Lecture 3
Efficiency, selectivity and resolution

The anatomy of a peak

In chromatographic theory the peaks are usually assumed to have (perfect) gaussian shapes.

Measures of resolution and chromatographic efficiency usually involve estimates of the chromatographic peak width

Peak widths can be estimated in several ways

Equations involving peak width come in many variants, depending on where the width is measured.

Always pay attention to whether the peak width is given as \( w_b \), \( w_h \), \( w_i \) (or something else).

Where to measure may also be given as fractions or percents of the peak height (\( w_h = w_{0.5} \)).

Use the following relationships to convert between equations:

\[
\begin{align*}
    w_i &= w_b \cdot 0.5 \\
    w_h &= w_b \cdot 0.589 \\
    w_{0.5} &= w_b \cdot 0.5 \\
    w_0.882 &= w_b \\
    w_1 &= w_b \\
\end{align*}
\]
Resolution (separation) between A and B is of course necessary for quantification of the compounds.

Chromatographic resolution

- Poor resolution
- Good resolution
- The resolution between two peaks, \( R_s \), is defined as:

\[
R_s = \frac{2 (t_{R(B)} - t_{R(A)})}{w_b(A) + w_b(B)}
\]

where

- \( t_{R(B)} \) is the retention time of peak B
- \( t_{R(A)} \) is the retention time of peak A
- \( w_b(A) \) is the width at baseline of peak A
- \( w_b(B) \) is the width at baseline of peak B

\( w_b \) is width at baseline and is defined as four times the standard deviation of the peaks.

Two factors affect resolution between peaks:
1. The distance between the peak maxima
2. The average peak width
Resolution (separation) between A and B is of course necessary for quantification of the compounds.

$$R_s = \frac{t_{R(B)} - t_{R(A)}}{\frac{1}{2} \left( W_{H(A)} + W_{H(B)} \right)}$$

There are variants of the equations when the peak widths are measured at half height.

If the resolution ($R_s$) is too poor there are two ways to improve it:

- Increase the distance between the peaks
- Reduce the peak widths

Chromatographic selectivity or relative retention between two peaks can be described by the separation factor, $\alpha$:

$$\alpha = \frac{k_B}{k_A}$$

Since $k$ is directly proportional to $t'_R$ (adjusted retention time)

$$\alpha = \frac{t'_{R(B)}}{t'_{R(A)}}$$

The unadjusted relative retention is calculated from the unadjusted retention times:

$$\gamma = \frac{t_{R(B)}}{t_{R(A)}}$$

Since $\gamma$ will depend also on $t_R$ it is not a pure estimate of selectivity. $\gamma$ may also be denoted $\alpha_G$.

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Chromatographic efficiency

Chromatographic efficiency is traditionally given as the plate number, \( N \), which is a measure of how narrow a peak is compared to its retention time.

\[
N = \frac{t_R}{\sqrt{H_w}} \quad \text{Eq (8)}
\]

Note that increasing the retention time or decreasing peak width (keeping the other constant) will increase \( N \).

Note: \( N \) is only meaningful as long as chromatographic conditions are kept constant during the run (mobile phase composition, temperature).

The plate height, \( H \), is a measure of the chromatographic efficiency per meter column. \( H \) should be minimized to achieve optimal conditions.

\[
N = \frac{t_R}{\sqrt{H_w}} \quad \text{Eq (9)}
\]

\[
H = \frac{L}{N} \quad \text{Eq (10)}
\]

The effective plate number applies \( t'_R \) instead of \( t_R \). Similarly, there is an effective plate height \( H_{\text{eff}} \) instead of \( H \).

\[
N_{\text{eff}} \quad \text{Eq (11)}
\]

The Purnell equation

The three factors leading to chromatographic separation, efficiency, selectivity, and retention, are summarized in the Purnell equation.

\[
R_s = \sqrt{\frac{N_{\text{eff}}}{4}} \left( \frac{1}{\alpha} - 1 \right) \quad \text{Eq (15)}
\]

If either of the terms is zero, resolution is zero. The equation may tell us where to put the effort if we need improved resolution.

Example: If \( \alpha = 2 \), you will get a 20% increase in resolution by doubling \( N_{\text{eff}} \), because the retention term increase from 0.667 to 0.800. If \( \alpha = 10 \) you will only get a 5% increase in resolution by doubling \( N_{\text{eff}} \) because the retention term increases from 0.91 to 0.95.
The Purnell equation

The three factors leading to chromatographic separation, efficiency, selectivity, and retention, are summarized in the Purnell equation.

\[ R_s = \sqrt{\frac{N}{4}} \left( 1 + \frac{1}{\alpha} \right) \left( 1 + \frac{k(B)}{1+k(B)} \right) \]

\[ \text{Eq (11)} \]

You may see variants of the equation above, referred to as the Purnell equation or under other names.

Resolution can also be expressed as a function of the unadjusted relative retention, \( \gamma \).

\[ R_s = \sqrt{\frac{N}{4}} \left( \gamma - 1 \right) \]

\[ \text{Eq (12)} \]

\( \gamma \) is a function of both retention and selectivity.

How to resolve peak overlaps

**Rules of thumb:**

- If the compounds differ in type or number of functional groups: try to change selectivity
- If the compounds are isomers: increase the efficiency
- In complex chromatograms: increase the efficiency
How to resolve peak overlaps

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- If the compounds differ in type or number of functional groups: try to change selectivity.
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- In complex chromatograms: increase the efficiency.

The maximum number of peaks that (in theory) can be separated by a method.

How to resolve peak overlaps

**Increase efficiency**
(same functional groups)

How to change selectivity in LC

- Change mobile phase composition
- Change type of stationary phase

Selectivity in GC

- Change mobile phase composition
- Change type of stationary phase

A gas has no selectivity.
How to change selectivity in GC

Example: fatty acids
Increasing polarity with increasing number of double bonds

20:0 (Eicosanoic acid)

20:5 n-3 (EPA)

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Apolar column: Separation according to boiling point

How to increase the efficiency?

→ Lecture 4
### Summary of equations

<table>
<thead>
<tr>
<th>Equation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>( R_s = 2 \left( \frac{t_R(B) - t_R(A)}{w_B + w_B} \right) )</td>
<td>Measured resolution</td>
</tr>
<tr>
<td>( \alpha = \frac{k(B)}{k(A)} )</td>
<td>Separation factor (relative retention)</td>
</tr>
<tr>
<td>( \gamma = \frac{t_R(B)}{t_R(A)} )</td>
<td>Unadjusted relative retention (( \gamma ) or ( \alpha ))</td>
</tr>
<tr>
<td>( N = 16 \left( \frac{t_R}{w_B} \right)^2 )</td>
<td>Plate number</td>
</tr>
<tr>
<td>( H = \frac{L}{N} )</td>
<td>Plate height</td>
</tr>
<tr>
<td>( N_{eff} = 16 \left( \frac{t_R}{w_B} \right)^2 )</td>
<td>Effective plate number</td>
</tr>
</tbody>
</table>

#### Resolution

- **Function of \( N \), \( k \), and \( \alpha \) (Purnell equation)**
  \[ R_s = \sqrt{N^4 \left( \alpha - 1 \right)^2} \left( \frac{k(B)}{1 + k(B)} \right) \]

- **Function of \( N \) and \( \gamma \)**
  \[ R_s = \sqrt{N^4 \left( \gamma - 1 \right)} \]