Increasing Purification Throughput Using a New Automated Flash Chromatographic System

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Abstract

Purification is the primary bottleneck in the synthesis process. Recent advancements focusing on ease of use utilizing a new automated flash chromatographic system (SP4™) are addressed in this poster. Results of this study show that at the system level the purification parameters, throughput, and sample variables can be automatically optimized.

In this poster, a mixture of compounds is purified demonstrating that an optimized purification method can be automatically achieved using this new purification system solely based on the solvent and retention factors from thin-layer chromatography (TLC)*.

Using the new SP4 system, sample throughput was significantly improved compared to a single-column system since each sample required less than fifteen minutes to purify using automated, optimized sample specific elution methods. No user intervention was required when switching between various solvents and samples. In addition, this paper shows how the system significantly simplifies the scale-up process when purifying milligram to gram scale samples.

* Patent pending
Biotage SP4™ Flash Purification System

- Automatically purifies 1 to 4 samples
- New Touch Logic Control™ graphic user interface simplifies system operation
- Automatically creates purification methods based on TLC data
- Four solvent inlets provide more flexible binary gradient capability
- Sequential purification of up to 4 samples using 12-65 mm cartridges
- Flow rate from 1 to 100 mL/min
- Variable dual-wavelength detector for added detection flexibility
Samples and Purification Cartridges

- Cartridges used in this study include:
  - Biotage Flash 12+™ M Si

- Test Samples:
  - Naphthalene
  - N-Phenylbenzylamine
  - 1-Nitronaphthalene
  - 2-Bromoacetophenone
  - Carbazole
  - 2-Bromo-4’-methoxyacetophenone
  - 2-Nitroanaline
  - 3-Phenoxybenzyl alcohol
  - 2-Nitroanaline
  - 3-Nitroanaline
Thin-Layer Chromatography Method (TLC)

- A variety of samples was first separated by TLC using 9:1 hexane/ethyl acetate, and then separated using the same solvents with Biotage Flash 12+M cartridge. Rf values, elution volume and the experimentally determined number of column volumes (CV) are listed as following:

### Samples

<table>
<thead>
<tr>
<th>Samples</th>
<th>Rf</th>
<th>mL</th>
<th>CV No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>0.53</td>
<td>19.8</td>
<td>2.0</td>
</tr>
<tr>
<td>N-Phenylbenzylamine</td>
<td>0.46</td>
<td>27.7</td>
<td>2.8</td>
</tr>
<tr>
<td>1-Nitronaphthalene</td>
<td>0.28</td>
<td>35.2</td>
<td>3.5</td>
</tr>
<tr>
<td>2-Bromoacetophenone</td>
<td>0.24</td>
<td>37.8</td>
<td>3.8</td>
</tr>
<tr>
<td>Carbazole</td>
<td>0.15</td>
<td>51.7</td>
<td>5.2</td>
</tr>
<tr>
<td>2-Bromo-4'-methoxyacetophenone</td>
<td>0.15</td>
<td>70.6</td>
<td>7.1</td>
</tr>
<tr>
<td>2-Nitroaniline</td>
<td>0.09</td>
<td>105</td>
<td>10</td>
</tr>
<tr>
<td>3-Phenoxybenzyl alcohol</td>
<td>0.05</td>
<td>194</td>
<td>19</td>
</tr>
<tr>
<td>3-Nitroaniline</td>
<td>0.04</td>
<td>252</td>
<td>25</td>
</tr>
</tbody>
</table>
The relationship between TLC Rf values and N or column volume (CV) that the compound elutes is best described by the formula:

\[ N = \frac{a}{R_f} \]

Generally “a” is a constant that approximately equals 1 depending on the experimental method, solvents, etc.

The Rf and CV relationship is further utilized in the new patent pending TLC-to-Gradient algorithm to automatically generate specific gradient purification conditions for individual compounds and solvent compositions.

CV is a dimensionless term that is related to any column length or diameter utilized in the new SP4 system that determines the appropriate flow rate and sample load based on the specified cartridge dimensions.

This plot compares the TLC retention factor (Rf) and experimentally determine Flash cartridge retention (CV) using the same solvent system.
Directly Associate Sample Size to Cartridge Dimension Based on TLC Rf factors

- Chemist enters TLC solvent system parameters
  - Solvents
  - Composition

- System creates purification method including
  - Gradient profile and appropriate flow rate for the specified cartridge

- Add Rf of the compound of interest and the Rf for any closely eluting impurities to determine appropriate cartridge size for the required sample load
Automatic gradient method calculated and displayed based on TLC data quickly creates optimal FLASH™ gradient conditions

- Gradient profile presented in CV
  - “On-the-fly” gradient extension is possible using touch screen

- SP4™ system automatically
  - Determines the weaker solvent
  - Forms the binary gradient from 4 possible solvents
  - Indicates the solvent location
7-Component Sample Separation using the SP4 FLASH Purification System

- TLC conditions
  - 80% Hexane (A)
  - 20% EtOAc (B)
  - Rf range: 0.07-0.86
  - Target (6) Rf: 0.14

- SP4 system’s algorithm automatically generates the gradient method based on using a FLASH 12+M KP-Sil cartridge
  - Automated Gradient Conditions
    - Segment 1: 5% B for 12 mL
    - Segment 2: 5-40% B over 120 mL
    - Segment 3: 40% B for 24 mL
  - Flow rate: 12 mL/min
  - Sample size: 150 mg

Chromatogram displayed on the screen including the gradient profile and solvent composition. Compound 7 does not elute.
Purification Profile and Data Review

- System provides single screen display of chromatogram overlaid with gradient profile and location of fractions

- Color highlighted fractions
  - Easily locate fractions by touch screen

- Dual wavelength monitoring with variable wavelength detector allows the chemist to reduce the sensitivity to maintain the peaks on scale

- System records purification profile and chromatograms in standard format that easily transfers for archiving or presentation
Multiple Fractionation Methods to Optimize Purity and Yield

- The SP4 purification system allows the chemist to choose various fractionation options to maximize product yield or purity
  - 4 preset collection routines
    - Collect all by fraction size
    - UV threshold triggered fractionation
    - Low Absorbance slope triggering
    - Medium Absorbance slope triggering
- Customized slope
- Graphic user interface screen displays:
  - Drop down screen choices for the fractionation method
  - System operating parameters including chemist, cartridge size, location, monitoring wavelengths and the fraction’s location
Summary

• The new Biotage SP4™ purification system significantly reduces the purification bottleneck by providing state of the art functionality

  – Automatic elution gradients based on TLC
  – Automatic cartridge selection
  – Automated suggested sample amount based on TLC data and cartridge choice
  – Solvent and waste level monitoring
  – A “smart” fraction collector
    ❖ Constantly monitors the arm position to prevent sample loss
    ❖ Monitors tray and waste container to prevent accidental spillage