Using Hexane Mixtures as Modifiers in SFC to Enhance Chiral Resolution of Racemic Pharmaceutical Compounds

Paul M. Lefebvre, Jeffrey P. Kiplinger, Michael J. Rego; *Averica Discovery Services Inc., One Innovation Drive, Three Biotech, Worcester MA 01605 USA*

INTRODUCTION

Hexane (or hexanes, heptane, pentane) is a common solvent in normal phase HPLC, but is infrequently used in SFC as its "solvent strength" is believed to be similar to that of super- or subcritical CO_2. Hexane has been used occasionally to increase the percentage of liquid in the SFC eluent and enable efficient fraction collection - it is often difficult to collect fractions efficiently at low liquid concentrations in preparative SFC, as the large amount of CO_2 may drive a fine mist of compound into gas exhaust lines. A combined liquid modifier in the eluent, e.g. 50:50 hexane:isopropanol, often results in a higher liquid concentration with equivalent elution profile, and a better fraction collection result.

In our laboratory, experimentation with hexane augmentation of common alcohols used as eluent modifiers has more often than not resulted in a change in chromatographic selectivity. We present examples where this change offers enhanced chromatographic performance and suggests that further experimentation is warranted and desirable.

EXPERIMENTAL

System: Thar Investigator SFC system (Milford, MA)

Columns: All 4.6 x 100 mm 5 µm

- (S,S) Whelk-O1 (Regis Technologies, Morton Grove, IL)
- RegisPack (Regis Technologies, Morton Grove, IL)
- LUX Cellulose-2 (Phenomenex, Torrance, CA)
- ChiralPak AD-H (Chiral Technologies, West Chester PA)

Method: 4 ml/ minute Gradient (unless otherwise noted)
5 – 65% Co-solvent over 3 minutes
65% Co-solvent for 30 seconds, then return to 5%
5 minute run time

Detection: UV 230 nm

Samples: Various racemic mixtures of small molecule drug-like compounds. Structures of compounds not revealed when proprietary.

WHEN HEXANE IS LIKE CO_2

Separations requiring low percentages co-solvent frequently give problems when scaling to purification. The small amount of liquid remaining to move the sample to the collection vessel, after the CO_2 is removed, can be insufficient to keep the material in solution.

If the hexane behaves similar to CO_2, then a mixture of 50:50 mix of hexane: co-solvent would required 2x the percentage for the same chromatography while doubling the amount of liquid collected.

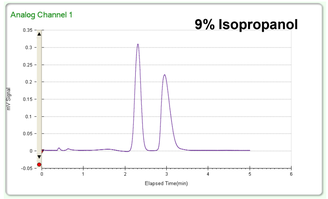

Figure 1: Isocratic separation of enantiomers on LUX Cellulose-2

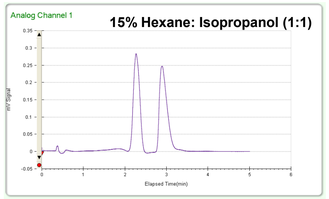

Figure 2: Same as Figure 1, using hexane to increase liquid co-solvent proportion

Doubling the amount co-solvent effectively yields the same separation. There is no increase or decrease in resolution when a portion of the CO_2 is replaced by hexane.

HEXANE IMPROVES RESOLUTION

As shown in the previous figures, hexane can displace CO_2 without impact to the separation. However, in some cases the addition of hexane can effect resolution. Figures 3 and 4 show an example in which a 1:1 mixture of Hexane: Isopropanol improves the resolution when the co-solvent percentage remains constant.

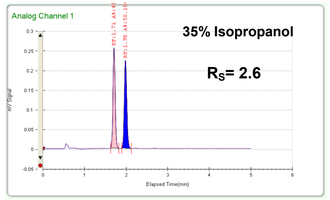

Figure 3: Isocratic separation of enantiomers on RegisPack

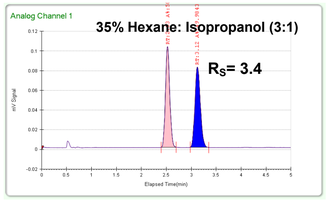

Figure 4: Same as Figure 3, using hexane to increase liquid co-solvent proportion and enhance resolution.

By replacing a portion of the isopropanol co-solvent with hexane, the resolution is improved.

Decreasing the co-solvent percentage of pure isopropanol (without adding hexane) increased retention time, but did not improve resolution (data not shown).

COMPLEX SEPARATIONS

Hexane can offer the same advantages when used in gradient separations, which in our lab are often performed for method development.

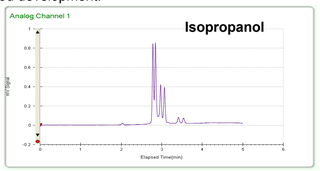

Figure 5: Gradient separation of enantiomers on Whelk-O1

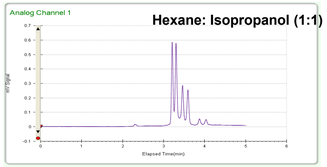

Figure 6: Same as Figure 5, using hexane to increase liquid co-solvent proportion. Retention time increases, but resolution is not significantly improved.

Figures 7 and 8 show a chiral separation of an impure sample. Note the impurity at 1.7 minutes is resolved by the addition of hexane.

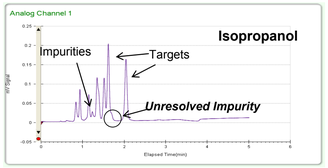

Figure 7: Gradient separation of enantiomers on Whelk-O1

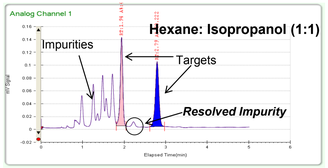

Figure 8: Same as Figure 7, using hexane to increase liquid co-solvent proportion and enhance resolution.

WITH METHANOL

Hexane can be added to methanol as well, even though these are not miscible liquids. Adding 3% isopropanol to any methanol:hexane mixture forms a homogeneous solution.

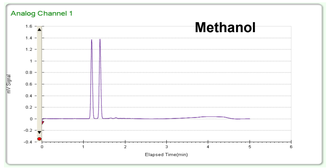

Figure 9: Gradient separation of enantiomers on RegisPack

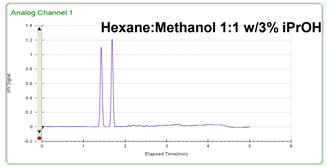

Figure 10: As in Figure 9, with a hexane admixture used to improve resolution and increase retention.

For some samples, the addition of hexane decreases the resolution in addition to increasing the retention time.

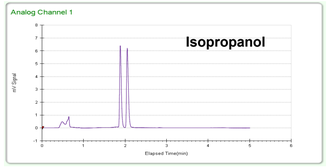

Figure 11: Gradient separation of enantiomers on ChiralPack AD-H

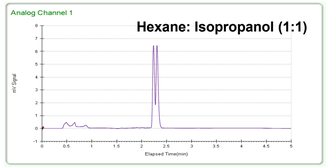

Figure 12: As in Figure 11, with hexane added to increase retention.

STACKED PREPARATIVE INJECTIONS

All separations shown have been successfully scaled to purification. Figure 13 shows an representative portion of a stacked injection chromatogram.

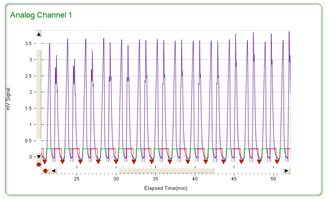

Figure 13: Isocratic preparative stacked injections on RegisPack at 35% Hexane : Isopropanol (1:1).

Preparative Benefits:

- Lower pressure drop across the column
- Faster dry-down with more volatile solvents

Considerations:

- Extra equilibration may be required for consistent retention times
- Lower grades of hydrocarbon solvents may leave residual material on dry-down

CONCLUSIONS

- Hexane improves collection efficiency for low co-solvent separations.
- In some cases, hexane acts just like CO2.
- Hexane can have various effect on peak separation. It can cause resolution lost or improved.
- Hexane : Methanol mixtures are possible by adding a small portion (3%) isopropanol.
- A lower pressure across the column is achieved by adding hexane to the co-solvent.
- Mixtures with hexane are regular used in the chiral screening process.
- Dry-down time is reduced.