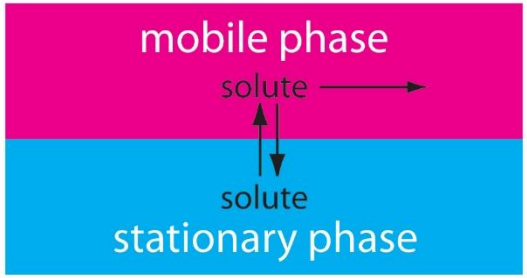
Theory of chromatography

What is chromatography?

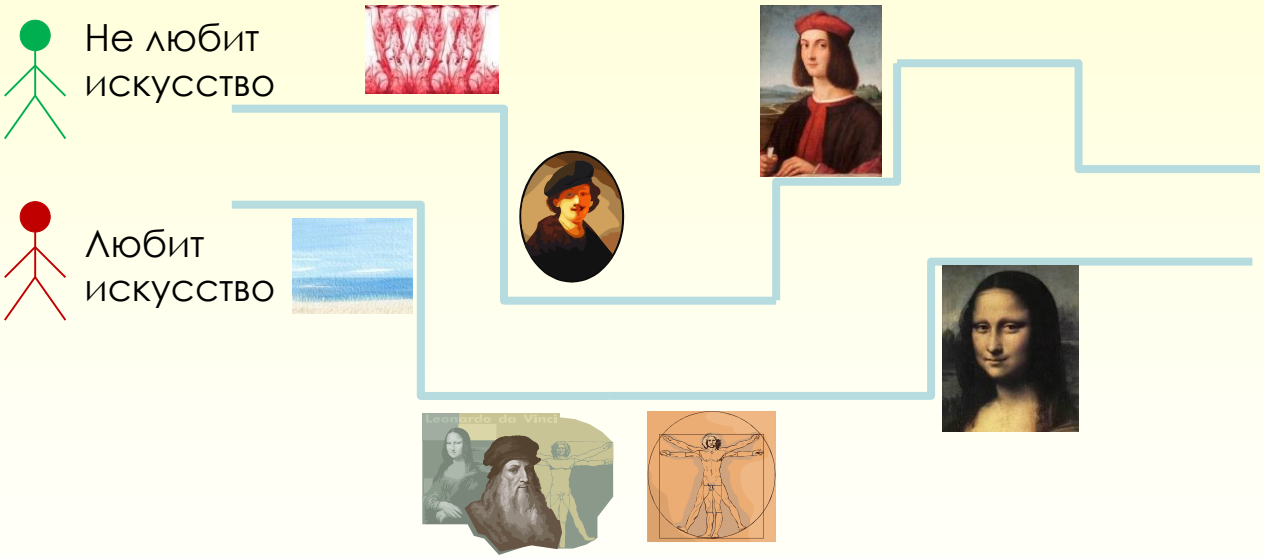
Chromatography – **physical separation method** in which compounds are partitioned between two phases - **stationary** and **mobile** (moving through stationary in a given direction)



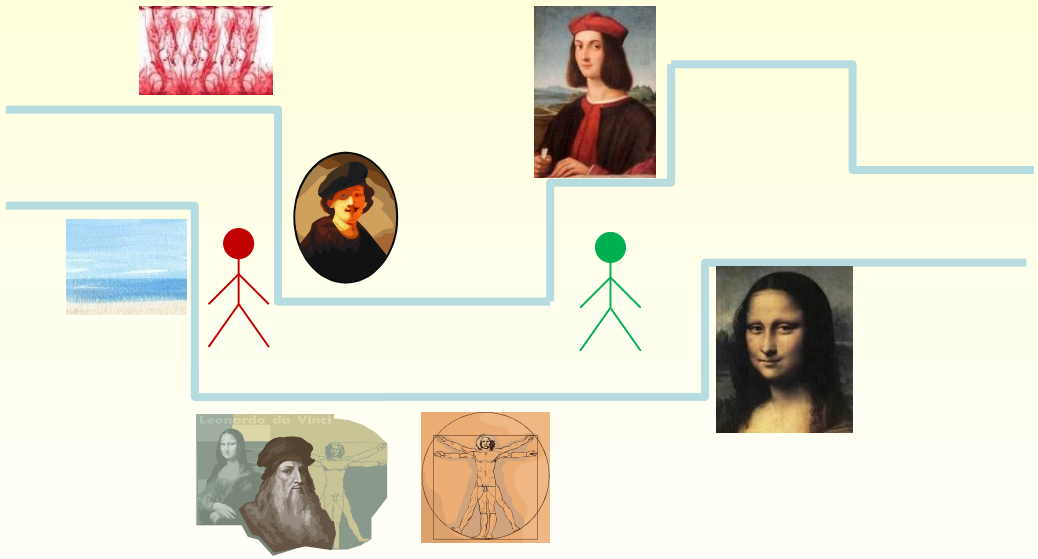
In chromatography we pass a mobile phase over a stationary phase. When we inject a sample into the mobile phase, the sample's components both move with the mobile phase and partition into the stationary phase.

Compounds stronger retained by stationary phase elute later.

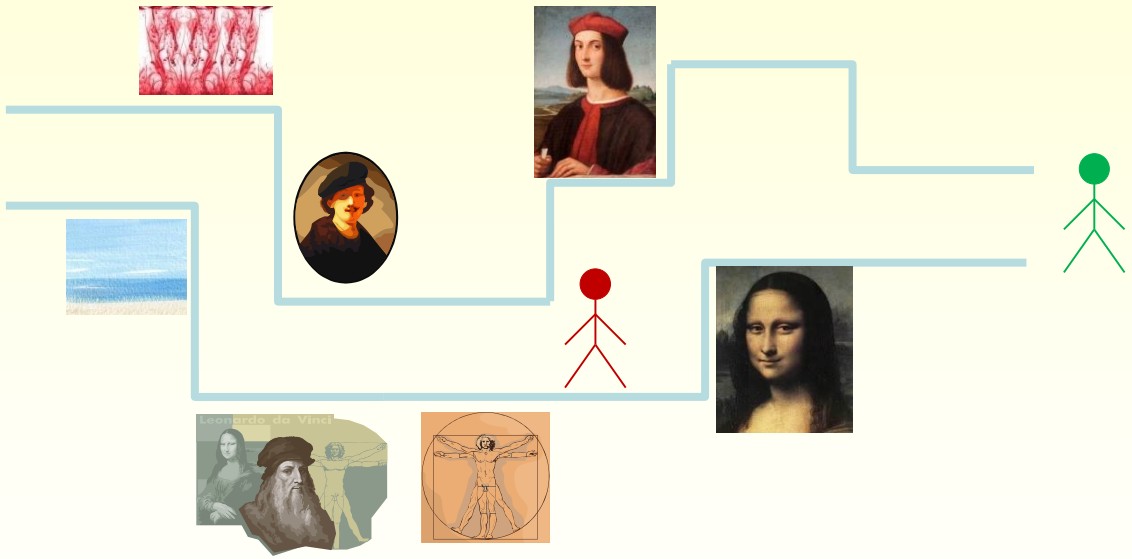
Example - Gallery



Example - Gallery



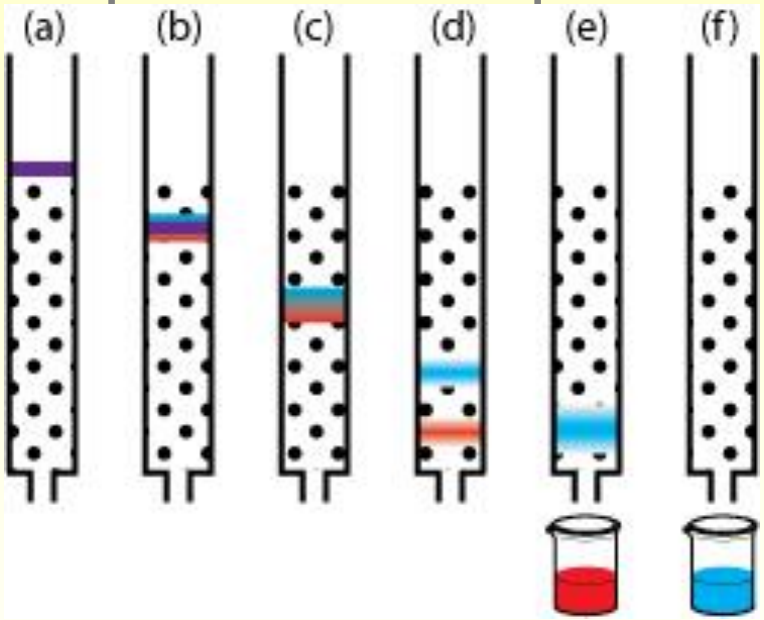
Example - Gallery



Types of Chromatography

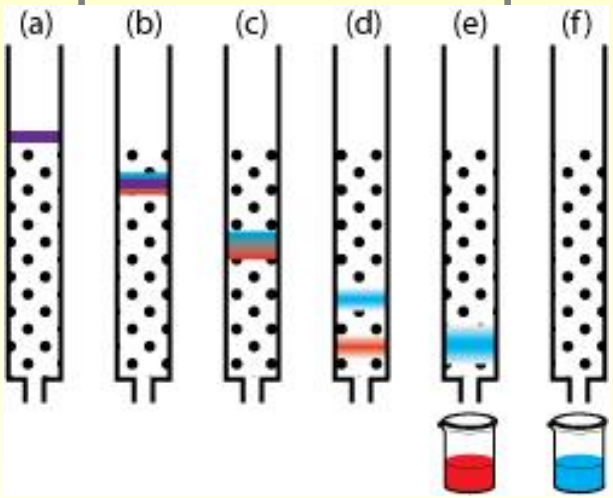
- by describing the physical state of the mobile phase and the stationary phase;
- by describing how we bring the stationary phase and the mobile phase into contact with each other (**column chromatography** or **planar chromatography**);
- by describing the chemical or physical interactions between the solute and the stationary phase.

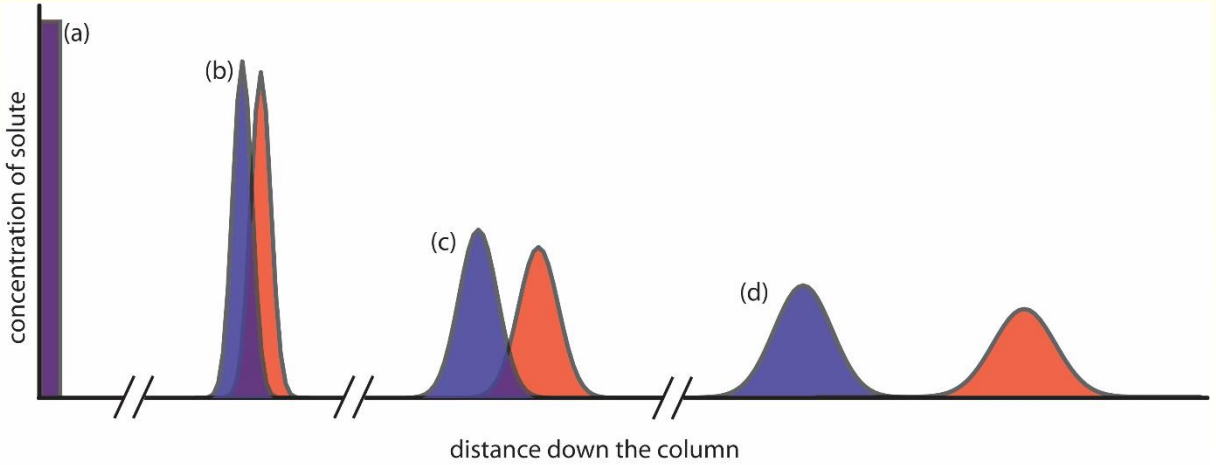
Column Chromatography separation process



Progress of a column chromatographic separation of a two-component mixture. In (a) the sample is layered on top of the stationary phase. As mobile phase passes through the column, the sample separates into two solute bands (b–d). In (e) and (f), we collect each solute as it elutes from the column.

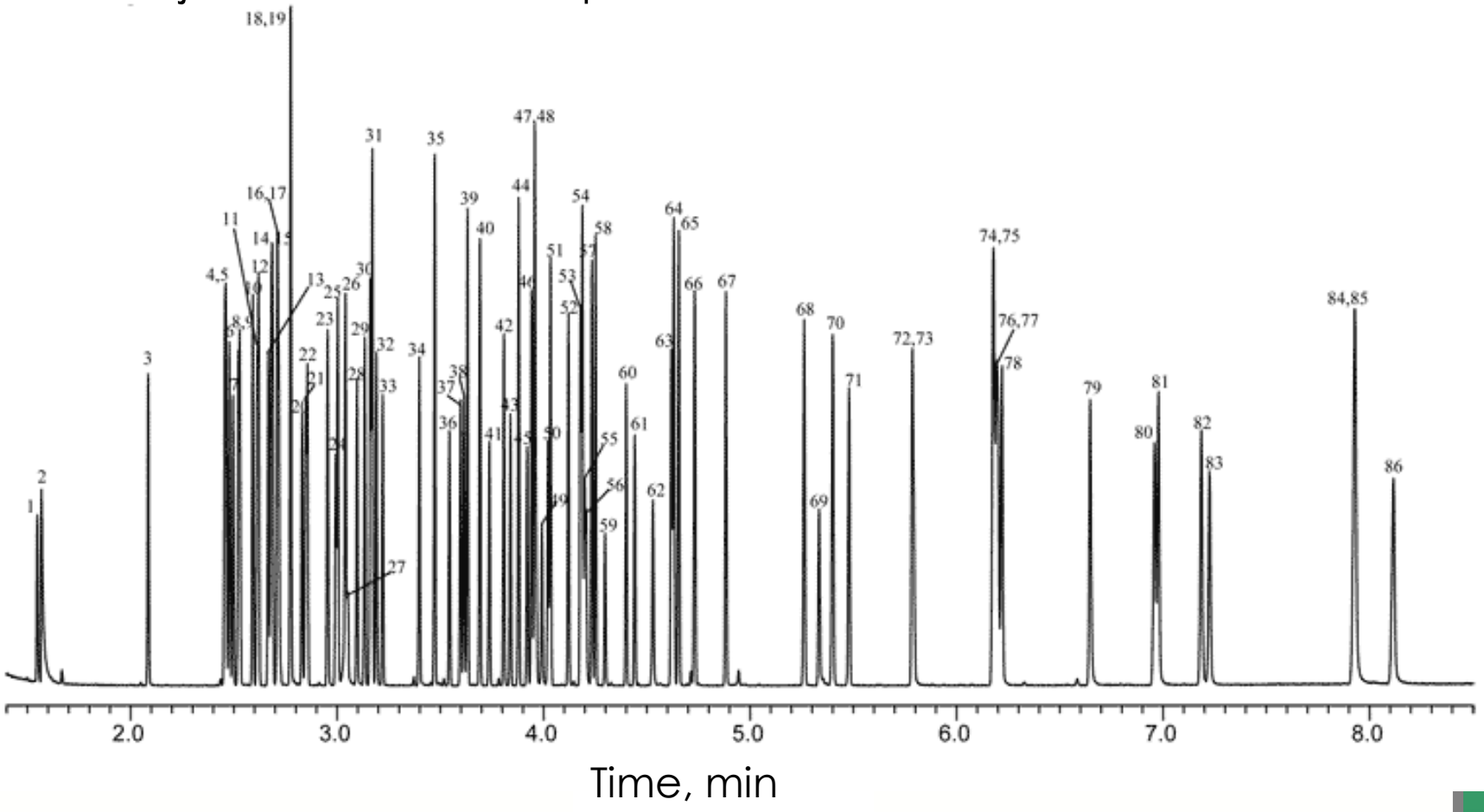
Column Chromatography separation process



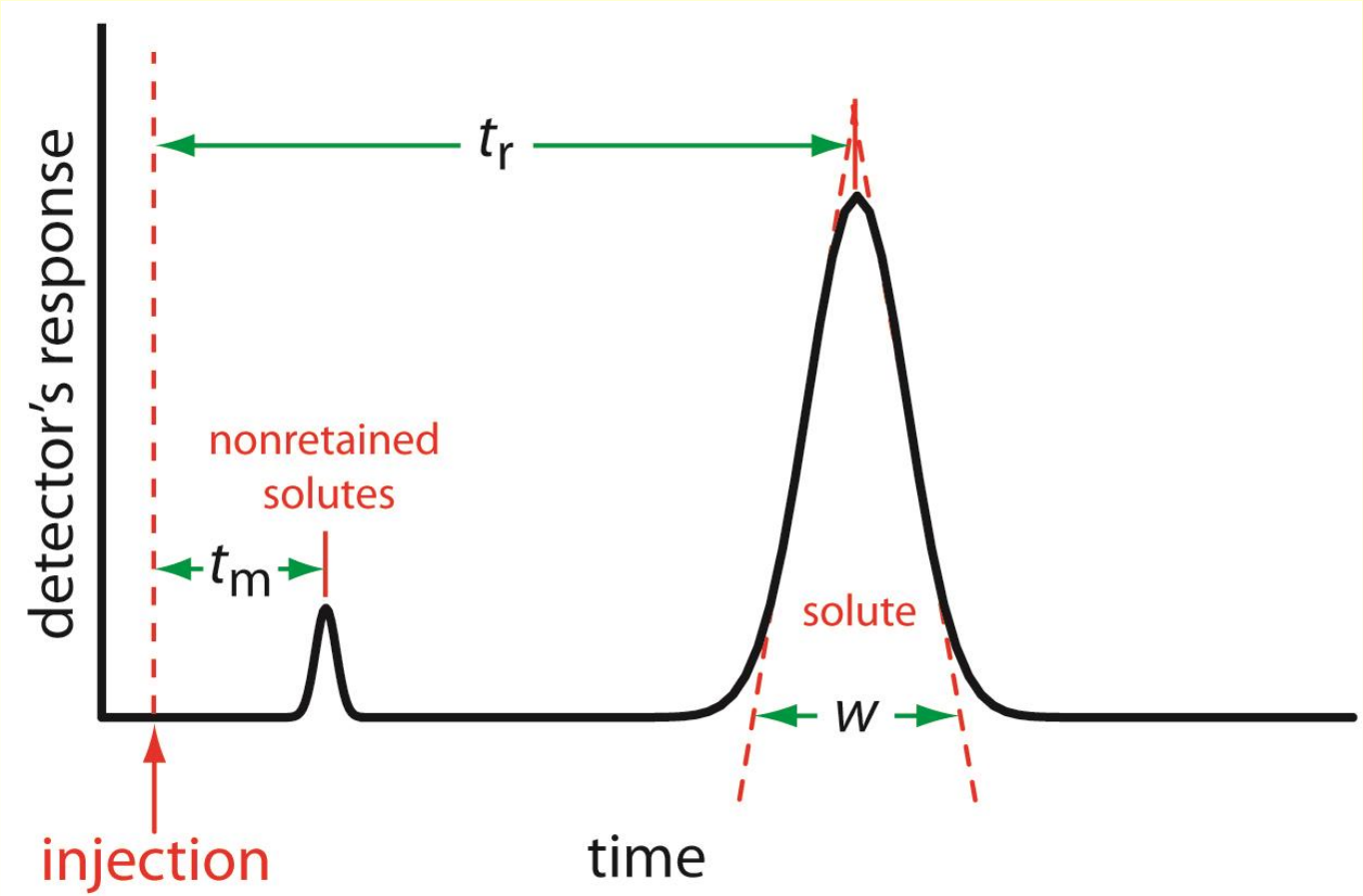


Chromatogram

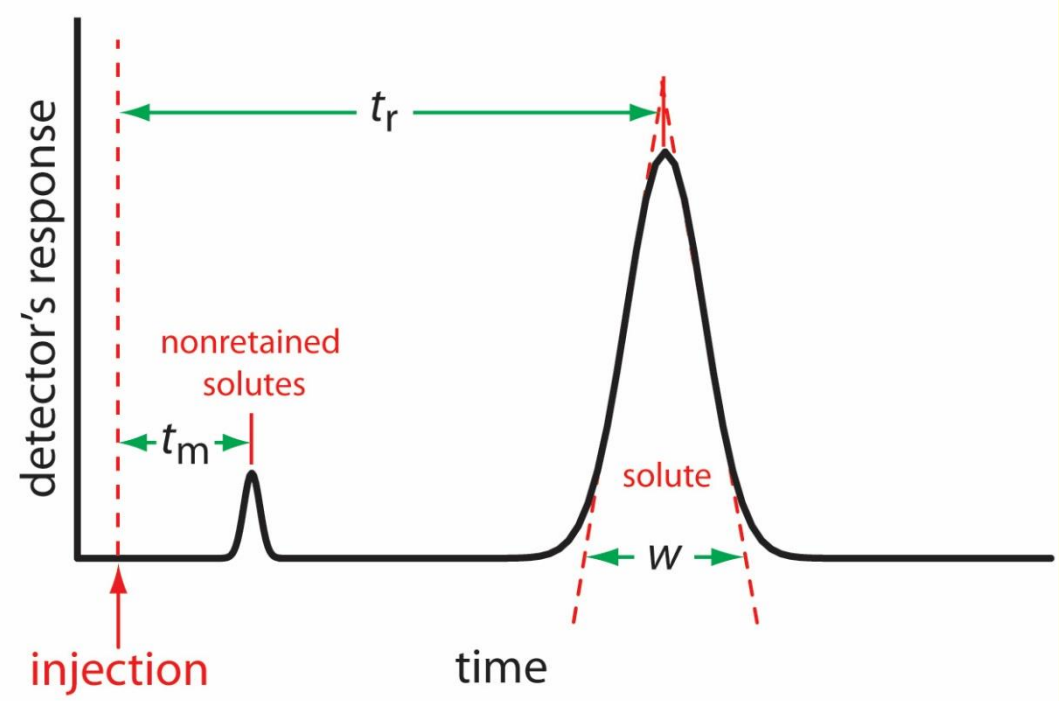
Chromatogram – dependence of a detector signal versus time after injection of the sample.



Main definitions



Main definitions



Retention time, t_r, is the time between the sample's injection and the maximum response for the solute's peak.

The time required to elute nonretained solutes is called the column's **void time, t_m.**

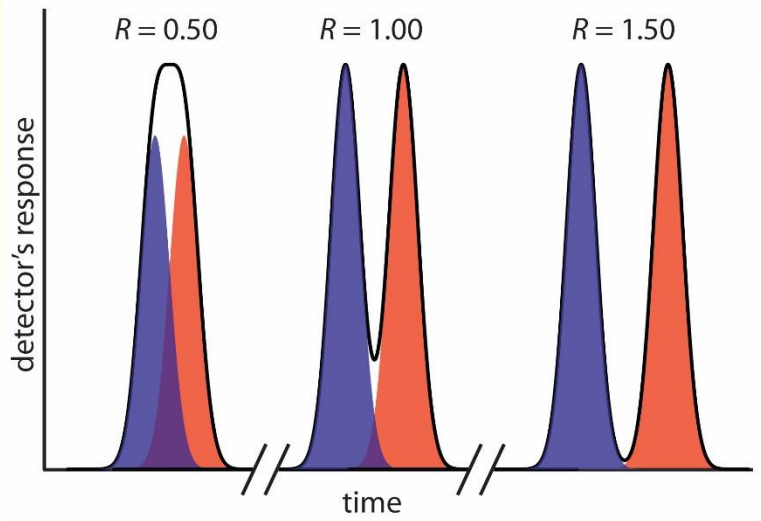
Chromatographic Resolution

Shows separation efficiency between two peaks

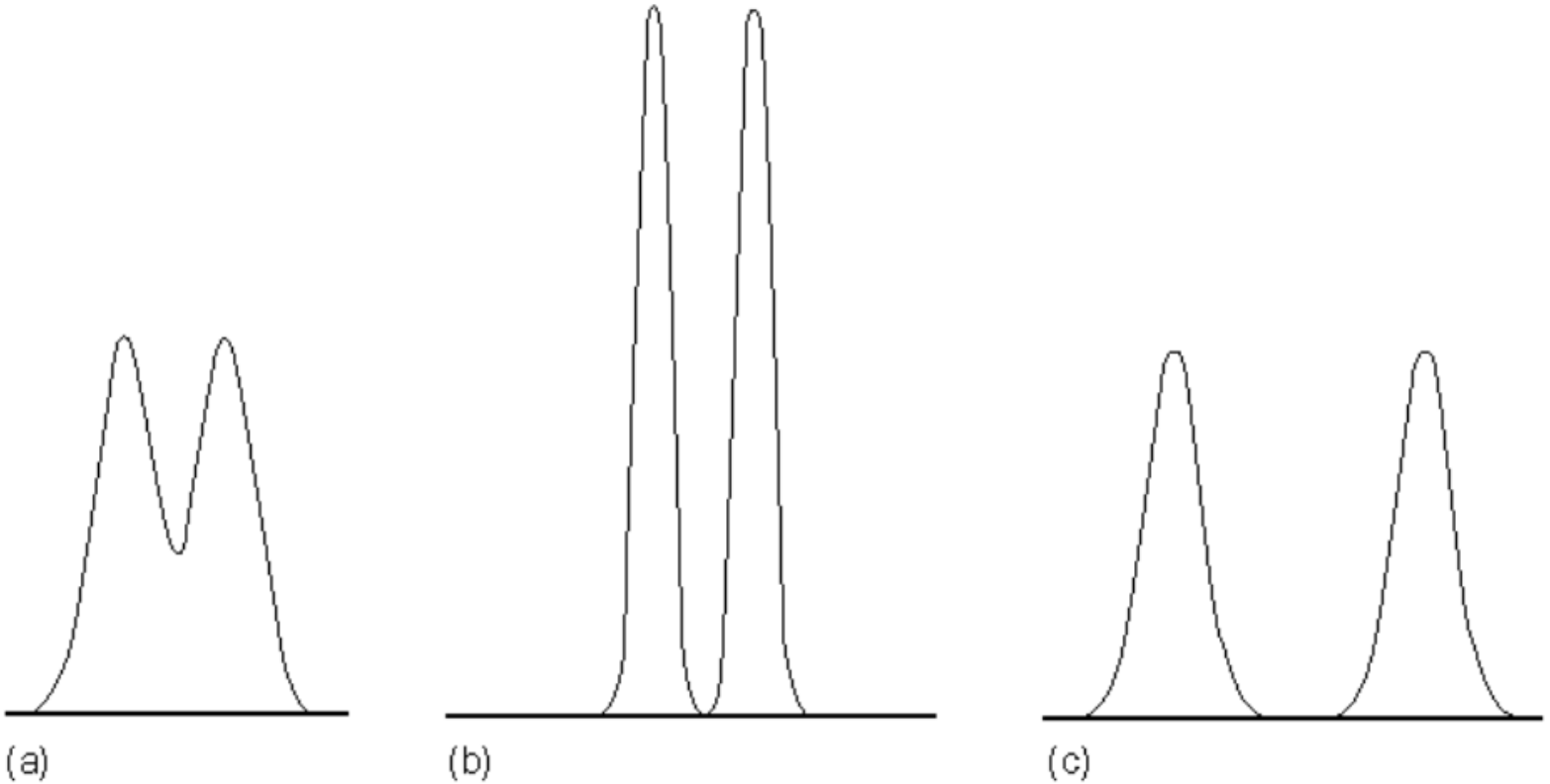
The **resolution** between two chromatographic peaks, R_{AB}, is a quantitative measure of their separation, and is defined as

$$R_{AB} = \frac{t_{r,B} - t_{r,A}}{0.5(w_B + w_A)} = \frac{2\Delta t_r}{w_B + w_A}$$

where B is the later eluting of the two solutes.



Improving separation



Chromatographic parameters

Retention factor – shows efficiency of analyte retention by a column

$$k = \frac{t_\mathrm{r} - t_\mathrm{m}}{t_\mathrm{m}} = \frac{t'_\mathrm{r}}{t_\mathrm{m}}$$

Column efficiency – provides a quantitative measure of the extent of band (peak) broadening

$$N = 16\frac{t_r^2}{w^2}$$

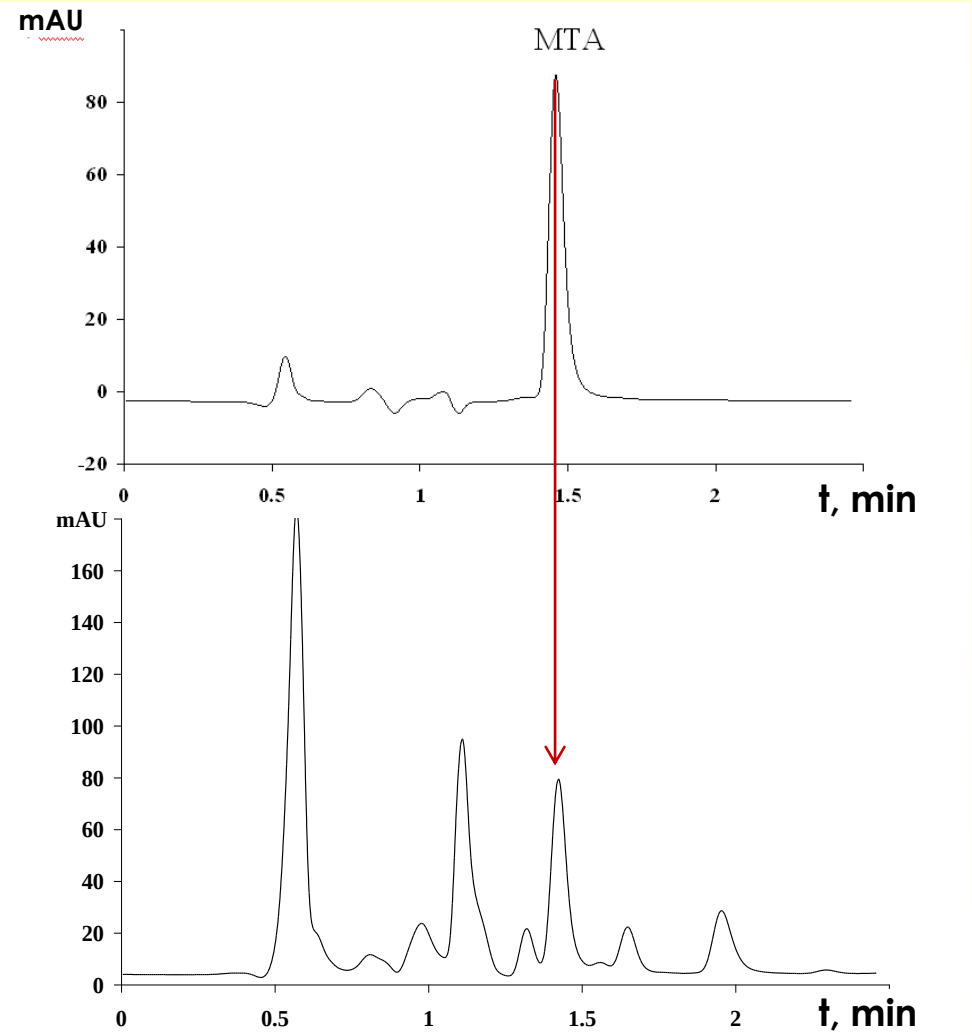
Qualitative analysis by chromatography

By retention times – comparison of retention times of individual compounds with retention times of chromatographic peaks (rule – retention time is constant at constant separation parameters)

By Kovat's retention index - calculation of Kovat's indices by the retention times of n-alkanes. Example: a substance with a Kovacs index of 1750 is on the chromatogram exactly between n-C_{17} and n-C_{18}

Using spectral data collected from spectral detectors – comparison of collected spectra with spectra of individual compounds using mass spectra library or database

Example of identification using retention time



Chromatogram of triazole standard; RT = 1.45 min

Chromatogram of water sample: triazole is detected by its retention time (1.45 min)

Kovat's retention index

$$RI = X \times 100 + \frac{(RT_A - RT\ (n - alkane\ X))}{(RT\ (n - alkane\ X) - RT\ (n - alkane\ X + 1))} \times 100$$

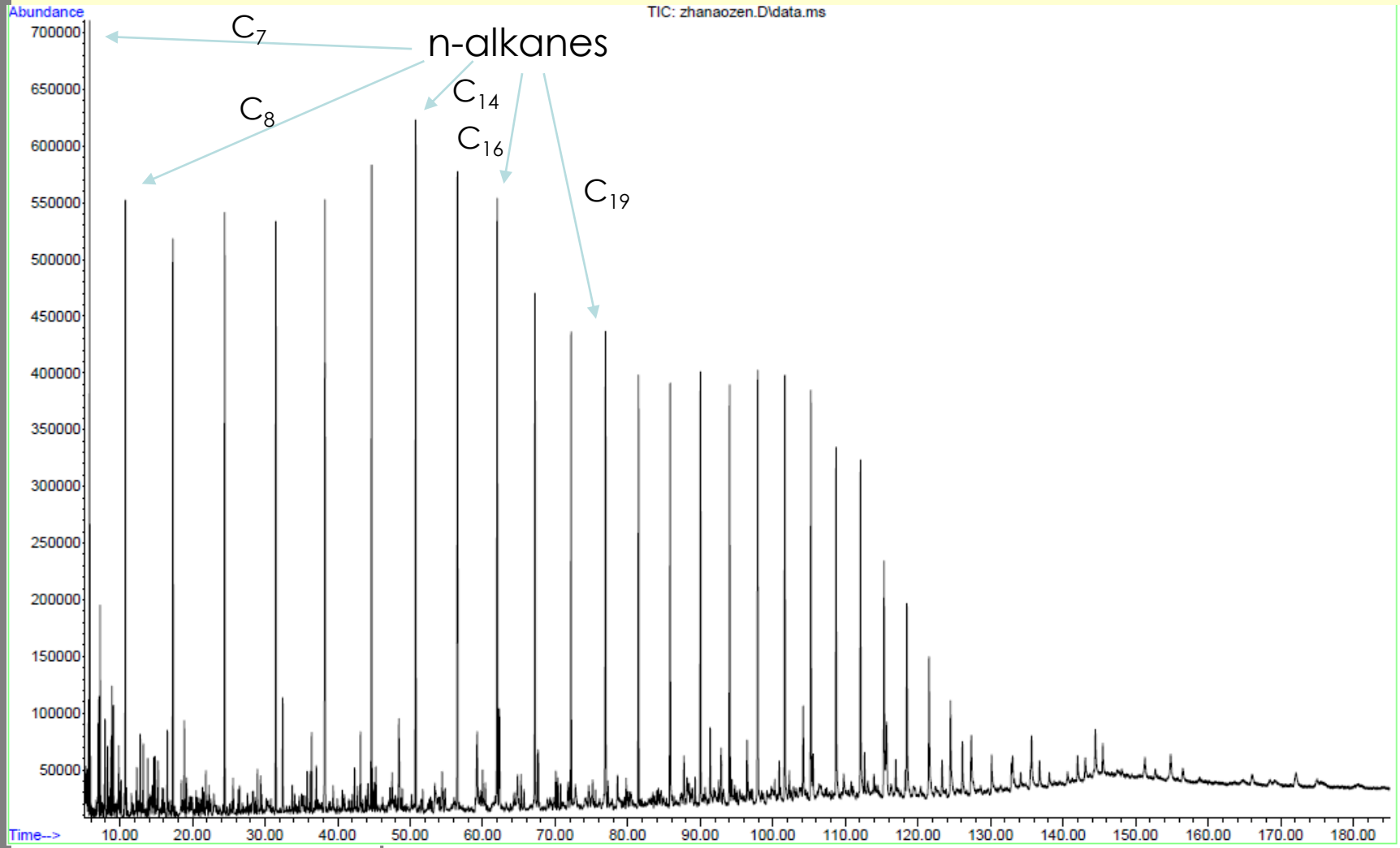
Where:

X – the number of n-alkane eluting immediately before the analyte

X + 1 – the number of n-alkane eluting immediately after the analyte analyte

RT - retention time, min

n-alkanes in the chromatogram of oil



Example 1

Retention time of unknown peak 14.44 min. This peak in the chromatogram is located between the peaks of n-C10 and n-C11, the retention times of which are 13.83 and 15.51 min, respectively. Find the unknown peak retention index.

$$RI = 100 \times 10 + \frac{14{,}44 - 13{,}83}{15{,}51 - 13{,}83} \times 100 = 1036$$

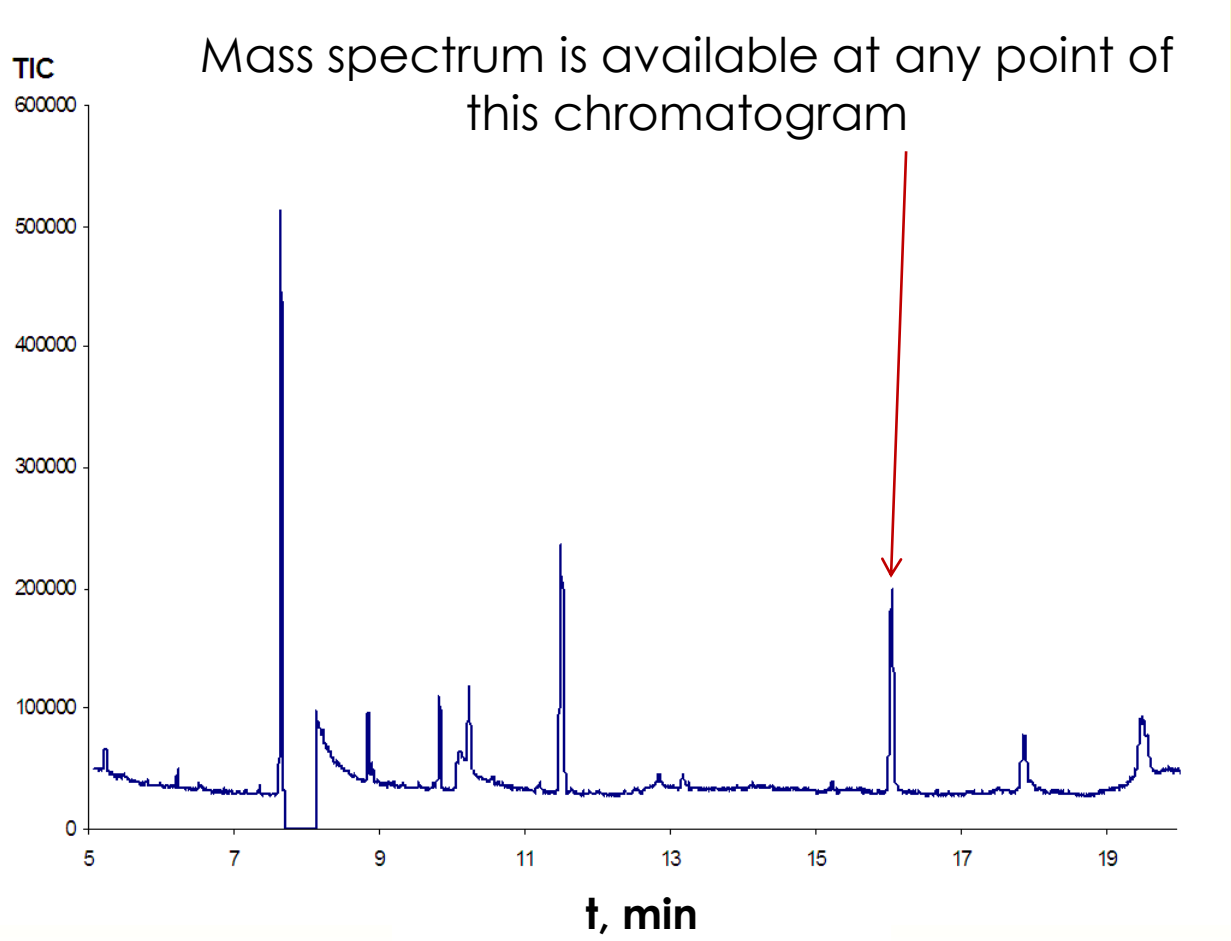
Example 2

According to the NIST library, the Kovat's index for phenol is 955. After analyzing the mixture of n-alkanes, the retention times of n-C9 and n-C10 were established, which were 9.55 and 11.32 min, respectively. Determine the estimated retention time of phenol.

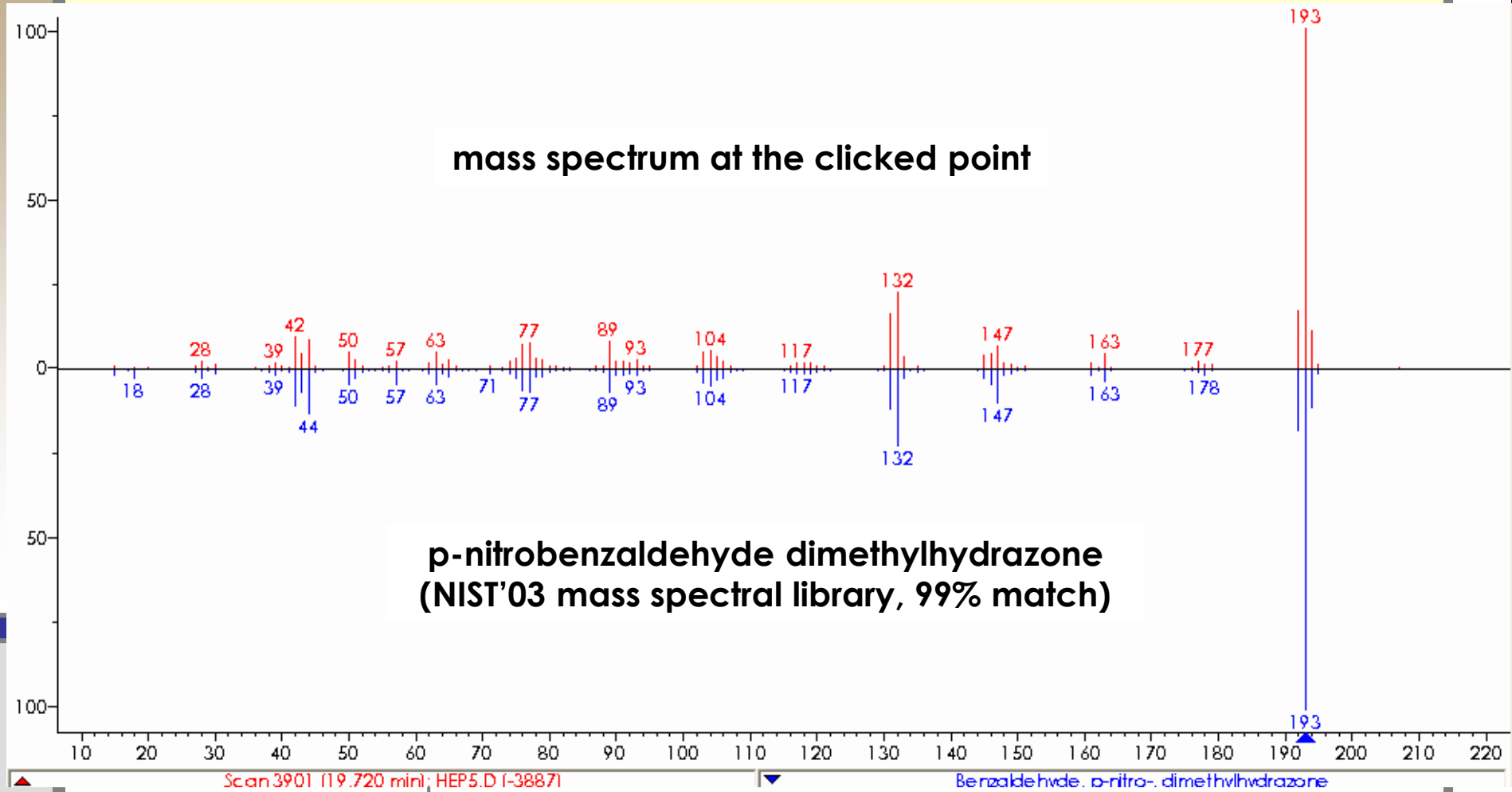
$$955 = 900 + \frac{X - 9{,}55}{11{,}32 - 9{,}55} \times 100$$

$$X = 0{,}55\,(11{,}32 - 9{,}55) + 9{,}55 = 10{,}52 \text{ мин}$$

Identification by spectral data

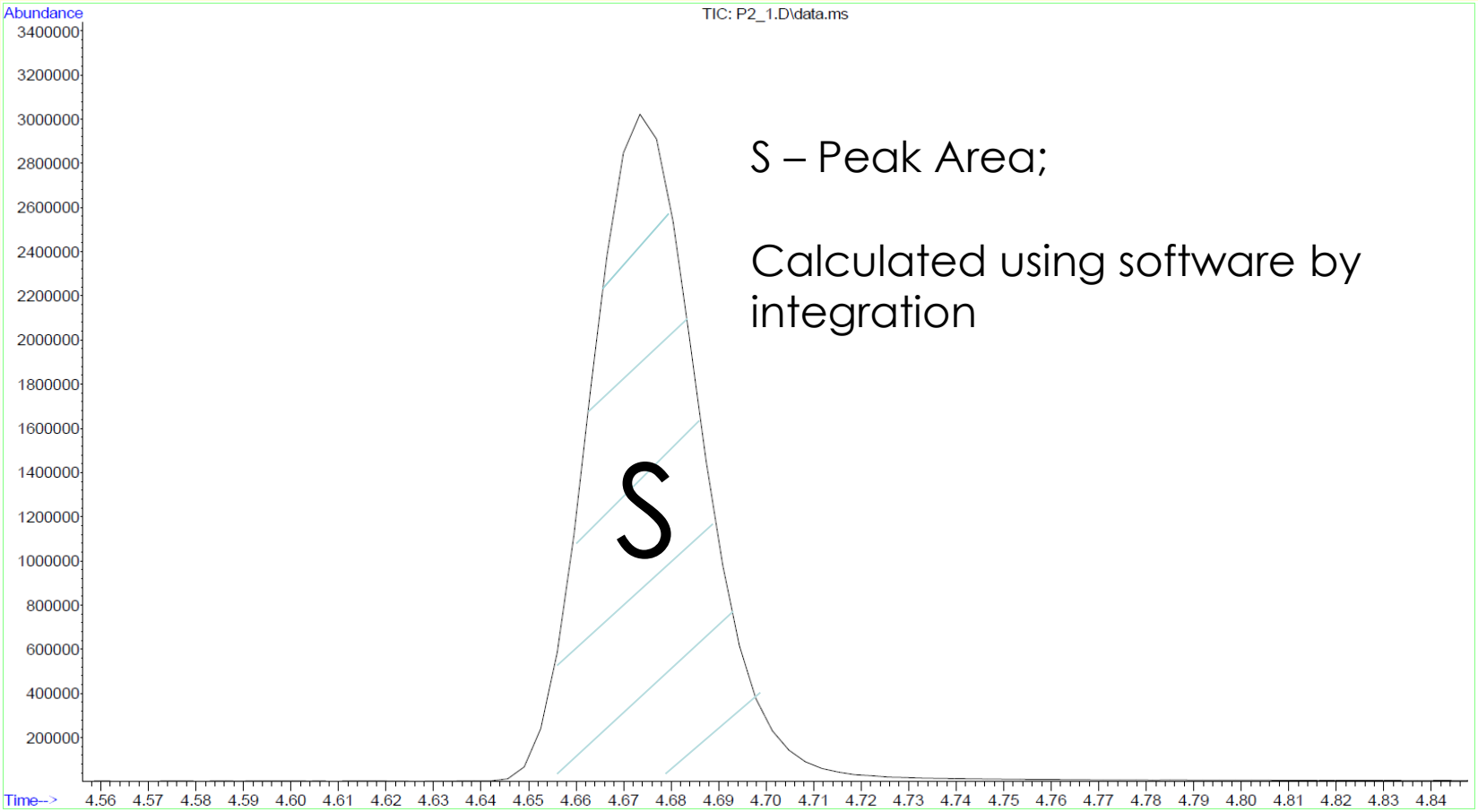


Identification by spectral data



Quantitative analysis by chromatography

By peak area – peak area is directly proportional to analyte concentration or mass of analyte that reached the detector



S – Peak Area;

Calculated using software by integration

Methods of quantitative analysis

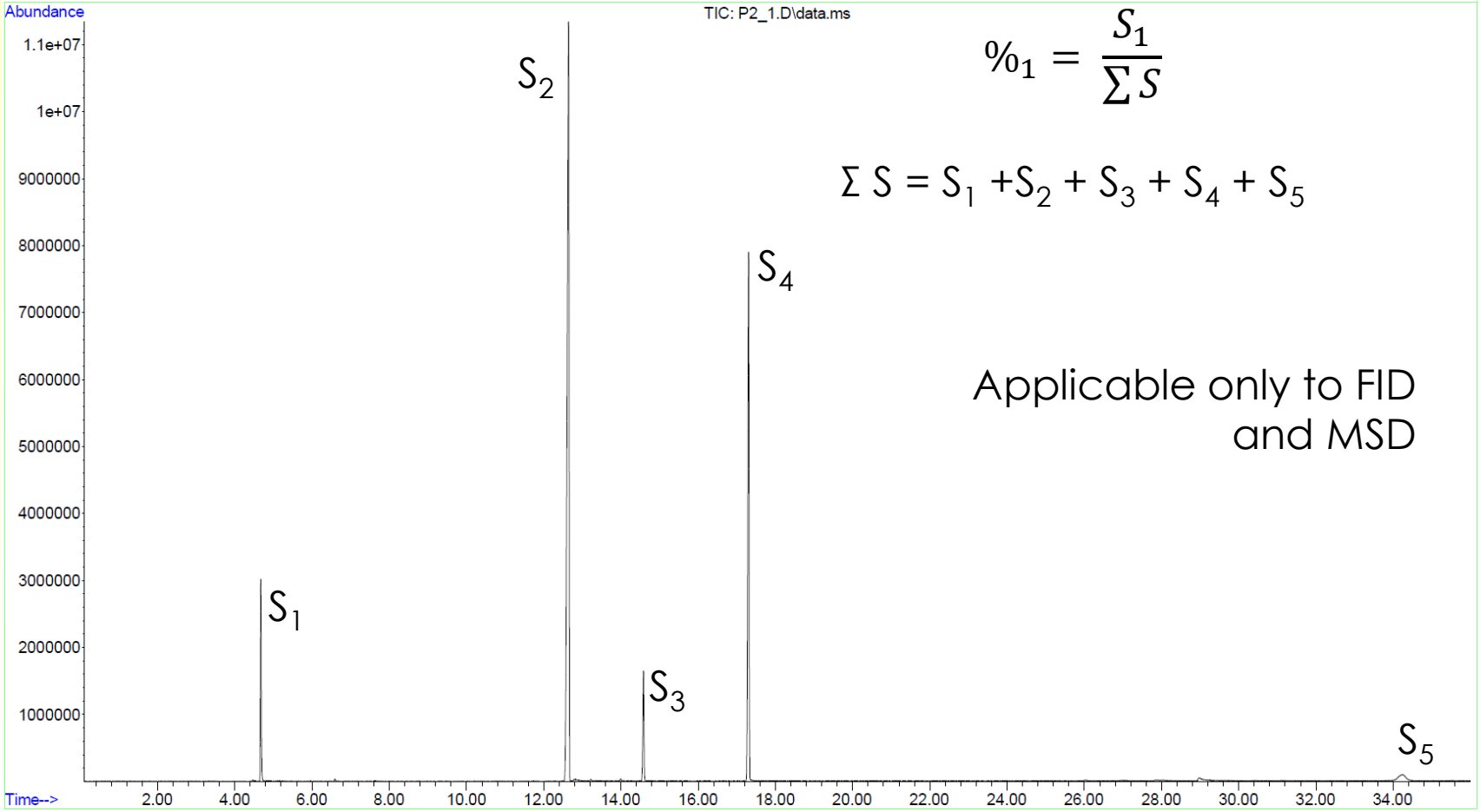
By normalization - percentage of analyte is calculated as its peak area divided by a sum of all the peaks detected on chromatogram

External standard calibration – different concentrations of analyte are analyzed to get a calibration curve: $S = f(C)$

Internal standard calibration – solutions having different ratios of analyte and internal standard concentrations are analyzed to get a calibration curve: $S/S_{is} = f(C/C_{is})$. In most cases C_{is} is constant during all analyses.

Standard addition – a known concentrations of analytes are introduced into the sample to get a calibration curve and measure the concentration of analyte. $S = f(C_{add.})$.

Normalization



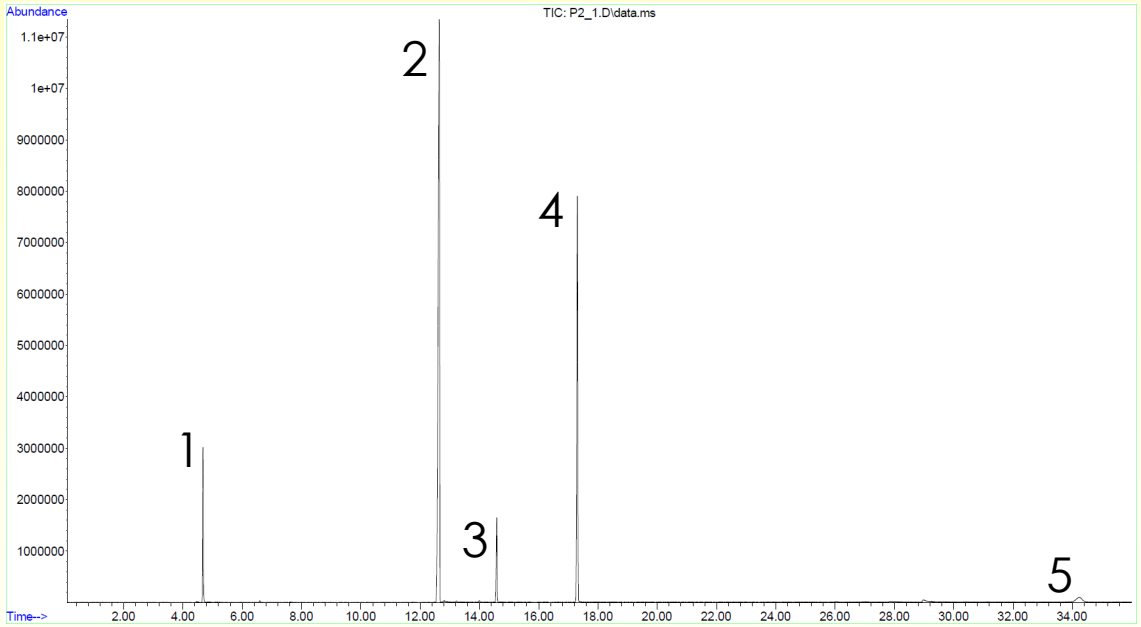
$$\%_1 = \frac{S_1}{\sum S}$$

$$\Sigma S = S_1 + S_2 + S_3 + S_4 + S_5$$

Applicable only to FID and MSD

Very simple and efficient method for analyzing unknown samples

Results of normalization



peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	4.675	1305	1318	1352	BB	2992968	49200040	12.88%	7.546%
2	12.645	3572	3606	3629	VB	11324431	382062343	100.00%	58.598%
3	14.585	4144	4163	4184	BB	1637784	33407127	8.74%	5.124%
4	17.306	4921	4945	4992	BB	7882056	175333879	45.89%	26.891%
5	34.251	9731	9812	9893	BB 6	90854	12002277	3.14%	1.841%

Quiz 1/4

What is the main goal of chromatography?

1 – to analyze substances

2 – to obtain spectra

3 – to determine peak areas

4 – to separate mixtures of chemical compounds

Quiz 2/4

What phase moves substances in chromatography?

1 – stationary

2 – solvent

3 – mobile

4 – water

Quiz 3/4

What chromatographic peak parameter is used for qualitative analysis?

1 – retention time

2 – peak width

3 – peak area

4 – peak height

Quiz 4/4

What parameter of the chromatographic peak is used for quantitative analysis?

1 – retention time

2 – peak width

3 – peak area

4 – peak height

Task 1

In a chromatographic analysis of lemon oil a peak for limonene has a retention time of 8.36 min with a baseline width of 0.96 min. γ-Terpinene elutes at 9.54 min with a baseline width of 0.64 min. What is the resolution between the two peaks?

Task 1

$$R_{AB} = \frac{2\Delta t_r}{w_B + w_A} = \frac{2(9.54\ \text{min} - 8.36\ \text{min})}{1.64\ \text{min} + 0.96\ \text{min}} = 1.48$$