Optimizing the Purification of a Standard Chiral Compound Utilizing a Benchtop, Multi-Purpose, Semi-Preparative to Preparative HPLC System

Application Note PHA0111

Keywords

Chiral, Enantiomer, HPLC, Normal Phase Chromatography, PLC 2020 Personal Chromatography System, Preparative Chromatography, Purification, Resolution, trans-Stilbene Oxide

Introduction

This study was presented at Pittcon 2011 held in Atlanta Georgia in March 2011.

There are often significant differences in the effectiveness and toxicity of drug enantiomers in biological systems (Cox, G. and Ellis, D., 2010; Li, B. and Haynie, D., 2006). This has led to the need for the separation of chiral drug candidates into their respective enantiomers for further testing. Semi-preparative and preparative chiral chromatography has become a common tool for the separation and purification of chiral compounds into their enantiopure form (Franco, P. and Zhang, T., 2010; Li, B. and Haynie, D., 2006). The use of several chiral phases and solvents of different polarities are often employed at this step. This may require the use of either normal phase or reverse phase chromatography during the optimization process. Using, learning and maintaining two different instruments or platforms to accomplish these separations can be both costly and time consuming.

The purpose of this application was to optimize the chiral separation of a standard compound using a stand alone, benchtop, preparative chromatography system (Figure 1) that is capable of performing separation by either normal phase or reverse phase chromatography with flow rates up to 100 mL/min. Percent recovery, loading capacity, fraction collection parameters, precision, and its percent impurity are reported for *trans*-Stilbene oxide using normal phase chromatography. In addition, a matrix was developed to assist a chemist with the determination of optimal sample loading parameters as well as methodology for performing simple fraction recovery for each enantiomer.

Gilson, Inc. World Headquarters
Middleton, WI 53562 USA
Telephone: 800-445-7661

Figure 1. Gilson PLC 2020 Personal Purification System (Part No. 21130000)

Materials & Methods

Materials

All solvents were distilled in glass suitable for GC, HPLC, LC/MS and spectrophotometry. All reagents were ACS grade quality or better. Hexane and Isopropyl Alcohol were purchased from Burdick & Jackson (part nos. AH212-4 and AH323-4). *Trans*-Stilbene oxide (98% purity) was obtained from Sigma Aldrich (Figure 2). The standard was prepared in HPLC mobile phase.

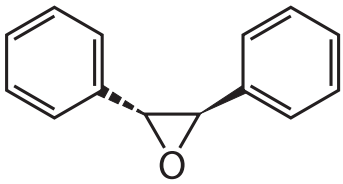

Figure 2. Chemical Structure of *trans*-Stilbene oxide

HPLC Conditions

HPLC purification was carried out using a Gilson PLC 2020 Personal Purification System. Separation was achieved using a 5 micron 10mm x 250 mm Phenomenex Lux® Cellulose-2 column at a flow rate of 5 mL/min. The isocratic mobile phase was 1:1 Hexane: Isopropyl Alcohol. Peaks were monitored with UV detection at 220 and 254 nm. See Figure 3 and Table 1 for complete details, including fraction collection parameters.

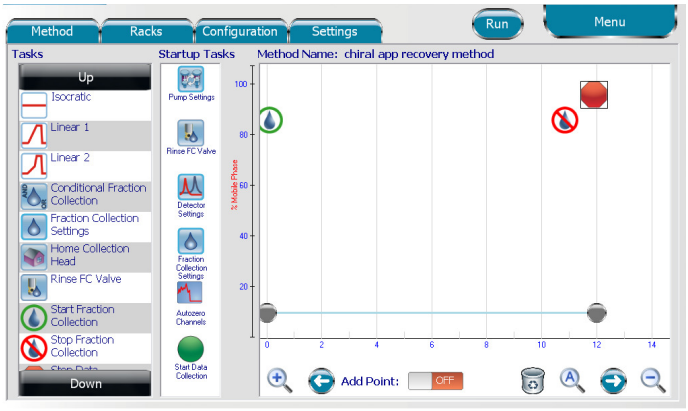

Figure 3. PLC 2020 Software Method (above) Used for the Chiral Separation of *trans*-Stilbene Oxide Enantiomers

Table 1. PLC 2020 Chiral Normal Phase Method

	Mobile Phase Solvent	Mobile Phase Gradient %A	Fraction Collection Conditions	Run Time (minutes)	Flow Rate (mL/min)	UV Detection (nm)
Normal Phase	**A = Hexane B = Isopropyl Alcohol**	**0-12 min = 90**	**Front Slope = 65 Back Slope = 65 Maximum Collection Volume per Tube = 20 mL**	**12.0**	**5.0**	**220 and 254**

Method Design

Percent recovery, loading capacity, fraction collection parameters, precision and % impurity were calculated for *trans*-Stilbene oxide from multiple injections (n=3) using a 100 µL total loop injection. The loading capacity was increased until a resolution of approximately 1 was achieved. This mg load was used for evaluating a single set of fraction collection parameters to determine fraction recovery using re-injection of each enantiomer (Peak 1 and Peak 2 seen in Figure 4).

Results

Chiral purification can be optimized by first performing a resolution study to determine the amount of compound that can be loaded onto the specific chiral column. The resulting matrix can assist the researcher with determining the best resolution for purification based on peak width and peak shape (see Table 2 and Figure 4 below). A resolution of 1.15 for the 1.8 mg *trans*-Stilbene oxide load was determined to be optimal and was used for this application.

Table 2. Resulting Resolution Matrix for *trans*-Stilbene Oxide

	trans - Stilbene oxide Peak 1		*trans* - Stilbene oxide Peak 2		
Column Loading Study (mg in 90:10 Hexane:IPA)	Retention Time (minutes)	Peak Width (minutes)	Retention Time (minutes)	Peak Width (minutes)	Resolution
0.5	4.60	0.67	7.06	0.83	3.29
0.6	4.55	0.73	7.05	0.84	3.19
0.7	4.40	0.75	6.70	0.91	2.77
0.8	4.66	0.77	7.27	0.98	1.88
1.5	4.64	0.82	7.17	1.15	1.39
1.8	4.65	0.91	7.16	1.22	1.15

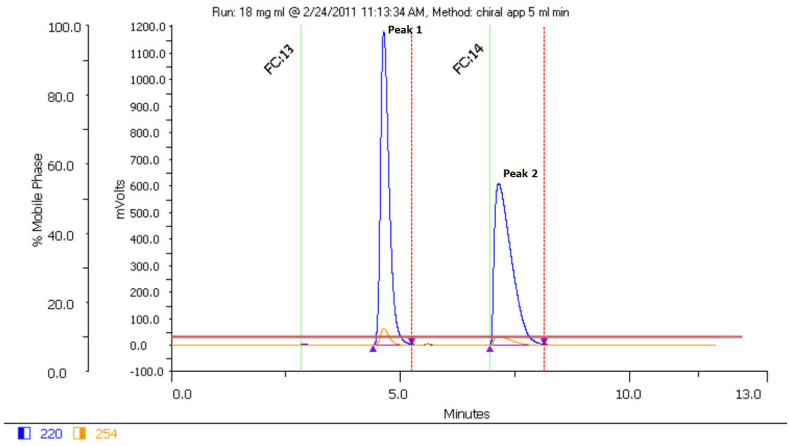

Figure 4. Chromatogram of *trans*-Stilbene Oxide at resolution = 1

The PLC 2020 Personal Purification System software allows the user to set both front and back fraction collection parameters (Table 1). This allows for accurate and optimal sample recovery when peak tailing or peak fronting is observed. It is effective for the collection of both large and small peaks. Sharp peaks with smaller peak widths will result in lower fraction collection fraction collection volumes that require less dry-down time. In this application, fraction volumes for *trans*-Stilbene oxide peaks 1 and peak 2 were 3.79 mL and 5.24 mL, respectively (Table 3). Fraction volumes were kept low as a result of narrow peak widths in combination with a 5 mL/min flow rate.

Triplicate injections were performed at the 1.8 mg level. Peak 1 and peak 2 were collected separately for each injection. Each of the triplicate fractions from peaks 1 and 2 were mixed manually prior to re-injection of the fractions. The chromatograms of the re-injected fractions are shown in Figures 5 and 6. Average recovery values were calculated at 103.17% and 99.70% for peak 1 and 2 respectively. Consistency in fraction collection was optimal at a load of 1.8 mg, with % CV values at < 1.5%.

Table 3. Comparison of *trans*-Stilbene Oxide – Peak 1 and Peak 2 Collected Fraction Volume and Re-injected Collected Fraction Recoveries

		Collected Fraction Volume *trans* - Stilbene oxide Peak 1			Re-injected Recovery *trans* - Stilbene oxide Peak 1		
Column Load	Resolution	Volume (mL) (n=3)	Standard Deviation	% CV	% Average Re-injected Recovery (n=3)	Standard Deviation	% CV
1.8	1.15	3.79	0.04	.01	103.17	0.01	1.17

		Collected Fraction Volume *trans* - Stilbene oxide Peak 2			Re-injected Recovery *trans* - Stilbene oxide Peak 2		
Column Load	Resolution	Volume (mL) (n=3)	Standard Deviation	% CV	% Average Re-injected Recovery (n=3)	Standard Deviation	% CV
1.8	1.15	5.24	0.05	.01	99.70	0.00	0.30

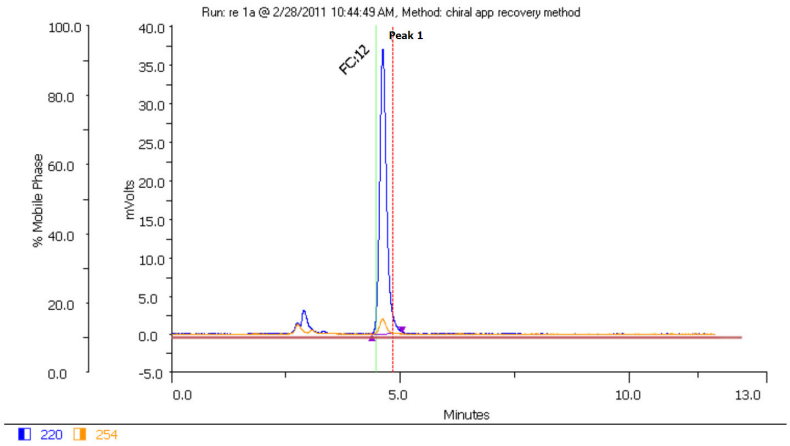

Figure 5. Re-injection Chromatogram for *trans*-Stilbene Oxide Peak 1

Chiral standards can often have impurities. A consistent impurity peak was visible at ~ 3 minutes in both injection and re-injection chromatograms. At a 1.8 mg *trans*-Stilbene oxide load, the average area impurity value was calculated to be 2.94% (Table 4). The area ratio of peak 1 and peak 2 in these same 1.8 mg load injections was calculated at 97.9%. Area averages of peak 1 and peak 2 and the impurity peak resulted in a final recovery value of 100.9%. This was consistent with the listed purity of the standard.

Table 4. *trans*-Stilbene Oxide % Ratio Area Average Comparison to Impurity Peak % Area Coverage

trans **- Stilbene oxide Peak 1/Peak 2 % Area Ratio Average (n=3)**	***trans*** **- Stilbene oxide Impurity Peak % Area Average (n=3)**	***trans*** **- Stilbene oxide Peak 1/Peak 2 % Ratio Area Average + Impurity Peak % Area Average (n=3)**
97.9	**2.94**	**100.9**

Summary

Optimizing HPLC purification of enantiomers using a benchtop solution that allows for front and back slope peak collection can greatly increase purification efficiency for a variety of separation conditions and applications and insures collection of the entire sample or peak of interest. In this application, a simple resolution matrix study enabled the maximum load that maintained adequate resolution. Fractions were re-injected onto the same column using the minimum volume possible and the same conditions as the original purification method for recovery verification. Consistent recovery and % CV values confirm the optimization of fraction collection volume and recovery rates.

References

Cox, G.B. and Ellis, D.W. (2010). Method Development for Analysis and Isolation of Chiral Compounds Using Immobilized Stationary-Phase Technology **Pharmaceutical Technology** Cphl/ICSE 2010, s14-s16.

Franco, P. and Zhang, T. (2010). Finding the Best Separation for Enantiomeric Mixtures. **LC GC North America 28** (9): 818 – 822.

Li, B. and Haynie, D.T. (2006). Chiral Drug Separation. In **Encyclopedia of Chemical Processing DOI: 10.1081/E-ECHP-120039232.** Taylor & Francis, p 449-458.