Inspiring Productivity with Modern Flash Chromatography

Delivering more chemical targets with less…

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Biotage®

Top 15 Pharma and Their R&D Efficiency

Investor Expectations Exceed Performance

Biotage

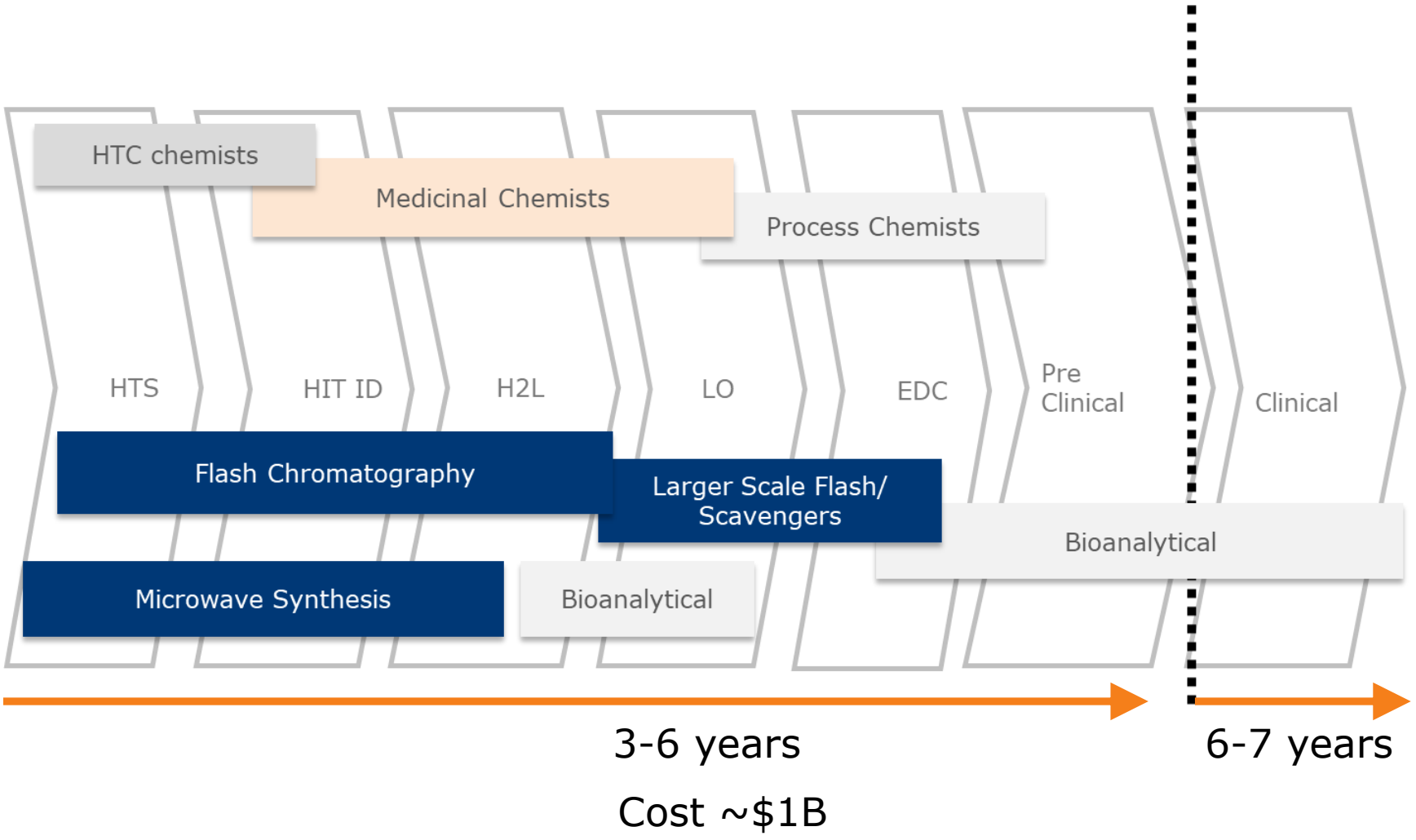
	2008-2017 sales (B)*	2008-2017 R&D (B)*	R&D spend as a % of sales	NME+BLA^	Efficiency ($/approval)
Pfizer	$ 477.096	$ 77.868	16%	7	$ 11.124
Novartis	$ 433.854	$ 81.404	19%	13	$ 6.262
Sanofi	$ 375.757	$ 62.376	17%	7	$ 8.911
Roche	$ 369.386	$ 83.534	23%	2	$ 41.767
Merck	$ 351.330	$ 75.614	22%	8	$ 9.452
GSK	$ 330.438	$ 52.854	16%	11	$ 4.805
Astra Zeneca	$ 272.597	$ 50.927	19%	8	$ 6.366
J&J	$ 270.205	$ 56.436	21%	10	$ 5.644
Lilly	$ 189.154	$ 45.441	24%	7	$ 6.492
Amgen	$ 177.887	$ 34.462	19%	5	$ 6.892
BMS	$ 165.531	$ 36.246	22%	7	$ 5.178
Teva	$ 164.492	$ 16.403	10%	2	$ 8.202
Gilead	$ 162.585	$ 22.361	14%	7	$ 3.194
Bayer	$ 153.498	$ 24.645	16%	7	$ 3.521
Takeda	$ 137.199	$ 33.040	24%	5	$ 6.608

» Investors expect a reasonable return on investment (ROI) provided by a high number of new molecular entities/biological license applications (NMEs/BLAs; new prescription drugs) approved and launched

*Pharmaceutical Executive Magazine Top 50 Pharma, annual issues from 2009-2018
^FDA CDER data

Drug Discovery Process

Finding Hits, Developing Leads, Selecting Candidates

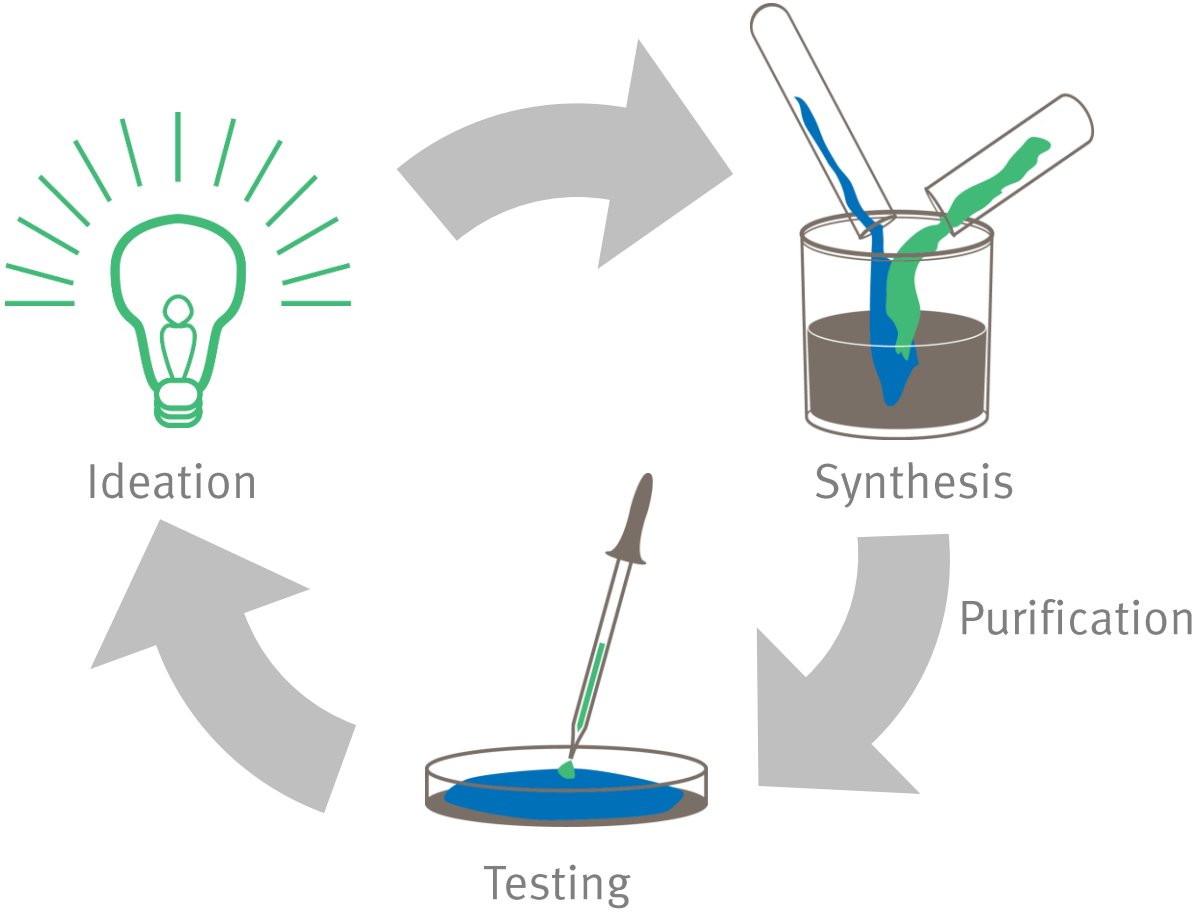


Drug Optimization Process

Iterative Process, Candidates Emerge in 3-6 Years

Biotage

Goal: Minimize the time between idea generation and biological testing



Quality in Discovery Research

Balancing Quality with Speed and Cost

- Three conflicting goals
  - High quality product
  - Low development cost
  - Fast development timeline
- Quality
  - #1 Priority, cannot be compromised
  - NMEs must meet the required needs to treat a specified disease
    - Safe, efficacious, bioavailable, etc.
- Cost
  - The cost required to discover a lead candidate is too high
    - Must be reduced
- Speed
  - The time it needed to discover new candidates is too long
    - Must be reduced

How can speed and cost improve without sacrificing quality?

Discovery Process Challenges

Synthesis, Purification - Difficult and Slow

- Synthesis
  - Complex reaction chemistries
    - Multiple reactions
      - Many by-products
      - Increasingly polar molecules
    - Often long in duration
    - Multiple work-up steps
    - Challenging purification

- Purification
  - NME intermediates (via normal-phase flash column chromatography)
    - Methods not optimized, inefficient
    - Gradient and load based on guesswork or familiarization/experience with a compound class
      - Results in long or overly-complex gradients and re-runs
    - Column selection inappropriate
      - Too big, too small, incorrect media, or solvent choice
  - Final NME clean-up (via reversed-phase preparative HPLC)
    - Reaction mix sent to the prep or analytical lab
    - More waiting, expensive, risk to compound yield

Fixing Discovery Process Challenges

Rethinking the Purification Approach

- Prep HPLC or flash chromatography?
  - Synthetic chemists prefer flash
    - Quick processing speed
    - Fast to dry down organic solvents
    - Retain compound control, mitigates yield loss risk
- Broad applicability with flash?
  - Useful for both intermediate and final NME clean-up?
  - Operate in both normal- and reversed-phase modes?
  - Complex reaction mixtures?

Flash Chromatography Evolution

A Remarkable Journey

- 1901 – Mikael Tswett performs first column chromatography separation
- 1978 - W. Clark Still publishes seminal paper on “flash” chromatography using granular 40-63 µm silica
- 1994 – Biotage® Flash 75 and Flash 150 pre-packed columns and pressurized systems launched. These are the first commercially available flash purification systems and columns
- 1997 – Argonaut Technologies launches first automated flash chromatography system (later acquired by Biotage) – Biotage® FlashMaster
- 2000 - Flash+® columns and Samplet® cartridges introduced by Biotage with direct scalability to Flash 75, Flash 150, and Flash 400 systems
- 2004 – The Biotage® SP1, the first fully automated flash system with touchscreen interface and predictive TLC to linear gradient software launched
- 2007 – Revolutionary Biotage® SNAP columns with removable cap for internal sample dry loading launched
- 2012 – Biotage® SNAP Ultra columns with spherical, ~25 µm, 750 m^2/g surface area silica launched increasing loading capacity up to 4x over conventional silica
- 2013 – Biotage® SNAP Ultra C18 (~27 µm, spherical) launched providing very high performance reversed-phase purification
- 2018 – Biotage® Selekt system launched. A 2-channel system that speeds up flash purification with high speed column equilibration and automatic, high speed normal-phase to reversed-phase (and back) solvent switching
- 2018 – High performance, high pressure Biotage® Sfär columns (spherical, 750 m^2/g, 20 µm and 60 µm silica columns launched). Increase load capacity and reduce purification time and solvent use compared to conventional columns

Flash Chromatography Methods

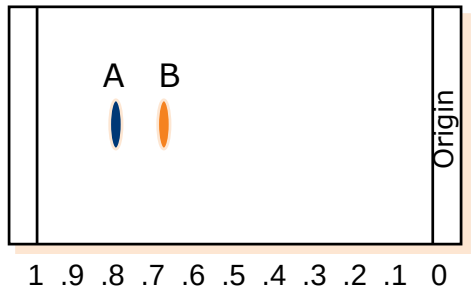
Early Days

- Column/load determination
  - The 1% rule
    - Choose a column that is 100 times larger than the amount of reaction mix to be purified
      - 100 mg requires a 10 gram column
      - 1 gram requires a 100 gram column
    - If the separation is poor, re-purify with a bigger column
- Gradients
  - 0-100% linear gradient (hexane/ethyl acetate)
  - 0-10% linear gradient (DCM/MeOH)
  - At best, using TLC Rf data to create a more optimized linear /step gradient
    - ΔRf used as a load guideline

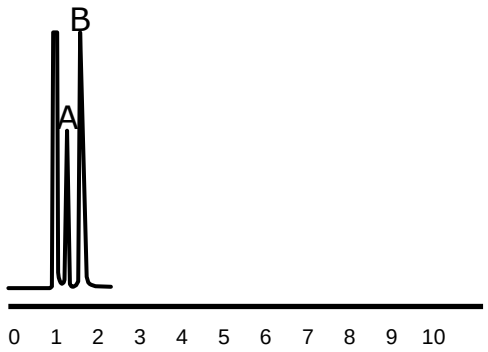
Flash Chromatography Methods

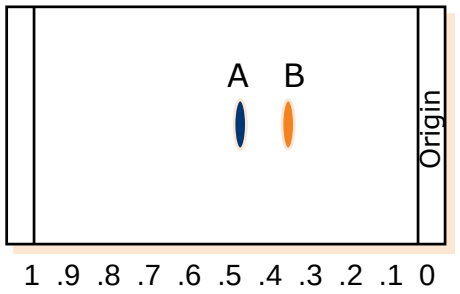
Today

- Determine sample load based on ΔCV rather than ΔRf
  - CV = 1/Rf
  - ΔCV = CV2-CV1

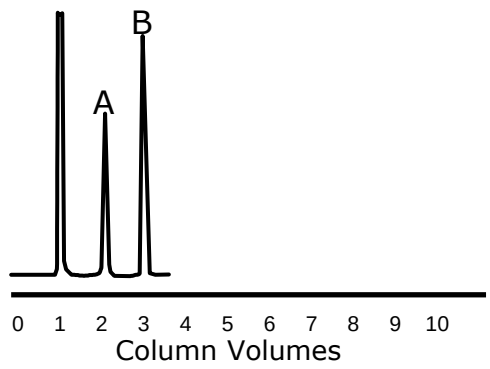


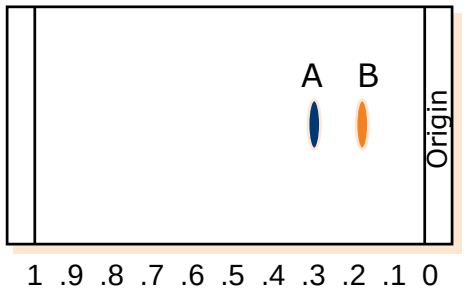
R_fA = .80 R_fB = .67 ΔR_f = .13
ΔCV = 0.24



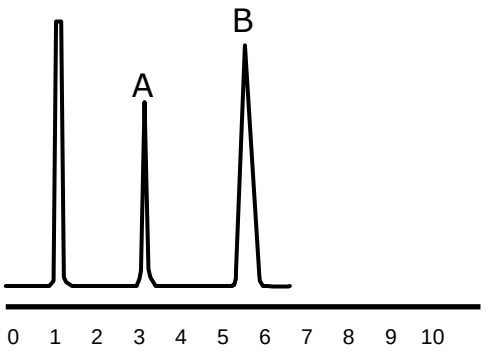


R_fA= .47 R_fB = .34 ΔR_f = .13
ΔCV = 0.81





R_fA = .32 R_fB = .18 ΔR_f = .14
ΔCV = 2.43

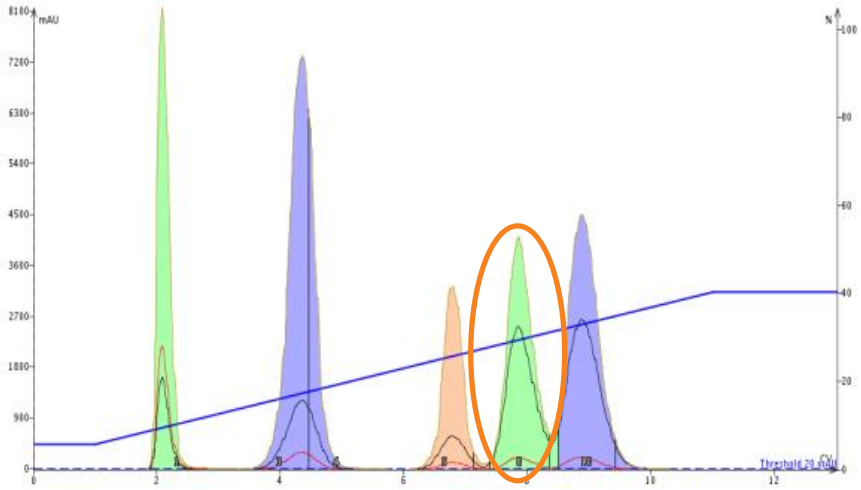


Flash Chromatography Methods

Today

- Use either one or two TLC plates to create a gradient
  - One plate
    - Flash system uses % strong solvent to create linear gradient
    - Uses Rf values (target compound and adjacent by-products) to determine CV and ΔCV
    - ΔCV used to determine the correct column size for the amount of RxN mix to be purified

TLC-based 13 CV linear gradient, 100 mg load



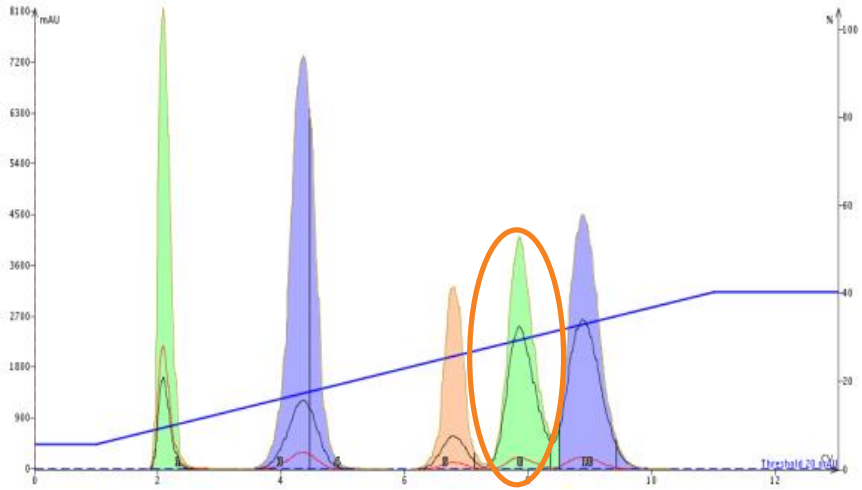
Flash Chromatography Methods

Today

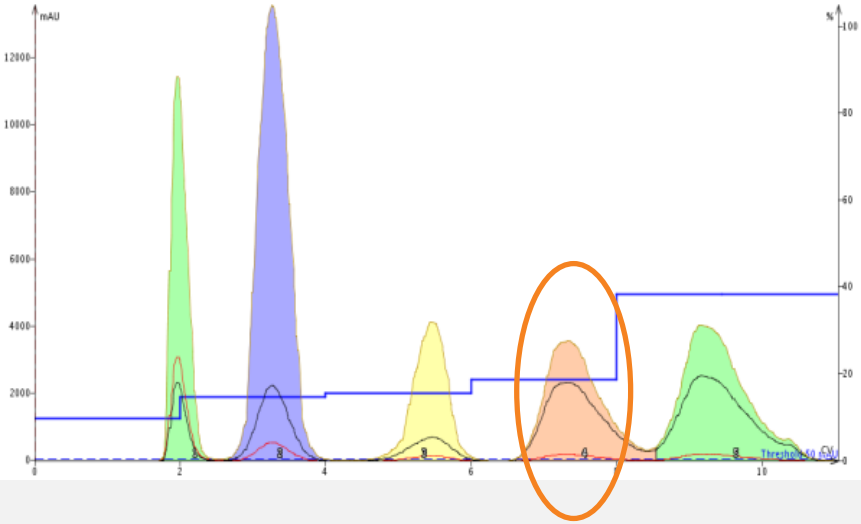
- Use one or two plates for gradient design
  - Two plates
    - Creates a step gradient
    - Optimized for your target of interest
    - Load amount doubled vs. linear gradient (in most cases)
    - Run time and solvent consumption reduced upwards of 50% compared to a linear gradient

- For more information on flash optimization listen to my webinar on this topic https://biotage.com/news/webinar-a-roadmap-to-successful-flash-chromatography

TLC-based 13 CV linear gradient, 100 mg load



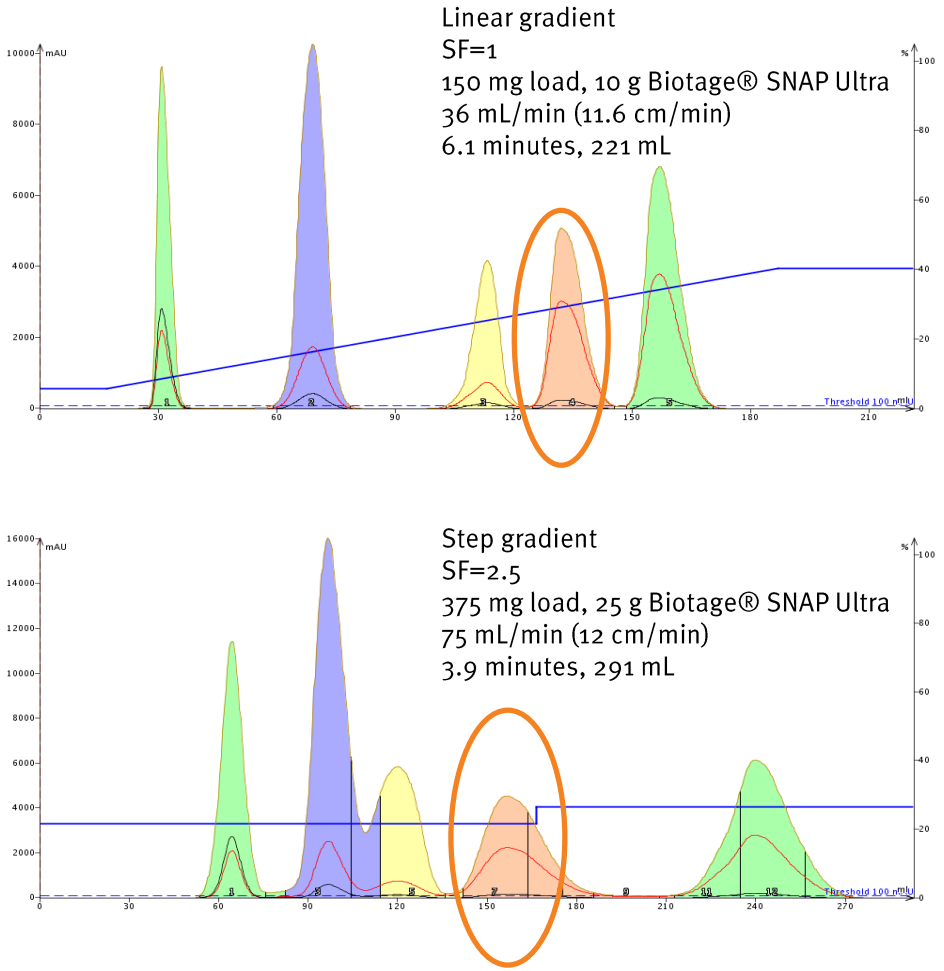
TLC-optimized step gradient, 200 mg load



Flash Chromatography Trends

System Advancements

- Smaller system footprints
  - Bench/hood space at a premium
- Faster flow rates
  - For larger columns/larger scale
  - Faster equilibration to reduce purification time
- Higher pressure tolerances
  - Reversed-phase compatible
- Advanced detection options
  - Diode array UV, UV-vis
  - Evaporative light-scattering (ELSD)
  - Mass (MS)
- Automated optimization
  - TLC to gradient with sample load estimation
  - ***Linear to step gradient*** conversion to minimize run time and solvent consumption, especially for ***scale-up***



Flash Chromatography Trends

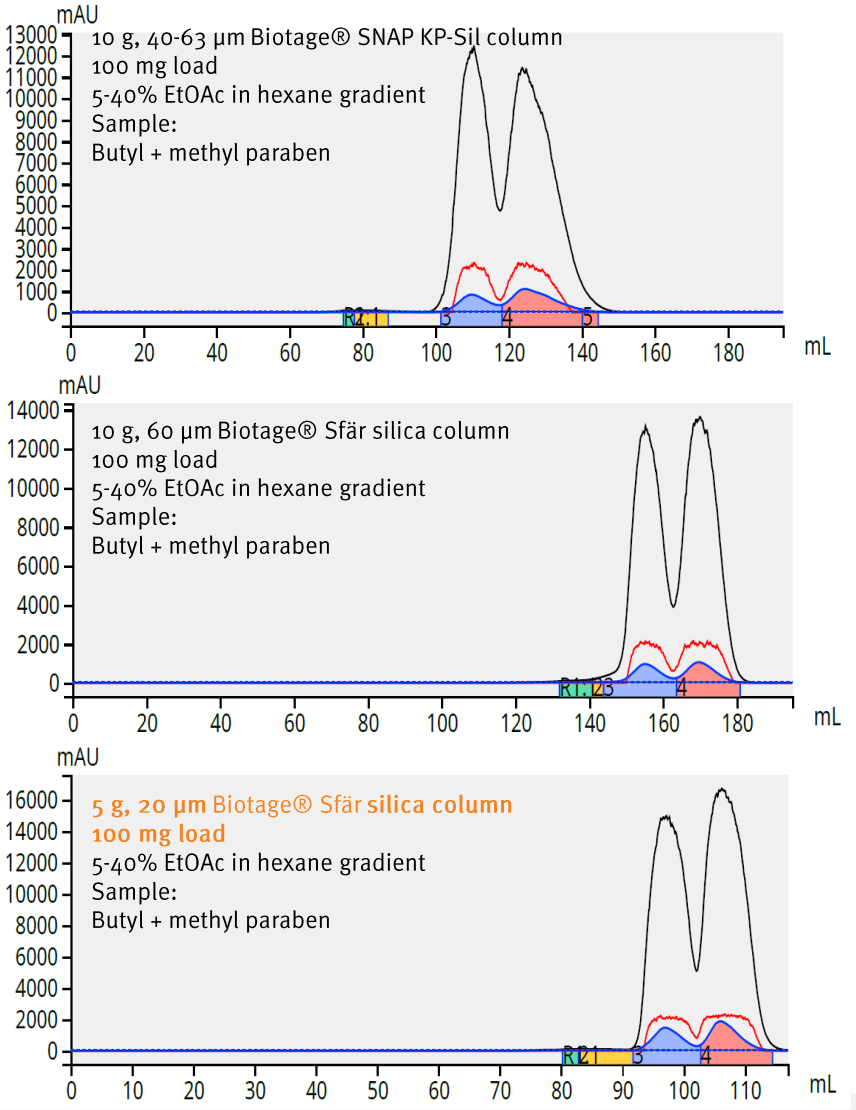
Column Advancements

- Advanced column technology
  - Smaller particle, spherical shape, higher surface area silica
    - Higher loading capacity
    - Higher fraction purity
    - Allows use of smaller columns, shorter gradients
    - Saves purification time and reduces solvent consumption
  - High performance reversed-phase columns
    - Small, spherical particles
    - Wide pore high performance reversed-phase columns for biomolecules
  - Higher pressure tolerance column bodies
  - Removable caps for internal RxN mix dry loading

Advancements in Flash Methodology

Yesterday vs. Today

- Standard flash chromatography-grade silica
  - Granular
  - 40-63 µm
  - 500 m^2/g
- Higher loading capacity silica
  - Spherical
  - 60 µm
  - 750 m^2/g
  - Better retention
  - Sharper peaks
- Highest performance silica
  - Spherical
  - 20 µm
  - 750 m^2/g
  - 2x loading capacity of 10 gram, larger particle columns!

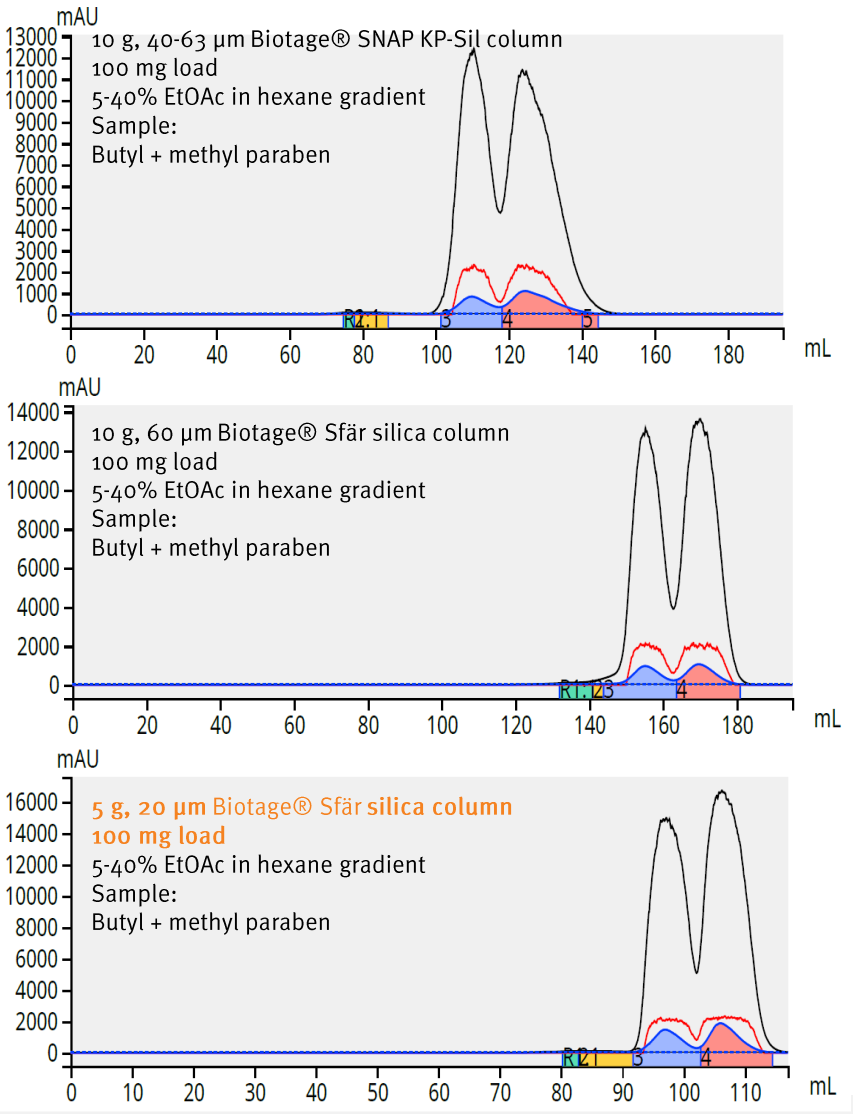


Flash Column Technology

An Efficiency Evolution

Parameter	10 g 40-63 µm	10 g 60 µm	5 g 20 µm
Column volume (mL)	15	15	9
Equilibration flow rate (mL/min)	36	150	150
Equilibration length (CV)	3	2	2
Equilibration volume (mL)	45	30	18
Equilibration time (min)	1.25	0.2	0.1
Run flow rate (mL/min)*	27	29	18
13 CV gradient run volume (mL)	195	195	117
13 CV gradient run time (min)	7.2	6.7	6.5
Total method volume (mL) (equil + gradient)	240	225	135
Total method time (min) (equil + gradient)	8.45	6.9	6.6
Total solvent savings (%)		**6**	**44**
Total time savings (%)		**18**	**22**

*Flow rates used provided equal linear velocities to match mass-transfer kinetics

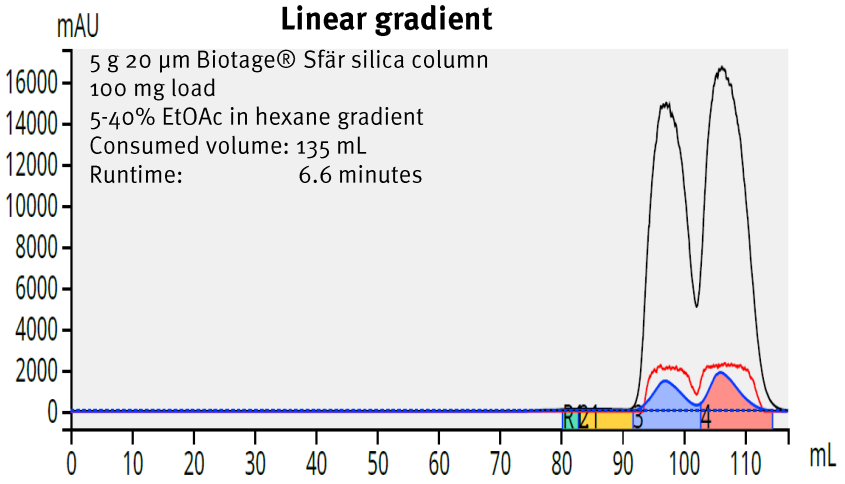


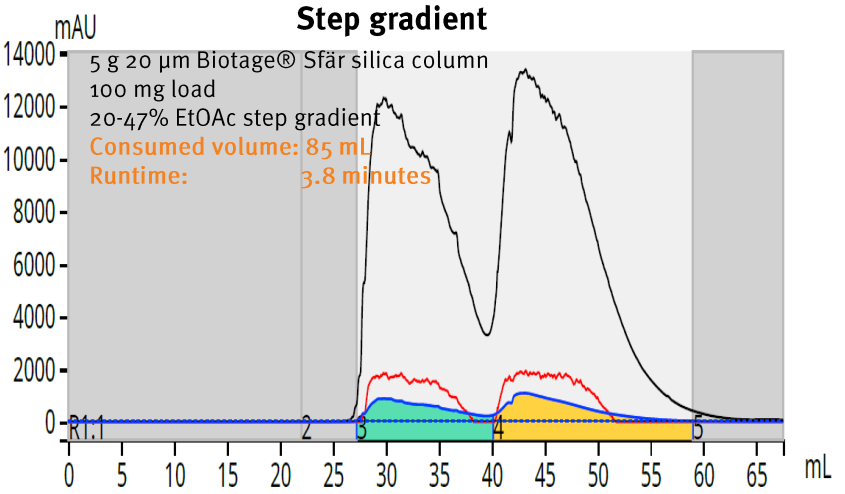
State-of-the-Art Columns Yield More

Same Column, Better Method, New Benefits

- Step gradient impact on productivity
  - 100 mg load
  - 5 gram, 20 µm column
- Linear gradient*
  - Fast – 6.6 minutes
  - Frugal – 135 mL
- Step gradient*
  - 42% faster – 3.8 minutes
  - 37% more frugal – 85 mL

* Includes equilibration volume and time



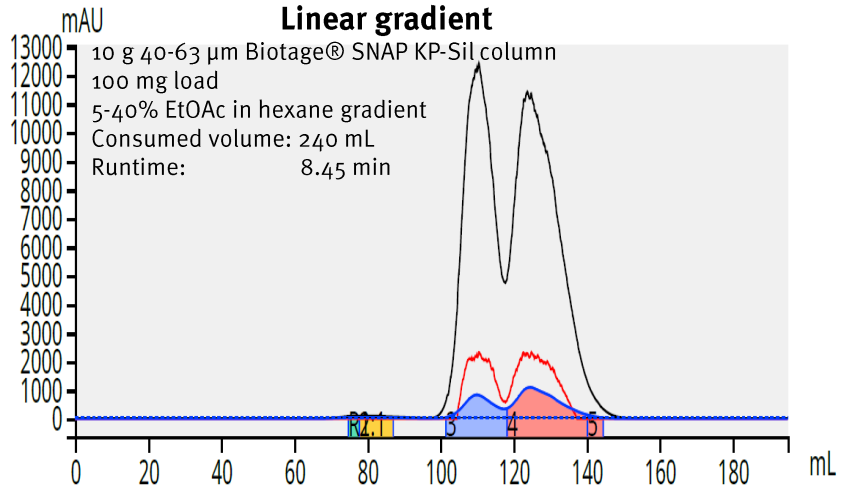


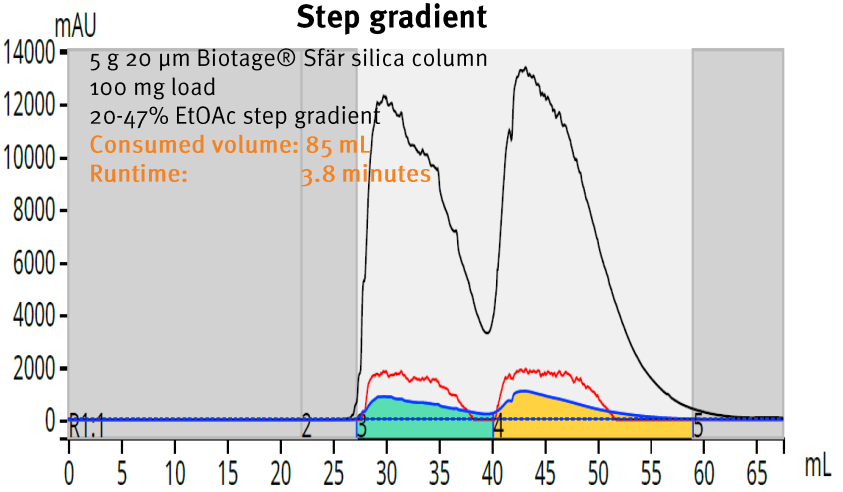
Achieving More with State-of-the-Art

Same Column, Better Method, Best Efficiency

Parameter	10 g 40-63 µm Linear	10 g 60 µm Linear	5 g 20 µm Linear	5 g 20 µm Step
Column volume (mL)	15	15	9	9
Equilibration flow rate (mL/min)	36	150	150	150
Equilibration length (CV)	3	2	2	2
Equilibration volume (mL)	45	30	18	18
Equilibration time (min)	1.25	0.2	0.1	0.1
Run flow rate (mL/min)*	27	29	18	18
Gadient run volume (mL)	195	195	117	67
Gradient run time (min)	7.2	7.3	6.5	3.7
Total method volume(mL) (equil + gradient)	240	225	135	85
Total method time (min) (equil + gradient)	8.45	6.9	6.6	3.8
Total solvent savings vs. 40-63 µm column (%)		**6**	**44**	**65**
Total time savings vs. 40-63 µm column (%)		**18**	**22**	**55**

*Flow rates used provided equal linear velocities to match mass-transfer kinetics





Overcoming Limitations

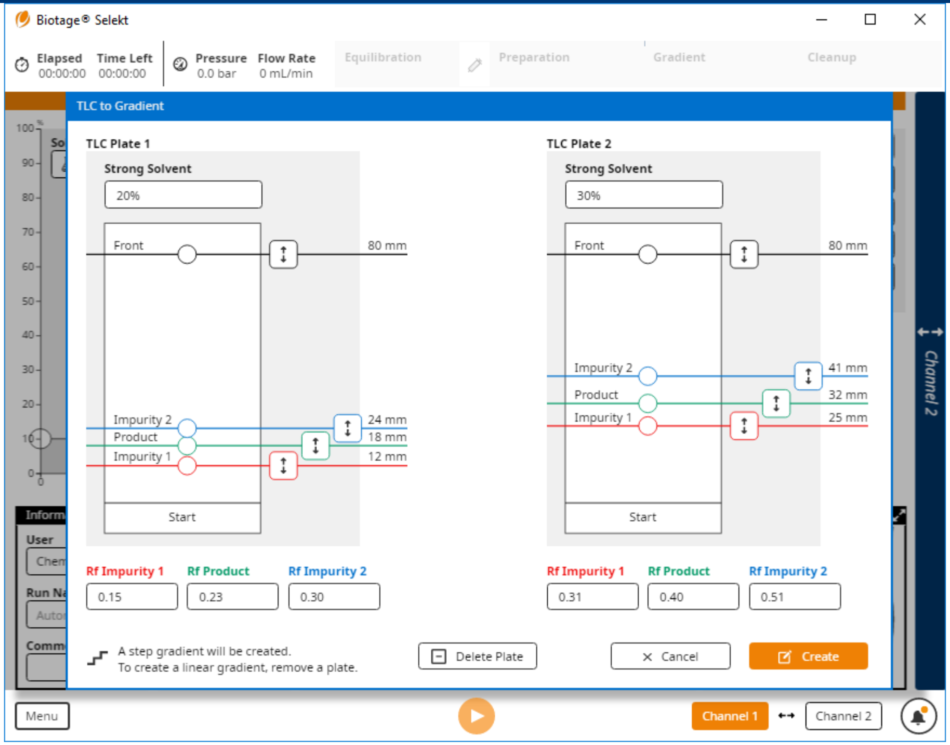
Modern Flash System Builds Optimized Methods from TLC Data

» *How are residual limitations of flash addressed?*
  - *Methods not optimized, inefficient*
  - *Guesswork or based on compound class familiarization*
  - *Poor column size/media choices/solvent choices, re-runs*
  - *Complex sample purification*

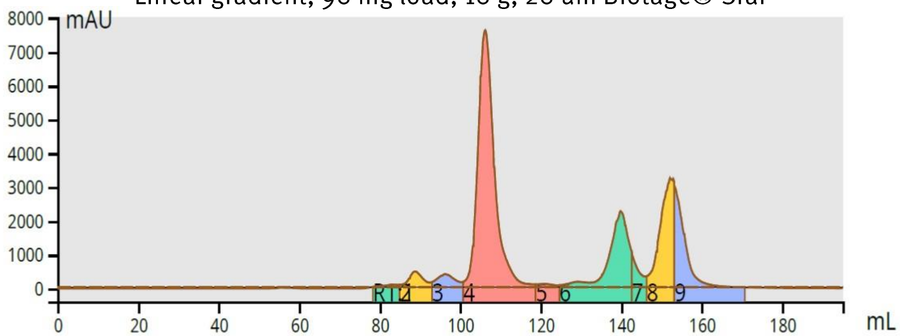
1. Data from 1 TLC plate
   - Converts your TLC data into a linear gradient
   - Suggests a column size appropriate for separating your product from the crude reaction mixture.

2. Data from 2 TLC plates
   - Creates a step gradient from two TLC plates with better results
   - Resulting method optimized for speed and resolving power
     - Fastest purification (by up to 2x)
     - Expects a maximum sample load (Enabled by Biotage® Sfär columns)

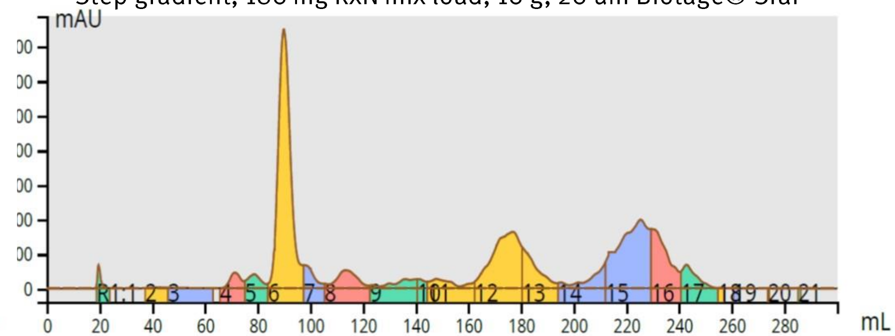
✓ **The Biotage® Selekt flash system is smart - builds optimal methods**



Linear gradient, 90 mg load, 10 g, 20 um Biotage® Sfär



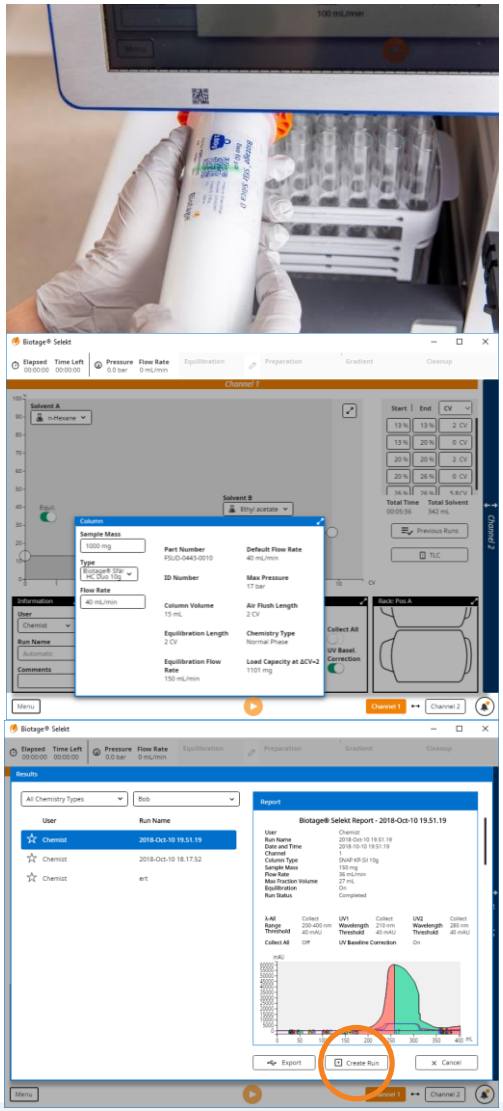
Step gradient, 180 mg RxN mix load, 10 g, 20 um Biotage® Sfär



Overcoming Limitations

Smart Capabilities Simplify Choices and Avoid Re-runs

- System IDs the Biotage® Sfär column and collection rack intended for use
  - Preloads pertinent information, including the sample load range and optimal flow rate
  - Media choices are easily determined from the accessible column selection flowchart
- Modern diode array detection corrects baseline for mobile phase UV absorption
- Creates a method from a previous result
  - The system allows a past run performed on a familiar chemical series is recalled from the list of results and the method used therein
  - No need to create a new method
- **The Biotage® Selekt flash system addresses re-run and incorrect choice concerns with smart, built-in capabilities**



Boosting Productivity

Modern Flash System - Speed Without Compromises or Worry

- ✓ **The Biotage® Selekt rapidly addresses time concerns**
  - » Rapid equilibration
    - Enables the user to load RxN mix within seconds, not minutes
  - » 2- channel sequential system
    - Walk-up system
    - Multiple users
    - Different methods
    - NP and RP on separate channels for convenience
      - – Intermediate purification on NP, final product purification RP
  - » Rapidly switches between RP-NP to free the users time
    - Eliminates hassle/know-how necessary for NP-RP switching
    - Allows for routine use of high load C18s for polar compounds and separation of complex mixes

Can speed and cost improve without sacrificing quality? YES

Discovery Process Challenges Solved

Modern Flash Chromatography for All Purifications

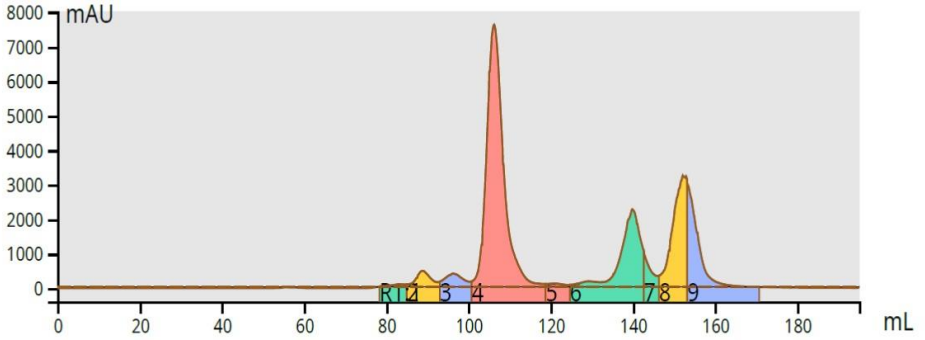
- Synthetic chemists prefer flash
  - It's quick chromatography
  - Fast organic solvent evaporation
  - You're in control
- Flash is broadly applicable
  - Useful for both intermediate (NP) and final NME products (RP)
- Flash limitations are resolved (with application of modern flash)
    - Methods can be optimized and are efficient
    - Guesswork and waiting are non-issues
    - Column size/media/solvent choices are simple, re-runs can be avoided
- Reduces time spent purifying
  - Complex mixtures included
- Reduces purification costs

Employing a Modern Flash System

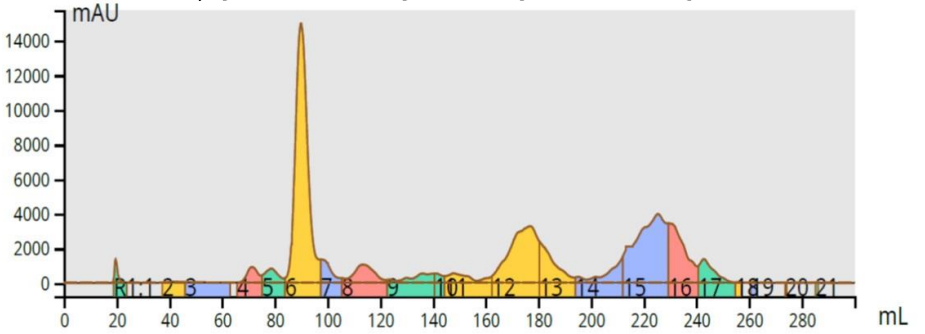
Tips for Achieving Productivity Gains

- To avoid guesswork on flash column choice, method setup, reruns, while reducing costs, employ an intelligent flash system that helps you to avoid mistakes…
  - *Optimizes conditions that take the least time and use the least solvent*
  - *Uses TLC data to generate both linear and step gradients*
  - *Determines an appropriate column choice*
  - *Auto-loads column parameters on column ID*
  - *Recalls previous run method conditions*
- To reduce waiting for flash system readiness, employ an intelligent flash system that rapidly flushes and equilibrates, requiring no additional user intervention
  - *Equilibrates columns in seconds*
  - *Phase changes (NP/RP) between channels in seconds, automatically*

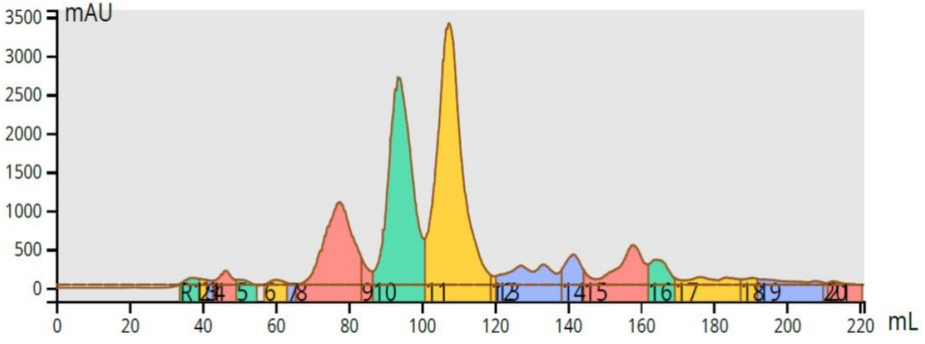
Linear gradient, 90 mg load, 10 g, 20 um Biotage® Sfär



Step gradient, 180 mg load, 10 g, 20 um Biotage® Sfär



Reversed-phase linear gradient, 90 mg load, 12 g, 27 um Biotage® Sfär C18



Employing Modern Flash Columns

Tips for Achieving Productivity Gains

1. *When using the same linear gradient method*, employ ½-size columns packed with high surface area, small particle size silica to…
    - Reduce purification time by 20%
    - Reduce solvent consumption by 40%+

2. *When using an optimized step gradient*, employ ½-size columns packed with high surface area, small particle size silica to…
    - Reduce purification time by 55%+
    - Reduce solvent consumption by 64%+

Inspired Productivity for Discovery

Benefits of Modern Flash Chromatography

Biotage

- *Discovery chemists get more from less*
  - *Run more syntheses per day with less effort due to faster purification*
  - *Obtain pure chemical targets in less time*
  - *Consume less solvent (less cost, less waste)*

- *So that chemists…*
  - *Meet their NME production goals sooner*

- *Such that . . .*
  - *NMEs move forward to biological testing*
    - *Drug discovery projects advance quicker!*

Thank you

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Biotage®