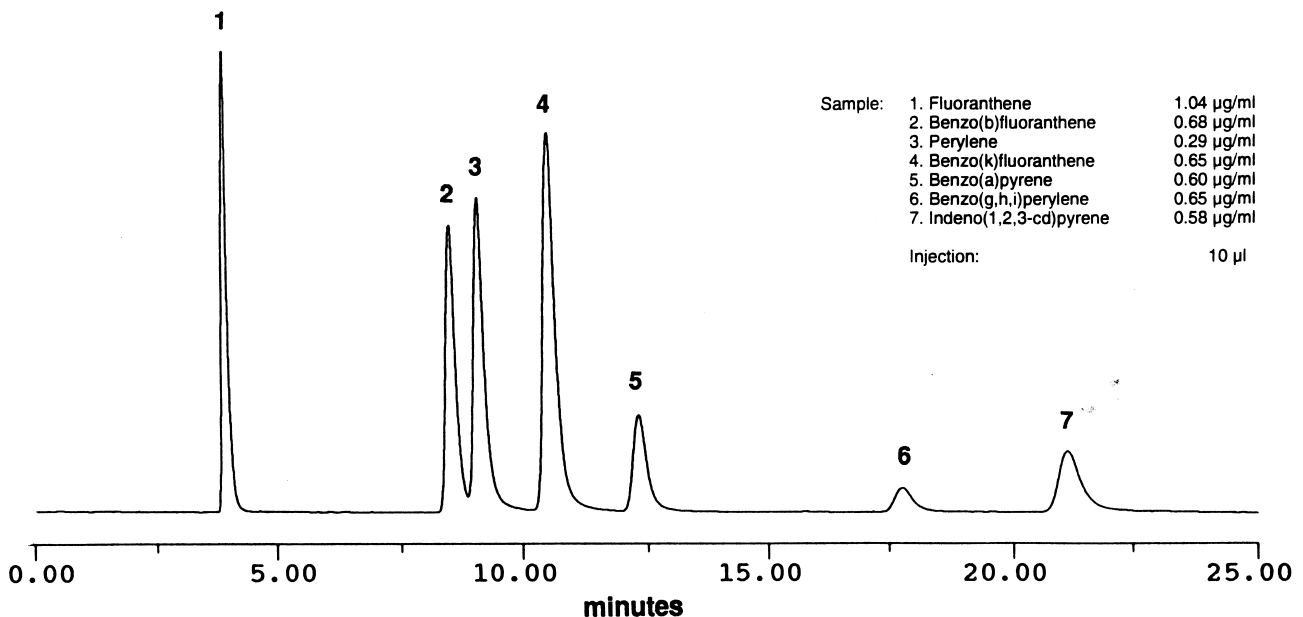


Figure 2: Separation of 7 PAH according to german tap water regulation.

Column: LiChroCART® 250-4 LiChrospher® PAH
 Eluent: Acetonitrile
 Flow rate: 1.0 ml/min

Temperature: 20 °C

Detection: Fluorescence Ex 365 nm, Em 470 nm
 (Detector: LiChroGraph® F-1080)





Handling of LiChroCART® HPLC cartridges

LiChroCART® cartridges are used with re-usable endfittings (manuCART®) which fit different cartridge lengths (75 mm–250 mm) and inner diameters (4.6 mm, 4 mm, 3 mm or 2 mm).

The endfittings (manuCART®) are designed to allow the cartridges to be hand-sealed at normal working pressures of 150 to 200 bar without the need for any tools. Only at higher pressures may further tightening with a wrench become necessary.

CARTRIDGE EQUILIBRATION

LiChroCART® cartridges were flushed with acetonitrile/water. As they can dry out during stockings and shipping they have to be activated by purging 10–20 column volumes of pure organic component (e.g. acetonitrile, methanol) before equilibrating them with the mobile phase. Please increase the flow rate gradually stepwise from 0 ml/min to the desired value. If the eluent contains a buffer salt, you have to flush the cartridges with water after the activating and before the equilibrating step.

Mounting the manuCART® to a LiChroCART® cartridge without precolumn

The manuCART® (Cat. No. 1.51486.0001) consists of the following pieces. Check that you have all of these pieces before you begin to assemble your column.

No.	Description	Quantity
1	Cap nut	2
2	Spin collet	4
3	End fitting	2

The following procedure describes how to fit the pieces to just one end of your cartridge. Repeat steps 1–4 for the other end.

1. Insert the cartridge through the end fitting, so that the outer thread is at the outermost end.
2. Place 2 split collets around the neck of the cartridge as shown in Figure 1.
3. Push the end fitting up over the 2 split collets.
4. Screw the end cap over the assembled endfitting. Tighten finger tight.
5. When you install your column into the flow path of your LC, tighten the end fittings leak tight using wrenches.

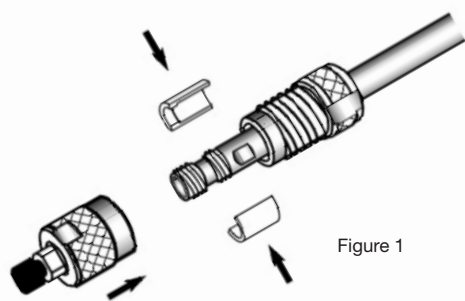


Figure 1

Mounting the manuCART® to a LiChroCART® cartridge precolumn

1. Insert the cartridge through the end fitting, so that the outer thread is at the outermost end.
2. Place 2 split collets around the neck of the cartridge as shown in Figure 2.
3. Place the guard column cartridge inside the space at the end of the collets.
4. Push the end fitting up over the assembled guard column cartridge.
5. Screw the end cap over the end fitting. Tighten finger tight.
6. When you install your combined column and guard column into the flow path of your LC, tighten the end fittings leak-tight using wrenches.

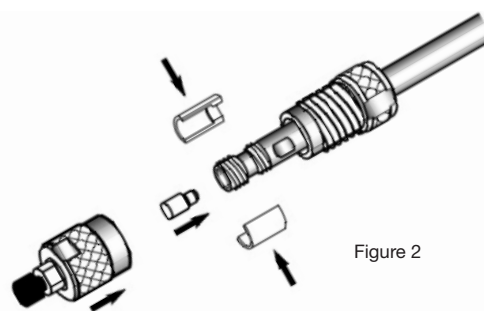


Figure 2

Exchanging the sieve and glass fiber Filter

Content of the tool for replacement of frits

(Cat. No. 1.15576.0001):

1 pin, 1 plastic tool, 1 centering sleeve

1. Using a wrench, loosen the cap nut from the end fitting.
2. Unscrew the cap nut, and remove the semicylindrical collets from the neck of the column cartridge. Put them aside for re-use later.
3. Using the pin, gently remove the sieve from the end of the column cartridge. (Figure 4)
4. Using a small spatula, remove the remains of the glass fiber filter and any soiled packing material.
5. Fill the void with freshly prepared packing material and smooth-off the surface.
6. Place a new filter on the open end of the cartridge. Do not attempt to push it into place with your fingers – it may wrinkle.
7. Using the plastic tool, push the filter inside the cartridge. (Figure 5)
8. Place the cylindrical sleeve over the open end of the cartridge.
9. Place a sieve inside the funnel.
10. Using the plastic tool, push the sieve firmly into place. (Figure 6)

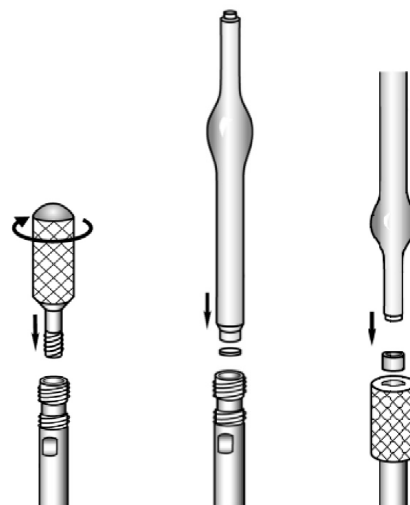


Figure 4

Figure 5

Figure 6

Made in Germany

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