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THE PRACTICAL ASPECTS OF NORMAL-PHASE HPLC

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OVERVIEW

In today's separation technology, reversed-phase liquid chromatography (RPLC) is the most widely used methodology due to its excellent resolving power and superior data reproducibility. However, normal-phase liquid chromatography (NPLC), as the first HPLC separation mode, is still very popular because of its unique selectivity, wide solvent choice, low system backpressure and easy solvent evaporation. A new normal-phase packing made from high purity silica with maximized surface area has been developed. Several applications are presented that address the unique selectivity of NPLC for isomers, steroids, and classical synthetic batch reactants in medicinal chemistry labs. The preparative NPLC columns are manufactured with Optimum Bed Density (OBD™) design, which ensures the direct scale-up from analytical to preparative columns. The OBD™ preparative columns also possess the advantages of higher mass loading and longer column lifetime. The impact of several commonly used solvents in NPLC separations is also studied from a practical view point. In addition, the equilibrium of different pore size silica normal phase particles in the presence of water is compared for a better understanding of particle design and use.

INTRODUCTION

- In Normal Phase Liquid Chromatography (NPLC), the stationary phase is polar in nature – either bare silica/alumina or a polar ligand bonded to the particle
- The separation is driven by the interaction of the polar functional group in the stationary phase with the polar functional group of the analytes
- NPLC is the combination of adsorption chromatography (silica or alumina) and partition chromatography (polar bonded phases)
- Retention characteristics:
  The more polar the analyte, the more retention in NPLC
  The more polar the solvent, the less retention in NPLC

SOLVENT POLARITY MAP

- In NPLC, utilizing a less polar solvent ***increases*** the retention of the analytes.
- Water is the strongest solvent in NPLC.

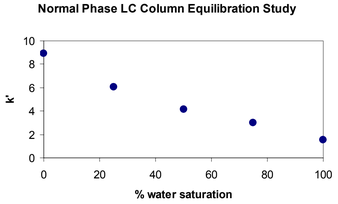
Solvents
Hexane
Heptane
Toluene
Acetone
Methylene chloride
Ethyl acetate
DMSO
Acetonitrile
Isopropanol
Methanol
Water

Weakest in NPLC

Strongest in NPLC

IMPACT OF H_2O IN NPLC

Column: SunFire™ Silica, 4.6 x 150 mm, 5 µm
Mobile Phase: Methylene chloride saturated to different degrees with water
Sample: 2-Phenyl-1-propranolol



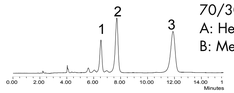
Note: methylene chloride 100% saturated with H_2O contains 0.15% of H_2O

- Existence of water impacts sample retention dramatically.
- Water replaces the solvents on the silica surface and binds strongly with the un-bonded silica surface.

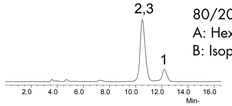
IMPORTANCE OF SOLVENT IN NPLC SELECTIVITY

Column: SunFire™ Silica, 4.6 x 150 mm, 5 µm
Mobile Phases: As indicated in the chromatograms
Flow Rate: 1.4 mL/min
Isocratic: As indicated in the chromatograms
Samples: Corticosterone, prednisone, prednisolone at 0.1mg/mL each
Sample Diluents: Same as the mobile phases used in the chromatograms
Injection Volume: 5 µL
Detection: UV @ 254 nm
Instrument: Waters Alliance™ 2695

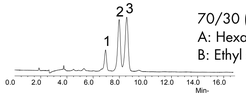
1. Corticosterone
2. Prednisone
3. Prednisolone



70/30 (A/B)
A: Hexane
B: Methylene chloride/Methanol (75/25)



80/20 (A/B)
A: Hexane
B: Isopropanol

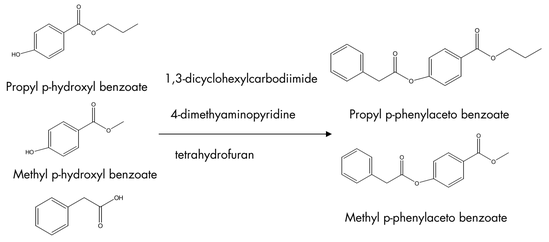


70/30 (A/B)
A: Hexane
B: Ethyl acetate

- Using solvents of various polarity results in different selectivity in NPLC.

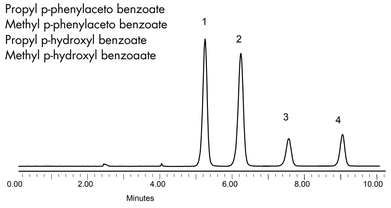
A POWERFUL TOOL IN SYNTHETIC LABS

In combinatorial synthesis, SunFire™ Silica OBD™ Prep columns are powerful tools in isolating and purifying intermediate or final synthetic compounds.



Column: SunFire™ Silica OBD™ Prep, 19 x 150 mm, 5 µm
Mobile Phase A: Hexane
Mobile Phase B: Ethyl acetate
Flow Rate: 18 mL/min
Isocratic: 80/20 (A/B)
Sample Load: 100 mg of crude from the above synthetic reaction batch
Injection Volume: 500 µL
Detection: UV @ 280 nm
Instrument: Waters Prep4000™ system

1. Propyl p-phenylaceto benzoate
2. Methyl p-phenylaceto benzoate
3. Propyl p-hydroxyl benzoate
4. Methyl p-hydroxyl benzoaate

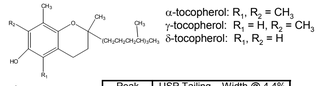


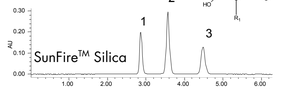
- Good resolution is achieved on this column.
- Sharp peaks ensure high mass loading on the column.
- Faster dry down in organic and low viscosity solvents to ease product treatment.

COMPARISON WITH OTHER SILICA NPLC COLUMNS

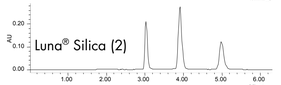
Columns: SunFire™ Silica, 4.6 x 150 mm, 5 µm;
Luna® Silica (2), 4.6 x 150 mm, 5 µm;
Zorbax® Rx-SIL, 4.6 x 150 mm, 5 µm;
Kromasil® Silica, 4.6 x 150 mm, 5 µm;
YMC-Pack™ Silica, 4.6 x 150 mm, 5 µm
Mobile Phase A: Hexane
Mobile Phase B: Isopropanol
Flow Rate: 1.0 mL/min
Isocratic: 98/2 (A/B)
Samples: α–Tocopherol, γ-tocopherol and δ-tocopherol at 200 µg/mL each in mobile phase
Injection: 5 µL
Detection: UV @ 295 nm
Teperature: Room temperature
Instrument: Waters Alliance™ 2695 with 2996 PDA

1. α-Tocopherol
2. γ-Tocopherol
3. δ-Tocopherol

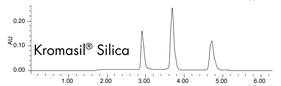




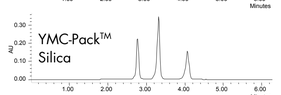
Peak	USP Tailing	Width @ 4.4%
1	1.12	0.17
2	1.09	0.21
3	1.07	0.27



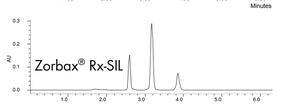
Peak	USP Tailing	Width @ 4.4%
1	1.2	0.16
2	1.22	0.22
3	1.23	0.29



Peak	USP Tailing	Width @ 4.4%
1	1.44	0.2
2	1.31	0.23
3	1.3	0.3



Peak	USP Tailing	Width @ 4.4%
1	0.88	0.17
2	0.84	0.21
3	0.84	0.26



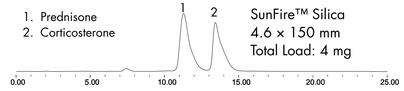
Peak	USP Tailing	Width @ 4.4%
1	1.1	0.12
2	0.95	0.17
3	1.01	0.21

- SunFire™ Silica column selectivity is comparable to the leading silica columns in the market.
- Nice and sharp peaks are observed on SunFire™ Silica column.

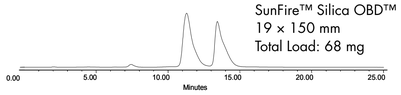
SCALABILITY

Columns: SunFire™ Silica, 4.6 x 150 mm, 5 µm;
SunFire™ Silica OBD™ Prep, 19 x 150 mm, 5 µm
Mobile Phase A: Hexane
Mobile Phase B: Isopropanol
Flow Rate: 1.0 mL/min (analytical); 17 mL/min (preparative)
Isocratic: 85/15 (A/B)
Samples: Prednisone and corticosterone
Sample Diluent: 85/15 (A/B)
Injection Volumes: 20 µL (analytical); 340 µL (preparative)

1. Prednisone
2. Corticosterone



SunFire™ Silica
4.6 x 150 mm
Total Load: 4 mg



SunFire™ Silica OBD™
19 x 150 mm
Total Load: 68 mg

- Linear scale-up is achieved from analytical column to preparative column.

CONCLUSIONS

SunFire™ Silica columns offer:

- Linear scale-up from analytical to preparative columns
- Excellent peak shapes
- High mass loading
- Comparable selectivity to other silica normal-phase columns in market
- Unique OBD™ prep column design to endure extended column lifetime and stability
- Low backpressure
- Available in 5 µm and 10 µm particle sizes

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