Accessing the Benefits of Polar Organic Mobile Phases on Cellulose-Based CSPs for Chiral HPLC

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Although the majority of chiral HPLC separations are done in normal phase mode (including SFC), mobile phases comprising polar solvents have advantages. They may offer different selectivity because of a conformational change of the CSP, better UV sensitivity, better solubility of the analyte, or LC-MS compatibility. The latter is a distinct advantage for bioanalytical applications. You can also use these mobile phases on instruments plumbed for reversed-phase, much more widely used than normal phase. Two mobile phase systems used in chiral HPLC are the POM (polar organic mode) and PIM (polar ionic mode). (In PIM, salts are added to facilitate ionization. In POM, if salts are used it is to suppress ionization.)

Mobile Phase Limitations of Cellulose-Based CSPs

Historically, the benefits of POM and PIM have been out of reach for the polysaccharide-based CSPs (cellulose, amylose) that dominate in chiral HPLC since they are often restricted to normal phase or SFC operation. In this short article, we will demonstrate high resolving power of the new Astec Cellulose DMP phase in polar organic mobile phases toward a set of basic pharmaceutical compounds. This unique column can operate in both normal phase and polar organic phase without detrimental effects, even with repeated cycles on the same column (1). We will also show its complementary character to the popular macrocyclic glycopeptide CSPs (Astec CHIROBIOTIC™).

Column Screening in NP, POM, and PIM Systems

Successful separation of several test compounds on Astec Cellulose DMP using a simple, LC-MS compatible mobile phase of 0.1% ammonium formate in methanol is shown in **Figure 1**. We then compared the selectivity of this column to Astec CHIROBIOTIC V2 (vancomycin-based) using the same LC-MS mobile phases, as well as normal phase on the cellulose column. Their performance was found to be complementary. **Table 1** summarizes the screening results of twenty-six basic racemic drugs. The best results for each compound are highlighted in red. In just three screening runs (one NP on Astec Cellulose DMP and one POM/PIM on both columns), twenty-two baseline separations were achieved, mostly in POM/PIM operation. The example of mianserin on the two columns and three mobile phases is shown in **Figure 2**.

Table 1. Screen Results on 26 Basic Molecules Using Astec Cellulose DMP and CHIROBIOTIC V2 Columns

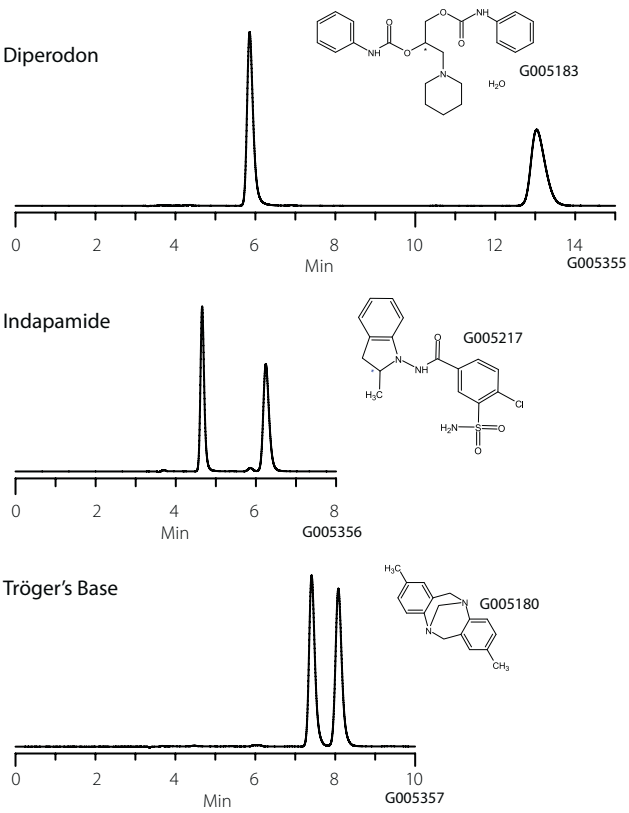
Conditions same as Figure 1 except:

column:	Astec Cellulose DMP, 15 cm x 4.6 mm I.D., 5 µm particles (51098AST) or Astec CHIROBIOTIC V2, 25 cm x 4.6 mm I.D., 5 µm particles (15023AST)
mobile phases:	
normal phase (NP):	10:90:0.1, IPA:Heptane:DEA
polar organic mode (POM):	0.1% w/v ammonium formate in methanol
polar ionic mode (PIM):	0.1% w/v ammonium formate in methanol
flow rate:	0.5 mL/min.
temp.:	25 °C
det.:	UV at 230 nm

Column: / Mode: / Compound	Astec Cellulose DMP normal phase (NP) k_1/selectivity	Astec Cellulose DMP polar organic mode (POM) k_1/selectivity	Astec CHIROBIOTIC V2 polar ionic mode (PIM) k_1/selectivity
Atropine	0.06/1.33	0.18/1.00	3.54/1.00
Bupivacaine	0.86/1.00	0.23/1.00	0.31/1.34
Citalopram	2.75/1.14	0.26/1.00	2.37/1.12
Clenbuterol	1.34/1.00	0.03/1.00	1.02/1.22
Diperodon	No elution	0.73/3.89	0.66/1.00
Disopyramide	1.65/1.07	0.11/1.02	1.08/1.14
Esmolol	3.36/1.57	0.09/1.25	1.34/1.12
Fluoxetine	1.09/1.08	0.07/1.02	2.00/1.24
Homatropine	2.40/1.62	0.08/2.04	0.13/1.00
Hydroxyzine	1.16/1.23	0.40/1.10	0.71/1.00
Indapamide	No elution	0.37/2.27	0.26/1.00
Ketamine	0.80/1.14	0.48/1.00	0.27/1.00
Ketoconazole	No elution	4.31/1.06	0.31/1.00
Mefloglumide	1.59/1.19	0.07/1.00	2.86/1.36
Methocarbamol	No elution	0.30/1/35	1.08/1.00
Methoxypheamine	0.86/1.21	0.07/1.00	1.52/1.16
Metoprolol	1.25/2.66	0.08/1.38	1.22/1.12
Mianserin	0.79/1.23	0.96/1.26	0.65/1.98
Ofloxacin	No elution	1.91/1.13	No Elution
Ondasetron	No elution	1.62/1.07	1.02/1.00
Promethazine	0.58/1.05	0.47/1.00	1.76/1.68
Propranolol	2.36/2.22	0.16/1.24	1.60/1.16
Ritalin	0.66/1.09	0.16/1.00	1.32/1.45
Thalidomide	No elution	1.20/1.00	0.47/2.97
Tolperison	0.41/1.00	0.27/1.00	1.14/1.24
Tröger's base	0.78/1.22	1.33/1.28	0.18/1.00

Figure 1. Separation of Basic Drugs Using Polar Organic Mobile Phase on Astec Cellulose DMP

column: Astec Cellulose DMP, 15 cm x 4.6 mm I.D., 5 µm particles (51098AST)
mobile phase: 0.1% w/v ammonium formate in methanol
flow rate: 0.5 mL/min.
temp.: 25 °C
det.: UV at 230 nm
injection: 5 µL
sample: 1 mg/mL in methanol



In conclusion, LC-MS compatible polar organic mobile phases (PIM/POM) can be applied to macrocyclic glycopeptide-based Astec CHIROBIOTIC CSPs and polysaccharide-based Astec Cellulose DMP columns for complementary results. The Astec Cellulose DMP is rugged enough to permit operation in both types of mobile phases. These findings demonstrate that polysaccharide-based CSPs can now extend their application areas to pharmacological/clinical trial studies where biological samples are routinely analyzed.

To learn more, request our brochures on Astec Cellulose DMP and Astec CHIROBIOTIC on the attached reply card. For complete information on all of our chiral HPLC and GC columns, visit our website *sigma-aldrich.com/chiral*.

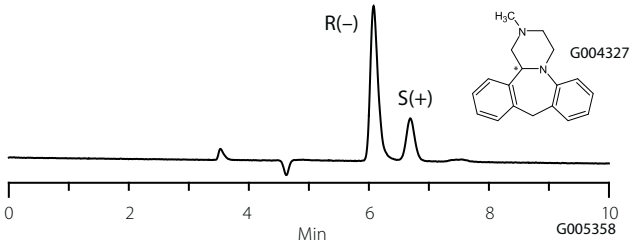
Reference

1. Lee, J. T.; Campbell, W., HPLC Enantiomeric Separations of Pharmaceuticals Using Polar Organic Mobile Phases. Presented at The Pittsburgh Conference, Atlanta, GA, March 17, 2011.

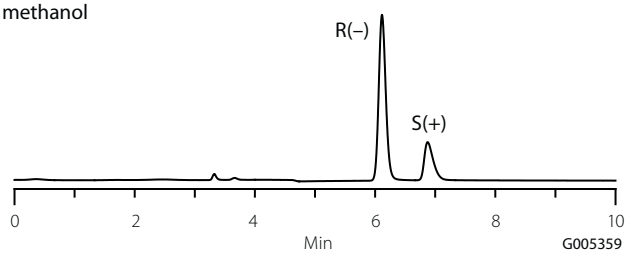
Figure 2. Example of Complementary Selectivity of Astec Cellulose DMP and Astec CHIROBIOTIC V2 in Various Mobile Phases (Mianserin Enantiomers)

Conditions (same as Figure 1 except):
columns: Astec Cellulose DMP, 15 cm x 4.6 mm I.D., 5 µm particles (51098AST) or Astec CHIROBIOTIC V2, 25 cm x 4.6 mm I.D., 5 µm particles (15023AST)
mobile phases:
normal phase, NP (a): 10:90:0.1, IPA:Heptane:DEA
polar organic mode, POM (b): 0.1% w/v ammonium formate in methanol
polar ionic mode, PIM (c): 0.1% w/v ammonium formate in methanol
flow rate: 0.5 mL/min. (a,b); 0.8 mL/min. (c)
sample: mianserin

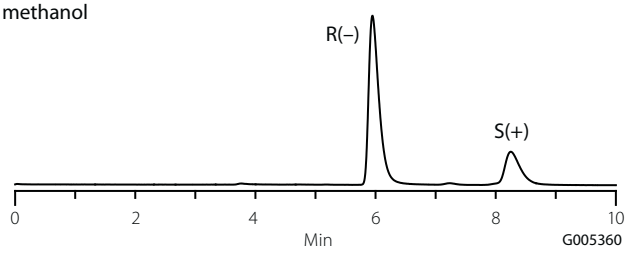
2a. NP on Astec Cellulose DMP, 10:90:0.1, IPA:Heptane:DEA



2b. POM on Astec Cellulose DMP, 0.1% w/v ammonium formate in methanol



2c. PIM on Astec CHIROBIOTIC V2, 0.1% w/v ammonium formate in methanol



Featured Products

Description	Cat. No.
Astec Cellulose DMP, 15 cm x 4.6 mm I.D., 5 µm particles	51098AST
Astec CHIROBIOTIC V2, 25 cm x 4.6 mm I.D., 5 µm particles	15023AST