Reversed Phase Chiral Method Development Using Polysaccharide-based Stationary Phases

by Liming Peng[1], Tivadar Farkas[1], Swapana Jayapaian[1], and Chankvetadze, Bezhan[2]

1. Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA (www.phenomenex.com)
2. Institute of Physical and Analytical Chemistry, Tbilisi State University, Tbilisi, Georgia

Developing simple and straightforward reversed phase chiral HPLC separations coupled with highly sensitive MS detection is highly needed for conducting drug metabolism and pharmacokinetic studies of stereoisomers in the drug development process. Polysaccharide-based chiral stationary phases (CSPs) are most prevalent in chiral LC due to their wide applicability and good selectivity. However, most chiral separations on polysaccharide CSPs are carried out under normal phase (NP) conditions with mobile phases (e.g. hexane/IPA) which may be incompatible with MS detection due to their high flammability in ion sources operated at high temperature. While separations conducted in polar organic (PO) mode utilize mobile phases that potentially prevent analyte ionization in the source, rendering them undetectable. The most promising separation mode for chiral LC/MS analysis is reversed phase (RP) LC. Moreover this separation mode is also complementary in selectivity to NP and PO separation modes (with either MS or UV detection).

We present results based on a study involving over 200 racemates of pharmaceutical interest on the CSPs cellulose tris(3,5-dimethylphenylcarbamate), cellulose tris(3-chloro-4-methylphenylcarbamate) and amylose tris(5-chloro-2- methylphenylcarbamate) in reversed phase mode with UV or MS detection.

Experimental Conditions:
Instrumentation:
HPLC System: Agilent 1100 series (www.agilent.com)

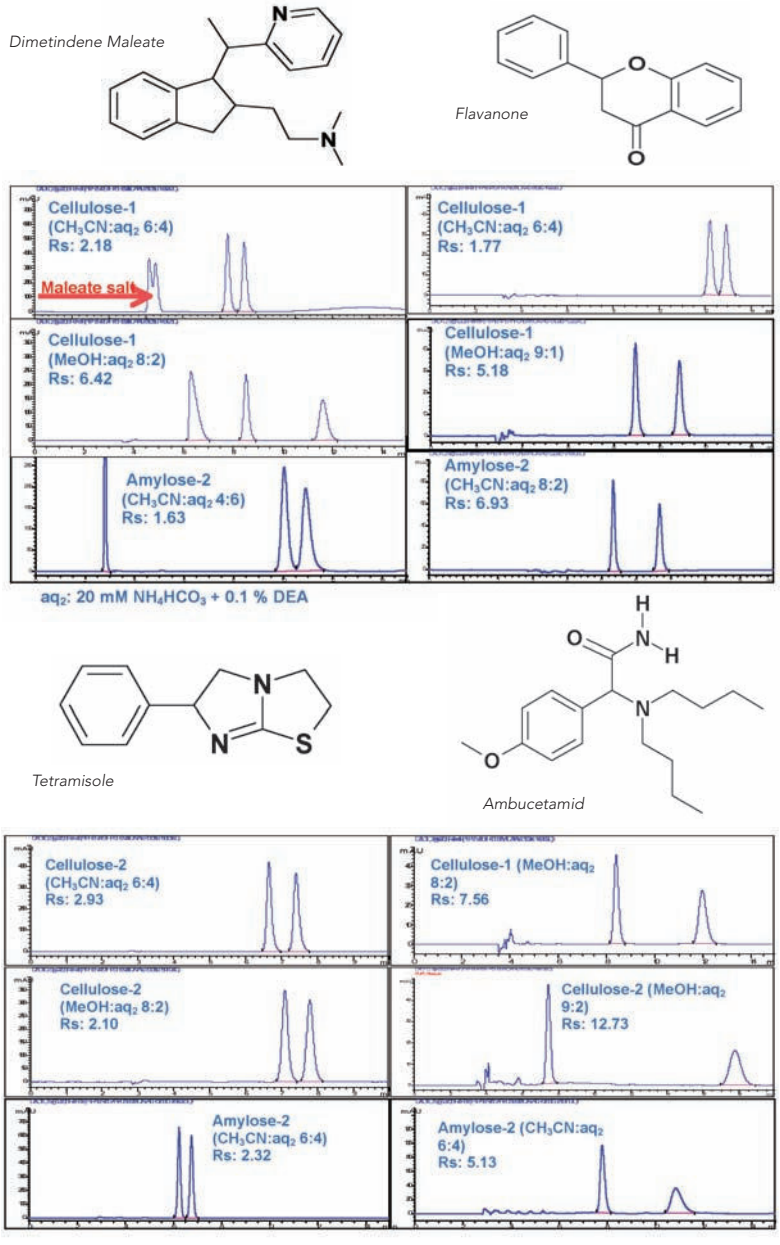
Pump: G1311A Quaternary Pump

Autosampler: G1313A ALS

UV Detection: G1315A Diode Array Detector

MS Detection:TurbolonSpray® - ESI, Positive Ion Mode; MRM; heater gas flow 5000 cc/min; heater temperature 400 °C

Figure 1: Representative Enantioseparations in RP



HPLC Conditions:

Flow Rate: 1.0 or 0.2 mL/min

Detection: UV @ 220 nm or MS/MS

Temperature: Ambient.

Injection Volume: 5 - 20 µL (depending on analyte response)

Sample Concentration: 500 µg/mL (UV); 200 ng/mL (MS/MS)

Columns: Lux™ Cellulose-1 5 µm
250 x 4.6 mm or 3 µm 150 x 2.0 mm
Cellulose tris (3,5-dimethylphenylcarbamate)
Lux™ Cellulose-2 5 µm
250 x 4.6 mm or 3 µm 150 x 2.0 mm
Cellulose tris (3-chloro-4-methylphenylcarbamate)
Lux™ Amylose-2 5 µm 250 x 4.6 mm
Amylose tris (5-chloro-2-methylphenylcarbamate)

Mobile Phase for basic or neutral compounds:

NH_4HCO_3; NH4Ac; or NH_4Ac + 0.1 % DEA; NH_4HCO_3 + 0.1 % DEA with CH3CN or MeOH

Mobile Phase for acidic and neutral compounds:

0.1 % HAc with CH_3CN or MeOH

(DEA = Diethylamine; HAc = Acetic acid; CH3CN = Acetonitrile; MeOH= Methanol)

Compound	Cellulose-1	Cellulose-2	Compound	Cellulose-1	Cellulose-2
Labetalol	X	X	Felodipine	X	√
Nifenalol	X	√	Ketamine	Partial	√
Benfluorex	X	X	Verapamil	√	X
Promethazine	Partial	X	Nicardipine	X	X
Bupropion	X	Partial	Dichloroisoproterenol	X	√
Trimipramine	X	√	Diltiazem	X	X
Metolazone	√	X	Nimodipine	X	X
Clenbutero	X	√	Sulpiride	X	X

Summary of Results

Resolution	Cellulose-1	Cellulose-2
Baseline resolution	2	6
Partial resolution	2	1
Total separations on both phases	Baseline resolution - 8 (50 %)	
√ : Baseline resolution: Rs > 1.5 X : No separation: Rs < 0.8	Partial resolution: 0.8 < Rs < 1.5	

Table 1: Enantioresolution of 18 Racemates on Cellulose-1 and -2 in RP

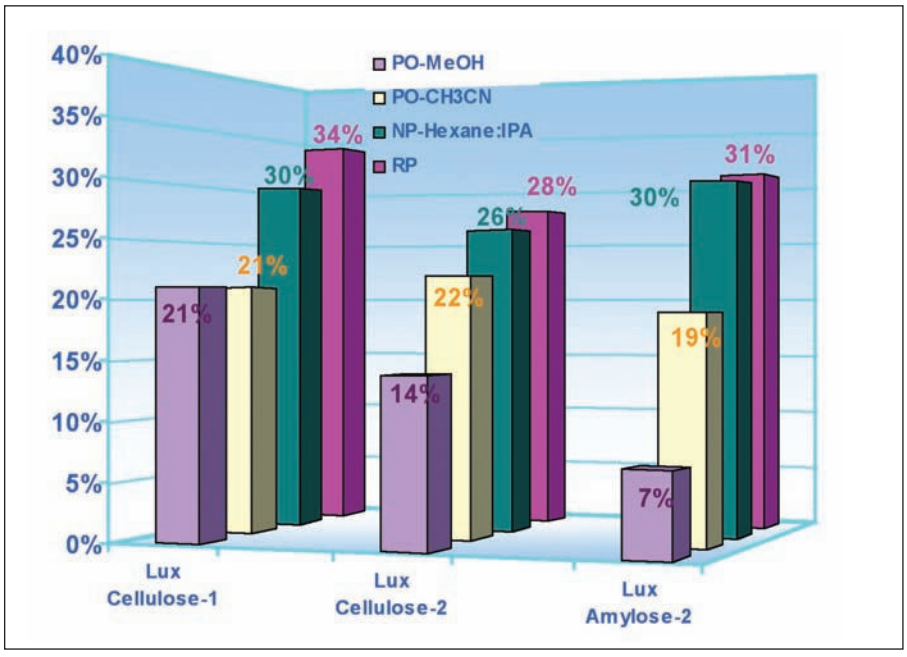


Figure 2: Success Rates of Lux™ Phases in RP for over 200 Racemates (Rs>1.5) – Various Conditions

Results and Discussion

Chiral LC(UV) and LC/MS/MS Applications

Three polysaccharide-based CSPs were explored in the reversed phase (RP) elution mode for over 200 compounds of pharmaceutical interest, in mobile phases made of ammonium bicarbonate with acetonitrile or methanol as organic modifier. Figures 1 and 4 demonstrate representative chiral separations on Lux™ CSPs. Most compounds were eluted in less than 15 min with baseline resolution in mobile phases of various eluting strength. The results show that Cellusose-1 was most successful in separating benzodizepines and ß-blockers, Cellusose-2 in separating imidazoles, and Amylose-2 in separating antihistamines and imidazoles. Comparing success rates in resolving racemates in RP, NP and PO elution modes revealed that RP elution mode using mobile phases compatible with MS/MS detection showed the highest potential for providing baseline resolution (Figure 2) and is suitable for MS/MS detection.

Effect of Mobile Phase Additives

The enantioresolution on Lux™ CSPs was evaluated in both CH3COONH4/CH3CN and NH4HCO3/CH3CN mobile phases (Figure 5). In general NH4HCO3 provided similar or occasionally superior resolution to CH3COONH4 as mobile phase additive. The addition of DEA to the mobile phase can improve resolution for very basic compounds (e.g. ß-blockers and tricyclic antidepressants), but it severely suppresses analyte response in ESI+ MS/MS even at levels as low as 0.025 % (Figure 5). However, DEA or acidic additives do not affect the enantioresolution of benzodiazepines, imidazoles or neutral stereoisomers. For all these compounds baseline separation can be achieved without DEA or acidic additives.

Effect of Organic Modifier

Decreasing the eluting strength of the mobile phase will increase retention and resolution as shown for nifenalol in Figure 5 and trimipramine and ketamine in Figure 3. However, once enantiomers elute later than 10 minutes with only partial resolution, baseline separation can be rarely achieved by further decreasing the mobile phase strength. In our study, acetonitrile provided more successful RP chiral separations than methanol on Lux™ CSPs.

Complementary Enantioselectivity in RP Mode

16 basic or neutral compounds not separated in NP and PO were screened in RP on Lux™ Cellulose-1 and Lux™

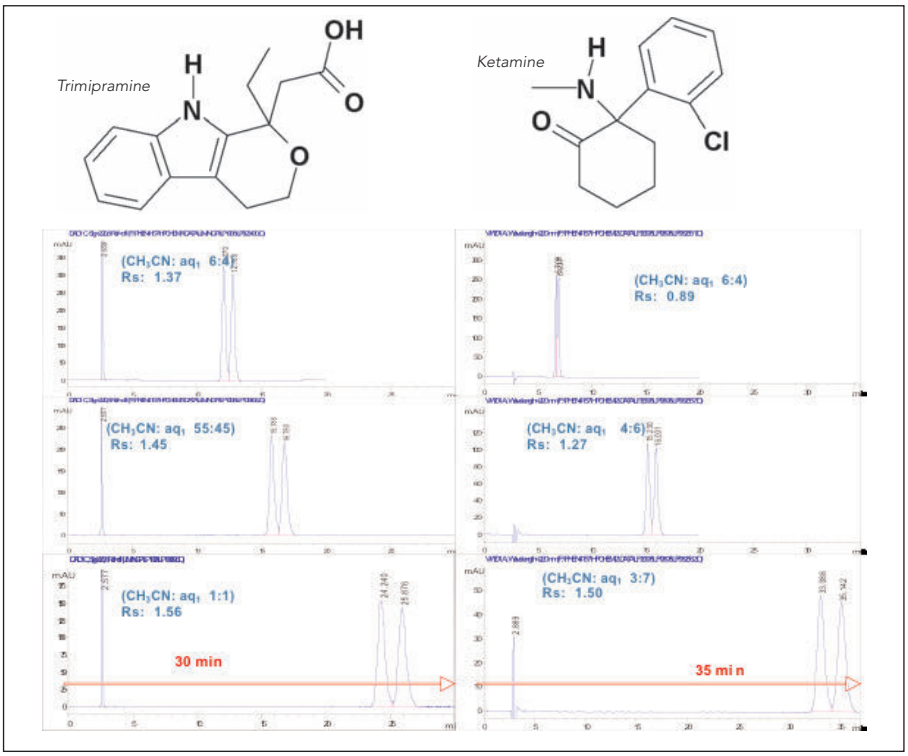


Figure 3: Effects of Organic Modifier on Enantioresolution in RP on Cellulose-2

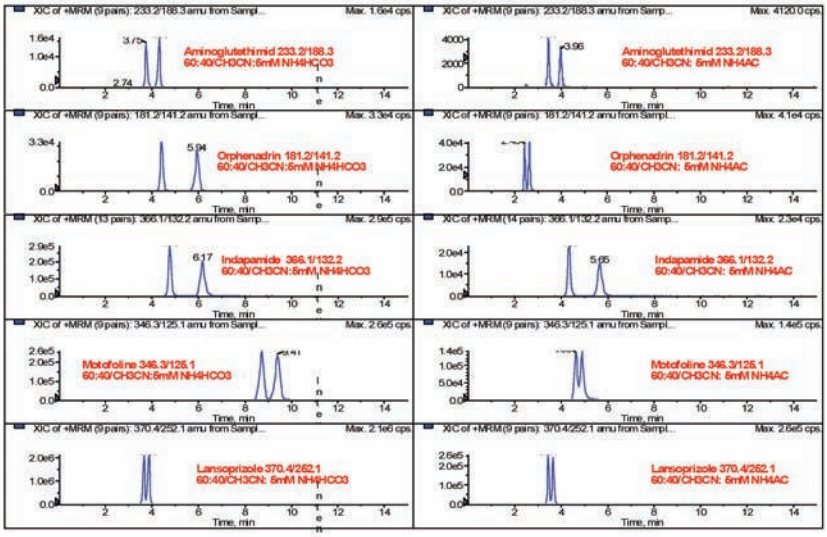


Figure 4: Effects of Buffers on Enantioresolution in RP LC/MS/MS on Cellulose-1

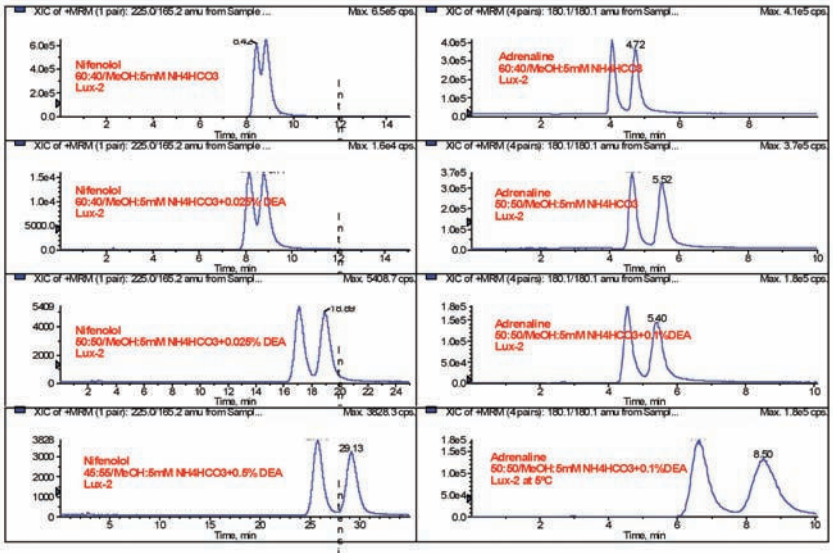


Figure 5: Effects of Additives and Column Temperature on Enantioresolution in RP LC/MS on Cellulose-2

Cellulose-2 (Table 1). Chiral recognition was observed for 63 % of these compounds with 50 % of them being baseline resolved (combined results for Cellulose-1 and -2).

Conclusions

- RP chiral LC on polysaccharide-based CSPs employing ammonium bicarbonate or acetate as mobile phase buffer salt is complementary to NP and PO separation modes. Furthermore, RP shows the highest potential for successful chiral LC/MS(MS) methods of analysis.
- Ammonium bicarbonate is the preferred buffer salt with ESI+ MS/MS detection for most basic pharmaceutical stereoisomers. Ammonium acetate is a viable alternative to ammonium bicarbonate but is less successful in providing baseline resolution.
- Diethylamine (as additional additive) can improve the chiral resolution of strong basic compounds, but it has a negative effect on analyte response in ESI+MS/MS even at low concentration levels (e.g. 0.025 %).
- Decreasing the mobile phase strength in RP (less % CH3CN or MeOH) has the expected effect: it increases retention and also enantioselectivity; adjusting % organic modifier is essential to optimizing chiral resolution.

Trademarks