Optimizing Chiral Separations and Purification of Enantiomers Through Selective Sample Slicing and Column Switching

Application Note PHA0113

Keywords

Enantiomers, Purification, Chiral Pharmaceuticals, GX-271 Semi-Preparative HPLC System, Column Switching, Sample Slicing, Sample Cleanup

Introduction

Pharmaceutical laboratories, as part of a chiral drug investigation, may be asked to provide separations of all chiral compounds to facilitate the testing of individual enantiomers (1). Sample cleanup prior to purification is a factor in reducing the cost and time of semi-preparative purification, resulting from the typical high cost and fragility of chiral sorbents. To eliminate exhaustive sample cleanup procedures, a complete automation of the analyte purification and chiral separation was developed. The application principle injected samples onto a separation column to cleave individual peaks and transfer these peaks onto secondary dual chiral columns for separation. Automated and complete purification of analytes with high resolution of the separated enantiomers provided speedy purification, efficient separation, and simplified collection of chiral analytes, eliminating the need for extensive sample cleanup.

Materials & Methods

Samples and Solvents

Note: All samples and solvents used were of HPLC grade.

- Guaifenesin
- Ephedrine
- Acetaminophen
- Hexane
- Ethanol

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System Plumbing Diagram (see Figure 1)

Blue Plumbing Diagram – TRILUTION® LC Software Control

- A Gilson GX-271 Liquid Handler with a direct injection module and solvent selection pump was used to perform the liquid handling and injection tasks.
- A Gilson 333 Pump was used to control the mobile phase flow through the separation column.
- Initial separation column from Keystone (20 x 150 mm; normal phase) was used post sample injection as the first separation column.
- A Gilson 155 UV/VIS Detector was used for monitoring compound peaks from the first separation column and is directly connected to the Gilson VALVEMATE® II.

Green Plumbing Diagram – Independent Keypad & Contact Closure Control

- A Gilson 333 Pump was used to control the mobile phase flow from the via the pump keypad (independent of the Blue Plumbing Diagram).
- Two Chiral Technologies (21.2 x 50 mm OD) columns were used in series to perform the chiral separation (green column image represents the two columns in series).
- A Gilson 155 UV/VIS Detector was used for monitoring compound peaks for the chiral columns in series.
- A Gilson FC204 Fraction Collector was used for enantiomer peak collection with collection via the keypad (independent of the Blue Plumbing Diagram).

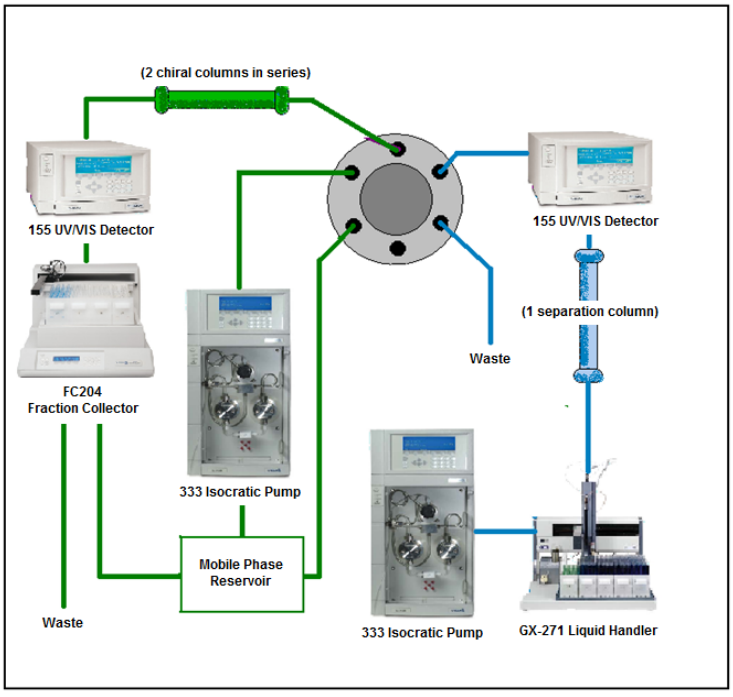

Figure 1: Chiral Purification System Plumbing Diagram

Gilson Chiral Switching Method:

- Mobile Phase:
  - Chiral columns: 70% hexane: 30% ethanol @ 20 mL/min chiral
    Normal phase column: 75% hexane :25% ethanol @ 15 mL/min
- UV Detection:
  - 210 nm
- Injection Volume:
  - Range of 100 to 1000 µL
- Sample:
  - A sample of Guaifenesin in cold remedy tablets with ephedrine and acetaminophen components was injected after being dissolved in ethanol.
  - The final concentration was 40 mg/mL Guaifenesin in 30:70 ethanol: hexane.
- Column Switch:
  - The Gilson FC204 controlled the switch of the VALVEMATE® II via a contact that was pulsed when the Guaifenesin peak reached a height of 5 mV.
  - Simultaneous to the valve switch, the mobile phase flow on the primary pump was diverted to the chiral columns.
  - Following peak collection from the chiral columns, flow was then diverted back to waste, and the chiral columns were switched to the secondary pump.

Results

The Guaifenesin peak was transferred to the chiral columns without loss of product. Resolution allowed for a clean cleavage of the peak from the mobile phase stream (see Figure 2). Any foreign compounds that were transferred along with the Guaifenesin were separated from the enantiomer peaks. The peak resolution on the chiral chromatogram was 1.9. The mobile phase that passed through the chiral column was recycled back to the reservoir. Using two columns allowed sample to be introduced immediately following the separation of the compounds on the normal phase column. The target peak was transferred to the chiral columns and injection of the new sample began while the enantiomers were separated, creating an efficient and automated purification process. Dual column switching using dual detectors allowed for maximum throughput via normal phase separation to occur while enantiomers are purified (see Figure 3).

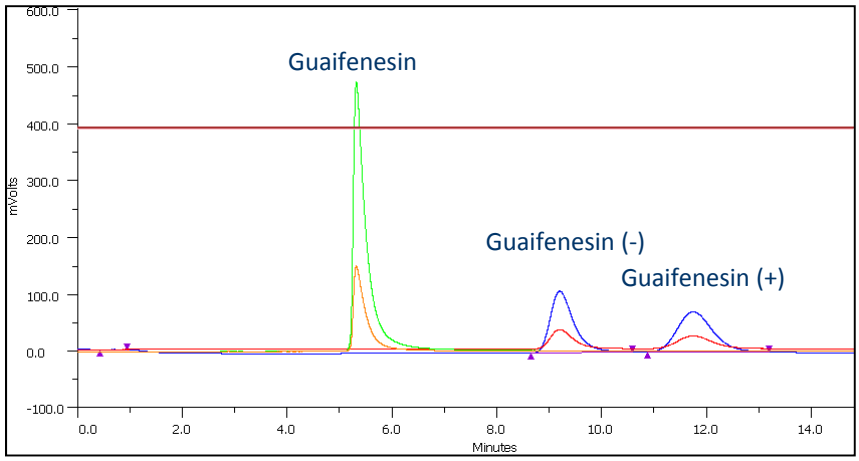

Figure 2: Initial Overlaid Guaifenesin Standard Chromatogram of Slice and Separation

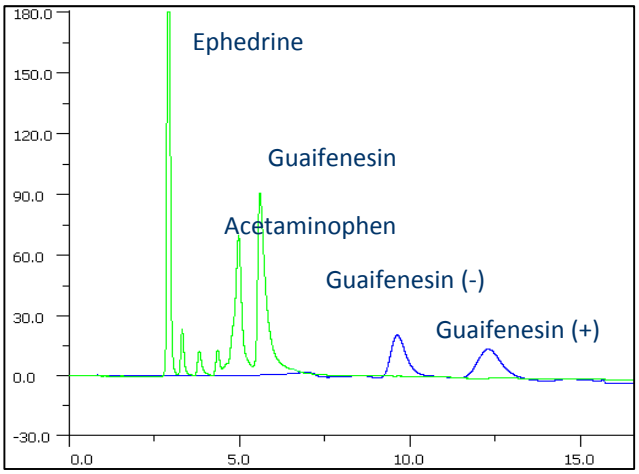

Figure 3: Dual Overlaid Sample Chromatogram of Slice and Separation

Summary

Flexibility in method development and optimization of methods were provided using flow rate and mobile phase ratios for each column. Automation of this dual column switching method provided accurate column switching of enantiomers once separated on the chiral columns without additional sample cleanup procedures.

References

1. D Muñoz-Torrero López-Ibarra. (2012). Recent Advances in Pharmaceutical Sciences 11: 115-134. **Transworld Research Network**.