Modifying Separation Selectivity by Selection of Fused-Core® Particle Bonded Phases

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Abstract

- Fused-Core® particles, with an overall diameter of 2.7 µm and a porous 0.5 µm thick shell, have demonstrated efficiencies comparable to those of sub-2-µm particles, but with significant lower column back pressure. Column efficiency (N) is an important parameter in the general resolution equation, yet resolution only increases by the square root of an increase in efficiency.

  Selectivity (alpha, α), on the other hand, has a direct linear relationship with resolution, and, thus, has a much more powerful influence on resolution. While the majority of applications developed today utilize C18 bonded phase chemistry, there are frequent instances when an alternative bonded phase yields superior results in terms of selectivity and, ultimately, resolution.

  Illustrative chromatograms using stationary phases with different selectivities, while maintaining the high plate efficiencies generated by the Fused-core particles, will demonstrate the effectiveness of such an approach for obtaining optimized HPLC separations. The value of using multiple column selectivities as part of a fast method development strategy using DryLab® 2010 software with Peak Match® will also be highlighted.

Most Effective Parameters to Change Selectivity

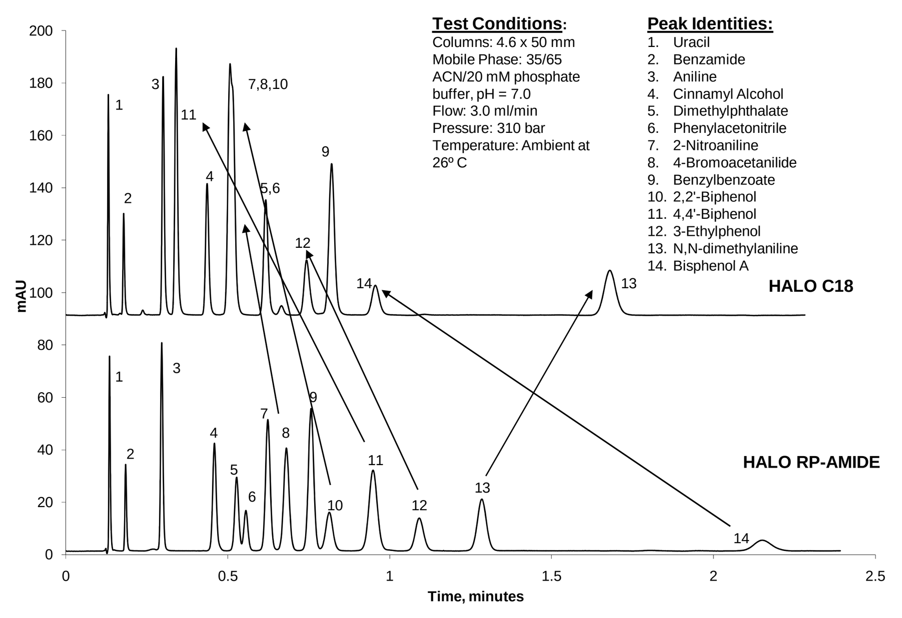
The analysis condition parameters that most affect selectivity, α are[1]:

Column type (C18, phenyl, amide, etc.)	++
B-solvent (acetonitrile, methanol, etc.)	++
Mobile phase pH	++
Ion-pair concentration	++
%B solvent/gradient steepness	+
Column temperature	+
Buffer concentration	+

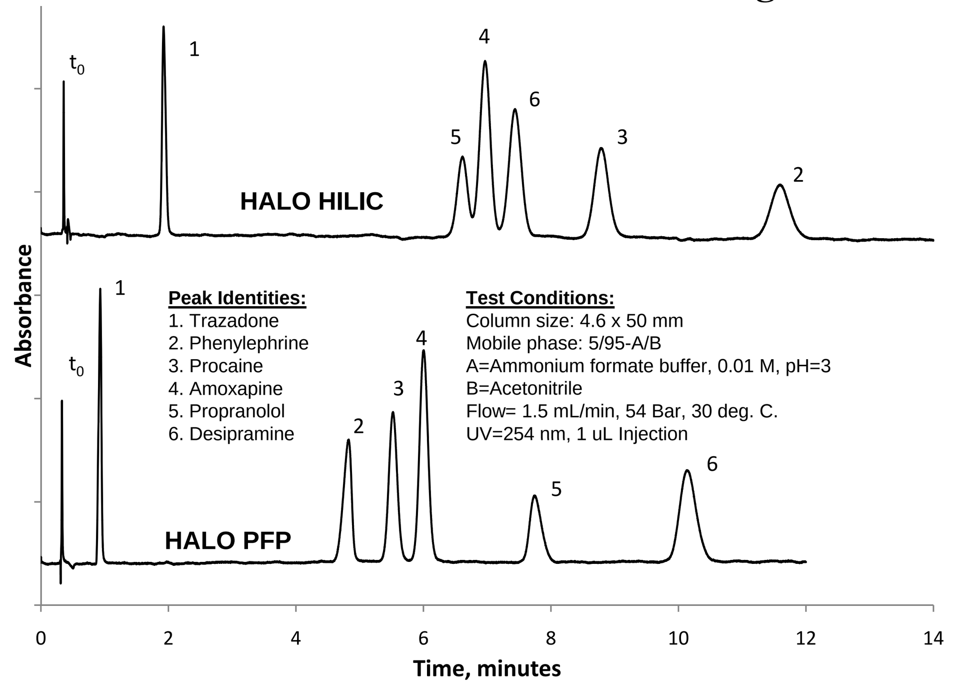
more effective ↑ less effective

[1]adapted from "Introduction to Modern Liquid Chromatography", 3rd Edition, L. R. Snyder, J. J. Kirkland, J. W. Dolan; p. 29, 2010, John Wiley & Sons, Inc.

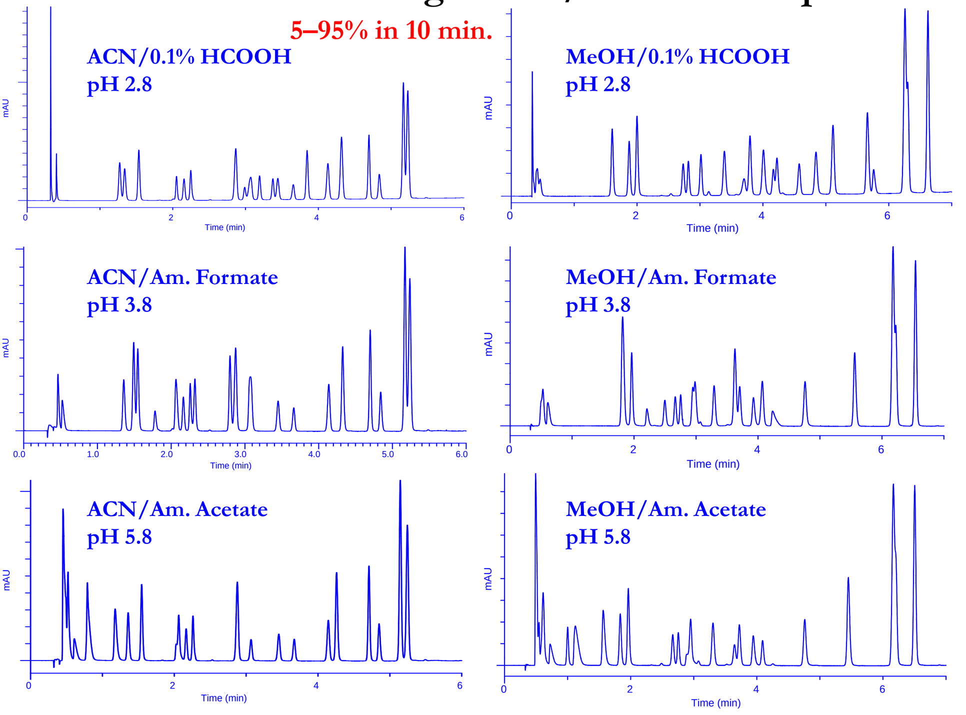
HALO C18 vs. RP-Amide: Polar Analytes



HALO PFP vs. HILIC: Basic Drugs



RP-Amide Screening: ACN/MeOH vs. 3 pHs



HALO Bonded Phase Characteristics

HALO Phase	Retention Mechanism	Retention Increased for	Best Application
C18, C8	Hydrophobic interactions	Lipophilic molecules, uncharged acids and bases, strong bases or acids in ion pairing mode	Analytes differing in hydrophobicity, homologues non-aqueous RPLC
RP-Amide	Hydrophobic, hydrogen bonding	Alcohols, acids, phenols	basic analytes, heterocycles, proton donors and acceptors, highly aqueous conditions
Phenyl-Hexyl	Hydrophobic, π–π	Electron-poor compounds, analytes with electron-withdrawing groups, (ketones, nitriles, alkenes, etc)	heterocycles, aromatics, highly aqueous conditions
PFP	Hydrophobic, π–π, hydrogen bonding, dipole-dipole	Electron-rich compounds, analytes with π bonds, electron delocalization and electron-donating groups, proton donors, analytes with different dipole moments	Bases, stereoisomers, steroids, taxanes, substituted aromatics, highly aqueous conditions, HILIC separations ≥ 80% ACN
Silica/HILIC	NPLC: analyte adsorption on silica and displacement by solvent molecules HILIC: partitioning of polar analytes between highly organic mob. phase and water layer near silica surface	NPLC: Polar vs. nonpolar analytes, planar vs. nonplanar HILIC: Polar vs. nonpolar analytes	NPLC: analytes with low or no water solubility, stereoisomers, HILIC Mode: polar acids, bases, and neutrals

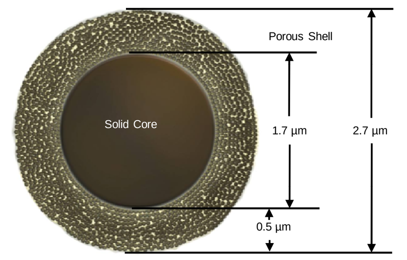
Fused-Core Particles

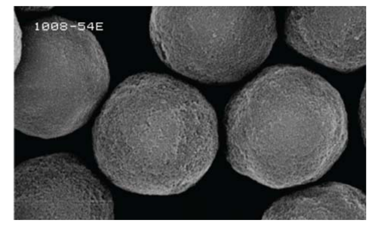
Particle Characteristics

- Silica: High purity, Type B
- Pore Size: 90 Å and 160 Å
- Particle Size Distribution: 5 % RSD
- pH range: 2–9
- Efficiency: 230,000 plates/m

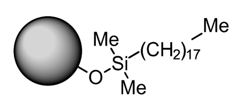
Features and Benefits

- Ultrafast separations save time and improve productivity
- UHPLC performance without the need for UHPLC equipment
- Low pressures enable the coupling of columns for high efficiency/high resolution

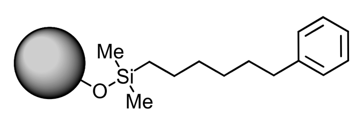




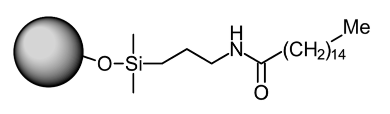
HALO Fused-Core Bonded Phases



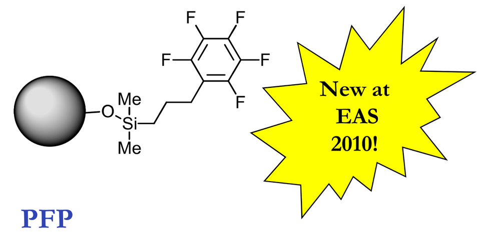
C18 (octadecyl)



Phenyl-Hexyl

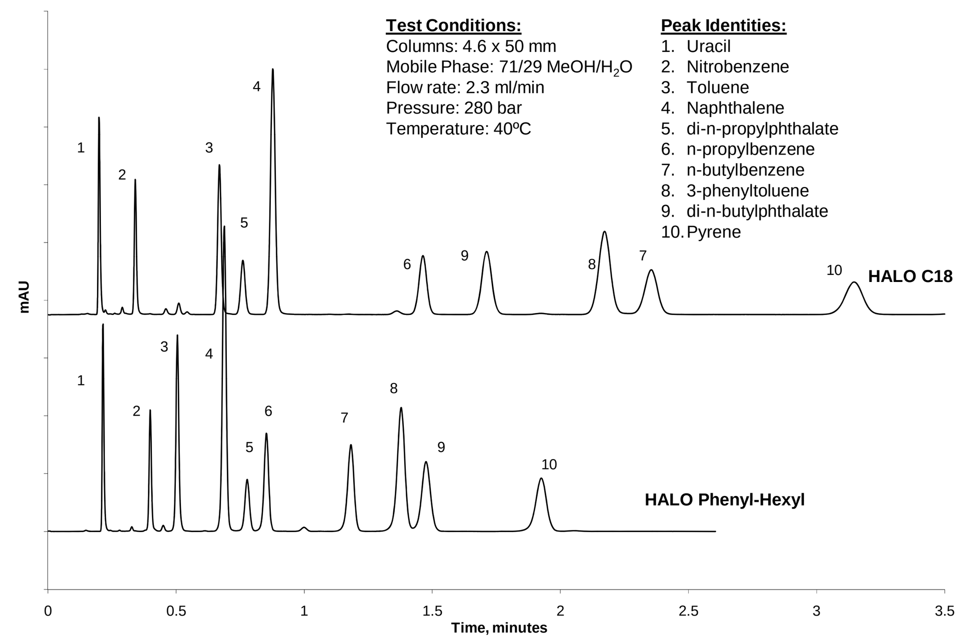


RP-Amide



PFP (pentafluorophenylpropyl)

HALO C18 vs. Phenyl-Hexyl: Aromatics



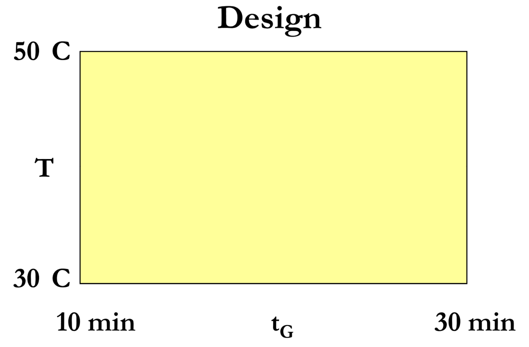
Example Method Development Strategy Using Multiple HALO Phase Selectivities

- Screen 1–4 column phases for analyte peak shape and selectivity using combinations of:
  - Column
    - HALO C18
    - HALO RP-Amide
    - HALO Phenyl-Hexyl
    - HALO PFP
  - Mobile Phase
    - Organic modifier
      - ACN
      - MeOH
    - Aqueous component
      - 0.1% HCOOH (pH 2.8)
      - 10 mM Ammonium Formate, pH 3.8
      - 10 mM Ammonium Acetate, pH 5.8
- Identify one or more combinations to investigate and optimize

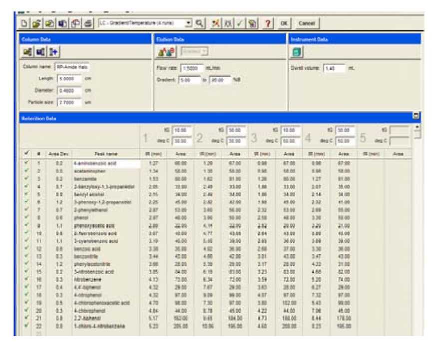
Optimization Approach

- 4.6 x 50 mm, 2.7 µm HALO RP-Amide
- 5–95% ACN/0.1% formic acid, 1.5 mL/min
- Gradient times of 10 and 30 min.
- Column temperatures of 30 and 50°C
- Input RTs and peak areas into DryLab® 2010

Design



DryLab Data Entry



Peak IDs assigned after optimization

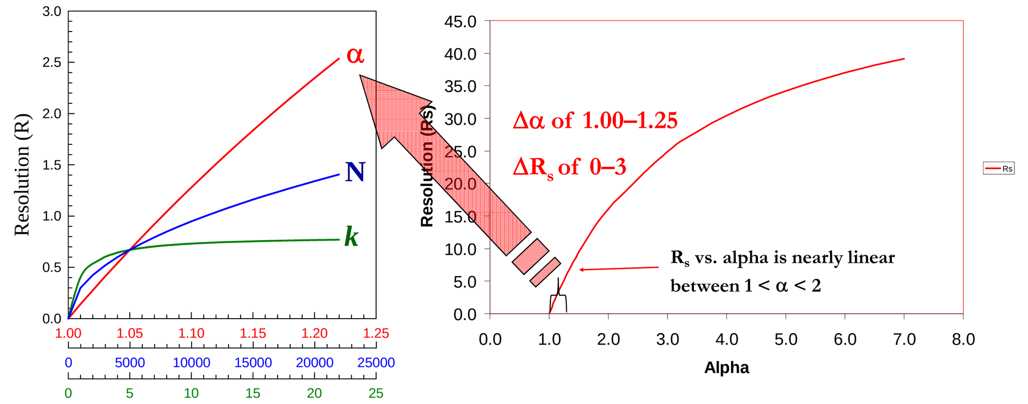
Summary

- Changing selectivity, α, is most effective way to improve resolution and adjust elution order.
- Column phases having different retention mechanisms are one of the top 3 parameters for changing selectivity of an HPLC separation.
- The HALO Fused-Core column family has a set of orthogonal phases to enable fast, effective method development.
- Combining HALO phase selectivities with different organic modifiers and blends over a broad pH range is an excellent approach for developing superior, robust and rugged RPLC methods.

Resolution Equation Shows that Selectivity is Most Effective Parameter to Change

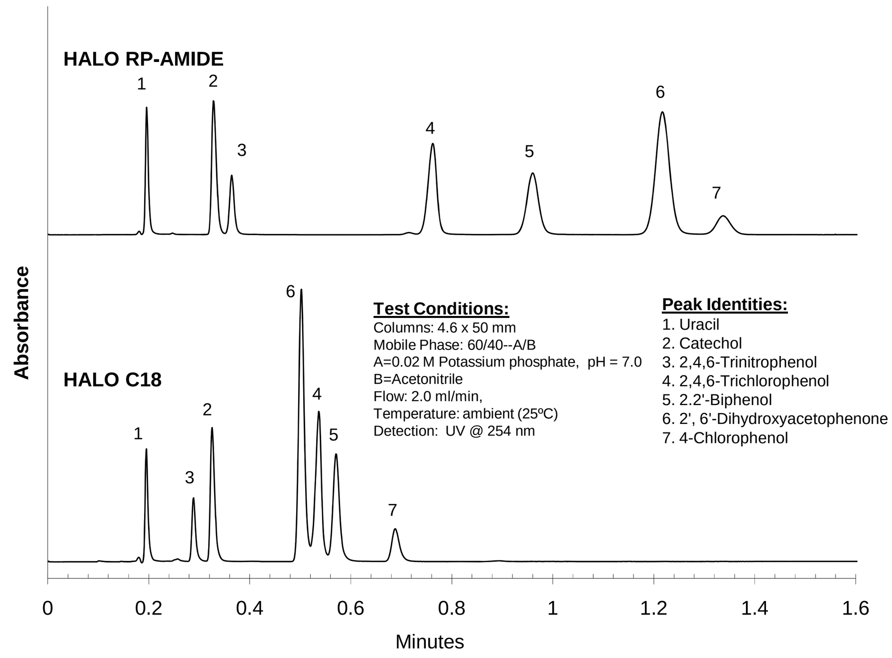
$$R_s = \left(\frac{1}{4}\right)\sqrt{N}\left[\frac{(\alpha - 1)}{\alpha}\right]\left(\frac{k_2}{(1+\bar{k})}\right)$$

Resolution is directly proportional to selectivity (α), but is only proportional to the square root of efficiency, N.

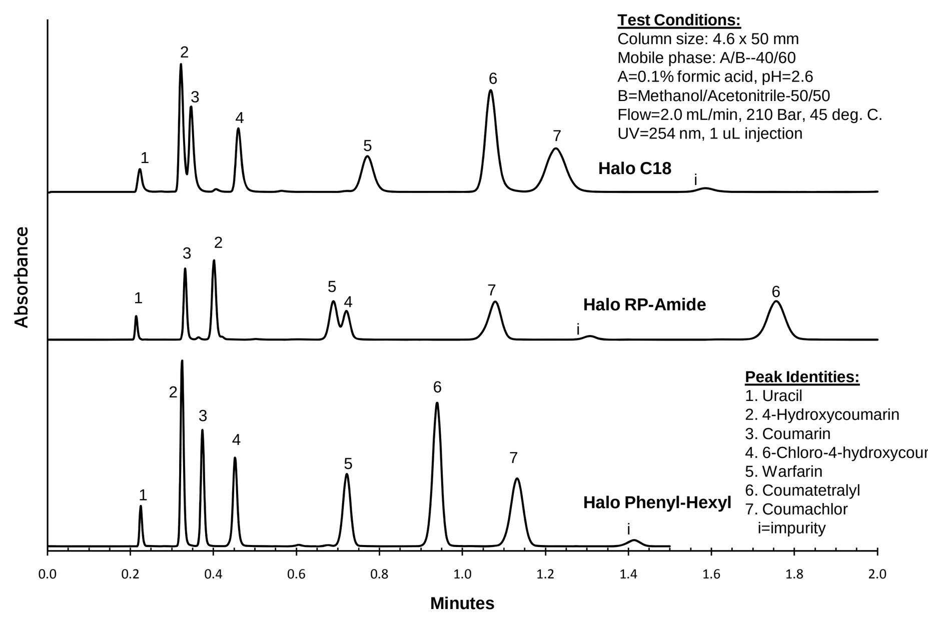


Source: Jun Mao, PhD Thesis with Professor Peter Carr, U. of Minnesota, 2001

HALO C18 vs. RP-Amide for Phenolics

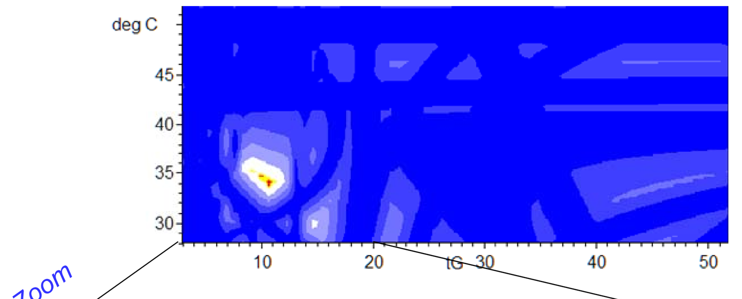


Fast Separation of Anticoagulants on HALO Fused-Core Packings

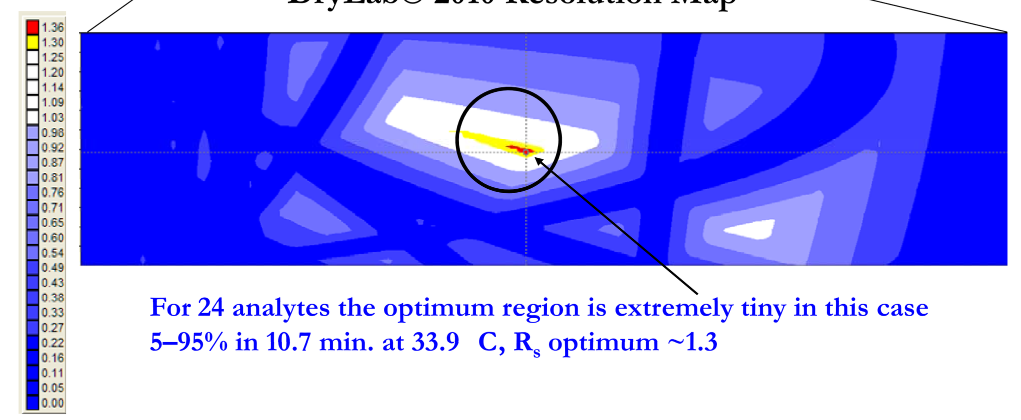


Column Phase Screening: Conditions

- Sample: mixture of 24 analytes
  - acids, alcohols, amides, amines, diols, nitriles, subst. benzenes, phenols
- Columns: 4.6 x 50 mm, 2.7 µm
  - HALO C18, RP-Amide, Phenyl-Hexyl, PFP
- Organic Modifier
  - ACN
  - MeOH
- Aqueous Modifier (pH)
  - 0.1% HCOOH (pH 2.8)
  - 10 mM Ammonium Formate, pH 3.8
  - 10 mM Ammonium Acetate, pH 5.8
- Gradient conditions: 5–95% organic in 10 min., 30°C
- Flow Rate and Linear Velocity: 1.5 mL/min, µ = 3.0 mm/sec
- Instrument: Agilent 1100 Quaternary, Delay Volume 1.4 mL
- Worst case back pressures @ 30°C
  - ACN: 132 bar (1920 psi)
  - MeOH: 210 bar (3051 psi)



DryLab® 2010 Resolution Map



For 24 analytes the optimum region is extremely tiny in this case 5–95% in 10.7 min. at 33.9 C, R_s optimum ~1.3