Available online at www.sciencedirect.com
ScienceDirect
Journal of Chromatography A, 1178 (2008) 56–59
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Optimization of steady state recycling parameters utilizing polarimetry in chiral separations

Mark E. Crawford [a,*], Joan M. Stevens [a], Luke Roenneburg [a], Gary Yanik [b]

[a] *Gilson Inc., 3000 Parmenter Street, Middleton, WI 53562, USA*
[b] *PDR-Chiral, 1331A South Killian Drive, Lake Park, FL 33403, USA*

Received 31 May 2007; received in revised form 26 October 2007; accepted 13 November 2007

Abstract

Processing gram to kilogram quantities of target analytes has led to the exploration of several high-throughput separation techniques. Among those investigated is steady state recycling (SSR). Similar to simulated moving bed (SMB) fractions are collected from the leading and trailing edges of a chromatographic profile while sample material is injected into the interior. Purifying large amounts of sample in the semi-preparative stage is ideal for these groups. SSR allows for development of methods capable of separating 50 g to kilograms and even greater amounts of product efficiently. Using polarimetry to optimize the SSR method further improves the efficiency of method development, providing comprehensive data leading to incisive development decisions. Accurate sample injection allowed continual 99% enantiomer separation after polarimetry optimization. We have developed an efficient SSR optimization methodology that offers rapid development of chiral separation by SSR.

Keywords: SSR; Closed loop; Preparative separations; Polarimeter; Optical rotation; Enantiomer; Chiral

1. Introduction

Steady state recycling (SSR) was introduced by Grill [1] in 1998. Similar to simulated moving bed (SMB), SSR injects sample into the interior of a chromatographic profile while collecting from the front and back of the edges of that profile. The difference between these two techniques is SMB is a continuous process and SSR is a discontinuous repetitive process that recycles the unseparated sample back into the column while inserting new sample into the interior of this recycled sample [2]. Recycling is not a new process. Mobile phase and sample have been recycled as a conservation measure as well as a separation procedure [3–5]. Porter and Johnson [6] reported a GC recycling procedure in 1959. Bailly and Tondeur [7] documented a recycling technique that involved mixing the unseparated sample with new sample and reinjecting it into the column. However, this latter approach did not preserve the profile of the unseparated sample as presented in Grill's method. SSR permits the uncollected portion profile to be reinjected into the column preserving its partial separation. Sample that has begun to separate is allowed to retain its state while the new sample is inserted into this recycled profile. Continuous reinjection allows the process to build into a steady state. Steady state provides a system with little manual intervention.

SMB systems allow large amounts of racemic compounds to be separated. With lower resolution of chiral stationary phases SMB throughput surpasses that of HPLC preparatory separations which rely on high resolution [8]. Drawbacks of the SMB are higher investment cost, higher complexity, and higher maintenance cost. Semi-preparative separation systems purify many compounds yearly, this leading to numerous developmental hours for an SMB and SSR systems. With fewer columns and valves the SSR system allow for efficient system equilibration. Less complexity leads to less hours when optimizing a new separation method. SSR systems perform similar to SMB systems in production rates, purities and recoveries [9–11]. In contrast SSR systems would require less investment cost, and less maintenance cost. All considered a less complex system than SMB. In laboratories where SMB costs are prohibitory, then SSR could be utilized to separate racemic compounds comparable to SMB without the high financial investment.

SSR is a binary collection process which lends itself to preparative enantiomeric separations of racemic compounds by

* Corresponding author. Tel.: +1 800 731 9873; fax: +1 608 831 4451.
E-mail address: mcrawford@gilson.com (M.E. Crawford).

0021-9673/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2007.11.073

collecting the leading and the trailing edge of the SSR profile. With the need for pharmaceutical companies to test the activity of each enantiomer within a racemic synthesis as mandated by the US Food and Drug Administration (FDA) [12] closed-loop SSR would provide a convenient method to process these chiral compounds.

The SSR system presented reduces the time involved in chiral method optimization by providing accurate and precise sample injection into the profile. Polarimetry has been used extensively in the separation, collection and identification of enantiomers [13–15]. In this method, polarimetry is used to provide the accuracy needed to locate the injection point within the SSR profile. The injection point corresponds to the zero point in optical rotation. A mixture of equal parts of an optically active isomer and its enantiomer is termed racemic and has a net rotation of plane polarized light of zero. Measuring optical rotation provides a method to access optical purity of a sample containing a mixture of enantiomers. When plane polarized light passes through the sample and the net optical rotation is measured as zero the injection of additional sample into the SSR profile is triggered. By injecting the sample into the profile at this point partially separated sample is preserved in the profile. Enantiomer collection is also monitored by the polarimeter. Sample collection is optimized by utilizing the maximum and minimum of the polarimeter's chromatogram. At these points the enantiomer percentage is changing significantly. Measurements taken from collections at these points provide the needed data to optimize collection parameters. We also have eliminated any mixing of the sample created by turbulence associated with employing an interior pump by placing the pump outside of the closed loop system. This provides further consistency in the SSR sample chromatographic profile. These changes allow for rapid optimization of our SSR system.

2. Experimental

2.1. Materials

Ibuprofen >98% (GC) (Sigma–Aldrich, St. Louis, MO, USA) was utilized as the sample in this chiral separation. The mobile phase consisted of HPLC-grade *n*-hexane provided by Burdick and Jackson (Muskegon, MI, USA) and HPLC-grade ethanol provided by (Sigma–Aldrich). The mixture of mobile phase was 98:2 v/v *n*-hexane–ethanol pumped at 8 ml/min. The instrumentation used was a GX-271 injection solvent selection liquid handler, 321 pump with two H1 heads, Valvemate II with a Valco 494C23006 two position valve, 155 UV detector, FC204 fraction collector all provided by Gilson (Middleton, WI, USA) PDR-chiral polarimeter equipped with a 25 mm flow cell 0.037 in. I.D. rated at 1000 psi maximum. (PDR-Chiral, Lake Park, FL, USA). Two Chiralcel OD columns 50 mm × 21 mm, 20 μm provided by Chiral Technologies (Exton, PA, USA) The analytical 250 mm × 4.6 mm OD-H column to test the collected fractions was also provided by Chiral Technologies 0.030 in. I.D. Stainless steel tubing was provided by Gilson. The data was compiled and chromatography was generated by Trilution® v1.4 software (Gilson, Middleton, WI, USA). The valve switching was controlled by FC204 firmware also from Gilson.

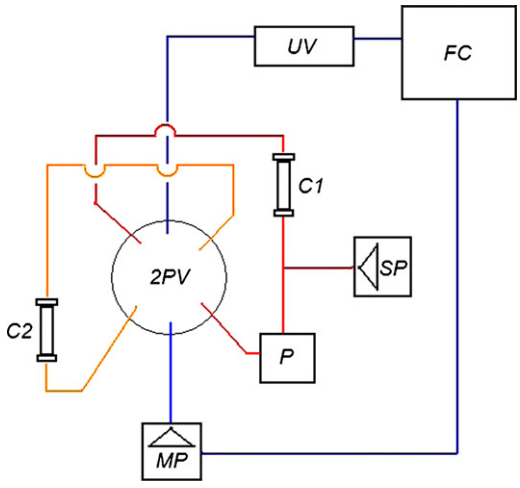

Fig. 1. SSR system components within the recycle loop: 2PV, two position valve; P, polarimeter, SP, sample pump; C1, column 1; C2, column 2; SSR, system components outside the recycle loop; UV, UV detector; FC, fraction collector; and MP, mobile phase pump.

2.2. SSR system

The SSR system was controlled through a two-position valve (see Fig. 1). This valve switched the flow from one column to the other cleaving off the leading and trailing edges of the SSR profile. The mobile phase pump was located outside the loop with column 1 and column 2 flanking it on the switching valve. A polarimeter and sample pump were placed inline before column 1 allowing the optical rotation of the SSR profile to be monitored and additional sample to be injected into the center of the SSR profile by the sample pump. Each column was reattached to the switching valve flanking the exit from the system. This leads to a UV detector. This would monitor the flow to the fraction collector. Data from the UV detector would allow precise collection of the enantiomers by identifying the leading and trailing edge of the chromatographic profile. Both the polarimeter and the UV detector were utilized to provide data that triggers fraction collection. The leading and the trailing edges of the profile detected by the UV detector triggered collection and stop collection. The data provided by the polarimeter is used to determine the start and stop collection times within the profile. Flow was then directed from the UV detector to the fraction collector. The low-pressure valve on the fraction collector was used to collect the enantiomers once the cleaved leading and trailing edges exited the system.

Flow within the system progressed through the polarimeter first. As the sample passed through column 1 the valve switched the sample stream from collection to column 2. Once the sample was passed to column 2 the valve then switched to column 1. This was timed so collection of the leading edge would occur. The center portion of the profile was then passed to the polarimeter. The sample detection took place and directed the injection of new sample into the SSR profile. The valve switched again to