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Journal of Chromatography A, 1046 (2004) 55–60
JOURNAL OF CHROMATOGRAPHY A
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Comparison of separation efficiency of early phase active pharmaceutical intermediates by steady state recycle and batch chromatographic techniques

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Received 8 March 2004; received in revised form 17 June 2004; accepted 25 June 2004

Abstract

Chromatographic resolution of enantiomers has become the accepted method for generation of active pharmaceutical intermediates (APIs) in early phase development. Continuous processes such as simulated moving bed (SMB) are recognized as alternative approaches for manufacturing of larger quantities of enantiomerically pure compounds. Steady state recycle (SSR) technology was initially described in 1998 [C.M. Grill, L. Miller, J. Chromatogr. A 827 (1998) 359] and has recently become a common technique in some laboratories. Batch chromatography, SSR, and SMB processes should be considered "*phase appropriate*" technologies. Phase appropriate technology refers to the scale of separation required in a step of the drug development process. SSR is phase appropriate technology for producing 100 g to several kilogram quantities of material. In this report, we will describe development and scale up of chiral separations utilizing the SSR technique for six different APIs utilizing several different chiral stationary phases and compare the efficiencies of both SSR and batch chromatographic techniques.

Keywords: Pharmaceutical analysis; Steady state recycling; Simulated moving bed; Chiral stationary phases, LC; Batch chromatography; Preparative chromatography

1. Introduction

The need for chiral drug candidates and the process of drug development in the pharmaceutical industry has been well documented [1,2]. The pharmaceutical development process consists of many steps require varying quantities of intermediates along the synthetic route. In discovery phase, milligrams to gram quantities are necessary for various biological testing protocols. When suitable candidates are identified in early development, gram to kilogram quantities are necessary. As compounds move into full development or manufacturing, kilogram to ton quantities are utilized to define and develop manufacturing processes. As quantity requirements change so should appropriate technology to generate these materials. This is referred to as phase appropriate technology. Chromatography has become the standard for rapid generation of quantities of pure enantiomers. Requirements for chromatographic resolutions include the ability to rapidly develop chiral separations. Many method development protocols for chiral HPLC and supercritical fluid techniques (SFC) have been published [3,4]. Rapid method development and subsequent ability to scale up separations are critical in developing cost factors to determine phase appropriate technology. Comparative cost data for operation of HPLC versus SFC have been published as well as comparative data from studies of HPLC versus steady state recycle (SSR) and simulated moving bed (SMB) for a single racemate [5–7]. These reports do not address the capital investment necessary to implement these technologies in a development environment.

Capital and facility cost for SMB or SFC may range from US$ 300 000 to US$ 1 000 000. Facility modification cost for large-scale evaporators (SMB) or carbon dioxide Dewar

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doi:10.1016/j.chroma.2004.06.103

system (SFC) can be substantial. Although both techniques require less solvent than traditional batch or SSR techniques, solvent usage is generally not an issue in early phase development. Development times tend to be longer with SMB methods and optimization of the technique requires more material than is generally available in early phase development studies. Few candidates in early development will reach manufacturing stage, thus development of manufacturing scale technology at this point in development is time consuming and not cost effective. Cost effective phase appropriate technologies are necessary at this point in pharmaceutical development. Batch chromatographic techniques such as shave and recycle chromatography or stacked injection techniques are often overlooked as viable methods. Based on our assessment of capital cost, development time, productivity for the technology, and material requirements, we determined that batch chromatography and SSR were phase appropriate techniques for production of the gram to kilogram quantities required for early phase development. Batch separations as well as SSR chromatography can be conducted on the same equipment. In this report we compare optimized batch chromatography to SSR for six active pharmaceutical intermediates (APIs) in terms of productivity (g/kg CSP/day) and solvent usage. We also present data on the chromatographic conditions including chiral stationary phase (CSP) and eluent used. Lastly we compare productivity with sample solubility in the chromatographic eluent.

2. Experimental

2.1. Materials

Fifty to five hundred gram quantities of APIs in this study were obtained from Lilly Research Labs. The structures are proprietary and thus are not published. All compounds were basic and contained single or multiple fused aromatic rings. Most had halogen substitution on the rings.

Twenty micrometer bulk Chiralpak AD, Chiralcel OD, OJ packing material and 150 mm × 4.6 mm analytical columns packed with 5 μm material were purchased from Chiral Technologies, Exton, PA, USA. Solvent compatibility data for the Chiralpak and Chiralcel columns is available on the Chiral Technologies technical data web page [8].

Heptane was obtained from Tedia, Fairfield, OH, USA. Methanol (MeOH), isoproanol (IPA), and acetonitrile (ACN) were purchased from Mays Chemical, Indianapolis, IN, USA.

2.2. Equipment

The analytical chromatography system used was a Shimadzu Scientific LCMS-QP8000 CL liquid chromatograph–mass spectrometer consisting of LC-10AD VP pumping system, SPD-M10A VP photodiode array detector, SIL-10AD VP Auto sampler, and six-port column switcher containing two FCV-14AH valves. Preparative chromatography systems were Novaprep 800 with Hitachi L7400 UV detector (Hitachi Instruments, Naperville, IL, USA). Prochrom LC80.VE.100 columns, 8 cm i.d., were obtained from Novasep, Boothwynn, PA, USA and packed to variable length as needed to accommodate approximately 1 kg of packing material. All preparative columns were prepared by slurring approximately 1 kg CSP in an appropriate amount of *n*-propanol to make a suitable solution for transfer to the Prochrom column skid. Pressure was applied to compress the bed to approximately 30 cm length. Pressure was continuously applied to the column bed during chromatography to maintain column efficiency. VAP-20 continuous rotary evaporators from Genser Scientific Instruments (Rothenburg o.T Germany), were used to concentrate fractions from the SSR processes.

The batch chromatography and SSR data were generated on the Hitachi instrument with LC Responder control software (Varian, Wakefield, RI, USA). Development of a SSR separation process involved generation of loading and recycle chromatograms on standard chromatography equipment. Recycle data from the first pass and second pass were graphically overlaid using Microsoft Excel to determine approximate position for isomer fraction cuts and midpoint of the profile for injection of racemate The SSR process was triggered by setting a detector response value for ascending height start, then relative timed events consisting of sample fraction cuts, recycle, and sample injection were determined. In the SSR process, sample concentration on the column is gradually increased until a constant sample profile has developed. During this process, fractions of isomers 1 and 2 are removed as racemate is added to maintain a constant concentration profile on the column. The concentration profile is based on ratio between solute in the CSP and solute eluent and will become constant or steady state after approximately 10–20 cycles.

3. Results and discussion

Development of efficient continuous preparative chromatographic separations requires methodology where sample solubility in the desired chromatographic eluent is maximized and retention times for separated isomers are less than 10 min. The short retention times are necessary for recycle chromatography. In our laboratories, we routinely examine separations extensively under polar organic mode to allow use of solvents such as acetonitrile or methanol and no heptane. Pharmaceutical intermediates tend to be more soluble in these solvent systems, allowing higher loading. Separation times are shorter, thus productivity can be higher by virtue of more injections in a given time. All APIs were screened on Chiralcel OJ, Chiralpak AD, and Chiralcel OD CSPs using methanol, acetonitrile, and ethanol eluent systems initially, then with alcohol–heptane systems when necessary to achieve a desired separation. An example of optimization of this type of separation is illustrated in Fig. 1 for API-1 on Chiralcel OJ. The Chiralcel OJ CSP with

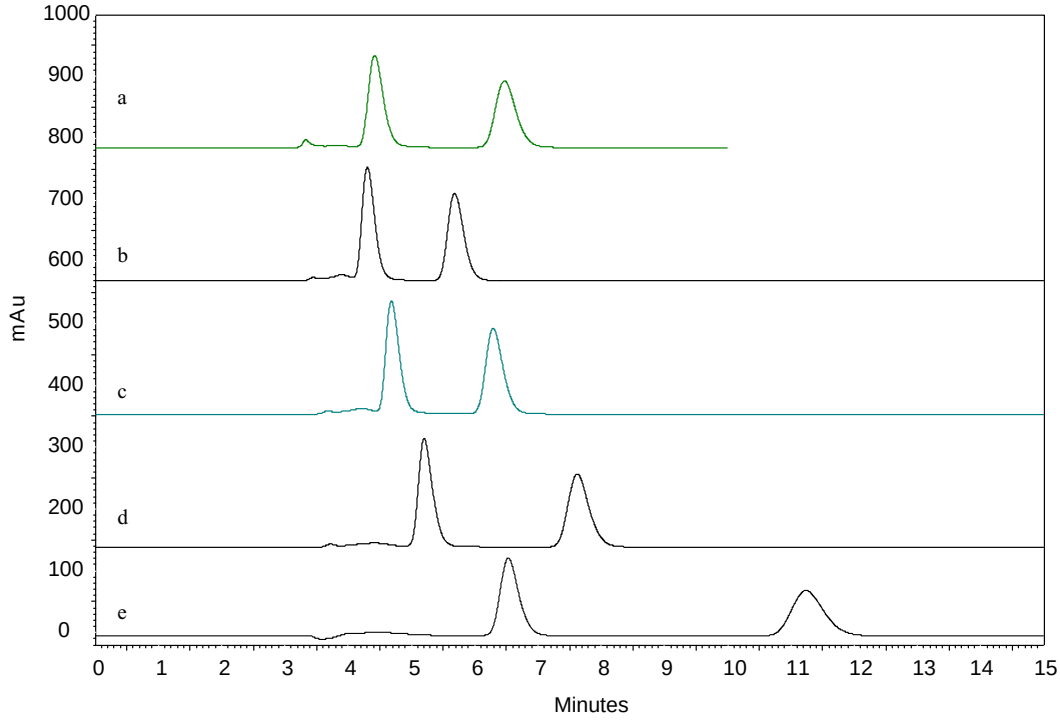

Fig. 1. Separation of API-1 on 4.6 mm × 150 mm Chiralcel OJ. Flow rate 0.6 ml/min, eluent: (a) 100% acetonitrile; (b) acetonitrile–methanol (80:20); (c) acetonitrile–methanol (60:40); (d) acetonitrile–methanol (40:60); (e) acetonitrile–methanol (20:80).

acetonitrile and methanol mixed eluent has proven a very good combination for resolving many pharmaceutical intermediates and for the follow-up SSR implementation. In the case of API-1, methanol alone produces a high α value and long retention time for separation that are unacceptable for developing a continuous process. Our experience with these types of compounds indicates the addition of acetonitrile usually decreases α and decreases total elution times.

Optimal analytical separation eluent for API-1 was chosen to be condition (c) in Fig. 1. This choice was based on total separation time less than 8 min and previous knowledge of better peak shape for these type compounds under overload conditions in this solvent system. Using the acetonitrile–methanol (60:40) system, a simple recycle experiment was performed on an 30 cm × 8 cm Chiralcel OJ column. A chromatogram from this experiment is illustrated in Fig. 2. Estimated sample loading was 3.0 g for this process. Graphically overlaying the initial column pass (A) and second pass (B) from this experiment is illustrated in Fig. 3. From this data, initial parameters for the SSR experiment can be determined. These initial parameters will be modified as a concentration profile builds on the column and may vary from compound to compound depending on the isotherm behavior of the two isomers. The overlay chromatograms approximate what will happen when two injections of API-1 are on the column simultaneously. This overlay may be viewed as three zones. Zone 1 containing isomer 1, zone 2 containing mixed fractions, and zone 3 containing isomer 2. The SSR process will be defined by five control events within these zones. Although estimation of these events can be made from this type graph, analytical monitoring of fractions taken and subsequent modification of event based on the analytical data is a necessary part of the SSR process development. On line techniques such as polarimetry may be useful in the future in making these type observations.

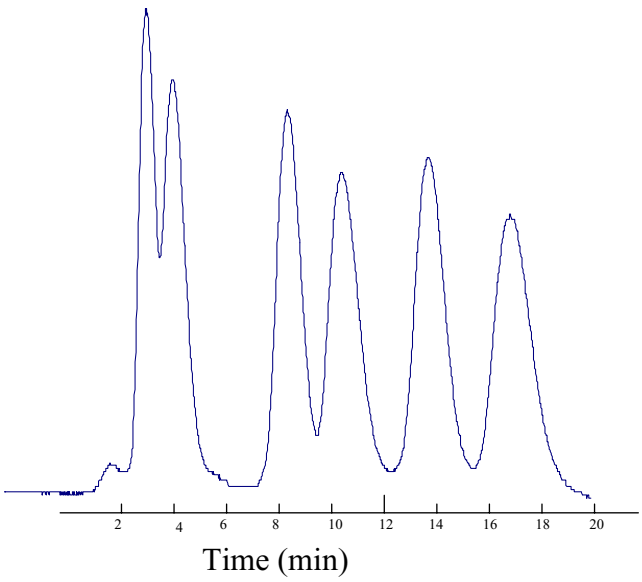

Fig. 2. Recycle experiment for API-1. Column dimensions: 30 cm × 8 cm Chiralcel OJ. Flow rate was 400 ml/min. Eluent was 60% acetonitrile in methanol.

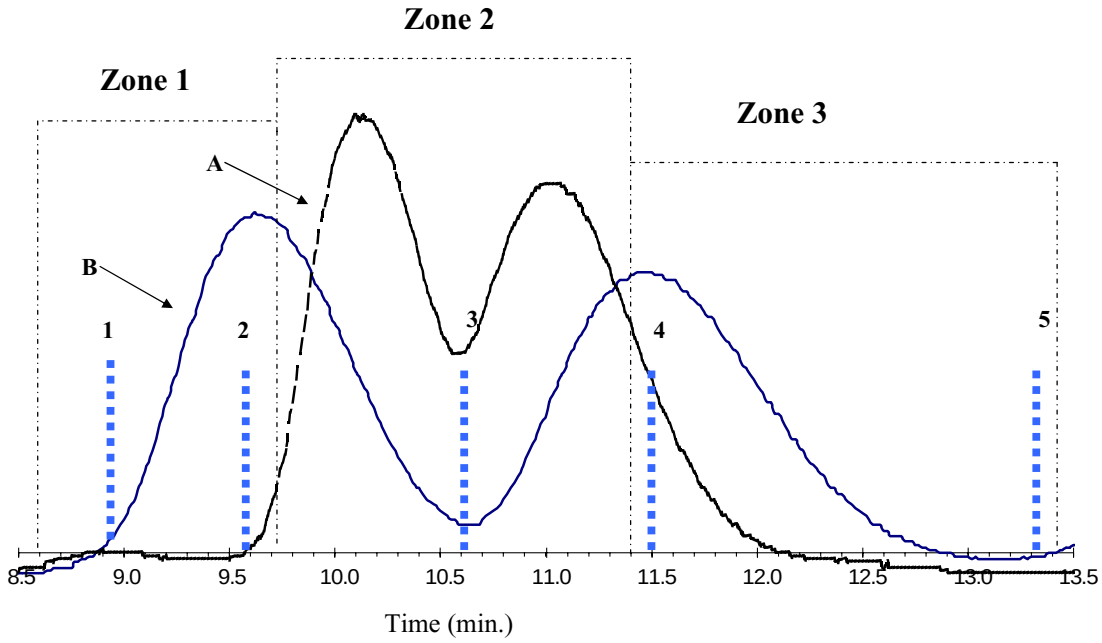

Fig. 3. Overlay chromatograms for estimation of SSR conditions. (A) Initial cycle and (B) second cycle, 1–5 are SSR control events. Explanation of events is in text.

Line 1 starts the process based on an ascending height from the baseline. All other events are triggered relative to this time point. The region between lines 1 and 2 is the area where isomer 1 should be pure, representing a time of 0.7 min for collection of fraction 1. Line 3 represents the midpoint of the profile and is the region for reinjection of racemate approximately 1.8 min after starting the process. Line 4 represents the region for fraction 2 collection at approximately 2.7 min. In this region, isomer 2 should be pure to the end of the process represented by line 5 approximately 4.6 min after process started. This represents a single cycle time of 4.6 min in the SSR process. In the course of developing a SSR process, slight modifications of any of the event points can have a dramatic effect on the concentration profile. We recommend changing single events and observing the resulting changes in profile after 5–10 cycles. This may lengthen the development time for a SSR method. However, following this approach, material is not lost and the need for reprocessing material that does not meet purity requirements is reduced.

Fig. 4 represents a series of four injections to generate a SSR chromatography profile for API-1 with 3.0 g loading. Analysis of collected fractions indicated the enantiomeric excess for isomer 1 was 99%. The enantiomeric excess for isomer 2 was also 99%, but isomer 1 was the desired product and

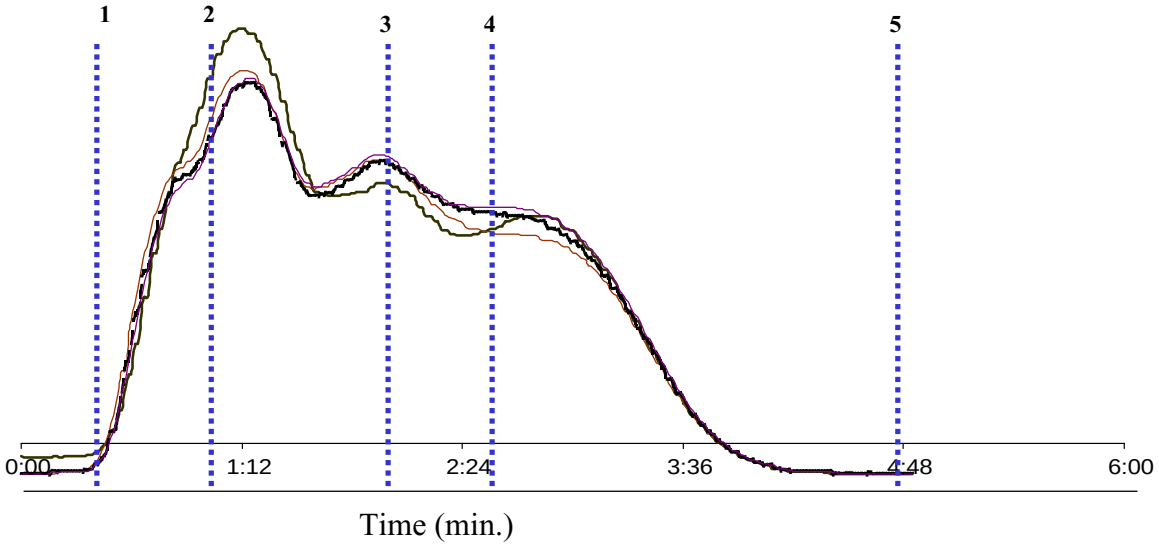

Fig. 4. SSR profile for API-1, four cycles. Loading was 3.0 g. Events 1–5 are explained in text. Enantiomeric excess for isomer 1 was 99%.

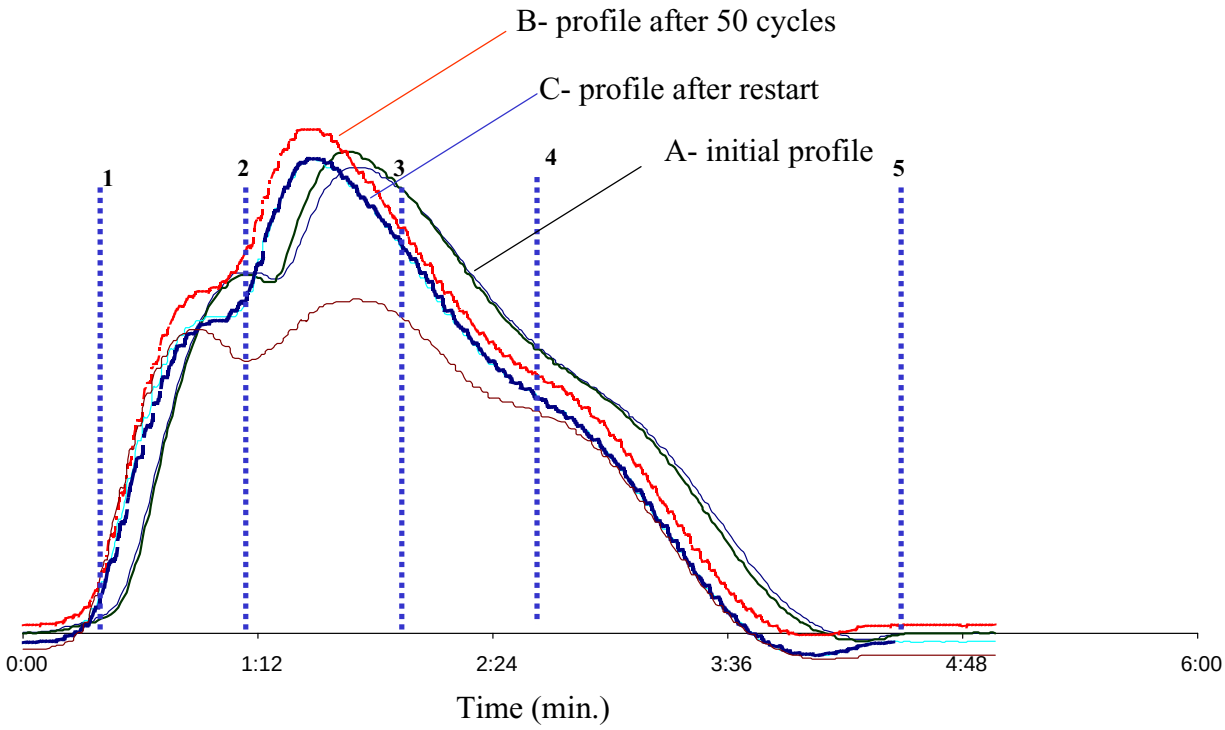

Fig. 5. SSR profile for API-1, six cycles. Loading was 4.5 g. Events 1–5 are explained in text. Enantiomeric excess for isomer 1 was 99%. (A) Profile for 40 cycles, (B) profile beginning after 50 cycles, (C) profile after flush and restart.

the SSR process was optimized for that compound. The five control events are the same as in Fig. 3. This is a typical profile from SSR chromatography and illustrates the zones that are observed in a real time process. In the case of API-1, solubility was quite high (120 mg/ml) in the eluent and we believed that loading and throughput could be improved. Loading was increased to approximately 4.5 g per cycle and the resulting profile is presented in Fig. 5. Representative chromatograms from 40 injections of 4.5 g loading are illustrated in profile A. This represents the processing of approximately 180 g of racemate. A shift in the profile between lines 1 and 2 indicated a possible decrease in purity of isomer 1. The shift is illustrated in profile B. This was confirmed analytically by analysis of factions. Profile B occurred after approximately 200 g racemate was processed. This type change in the profile occurs in the SSR process when one isomer concentration builds up at a higher rate than the other due to differences the sampling or shifting due to isotherm behavior. Isotherm behavior may be different for each isomer. In this case, more isomer 1 was being removed by fractionation than isomer 2. Thus, higher concentrations of isomer 2 on column caused a shift forward and decrease in the purity of isomer 1. At this point, the enantiomeric excess of isomer 1 had dropped to about 95%. The system was flushed and restarted with fresh racemate. Cleaning the system resulted in reproduction of the plateau between lines 1 and 2 as illustrated in profile C. The observation of this plateau similar to the starting profile indicated that enantiomeric excess of 99% for isomer 1 should be possible. Subsequent analysis indicated that enantiomeric purity of isomer 1 had been restored. Productivity, solvent usage and comparison of purification by batch chromatography for 6 APIs are detailed in Table 1. Four hundred seventy six grams of API-1 racemate was processed by this method. Two hundred grams of isomer 1 with an enantiomeric excess of 99% was recovered. This represents an 84% recovery of material from racemate.

Batch methods were performed under the same column and eluent conditions as SSR methods. Shave and recycle procedures were used for batch methods whenever possible to improve efficiency. In all cases studied, solvent usage decreased and improvement in productivity was observed when SSR was used. In our experience, development of an SSR separation system takes about one half to one full day of optimization. A batch process can be developed in 1 h or less time. Based on the data in Table 1, it is worth the effort to develop a SSR method when quantities of 50 g or more are

Table 1
Comparison of SSR to batch chromatography

Compound	SSR productivity*	Batch productivity	SSR solvent**	Batch solvent
API-1	1351	228	0.21	2.5
API-2	648	90	0.68	6.4
API-3	67	24	5.60	12.0
API-4	132	36	3.64	10.15
API-5	130	34	3.02	9.06
API-6	115	33	3.86	11.30

* Productivity data are listed as (g/kg CSP/24 h).
** Solvent data are listed as (l/g).

Table 2
Chromatography conditions

Compound	CSP*	Eluent	Solubility (mg/ml)
API-1	Chiralcel OJ	ACN–MeOH (60:40)	120
API-2	Chiralcel OD	ACN–MeOH (50:50)	98.7
API-3	Chiralcel OJ	ACN–MeOH (10:90)	12.5
API-4	Chiralpak AD	MeOH	20
API-5	Chiralcel OD	Heptane–IPA (97:3)	20
API-6	Chiralcel OD	Heptane–IPA (95:5)	20

* All columns contained 1 k CSP. Column dimensions were 30 cm × 8 cm.

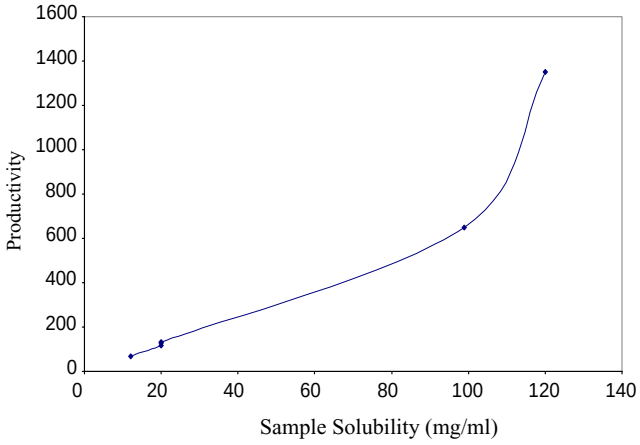

Fig. 6. Productivity as function of sample solubility.

purified. Based on our experience using the 8 cm column system and Novaprep 800 system, quantities of at least 50 g or more of material are needed for SSR methodology to be advantageous over batch purification. This amount is necessary due to number of cycles typically required to reach a steady state on this system.

The chromatographic conditions as well as solubility data for these APIs are listed in Table 2. Fig. 6 illustrates the effect of sample solubility on productivity for these SSR processes. These data represent productivity data versus solubility for each of the six APIs in this study. In any single case, the data should have a plateau when saturation capacity for the chiral stationary phase is reached. These data point to development of methods that maximize sample solubility in the eluent system used for a chromatographic process.

4. Conclusions

These data illustrate the advantages of SSR over batch chromatography for purification of quantities of materials typically needed for early phase development. SSR is a cost effective phase appropriate technology and should be investigated whenever quantities of 50 g to kilograms of materials are purified. Productivity is improved and solvent usage is lower, many times by a factor of 10. Productivity improvements can reduce purification times by days or weeks in many cases. SSR equipment may be utilized for batch chromatography when scale up of difficult separations is required, thus eliminating the need to purchase additional equipment and reduce capital expenses. Lastly, these data illustrate the need to develop methodology where sample solubility is maximized in the chromatographic eluent. Since continuous methods such as SSR do not require the same resolution as batch chromatography, the task of matching higher sample solubility to polar organic mode of separation is more feasible.

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