INTRODUCTION

The development of methods allowing improved chiral separation and purification of therapeutic agents is crucial as the pharmacokinetic and pharmacological behavior of both enantiomers and also of diastereomers can vary significantly from the active pharmaceutical ingredient. Recently Stationary-Phase Optimized Selectivity Liquid Chromatography (SOS-LC) has been successfully further developed and progressively used for isocratic and gradient liquid chromatography separations. Until now the potential of this methodology to facilitate the separation and purification of chiral isomers has not yet been investigated, which could speed up the purification process or improve the overall yield of the process.

EXPERIMENTAL

1. Chemicals and reagents
Hexane, 2-Propanol, ethanol chiral mixture (trans-Stilbene oxide, 1,2,3,4 Tetrahydro-1-naphthol, 4-phenyl-1,3-dioxane and hexobarbital).

2. Chromatographic conditions
a. Instrument: Agilent 1260 HPLC system with (VWD);
    b. Mobile Phase: A. Hexane, B. IPA , C. Flow: 0.5 ml/min; d. Detector Wavelength: 210 nm; e. Column Oven: 25°C; f. inj. Vol.: 2µl; g. Lux 3u Columns (50 mm x 4.6 mm, 3µm): Amylose 2, Cellulose 1, Cellulose 2, Cellulose 3 and Cellulose 4 (Phenomenex, USA) with Lux Amylose 2 (4 x 3.0 mm) pre-column.

3. Separation of the chiral mixture on individual column segments.

4. Optimization of stationary phase

RESULTS & DISCUSSION

A. Isocratic Chiral SOS-LC

The possible column combinations were simulated with the straightforward isocratic algorithm (based on equation 1) and ranked in order of decreasing selectivity of the critical pair, which provides the optimal column combination composed of Amylose 2, Cellulose 2, Cellulose 3 and Cellulose 4 columns (assembled in random order).

B. Gradient Chiral SOS-LC

1. Concept of the prediction algorithm for retention times under linear gradient conditions on combined column segments.

For chiral gradient SOS-LC a discontinuous algorithm based on numerical integration was developed allowing the prediction of retention times on columns with serially connected chiral stationary phases. A linear gradient was thereby considered as the sequence of small isocratic stages, which correspond to small time intervals. This discontinuous approach allows for the determination of the intermediate migrated distance through the column at each time point during the analysis. This is a useful approach when very coupled columns with different stationary phases are considered.

2. Calculations of nonlinear regressions based on the quadratic relationship between ln(k) and φ ([ln(k)] = aφ + bφ + c). The obtained relationships allowed for visualization of the retention behavior of each compound with each stationary phases.

3. Figures of merit of the chiral gradient approach

The effectiveness of the above methodology for the prediction of retention times on a set of commercially available columns has been proven for trans-Stilbene oxide on a number of random column combinations represented in Figure 5. An overall gradient of all column combinations (for annotations refer Table 1).

The complete chiral SOSLC methodology was subsequently performed on all 8 solutes on all possible column combinations for a fixed gradient of 1 to 20% IPA in 60 min. From the 325 possible solutions (columns combinations) the optimal combination consisting of the Cellulose 3 column at the inlet, followed by the Cellulose 2 phase and ending with the Amylose 2 column. This resulted in a total column length of 15 cm and corresponded to the highest value for the retention time difference of the critical peak pair of all combinations, while enabling analysis within a stipulated maximum time limit of 60 min. (Figure 4)

4. Predicted (A) & experimental chromatogram (B) of the chiral mixture. (For annotations refer Table 1)

CONCLUSION

- Stationary phase optimized selectivity approach is transferable to chiral separations both under isocratic and gradient conditions on a set of commercially available columns.
- Retention time predictions on combined polysaccharide phases are achievable with good predictive accuracies.
- Baseline separation of 4 chiral pairs could be achieved for a mixture of solutes of varying polarity through gradient analysis.
- The study also indicated that the elution order of chiral pairs is not constant on all columns and that appears to be highly depending on the type of solute.

REFERENCES


GHENT UNIVERSITY