# Automating TLC to Flash Purification Gradient Methods Containing Polar Solvents*

**Sjaan Armentrout, Jack Liu and Peter Rahn**
**Biotage**
**Discovery Chemistry Group**
**1725 Discovery Drive**
**Charlottesville, VA 22911**

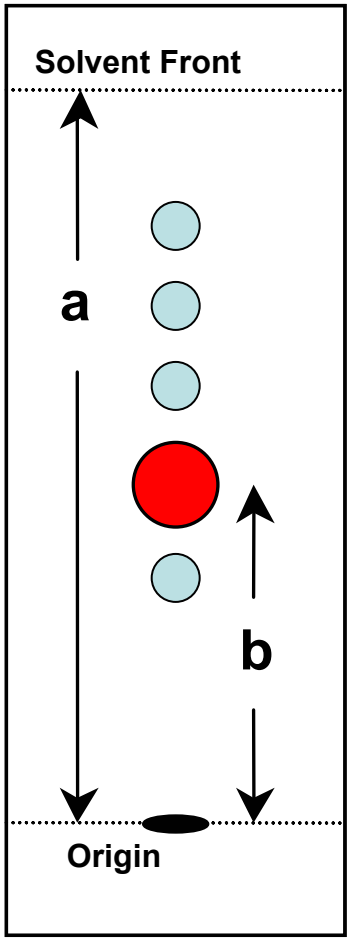
* Patent Pending

Biotage

# Abstract

- **Biotage SP1 and SP4 systems automatically produce appropriate optimized Flash gradient profiles from TLC data**
- **These automated gradient methods are applicable for a wide range of solvent systems including those containing acetonitrile or alcohols**
- **Examples of reductive amination products utilizing TLC data to produce successful Flash gradient purification separations are presented**

# TLC Data Gathering




- **New Biotage Flash systems automatically utilize TLC data to generate optimized Flash purification conditions**
- **Eliminates need to optimize TLC separation**
- **Chemist empirically determines suitable solvent composition to separate target compound from nearest impurity**

**Calculate Rf = b/a**
**Identify Solvent and ratio X:Y**



Biotage

# New Automated Biotage Flash Systems

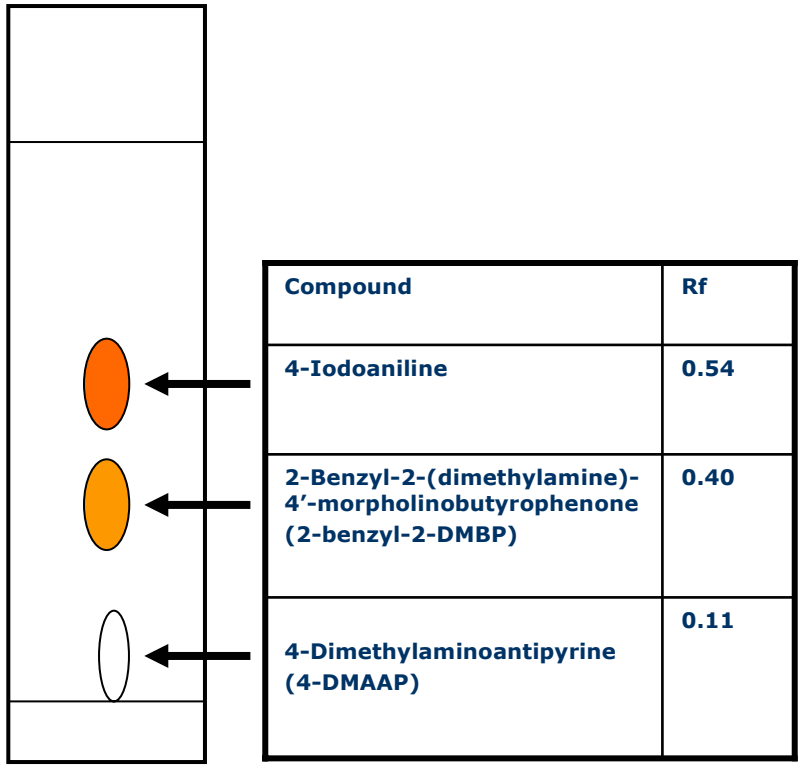

- **Chemist Inputs the following TLC Data**
  - **Rf of selected compound**
  - **Rf of additional compounds**
    - If Rf of more than one compound is entered system determine sample load or cartridge size)
  - **Solvent identity and ratio**
- **Automatic Flash System Setup***
  - **Identifies weaker solvent**
  - **Produces appropriate gradient profile including initial hold, gradient and final hold**
  - **System suggests altering solvent composition from TLC concentration if selected solvents exceed a specified solvent strength threshold**

*** Patent Pending**



Biotage

# Automatic Optimization of Purification Conditions for Any Component In a Mixture



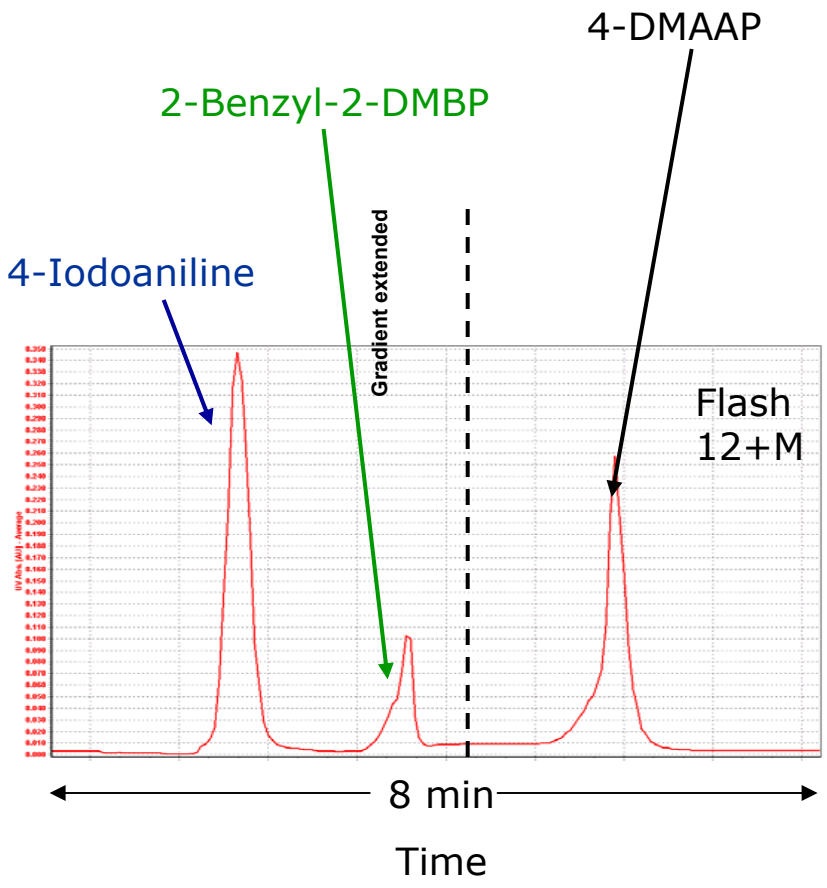
| Compound | Rf |
|---|---|
| 4-Iodoaniline | 0.54 |
| 2-Benzyl-2-(dimethylamine)-4'-morpholinobutyrophenone (2-benzyl-2-DMBP) | 0.40 |
| 4-Dimethylaminoantipyrine (4-DMAAP) | 0.11 |

**TLC Conditions**

**DCM:MeOH 98:2**

- **Algorithm works for Non-Polar and Polar Solvents**
  - **MeOH was required to separate these polar compounds**
  - **If TLC solvents include polar solvents such as acetonitrile, methanol, ethanol, isopropanol etc. the system automatically notifies chemist to adjust the solvent mixture to an appropriate value**
- **Rf factors between 0.05 and 0.9 are suitable**
  - **Eliminates the need to optimize Rf between 0.15 and 0.3**
- **Automated Sp1 System optimizes gradient conditions to purify identified product**
  - **Includes initial, gradient and final hold conditions**
  - **Suggests adjustments in mobile phase composition**
  - **Suggests column load or column size if more than one Rf is entered**

# Optimized Flash Gradient Profile to Purify 4-Iodoaniline




- **Rf of 4-Iodoaniline= 0.54 entered into SP1 system and DCM:MeOH solvent system identified and Flash 12+ cartridge specified**
- **Automatic gradient profile generated:**
  - **Initial conditions = 18 mL at 0% B**
  - **Linear gradient = 120 mL 0-46%B**
  - **Final conditions = 36 mL at 46% B**

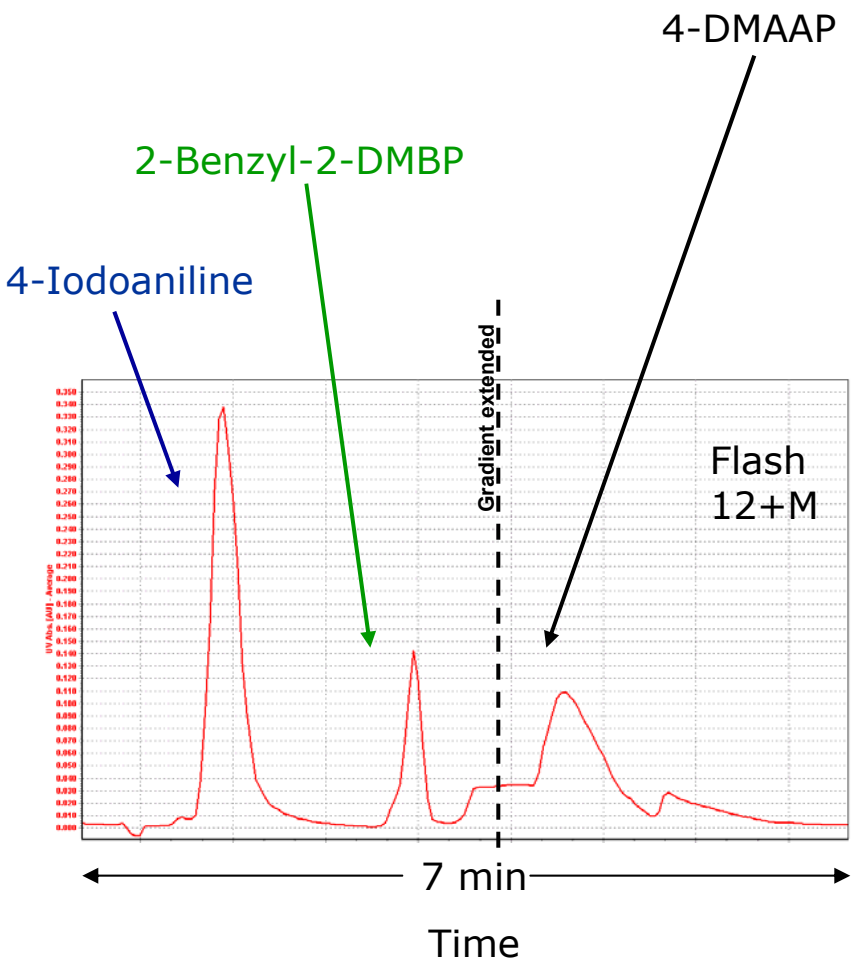
**Resolves 4-Iodoaniline and 2-Benzyl-2-DMBP**

**Solvent strength wasn't sufficient to elute 4-DMAAP- required only 1 automatic extension ( ------ )**



Biotage

# Optimized Flash Gradient Profile for 2-Benzyl-2-DMBP

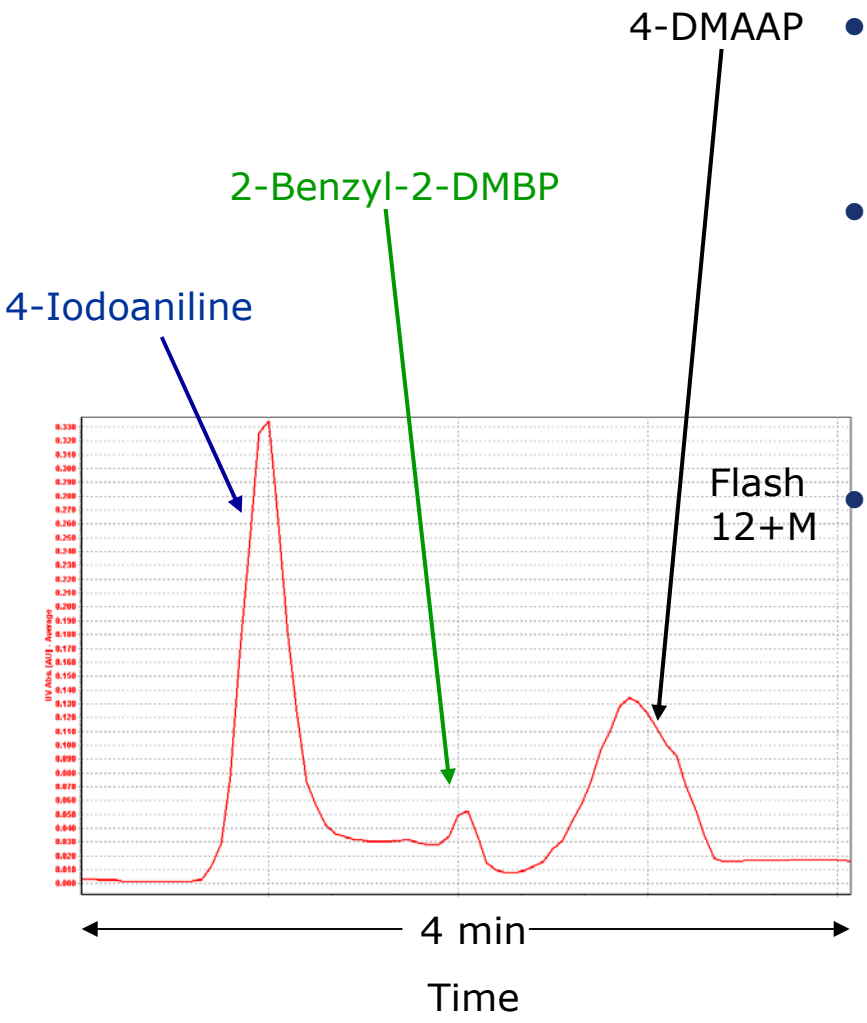



- **Rf=0.40 for 2-Benzyl-2-DMBP entered into SP1 system**
- **Resulting gradient:**
  - **Initial conditions = 18 mL at 0% B**
  - **Linear gradient = 120 mL 0-60%B**
  - **Final conditions = 36 mL at 60% B**
- **Achieved excellent resolution of first 4-Iodoaniline and 2-DMBP**
- **Solvent strength still not sufficient to elute 4-DMAAP and an automatic gradient extend was initiated (------)**



Biotage

# Optimized Flash Gradient Profile to Purify 4-DMAAP

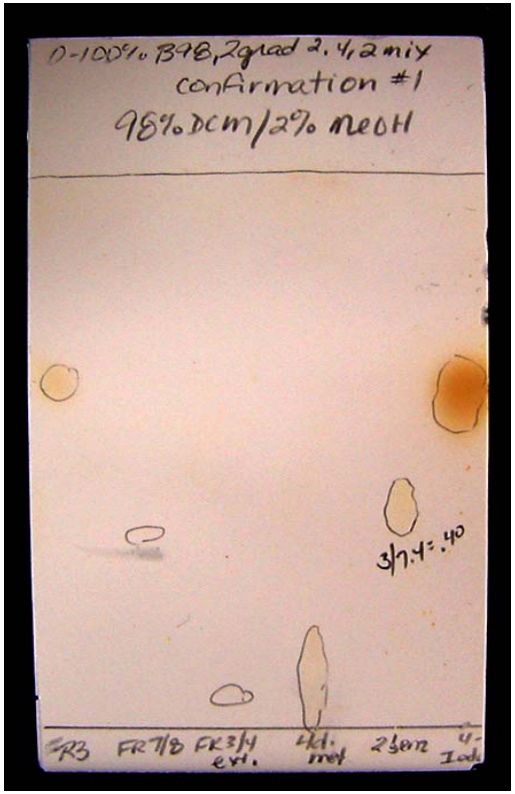



- **Rf=0.11 for 4-DMAAP (compound 3) entered into SP1 system**
- **Resulting automatic gradient profile:**
  - **Initial conditions = 18 mL at 0% B**
  - **Linear gradient = 120 mL 0-89%B**
  - **Final conditions = 36 mL at 89% B**
- **Good resolution of all three compounds, no gradient extension required**
  - **Even with this stronger solvent mixture all the polar compounds remained resolved**



Biotage

# SP1 Flash Purification Results Using Automated Purification Conditions for Polar Compounds




Fr 2/3 Fr 7/8 Fr 10/11 4-I DMPB DMAAP

**TLC Conditions**

**DCM:MeOH 98:2**

- **TLC shows fraction analysis after Automated Flash Purification using DCM:MeOH**
- **Algorithm works for Rf factors between 0.05 and 0.9**
- **Automated Sp1 System optimizes gradient conditions to purify identified product**
  - **Includes initial, gradient and final hold conditions**
  - **Suggests adjustments in mobile phase composition**
  - **Suggests column load or column size if more than one Rf is entered**
- **MeOH was required to separate these polar compounds**
  - **System automatically notifies user to modify polar solvent ratio**
- **Examples using this patented technology for the purification of amination products in shown in the next section of this poster**



Biotage

# Synthesis and Purification of Amination Products using Biotage Initiator™ Microwave Synthesis and Biotage SP1™ Flash Purification Systems

- **Biotage Initiator™ 60 Microwave System was equipped with the following:**
  - **Compact footprint**
  - **Touch screen control**
  - **Four different vial sizes in any order or combination at any time without system modifications for greater flexibility and direct scale-up of milligrams to grams**
  - **60-position sample bed**
  - **Best-in-class safety**

- **The 2-5 mL reaction vials containing the solid supported reagent, solutions and enclosed magnet to stir reaction mixture during synthesis**

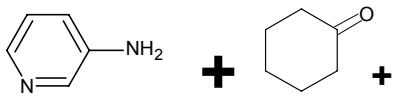
- **Purifications performed on the compact SP1 system using the new TLC to gradient algorithm**
  - **Accommodates the Biotage FLASH 12–40 cartridges without external stands**
  - **Solvent and waste monitoring**
  - **Variable dual-wavelength detector**
  - **Auto-continue feature**



Biotage

# Microwave Synthesis and Purification of Reductive Amination Product

**Step 1**




1 equivalent

+



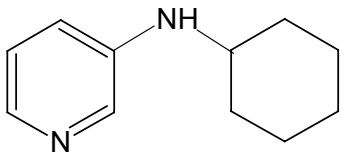

1.2 equivalent

+ **2 mL 20% HOAc in THF**
**1 equivalent Si-NaCNBH$_3$**

**Step 2** Microwave 5 minutes @ 150 ºC

**Step 3** Confirm product formed by TLC evaluation

**Step 4** FLASH chromatography with catch and release using Syntage TsOH Samplet and DCM:Methanol gradient




- **Starting amine and ketone materials placed in reaction vessel with 20% HOAc in THF as solvent and Syntage Cyanoborohydride reagent**
- **Reaction mixture heated and stirred in Initiator microwave at 150°C for 5 minutes**
  - **Reaction mixture tested by TLC**
- **Reaction mixture solution containing THF directly added to 12+M cartridge containing a TsOH Samplet**
- **From TLC input Rf data for compound of interest**
- **Product automatically and successfully purified on SP1 Flash system using catch and release technique**



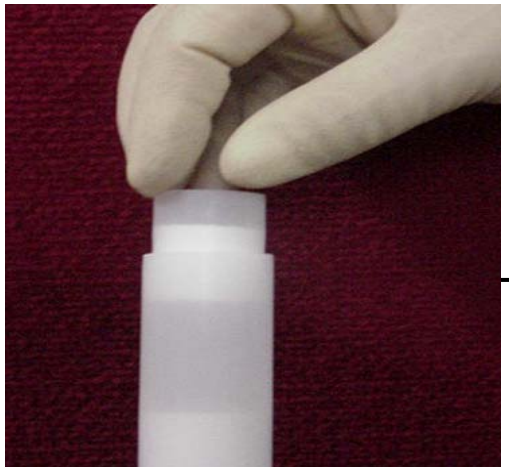
Biotage

# Syntage TsOH Samplet Catch and Release Procedure

**Step 1**

**Add reductive amination reaction mixture in THF to Samplet/ Cartridge**

**Biotage Si 12+M cartridge containing TsOH Catch and Release Scavenger Samplet**



**Step 2**

**Capture product and wash out THF with DCM**

Discard THF washed from column with DCM

Reaction product (amine) bound to TsOH Samplet

**Step 3**

**Release reaction product** and impurities from Syntage Samplet with 0.5 mL 2.0M $NH_3$ in MeOH

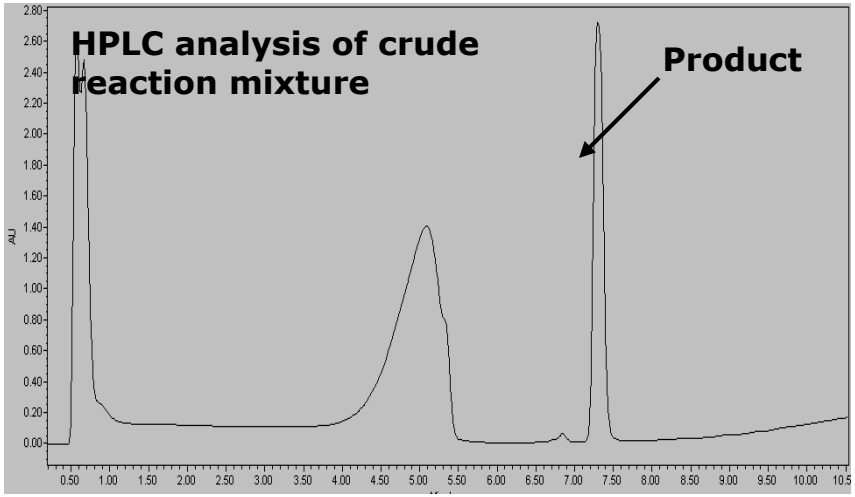
**Step 4**

Purify selected compounds with specified DCM:MeOH gradient



Biotage

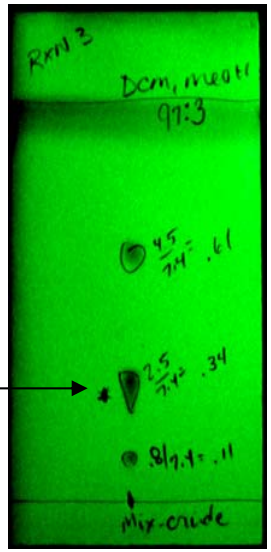
# TLC and Purification Data for Reductive Amination Product with DCM and MeOH




**HPLC Conditions**

| | |
|---|---|
| Column: | $C_{18}$ 5µm 4.6 x 50 mm |
| Mobile Phase: | A. $H_2O$:ACN (95:5) + 0.1% TFA |
| | B. ACN:$H_2O$ (98:2) + 0.1% TFA |
| Gradient: | 0-100% B in 8 min, hold 2 min |
| Flow Rate: | 1.5 mL/min |
| Detection: | 254nm |

**TLC analysis of crude reaction mixture**




Product →

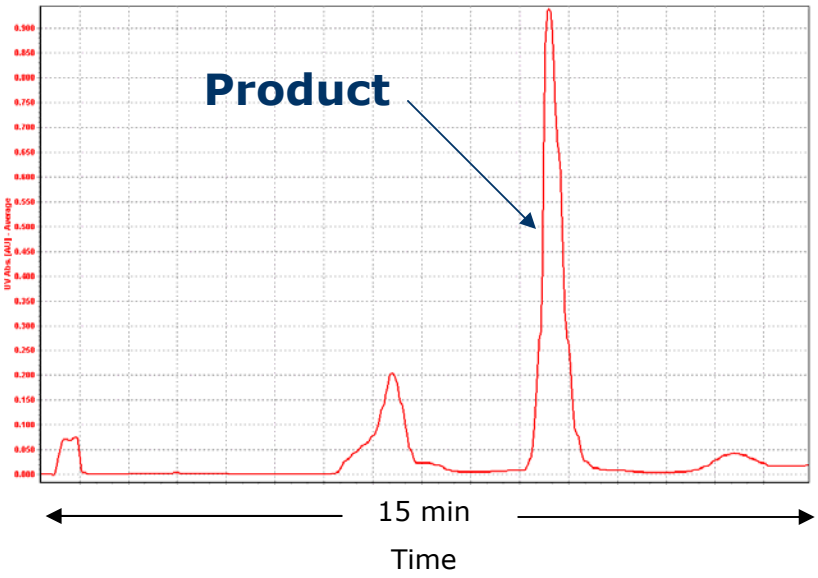
| Rf |
|---|
| 0.61 |
| 0.34 |
| 0.11 |

- **TLC solvent system was DCM:MeOH 97:3**
  - **Product Rf 0.34**
- **Gradient profile automatically generated by Flash system**
  - **Solvent A: DCM**
  - **Solvent B: DCM:MeOH (94:6)**
  - **Initial hold for 18 mL at 0% B**
  - **120 mL 0-66% B linear gradient**
  - **Final hold for 36 mL at 66% B**

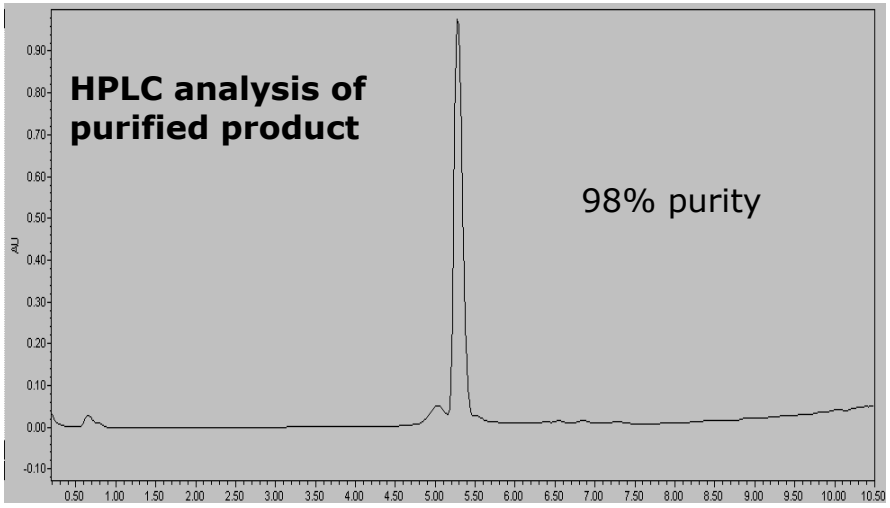


Biotage

# Reductive Amination Product Purification Results with DCM and MeOH




- **Purification with Biotage Flash SP1 system using Automated DCM/MeOH gradient**
  - **Flash conditions: Flash 12+M cartridge loaded with 110 mg crude**
    **Solvent A: DCM**
    **Solvent B: DCM:MeOH (94:6)**
    **Gradient: Initial hold for 18 mL at 0% B**
    **120 mL 0-66% B linear gradient**
    **Final hold for 36 mL at 66% B**
  - **Total Purification Time 15 minutes**




**HPLC Conditions**

| | |
|---|---|
| **Column:** | **C8 4.6 x 50 mm** |
| **Mobile Phase:A:** | **$H_2O$:ACN (95:5) + 0.1% TFA** |
| **Mobile Phase B:** | **ACN:$H_2O$ (98:2) + 0.1% TFA** |
| **Gradient:** | **5-100% B in 8 minutes, hold 2 min** |
| **Flow Rate:** | **1.5 mL/min** |
| **Detection:** | **254nm** |

Biotage

# Advantages of New Flash Automated Gradient Technique

- Accelerates Solvent System Selection
  - Eliminates guessing the solvent mixture that produces both an acceptable separation and a Rf within a narrow band
  - Eliminates need to perform multiple TLC separations
  - Technique applicable when Rf ranges from 0.05 to 0.9
  - New Algorithms applicable for all solvents
- Provides a quick, easy solution for alcohol containing gradients
  - Good for very polar compounds that require alcohol
  - System automatically adjusts conditions using algorithms when solvents exceed a predetermined solvent strength value with no user intervention
- Eliminates remixing solvents to elute different compounds
  - User optimizes separation of mixture components by inputting different Rf values
- Accelerates Clean-up and Purification
  - Biotage catch and release scavenger Samplets eliminate time-consuming clean-up of reaction mixture prior to purification
  - Catch and release technique ensures polar solvents do not cause co-elution or early breakthrough of desired compounds