Questions from the "PAL Prüfungsbuch"
Application of Chromatographic Methods
All tasks are to be scored with 10 to 0 points

The following information correspond to the IUPAC recommendations "Nomenclature for Chromatography" and serve as a formulary for solution of the subsequent tasks.

$$\bar{u} = \frac{L}{t_M} \qquad k' = \frac{t_R - t_M}{t_M} = \frac{t'_R}{t_M} \qquad \alpha = \frac{t'_R\ (Peak\ b)}{t'_R\ (Peak\ a)}$$

$$R_s = \frac{1.177 \times (t_{R2} - t_{R1})}{w_{h1} + w_{h2}} \quad \text{or} \quad R_s = \frac{2 \times (t_{R2} - t_{R1})}{w_{b1} + w_{b2}}$$

$$N = 5.545 \times \left({}^{t_R}/_{w_h}\right)^2 \quad \text{or} \quad N = 16 \times \left({}^{t_R}/_{w_b}\right)^2$$

$$H = \frac{L}{N} \qquad MF = \frac{m_{Kal-STD} \times A_{ISTD}}{m_{ISTD} \times A_{Kal-STD}} \qquad w_{PR} = \frac{MF \times m_{ISTD} \times A_{Pr}}{A_{ISTD} \times m_{Pr}}$$

$$\beta_{Pr} = \frac{\beta_{STD} \times A_{Pr}}{A_{AUFG} - A_{Pr}}$$

t_M	Hold-up time (column dead time)	t_R	Retention time
t'_R	Reduced retention time	w_b	Peak width at base line
w_h	Peak width at half height	$m_{KAL-STD}$	Mass of calibration standard
m_{ISTD}	Mass of internal standard	m_{Pr}	Mass os sample
L	Length of the chromatographic system	A_{Pr}	Peak area of the sample peak
b_{Pr}	Mass concentration in sample	A_{AUFG}	Peak area of spiked sample peak
b_{STD}	Mass concentration of standard		

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1:

A sample consists of

- Water
- Ethen-1,2-diol (glycol)
- Propane-1,2,3-triol (glycerin)

(in approx. equal parts)

1. Suggest a chromatographic method to separate the mixture
2. Explain your selection
3. Choose a detector which is suitable for the quantitaive determination of **all three** analytes

2:

In a preliminary HPLC investigation it was found that parts of the sample matrix disrupt the chromatographic separation of the analytes by superposition of peaks. Name two measures that can be taken so that the analytes nevertheless can be faultlessly quantified.

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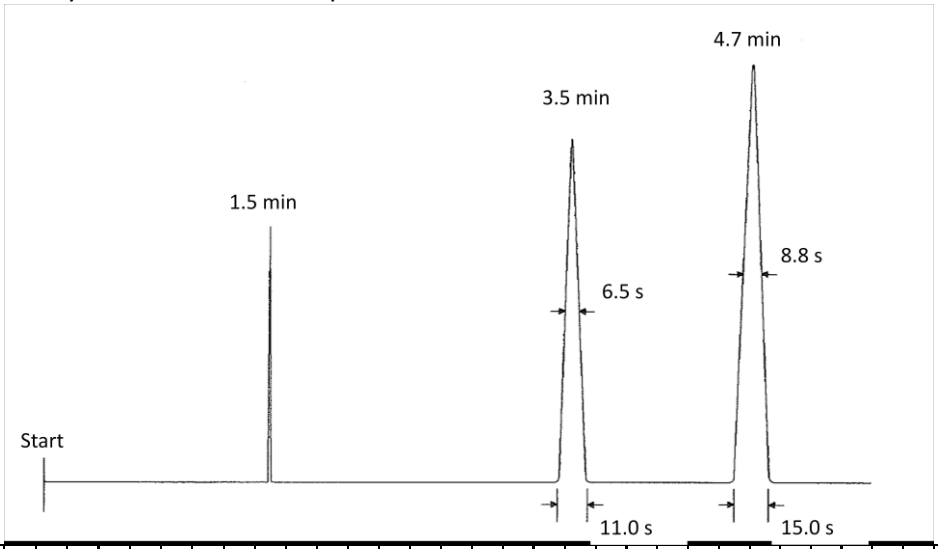
3:

The pictured chromatogram was taken with a 12.5 cm long HPLC-column. The inlet from the injector to the column is 2.5 cm, the outlet from the column to the detector is also 2.5 cm. A hold-up time marker was added to the two component sample.

Calculate the following parameters:

1) Reduced retention time of peak number 2
2) Average linear flow velocity
3) Resolution between peak 2 and 3
4) Theoretical plate number regarding peak 3
5) Selectivity coefficient between peaks 2 and 3



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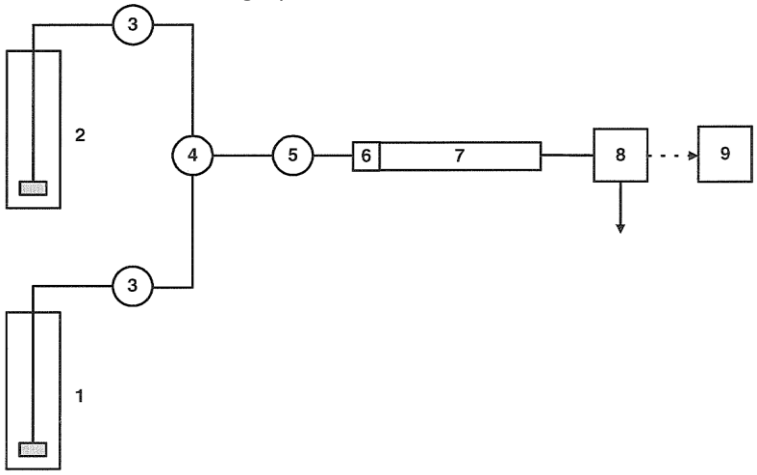
4:

The following design shows a chromatograph



1. Name the type (working technique) of HPLC, for which such device configuration is normally applied.
2. Name the contents of vessels 1 and 2 and explain the role (function) that these matters undertake.
3. Label parts 3 to 9 in the below given table and give a short description of their function.

1

2

3

No.	Part	Function
3		
4		
5		
6		
7		
8		
9		

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5:

1. Give a short description of the principle of a chromatographic analysis with an internal standard
2. In which case the method of the internal standard must be used? Name **two** major reasons.

6:

Name a cause of **each** „Tailings“ and „Frontings“ in a GC- or HPLC-chromatogram

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7:
In a

- Gas chromatography (with thermal conductivity detector) or
- HPLC (with UV-detector)

the column length is increased.
Explain how this affects the parameters that are specified in the table

Note:
Fill in the predefined answers given below **either** for gas chromatography **or** HPLC:

increases
decreases
remains constant

Hold-up time t_M	**Retention time t_R**	**Theoretical plate number *N***	**Plate height H**	**Peak area *A***

8:
Explain the terms given in the table below.

Term	**Explanation**
Selectivity coefficient $\alpha = 1$	
Resolution $R_s = 1.5$	
RP 18	
Headspace	
Permeability	

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10:

In a HPLC-two-point calibration the following data were determined:

Weighed portion of analyte	Peak area
2.00 mg	23400 counts
4.00 mg	44510 counts

2.50 mg pure analyte was added to the pure sample matrix without analyte. The mixture was then analyzed. A peak area of 28400 counts was obtained.
Calculate the recovery rate RR (in %).

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