PREPARATIVE CHROMATOGRAPHIC PROPERTIES OF NEXT GENERATION HYBRID HPLC MATERIALS

Waters

Jie Y. Cavanaugh, Fang Xia, Diane M. Diehl, Jeffrey R. Mazzeo
Waters Corporation, Milford, MA 01757
(Send correspondence to jie_cavanaugh@waters.com)

OVERVIEW

Preparative HPLC has become increasingly important in high-throughput screening and large-scale product purification in the pharmaceutical industry. With all the available column chemistries in the market, it is very difficult for chromatographers to pick the best column and the best overall chromatographic conditions for their separation.

The next generation of hybrid LC particles with unique bridged-ethyl chemistry enables robust chromatographic separations in the pH 1-12 range, while exhibiting excellent mechanical strength and efficiency.

Selectivity, scalability and loadability are critical for the success of preparative HPLC separations. There are several variables which can be manipulated to achieve the goal of loading as much analyte as possible on the column without sacrificing resolution. Solvent type, column chemistry and buffer pH are the most common factors used to control selectivity. In this study, we demonstrate an efficient method development approach which utilizes these three factors to optimize selectivity and then scale-up to the preparative column under the best conditions.

LIGAND TYPES

Figure 1. Four different XBridge™ chemistries : trifunctional C_{18}, C_8, Phenyl and monofunctional Shield RP_{18}.

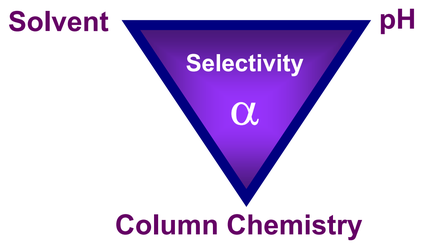

Figure 2. Selectivity variables.
It is important to consider all the variables in method development, especially the combined effect of solvent, pH and column chemistry.

MOBILE PHASE PH AND LOADABILITY

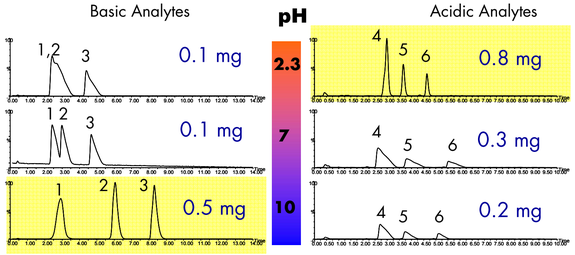

Figure 3. Mobile phase pH and loadability.
All data on XBridge™ C_{18} 4.6 x 50 mm, 5 µm column. Left: High pH for basic analytes results in the best loadability, as well as the best retention and separation (Analytes: 1. Nordoxepin, 2. Doxepin, 3. Amitriptyline). Right: Low pH for acidic analytes results in the highest loadability, as well as the best retention and separation (Analytes: 4. Oxacillin, 5. Cloxacillin, 6. Dicloxacillin).

SCOUTING CONDITIONS FOR COLUMN CHEMISTRY AND ORGANIC MOBILE PHASE

Columns: 4.6 x 50 mm, 5 µm
XBridge™ C_{18}
XBridge™ Shield RP_{18}
XBridge™ Phenyl

Mobile Phase A: Water with 0.1% NH_4OH
Mobile Phase B1: MeOH with 0.1% NH_4OH
Mobile Phase B2: MeCN with 0.1% NH_4OH
Flow Rate: 1.0 mL/min
Gradient: 10-min gradient from 5% to 100% B
Sample Concentration: 500 µg/mL each
Sample Diluent: DMSO
Injection Volume: 10 µL
Temperature: 30 °C
Detection: UV @ 254 nm
Instrument: Waters Alliance® 2695 with 2996 PDA

Since the analytes are bases, we choose to use high pH for best peak shape and loadability

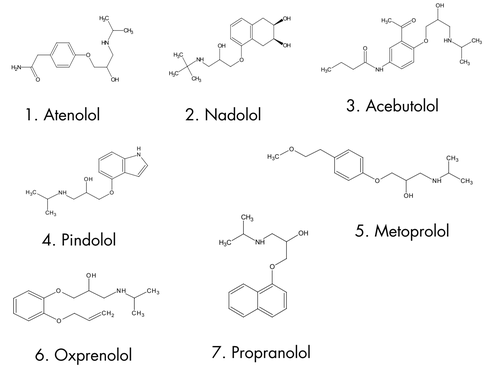

Figure 4. Analyte structures for β-blockers (bases).

SCOUTING RESULTS

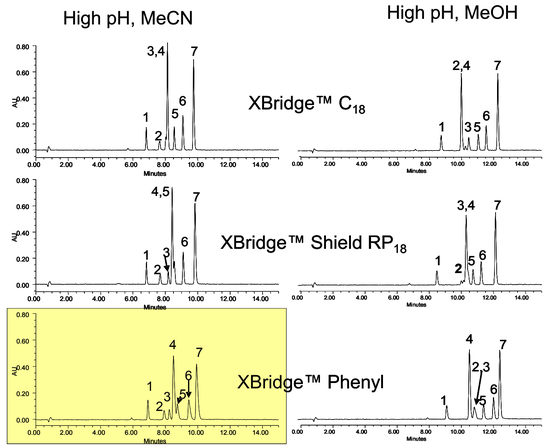

Figure 5. Scouting results.
The seven analytes exhibit different selectivity under various chromatographic conditions. XBridge™ Phenyl with MeCN offers the best set of starting conditions to separate the seven β-blockers.

OPTIMIZED CONDITIONS AND SCALE-UP

Columns: XBridge™ Phenyl, 4.6 x 50 mm, 5 µm
XBridge™ Phenyl OBD™, 19 x 50 mm, 5 µm
Mobile Phase A: Water with 0.1% NH_4OH
Mobile Phase B: MeCN with 0.1% NH_4OH
Flow Rate: 1.4 mL/min (analytical); 23.9 mL/min (preparative)
Gradient: 10-min gradient from 10% to 70% B
Sample Concentration: 36 mg/mL total in DMSO
Injection Volume: 100 µL (analytical); 1700 µL (preparative)
Detection: UV @ 280 nm
Instrument: Waters FractionLynx™ UV AutoPurification™ System

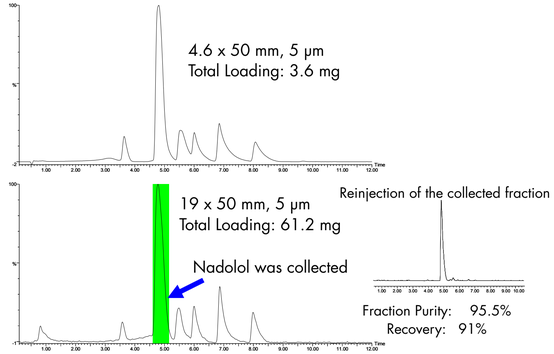

Figure 6. Optimized conditions, scale-up, and fraction collection.
Linear scale-up from the XBridge™ analytical column to preparative column was achieved. UV directed fraction collection results in high purity and recovery for target compound.

CONCLUSIONS

- XBridge™ columns offer different chemistries that can be used in method development for diverse applications
- An approach to a successful separation was developed
  - Run scouting study to select column chemistry and mobile phase conditions
  - Optimize the chromatographic conditions to get best separation on analytical column
  - Perform a loading study on analytical column
  - Scale from analytical to preparative columns
- The UV/MS directed fraction collection system enables highly pure collected samples

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