HALO® Fused-Core® Columns: Fast Method Development Using Conventional Instrumentation

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Abstract

Improved productivity is the goal of most chromatographers in the pharmaceutical, environmental, chemical, and biochemical laboratories in the 21st century. The development of a reversed phase LC method for the analysis of an active ingredient, related substances, or trace-level impurities can be an extremely challenging and time-consuming venture. In addition, many analytical experts recommend that some form of method quality assessment or preliminary method validation should be carried out throughout the method development and implementation process. The U.S. FDA has called for improvements in both product quality and new drug applications by strongly recommending that applicants use a Quality by Design (QbD) approach for all stages of pharmaceutical development and registration. It's been well documented that the first registrant for a generic pharmaceutical often reaps 80% or more of the lifetime sales versus other generic drug applicants. For all of these reasons, the capability to develop LC methods faster and to deliver methods that can produce high quality results with high productivity is urgently sought.

With the advent of HALO® Fused-Core® columns those with conventional instrumentation can develop methods more quickly by their ability to rapidly screen various column phases under different conditions of mobile phase composition, temperature and pH so that robust and rugged conditions can be identified and applied. An example of a systematic approach for the selection of stationary phase and robust separation conditions for a rapid separation of a mixture of ~20 acidic, basic, and neutral pharmaceuticals and chemicals will be demonstrated in a step-by-step fashion. The usefulness of DryLab® 2010 software for method optimization will also be demonstrated.

RPLC Method Development

- Usually expensive, and time- and resource-intensive
- Strategies for method development can be simple or complex
- Method performance objectives should be set before starting method development
- Careful consideration and evaluation of important separation selectivity parameters are effective and critical for final method quality
- Use of multiple column phase selectivities helps to ensure separations accomplish their goals
- Development of a robust and rugged separation following QbD (Quality by Design) approach helps to minimize or avoid future problems and builds in quality.

Method Performance Goals

Performance Criterion	Measure	Target
Analysis Speed	Run time	≤ 10 min. assay ≤ 20 min. impurities
Resolution	Minimum R_s critical pair	≥ 2.0
Peak Shape	USP Tailing Factor	≤ 1.5
Robustness	Resolution	± 15% relative with small change in conditions
Instrument Limitation	Maximum backpressure	80% Instrument Maximum

RPLC Method Selectivity Parameters

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

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

Note: parameters in blue font are varied in this work

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

Method Development Strategy Varies Depending on Need

Simple Approach (isocratic separation)

1. Select high quality column: reproducibility, efficiency, and peak shape, and stability
2. Choose 'mobile phase A' pH, and buffer conc.; choose 'mobile phase B' (organic modifier); select column temperature
3. Start at 100% B or highest %B that buffer conc. solubility allows.
4. Decrease %B successively by 10% (v/v) until last peak has k ~10 and first peak has k ~0.5-1. If not adequate, change column or phase.
5. Select best result and evaluate selectivity and robustness vs. %B; then pH, temperature, etc.

More Complex Approach (gradient separation)

1. Select high quality column (see above)
2. Choose combinations of two or more organic modifiers, several pHs and buffers
   - a. "Mobile Phase A": ACN vs. Methanol
   - b. "Mobile Phase B": pH 2.5 or 3.0 vs. pH 4.75 or pH 7.0 (or possibly higher)
   - c. Run gradients to compare selectivity, peak shape
3. Select best combination of 'A' and 'B', optimize gradient, evaluate selectivity and robustness vs. gradient slope and endpoints: then optimize pH, temperature, etc.

Complex Method Development Strategy for Related Substances Method

1. Choose 4–8 columns having different selectivities(for example, C18/C8, phenyl, polar-embedded, cyano, etc.)
2. Choose representative sample(s)
   - a. Active ingredients
   - b. Suspected and known impurities
   - c. Degradants from accelerated stability study experiments
     - a. Hydrolysis products
     - b. Oxidation and reduction reaction products
     - c. Products from acid, base, heat, and light exposure
3. Carry out gradient separations and compare results (T_r, N, α, R_s)
   - a. 2-3 organic modifiers or modifier blends
   - b. 2-4 different pHs for aqueous component
   - c. 2-3 gradient slopes
   - d. Compare best condition(s) at multiple temperatures
4. Use computer simulation software to determine most robust combination and conditions.

Fast Method Development Strategy Used

1. Screen short, high efficiency HALO phases with fast gradients at room temperature.
2. Select 3 x 50 mm size—similar in efficiency to 4.6 x 150 mm, 5 µm column by experts.
3. Use 2 different organic modifiers: ACN and MeOH
4. Use 2 different LC/MS-ready aqueous components
   - a. 10 mM ammonium formate (pH 3.0)
   - b. 10 mM ammonium acetate (pH 6.8, unbuffered)
5. Choose best 1 or 2 combinations of stationary phase and mobile phase (organic modifier/pH)
6. Generate input data for DryLab 2010 with 2 different gradient steepnesses at 2 temperatures (t_G x T expt.)
7. Identify optimum(a) and assess robustness
8. Run optimized conditions to verify performance
9. Compare performance vs. method goals

HALO Fused-Core Stationary Phases Column Comparison Function Values

- C18
- C8
- Phenyl-Hexyl
- RP-Amide

Fs Values[1] from ColumnMatch

30% Organic	pH 7.0
0.0	Halo-C18
8.2	Halo-C8
18.0	Halo Phenyl-Hexyl
38.7	Halo RP-Amide

Fs Values at low pH

30% Organic	pH 2.5
0.0	Halo-C18
9.5	Halo-C8
18.3	Halo Phenyl-Hexyl
51.3	Halo RP-Amide

Fs values > ~10 indicate relatively different selectivities

1 Fs values are column comparison function values from the Hydrophobic Subtraction model developed by L. R. Snyder, J. W. Dolan, P. W. Carr and others. ColumnMatch software is available on the USP web site and is also bundled with DryLab® 2010 software sold by the Molnar-Institute.

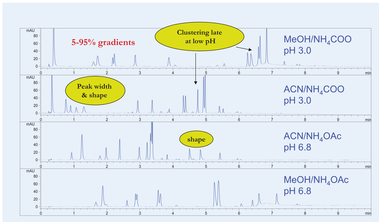
Experimental

Instrument	Agilent 1100 Quaternary
Configuration	Shortest length 0.005" ID tubing between modules, 1.6 µL heat exchanger, Semi-micro flow cell bypassed (1 < V_{cell} < 5 µL)
Injection Volume	1.0 µL
Column Temperature	25 °C for screening gradients 25 ° and 50 °C for optimization gradients
UV Detection	254 nm, 1100 VWD
Response time	0.0625 sec.
Data Rate	13.7 Hz
Extracolumn Volume	10.8 µL

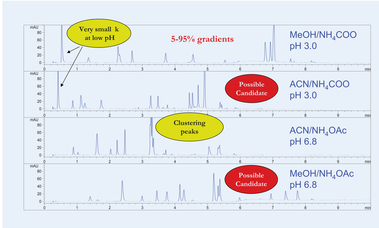
Acids	Bases	Neutrals
4-nitrophenol	3-pyridylacetonitrile	4-nitroanisole
acetaminophen	4-nitroaniline	4-chloro-3-nitroanisole
fenoprofen	amitriptyline	benzonitrile
ibuprofen	diphenhydramine	estrone
ketoprofen	famotidine	
m-toluic acid	nizatidine	
sodium naproxen	nortriptyline	
sodium tolmetin	ranitidine	

50 µL aliquots of stock solutions were combined to give ~ 1 mL composite

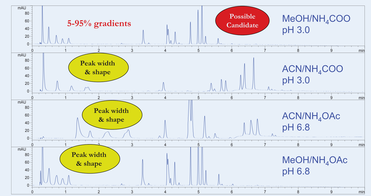
HALO C18 Screening Gradients: 8 min.



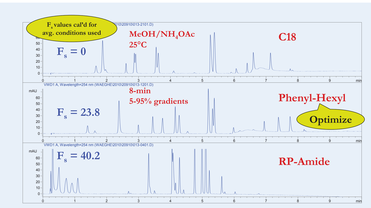
HALO Phenyl-Hexyl Screening Gradients: 8 min.



HALO RP-Amide Screening Gradients: 8 min.

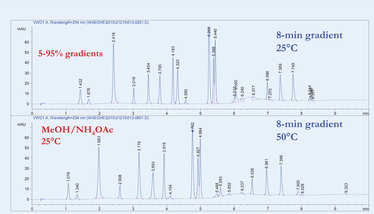


HALO C18, Phenyl-Hexyl, RP-Amide Selectivity and Peak Shape Comparison

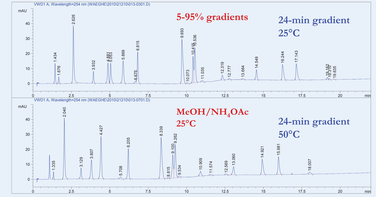


Selectivity differences and peak width and shape differences among all 3 phases

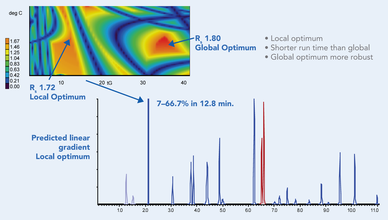
Phenyl-Hexyl DryLab Input Runs, 8-min.



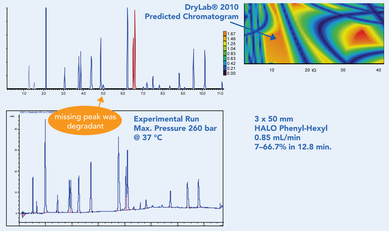
Phenyl-Hexyl DryLab Input Runs, 24-min.



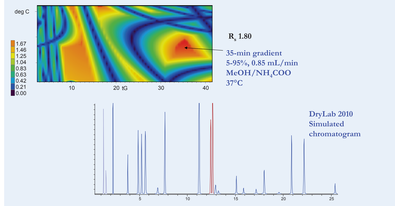
DryLab Resolution Map Local Optimum Predicted Separation



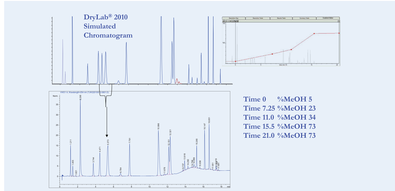
Comparison: Predicted vs. Actual for Local Optimum



HALO Phenyl-Hexyl Global Optimum, Linear Gradient



HALO Phenyl-Hexyl Global Optimum, [red] Gradient



Time Comparison for Fast Method Development HALO vs. Conventional Column

Column	3 x 50 mm, 2.7 µm HALO	4.6 x 150 mm, 5 µm Conventional
Reduced Plate Height (h)	1.6	2.5
Theoretical Plates (N)	11500	12000
Flow Rate (mL/min)	0.85 (optimum µ)	1.00 (above optimum ~0.75)
Screening Runs	204 min.	576 min.
Optimization Runs	225 min. (worst case) 121 min. (best case)	672 min. (worst case) 360 min. (best case)
DryLab Simulation	60 min. (worst case) 30 min. (typical)	60 min. (worst case) 30 min. (typical)
Total Time	490 min. (worst case) 355 min. (best case)	1308 min (worst case) 966 min. (best case)
Speed Advantage	2.7-fold	16-21 hours!

highest backpressure only 260 bar (MeOH/water, 25 °C

NOTE: The 30- and 90-min optimization runs for 150 mm,5 µm column have only 50% of k* value the 8- and 24-min runs for 3 x 50 mm HALO column.

Worst Case: 1 blank run precedes each optimization input run. Samples are run only once, except very 1st short-est run.

Best Case: 1 blank run precedes only 1st run of optimization runs.

Summary and Conclusions

- Fast development of robust RPLC separations on conventional instruments is made easier with a systematic experimental strategy that includes:
  - Columns with different selectivities such as HALO C18 and C8, Phenyl-Hexyl, and RP-Amide phases in 3-mm ID in short lengths such as 50 and 75 mm
  - Prudent choices of organic modifiers and aqueous phases at several pHs and temperatures
- Significant time savings are achieved compared to conventional column sizes while maintaining excellent resolving power.
- Methods developed on HALO® columns can be transferred among conventional, UHPLC, and UPLC® systems