Testing Conventional Wisdom for Polysaccharide Chiral HPLC Columns

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Scope of Work

This work examines the ability of polysaccharide chiral HPLC columns to be converted between normal phase and reversed phase HPLC conditions. It also investigates the conversion between acidic and basic eluents under reversed phase HPLC conditions.

Introduction

Chiral HPLC columns are amazing pieces of technology. They are able to separate enantiomers, which are essentially the same chemical compounds but are mirror images of each other. This amazing technology typically comes with a higher price tag than a typical achiral HPLC column and this can make the chromatographer very cautious.

Conventional wisdom says that "a polysaccharide chiral HPLC column is delicate and should be dedicated to a single type of chromatography". The work presented here will describe an investigation of this wisdom. The results for the same polysaccharide columns being tested under multiple conditions, typically used for chiral HPLC methodology, are presented. These conditions include converting columns back and forth between reversed phase and normal phase applications, and the effects of switching between different eluent pH modifiers.

Materials and Methods

Reagents and Chemicals

All chemicals and reagents were obtained from the Sigma-Aldrich Company (St. Louis, MO, USA). Water purification via Sartorius® arium® Comfort II (Goettinger, Germany).

Instrument Description

Agilent® 1100 HPLC system

HPLC Column Descriptions

Column: Lux® 3 µm Cellulose-1
Dimensions: 50 x 2.0 mm
Part No.: 00B-4458-B0

Column: Lux 3 µm Amylose-1
Dimensions: 50 x 2.0 mm
Part No.: 00B-4729-B0

Column: Lux 3 µm Cellulose-2
Dimensions: 50 x 2.0 mm
Part No.: 00B-4456-B0

Column: Lux 3 µm i-Amylose-3
Dimensions: 50 x 2.0 mm
Part No.: 00B-4778-B0

Column: Lux 3 µm Cellulose-3
Dimensions: 50 x 2.0 mm
Part No.: 00B-4492-B0

Column: Lux 3 µm i-Cellulose-5
Dimensions: 50 x 2.0 mm
Part No.: 00B-4755-B0

Column: Lux 3 µm Cellulose-4
Dimensions: 50 x 2.0 mm
Part No.: 00B-4490-B0

Normal Phase / Reversed Phase Conversion Conditions

Normal Phase Methodology
Column: 3 µm, 50 x 2.0 mm - See HPLC Column Descriptions
Isocratic Mobile Phase: 90 % Hexane, 10% Isopropanol
Flow Rate: 0.5mL/min
Temperature: Ambient
Injection Volume: 0.2 µL
Detector: UV @ 245 nm
Run Time: 5 min

Phase Conversion Methodology
Column: 3 µm, 50 x 2.0 mm - See HPLC Column Descriptions
Isocratic Mobile Phase: 90 % Methanol, 10% Ethanol
Flow Rate: 0.5mL/min
Temperature: Ambient
Injection Volume: 0.2 µL
Detector: UV @ 245 nm
Run Time: 16 min

Reversed Phase Methodology
Column: 3 µm, 50 x 2.0 mm - See HPLC Column Descriptions
Isocratic Mobile Phase: 55 % Water, 45% Acetonitrile
Flow Rate: 0.5mL/min
Temperature: Ambient
Injection Volume: 0.2 µL
Detector: UV @ 245 nm
Run Time: 8 min

Acid Modifier / Base Modifier Conversion Conditions

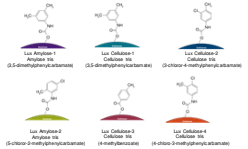
Acidic Reversed Phase Methodology
Column: 3 µm, 50 x 2.0 mm - See HPLC Column Descriptions
Isocratic Mobile Phase: 55 % Water (0.1 % Trifluoroacetic Acid), 45% Acetonitrile
Flow Rate: 0.5mL/min
Temperature: Ambient
Injection Volume: 0.2 µL
Detector: UV @ 245 nm
Run Time: 8 min

Acidic / Basic Conversion Methodology
Same as Phase Conversion Methodology above

Basic Reversed Phase Methodology
Column: 3 µm, 50 x 2.0 mm - See HPLC Column Descriptions
Isocratic Mobile Phase: 55 % Water (0.1 % Diethylamine), 45% Acetonitrile
Flow Rate: 0.5mL/min
Temperature: Ambient
Injection Volume: 0.2 µL
Detector: UV @ 245 nm
Run Time: 8 min

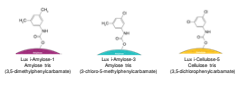
Lux Polysaccharide Based CSPs

Coated



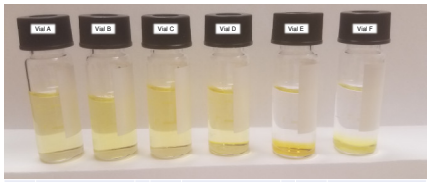
Compatible Eluents for Coated CSPs
- Normal Phase
  - Mixtures of an alkane and a miscible alcohol
- Polar Organic
  - Mixture of acetonitrile and alcohol
- Reversed Phase
  - Mixture of water and alcohol or acetonitrile
- Supercritical Fluid Chromatography
  - Mixture of carbon dioxide and alcohol or acetonitrile

Immobilized



Compatible Eluents for Immobilized CSPs
- Normal Phase
  - Mixture of an alkane and any miscible HPLC solvent
- Polar Organic
  - Mixture of any polar HPLC solvents
- Reversed Phase
  - Mixture of water and any miscible HPLC solvent
- Supercritical Fluid Chromatography
  - Mixture of carbon dioxide and suitable organic

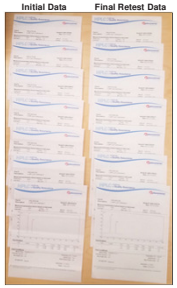
Eluent Miscibility for Conversion



Vial A	10:90 Hexane / Methanol	Vial C	10:90 Hexane / Alcohol Mix (90:10 Methanol / Ethanol)	Vial E	90:10 Hexane / Methanol
Vial B	10:90 Hexane / Acetonitrile	Vial D	90:10 Hexane / Alcohol Mix (90:10 Methanol / Ethanol)	Vial F	90:10 Hexane / Acetonitrile

Converting an HPLC column between normal phase and reversed phase, is successfully accomplished by using an eluent that is miscible with both conditions. Miscibility can be demonstrated with vials containing a mixture of solvents. To help visualize miscibility, a small amount of die is added to the vials. The die is not soluble in hexane. Vials A and B show that a small amount of hexane is miscible with methanol and acetonitrile, since both vials have a single layer. Vials E and F show that small amounts of methanol and acetonitrile are not miscible with hexane, since both vial shows a small but separate layer at the bottom. Vials C and D show that a mixture of 90:10 Methanol / Ethanol is miscible with small and large amounts of hexane.

Initial QC and Retest Column Data



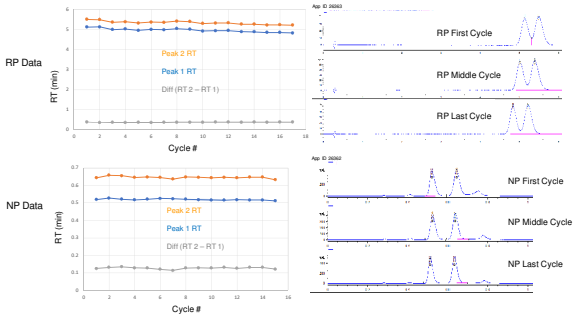
These specific columns were initially tested and then retested by the Phenomenex QC Department. Trans Stilbene Oxide (TSO) was used as the test probe and critical parameters are tabulated to compare the initial test results with the final retest results.

		Separation (RT 2 - RT 1) (min)	Efficiency (Plates/m)	Peak Asymmetry	Pressure (psi)
Lux Cellulose-1	Initial	0.73	99800	1.32	249
	Final	0.92	93360	1.21	236
	Change (Final-Initial)	0.19	-6440 (6%)	-0.11	-13
Lux Cellulose-2	Initial	0.72	95800	1.13	263
	Final	0.86	101360	1.09	240
	Change (Final-Initial)	0.14	5560 (6%)	-0.04	-23
Lux Cellulose-3	Initial	0.26	91660	1.39	236
	Final	0.34	94740	1.23	216
	Change (Final-Initial)	0.06	3080 (3%)	-0.16	-20
Lux Cellulose-4	Initial	0.57	90720	1.07	266
	Final	0.79	106920	1.11	247
	Change (Final-Initial)	0.22	16200 (18%)	0.04	-19
Lux Amylose-1	Initial	1.26	97420	1.07	218
	Final	1.14	103960	1.02	283
	Change (Final-Initial)	-0.12	6540 (7%)	-0.05	65
Lux i-Amylose-3	Initial	0.31	103060	1.09	330
	Final	0.34	106260	1.08	334
	Change (Final-Initial)	0.03	3200 (3%)	-0.01	4
Lux i-Cellulose-5	Initial	0.26	95820	1.20	398
	Final	0.31	99880	1.19	388
	Change (Final-Initial)	0.05	60 (0.1%)	-0.01	-10

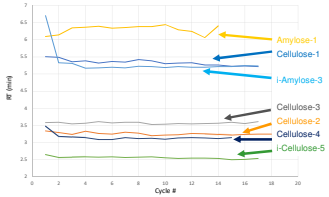
The changes between the 2 values are also presented as, final value minus the initial value. If the final value increased, the difference is presented in green. If the final value decreased, the difference is presented in red.

Normal Phase / Reversed Phase Conversions

Lux Cellulose-1 Data



RP Peak 2 Retention Time Data from All Columns

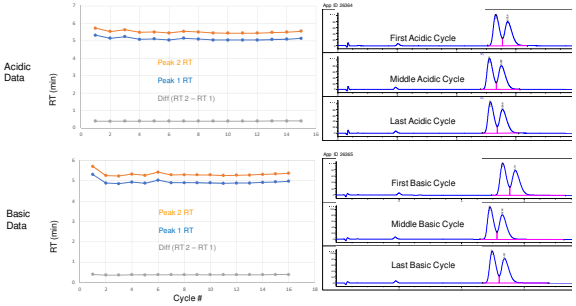


Presented here is data for Lux Cellulose-1 being cycled back and forth between NP and RP. The graphs on the left plot the retention times for both peaks in the TSO sample for each eluent cycle. Each graph also contains the difference in retention time between the two peaks. To help visualize the changes that occurred during this experiment, TSO chromatograms from the initial cycle, a middle cycle and the final cycle are also presented

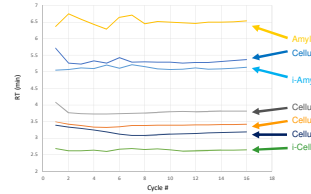
The RP data was used to track changes in the TSO chromatography. The graph on the right plots the retention time for the second TSO peak observed during each cycle of every column investigated.

Acidic Modifier / Basic Modifier Conversions

Lux Cellulose-1 Data



Base Peak 2 Retention Time Data from All Columns



Presented here is data for Lux Cellulose-1 being cycled back and forth between acidic RP eluent and basic RP eluent. The graphs on the left plot the retention times for both peaks in the TSO sample for each eluent cycle. Each graph also contains the difference in retention time between the two peaks. To help visualize the changes that occurred during this experiment, TSO chromatograms from the initial cycle, a middle cycle and the final cycle are also presented

The RP data was used to track changes in the TSO chromatography. The graph on the right plots the retention time for the second TSO peak observed during each cycle of every column investigated.

Discussion

Polysaccharide columns are the work horses for HPLC and SFC chiral methodology. A small set of columns can be used to separate a large number of biologically relevant small molecule compounds. This is particularly true when the different modes of chromatography are effectively employed. The conventional wisdom for polysaccharide chiral columns, has always been to take a cautious approach. There were real issues in the past for this type of chromatographic media, but many improvements have been made over the past 15 to 20 years.

In the work presented here, TSO was used as a test probe to evaluate changes in chiral selectivity. There were only minor changes observed in column selectivity after. This might be from TSO not being very sensitive to minor changes in the chiral stationary phase. TSO is not complicated and does not have any acidic or basic character. Different choices for test probes, might have shown more pronounced changes in selectivity.

When converting a chromatography column from one mode to another, there are several important factors. The miscibility of the mobile phase is a significant parameter. If the mobile phase becomes two separate phases, there will be surface tension at that interface, and this could cause damage to the polysaccharide stationary phase under pressure and flow within the column. When the eluent forms two phases, one of the components can become trapped inside the pores within the media and that will alter the performance of the column. The work presented here used 90:10 methanol/ethanol as the conversion eluent. Isopropanol is a typical choice for this task, and it works well based on solvent miscibility. However, isopropanol has a relatively high viscosity, can generate high backpressures and can be slow to penetrate all of the small pores within the stationary phases.

All of the columns tested in this project were tested initially and retested at the end by the Phenomenex QC Department. By comparing the retested results with the initial results, many attributes improved. Some of the attributes degraded slightly, but there were no significant negative changes.

This work looked at two different forms of chiral column conversion. The first was to convert columns between normal phase and reversed phase and the second was to convert columns between acidic and basic eluent modifiers. Each column was cycled a minimum of 15 times for each form and the observed changes were minimal. There was the most variability in the early cycles and this is attributed more to equilibration than a change in the stationary phase.

Does this mean we can switch chiral columns back and forth without limits? Not necessarily. There is more work to do. Other conversion eluents like isopropanol and neat ethanol should be evaluated. Additional acidic and basic modifiers and more difficult test probes, particularly with acidic functional groups need to be evaluated. This work does show that we can switch columns between chromatographic modes but there is more work to be done before we can completely disregard the conventional wisdom.

Conclusion

We have demonstrated that Lux Polysaccharide Chiral HPLC columns can be successfully switched between RP and NP HPLC applications without drastic changes to the chiral selectivity. We have also shown that switching between TFA and DEA can be accomplished without significant loss of column performance. This means that the conditions demonstrated here will allow for switching back and forth. This data does not imply that all conditions can be switched back and forth.

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