Lab scale isolation of compounds by Flash Chromatography

- Properties of Silica Gel
- Using Thin Layer Chromatography (TLC) for chromatographic method development
- Transfer from TLC to Flash Chromatography
- Applications
Silica Production Process

Hydrogel
- (raw silica powder, particle size 0.5 - 6 mm)
- temperature control
- drying temperature control

Xerogel
- (raw silica powder, particle size 0.5 - 6 mm)
- washing pH-control, ion content

Ground silica gel
- (dp 5 - 500 µm)
- washing pH-control, ion content

Hydrosol
- (oligosilicic acid, orthosilicic acid)
- control of temperature and pH

\( \text{Na}_2\text{SiO}_3 \) (water glass) + \( \text{H}_2\text{SO}_4 \)

Classified silica gels
- control of temperature and pH
- drying temperature control
- washing pH-control, ion content

Production Plant

... the biggest chromatographic silica gel plant in the world,
Production Plant

Three reactors for silica gel production,

... Large scale production, batch size: several tons

Standardized Silica Gels

<table>
<thead>
<tr>
<th>LiChrosorb Si60</th>
<th>5, 10µm</th>
</tr>
</thead>
<tbody>
<tr>
<td>LiChroprep Si 60</td>
<td>15-25 µm</td>
</tr>
<tr>
<td></td>
<td>25-40 µm</td>
</tr>
<tr>
<td></td>
<td>15-40µm</td>
</tr>
<tr>
<td>Silica Gel 60</td>
<td>40-63µm [9385]</td>
</tr>
<tr>
<td>+ Silica Gel for TLC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>below 63µm</td>
</tr>
<tr>
<td></td>
<td>63-100µm</td>
</tr>
<tr>
<td></td>
<td>63-200µm [7734]</td>
</tr>
<tr>
<td></td>
<td>200-500µm</td>
</tr>
</tbody>
</table>

All from one Production Process

CONSTANT SELECTIVITY
### Factors influencing Selectivity and Usability

<table>
<thead>
<tr>
<th>Form</th>
<th>irregular / spherical</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface</td>
<td>Modification</td>
</tr>
<tr>
<td>Area / Pore Size</td>
<td>Selectivity</td>
</tr>
<tr>
<td>Purity</td>
<td>Loadability</td>
</tr>
<tr>
<td>pH</td>
<td>Selectivity, Resolution</td>
</tr>
<tr>
<td>Water Content</td>
<td>Selectivity</td>
</tr>
<tr>
<td>Stability</td>
<td>Life Time</td>
</tr>
<tr>
<td></td>
<td>CIP - Conditions</td>
</tr>
</tbody>
</table>

- Efficiency, Pressure Drop
- Selectivity
- Loadability
- Selectivity, Resolution
- Selectivity
- Life Time
- CIP - Conditions

### Separation Optimization

**Resolution** - the key parameter for preparative chromatography

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

- maximize through sorbent screening
- minimize - keep cycle time short
- maximize - high efficiency sorbent
Method Development – using Thin Layer Chromatography

• You have to consider three phases in TLC:
  - Gas phase
  - Sample
  - Stationary phase
  - Liquid phase

Method Development – using Thin Layer Chromatography

• Several parameters have to be considered when developing a TLC method:
  - Activity of the stationary phase
  - Conditioning / preparation
    1. methanol / isopropanol (plate developing or dipping)
    2. drying (30 min @ 120 °C)
  - Support (plastic, aluminium, glass)
  - Binder
  - Fluorescent indicator
  - Manufacturer / lot reproducibility
  - Manufacturer / comparability with Flash / column chromatography
Method Development – using Thin Layer Chromatography

- The mobile phase should:
  - Dissolve the sample
  - Desorb the sample from the stationary phase
  - Transport the sample over an acceptable distance

- The mobile phase should in general be:
  - Simple (maximum 4, better 2 components)
  - Non-toxic
  - of chromatographic quality
  - Not cause any secondary reactions
  - Not demixing
  - Of favourable low viscosity

Solvents classification: Snyder triangle

- proton donor
- proton acceptor
- dipole interaction

Method Development – using Thin Layer Chromatography
TLC/HPLC - Conversion

\[ R_f = \frac{d_{as}}{d_{af}} \]

\[ k'_{DC} = \frac{1}{R_f} - 1 \]

ATTENTION: the migration distance is measured from the sample spot.

TLC - equipment

- CAMAG – vario chamber → parallel use of 6 different solvents or solvent mixtures
Thin Layer Chromatography (TLC) as pilot technique

**Some general rules:**
- Adjust polarity to lower retention values on TLC plates
- Try to maintain the same relative activity on the plate and on the column
- Do not use volatile mobile phase components

**Literature:**
TLC as a pilot technique for transferring retention data to HPLC

**TLC/HPLC - Conversion**

Place a spot of the sample on a TLC-plate
Develop chromatogram with mobile phases of different elution strength

<table>
<thead>
<tr>
<th>ε^0 (elution strength)</th>
<th>far too low</th>
<th>too low</th>
<th>perfect</th>
<th>too high</th>
</tr>
</thead>
</table>

Literature: E.Stahl, Chemiker-Ztg. 82 (1958) 323
TLC/HPLC - Conversion

Apolar Compound
- n-Heptane: 0.01
- Cyclohexane: 0.04
- MTBE: 0.38
- Dichloromethane: 0.42

Polar Compound
- MTBE: 0.38
- Dioxane: 0.56
- THF: 0.57
- Etat: 0.58
- IPA: 0.82
- EtOH: 0.88
- MeOH: 0.95
- ACN: 0.95

TLC-Approach 1 (Solvent Screening model J.Dingenen, Janssen Pharmaceutica)

1) Two series of neat solvents covering a broad polarity and selectivity range

Run 1
- MTBE
- Toluene
- CH₂Cl₂
- CH₃CN
- EtAc
- THF

Run 2
- MeOH
- EtOH
- IPA
- Acetic acid
- 2-propanone
- 2-butanone

2) Solvent series based on dichloromethane – methanol mixtures

Run 3
<table>
<thead>
<tr>
<th>Lane</th>
<th>N-Hexane 50%</th>
<th>MeOH 2%</th>
<th>MeOH 5%</th>
<th>MeOH 10%</th>
<th>MeOH 20%</th>
<th>MeOH 50%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>48%</td>
<td>2%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>46%</td>
<td>4%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>44%</td>
<td>6%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>42%</td>
<td>8%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>40%</td>
<td>10%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
TLC-Approach 1 (Solvent Screening model J. Dingenen, Janssen Pharmaceutica)

2) Solvent series based on dichloromethane – methanol mixtures

<table>
<thead>
<tr>
<th>Eluent front</th>
<th>Preparative gradient % methanol</th>
<th>Start</th>
<th>End</th>
</tr>
</thead>
<tbody>
<tr>
<td>hRf &gt; 50</td>
<td></td>
<td>0 %</td>
<td>5 %</td>
</tr>
<tr>
<td>30 &lt; hRf &lt; 50</td>
<td></td>
<td>0 %</td>
<td>10 %</td>
</tr>
<tr>
<td>hRf &lt; 30</td>
<td></td>
<td>0 %</td>
<td>20 %</td>
</tr>
</tbody>
</table>

Start experiments on the preparative chromatography column

1) Good selectivity for one of the tested neat solvents or for one of the dichloromethane – methanol mixtures

2) Good selectivity for one of the tested neat solvents but Rf values to high
   Addition of an apolar solvent to adjust the Rf values between 0.10 and 0.35
   (corresponding to a K' value between 1.85 and 9)

3) Unsatisfactory results
   On the basis of the observed spot shapes in the experiments with neat solvents the most suitable solvent combinations (binary – ternary) are chosen and further investigated
TLC-Approach 1 (Solvent Screening model J. Dingenen, Janssen Pharmaceutica)

Binary solvent mixtures: THF/acetone or Ethanol/Acetone

Ternary solvent mixtures
TLC-Approach 2
(EtAc/Heptane-Gradient, Solvias)

TLC-Screening with Ethyl acetate / n-hexane (1:4)

Segment 3
(Rf=0.60-0.90)

Segment 2
(Rf=0.25-0.60)

Segment 1
(Rf=0.05-0.25)

Transfer to preparative normal phase silica, according to segment

Transfer to LiChrospher Si 60, 12 µm packed into Selfpacker columns 125 mm length and 25 or 50 mm I.D.

Exponential gradient:

\[ c(EtAc) = \frac{(e^{aT} - 1) \cdot (1 - K)}{e^a - 1} + K \]

T=length of gradient(min), K=c(EtAc) at t=0, a=exponential gradient parameter, t=runtime

<table>
<thead>
<tr>
<th>Rf</th>
<th>0.05-0.25</th>
<th>0.25-0.60</th>
<th>0.60-0.90</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>7</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>K</td>
<td>0.15</td>
<td>0.09</td>
<td>0.05</td>
</tr>
<tr>
<td>T</td>
<td>8</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>

Literature:
Automated normal-phase preparative HPLC as a substitute for flash chromatography in the synthetic research laboratory
P.Renold, E.Madero, Th.Maetzke, J.Chromatogr. 2001 (908), 143-148
**TLC-Approach 2**

(EtAc/Heptane-Gradient, Solvias)

- **OA / hex = 1:4**
- 0.70 Benzyl
- 0.60 Ethylbenzoate
- 0.54 Dibutylphthalate
- 0.50 Acetophenone
- 0.43 Diethylphthalate
- 0.33 Dimethylphthalate
- 0.22 Benzylalcool
- 0.05 Acetanilide

**Example for TLC method development (Diastereomers + impurities)**

- 100 % n-Heptan
- 95 % n-Heptan, 5 % Ethylacetat
- 90 % n-Heptan, 10 % Ethylacetat
- 85 % n-Heptan, 15 % Ethylacetat
- 80 % n-Heptan, 20 % Ethylacetat
- 75 % n-Heptan, 25 % Ethylacetat
Example for TLC method development (Diastereomers + impurities)

Example for TLC method development (Diastereomers + impurities)

HPLC chromatogram

TLC chromatogram
Comparison of TLC and HPLC

**Used stationary phases:**

**TLC**
- HPTLC KG 60
- HPTLC RP-8
- HPTLC RP-18
- HPTLC CN
- HPTLC NH₂
- HPTLC Diol

**HPLC**
- LiChrospher Si 60
- LiChrospher 100 RP-8, LiChrospher 60 RP select B
- LiChrospher 100 RP-18
- LiChrospher 100 CN
- LiChrospher 100 NH₂
- LiChrospher 100 Diol

Comparison of TLC and HPLC

**Example 1: Triazines**

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>hRf</th>
<th>K'</th>
<th>K'(RP-8)</th>
<th>K'(select B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terbutryn</td>
<td>19.3</td>
<td>4.18</td>
<td>15.70</td>
<td>3.20</td>
</tr>
<tr>
<td>Prometryn</td>
<td>21.4</td>
<td>3.67</td>
<td>14.18</td>
<td>2.90</td>
</tr>
<tr>
<td>Terbutylazin</td>
<td>27.1</td>
<td>2.69</td>
<td>2.10</td>
<td>1.91</td>
</tr>
<tr>
<td>Propazin</td>
<td>30.0</td>
<td>2.33</td>
<td>1.89</td>
<td>1.71</td>
</tr>
<tr>
<td>Sebutylazin</td>
<td>30.0</td>
<td>2.33</td>
<td>1.85</td>
<td>1.68</td>
</tr>
<tr>
<td>Atrazin</td>
<td>35.7</td>
<td>1.80</td>
<td>1.46</td>
<td>1.31</td>
</tr>
<tr>
<td>Methribuzin</td>
<td>45.7</td>
<td>1.19</td>
<td>1.06</td>
<td>0.94</td>
</tr>
<tr>
<td>Simazin</td>
<td>45.7</td>
<td>1.19</td>
<td>1.10</td>
<td>1.01</td>
</tr>
<tr>
<td>Cyanazin</td>
<td>47.1</td>
<td>1.12</td>
<td>0.95</td>
<td>0.85</td>
</tr>
<tr>
<td>Hexazinon</td>
<td>52.1</td>
<td>0.92</td>
<td>0.81</td>
<td>0.79</td>
</tr>
<tr>
<td>Desethylatrazin</td>
<td>55.0</td>
<td>0.82</td>
<td>0.66</td>
<td>0.62</td>
</tr>
<tr>
<td>Metamitron</td>
<td>60.7</td>
<td>0.65</td>
<td>0.61</td>
<td>0.54</td>
</tr>
<tr>
<td>Desisopropylatrazin</td>
<td>61.4</td>
<td>0.63</td>
<td>0.52</td>
<td>0.49</td>
</tr>
</tbody>
</table>
Example 1: Triazines

Comparison of TLC and HPLC

a1) RP-8 (without Terbutryn and Prometryn): $r = 0.996; K'_S = 0.11 + 0.75 K'_P$

Comparison of TLC and HPLC

a2) RP-select B: $r = 0.996; K'_S = 0.03 + 0.75 K'_P$
Comparison of TLC and HPLC

Transfer of a real sample (salad)

HPTLC-Chromatogramm
(λ = 200 nm)

HPLC-Chromatogramm
(λ = 200 nm)

Correlation coefficient between TLC and HPLC

- All samples, except Phenoxy-carbonic acids
- Real sample (Salad, spiked)

Silica  | CN    | Amino | Diol | RP-8 | RP-18

Correlation coefficients:
- 0,97
- 0,975
- 0,98
- 0,985
- 0,99
- 0,995
- 1
Transfer to Flash Chromatography

- We now have a complete range of Flash Cartridges:

  - 2.5g
  - 10g
  - 15g
  - 25g
  - 30g
  - 70g
  - 90g
  - 200g
  - 300g
  - 150g
  - 200g
  - 300g
  - 400g
  - 600g
  - 800g

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Transposition, example 1

- In ideal conditions, transposition from TLC to Flash Chromatography should give such results
How to increase performance...

- .. by using smaller particles, but:

  Smaller granulometry ⇔ Higher pressure !!!

  Ex : Back pressure in Cyclohexane 90 / 10 Dioxane
  Bed height = 100 mm  Flowrate = nominal

  Si60 63-200 µm  ⇒ 0,4 bar
  Si60 40-63 µm  ⇒ 1,2 bar
  Si60 15-40 µm  ⇒ 4,3 bar

  ⇒ Need for pressure stable flash cartridges

Particle size comparisons

- Test silice Si60 40-63 µm
- Test silice Si60 63-200 µm
- Test silice Si60 15-40 µm

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**Efficiency depending on particle size**

- Comparison between 15-40, 25-40 spherical and 40-63 µm

**Biotage 40-63 µm**

**Pharmprep CC 25-40 µm**

**Efficiency depending on particle size**

- Comparison between 15-40, 25-40 spherical and 40-63 µm

**Biotage 40-63 µm**

**LiChroprep 15-25 µm**
Difficult separations – cis/trans-Phytol

Biotage 40-63 µm
Supervarioprep 15-40 µm

HPLC-Chromatogram

Difficult separations – Citrus-Oil

Biotage 40-63 µm
Supervarioprep 15-40 µm

HPLC-Chromatogram
Dry loading

- **Dry loading**: Another advantage of pre-packed cartridges
  - In TLC, the sample is always deposited as a "Dry loading".
  - In Flash, it depends on the solubility of the sample in the eluent.

But... the sample does not always behave identically if injected in solution, or as a Dry loading.

In order to obtain good transposition to Flash on basis of TLC results (especially in Anti-Langmuir because of low solubility in solvent):

- Check loading in TLC.
- Use Dry loading.

Our cartridges have special empty volume for this Dry loading:
Conclusion

• New Flash cartridges packed with Si60 15-40 µm offer:
  – Easy transposition TLC / Flash.
  – Very good purification efficiency.
  – Quicker purifications than Glass columns.
  – Smaller fractions to evaporate.
  – Secure working area (no glass breakage risk).
  – Easy Dry loading directly on-column.

Contact

• We remain at your service for any question regarding Flash Chromatography:
  – Products presentation
  – Tests on your products
  – Purification strategy
  – Trainings
  – Etc…

Thomas PONCET  Process Separations  Merck Chimie SAS
thomas.poncet@merck.fr