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Simulated moving columns technique for chiral liquid chromatography

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Abstract

Enantioselectivity of chiral selectors is often relatively low in chiral HPLC. For difficult chiral separations, often only partial resolution is obtained rather quickly by column and mobile phase screening, and, by trial-and-error, additional method optimization is required to achieve complete resolution. This paper describes the development of a novel column-switching technique called "simulated moving columns" (SMC) to quickly achieve complete chiral resolution on columns with limited enantioselectivity. The simulated moving columns (SMC) technique uses two (2) or three (3) short chiral HPLC columns connected in series, and forces the unresolved enantiomers to recycle exclusively through the columns until sufficient resolution is attained. In effect, SMC helps to achieve chiral resolution by virtually multiplying the column length, thus enhancing separation efficiency and resolution, without increasing backpressure. Comparison of the standard non-SMC approach with SMC, and selected applications of chiral separations of pharmaceutical drug molecules are presented. Through measurement and calculation, evaluation of off-column band broadening resulting from a two-column SMC system is provided. The results clearly indicate that SMC eliminates the significant band broadening that is inevitable in the closed-loop recycling techniques currently used in preparative chromatography. Furthermore, SMC is not only useful to enhance resolution for analytical and preparative chiral separation, but also has great potential to enhance recovery and purity for difficult chiral preparative chromatography.

Keywords: Simulated moving columns; Enantioselectivity; Recycling chromatography; Chiral stationary phases, LC; Preparative chromatography; Recycling chromatography

1. Introduction

During the last decade, the pharmaceutical industry has raised emphasis and expectations on discovering and developing enantiomerically pure compounds in the search of therapeutic benefits to develop safer and more efficacious drugs. The majority of today's top-selling drugs are marketed as single enantiomer [1]. Enantioselective chromatography on a chiral stationary phase (chiral selector) has become an important tool to separate enantiomers. As chromatographic methods usually furnish both enantiomers in high optical purity within a relatively short time period, they have been the most heavily utilized techniques to separate, isolate and analyze pure enantiomers particularly at the stages of drug discovery and early development [2]. Fast method development for complete enantiomeric resolution is crucial to the continued success of chiral chromatography. However, despite major advances in the development of hundreds of chiral stationary phases over the past decades [3,4], finding the optimal column and mobile phase combination for complete resolution of a specific pair of enantiomers remains largely a time consuming "trial-and-error" process [5–7]. Often only partial resolution can be attained rather quickly without further extensive column scouting and mobile phase optimization. Thus, the aim of our research was to develop techniques that can speed up method development and help achieve complete resolution under column and mobile phase conditions that nominally provide only partial enantiomeric separation.

In preparative chiral chromatography, recycling techniques have long been recognized as a useful method to improve recovery yield, production rate and enantioseparation [8,9]. Although there are many different modes of recycling techniques including the innovative closed-loop steady-state recycling (SSR) [10], all of the techniques used for HPLC published over the past thirty years relies on a single column and the enantiomers are recycled through the entire HPLC system including the pump and detector.

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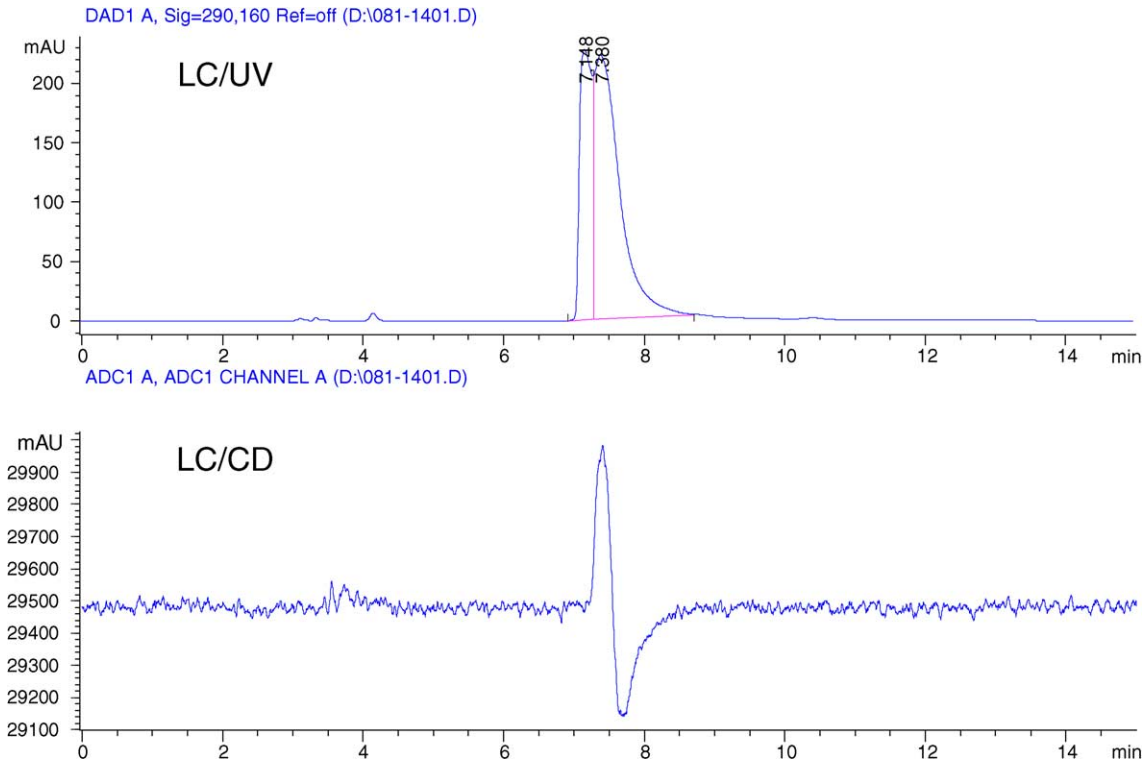

Fig. 1. LC/UV and LC/CD showing the limited chiral separation of a Wyeth discovery compound after an initial column and mobile phase screening (five columns and four normal and polar organic phases). Chiralpak AS-H 4.6 mm × 250 mm, 5 μm; mobile phase: 50% IPA/50% hexane; flow: 1 ml/min; pressure 75 kg/cm^2. Further experimentation is required for baseline separation.

In addition to the large void volume, mixing occurs in the pump. As a result, significant off-column dispersion occurs and a large part of the separation attained in the column is destroyed during the closed-loop recycling [11]. A novel column switching technique called "simulated moving columns (SMC)" has been developed and described herein that uses two (2) or three (3) short columns, and allows the enantiomers to recycle exclusively through the columns to improve the performance of chiral chromatography with insignificant off-column band broadening.

Although SMC is a multi-column chromatography technique similar to simulated moving bed (SMB) chromatography, it is fundamentally different from SMB. SMB was invented in the early 1960s by Broughton et al. [12]; it simulates the counter-current movement of the packed bed (stationary phase or columns) and the mobile phase by switching

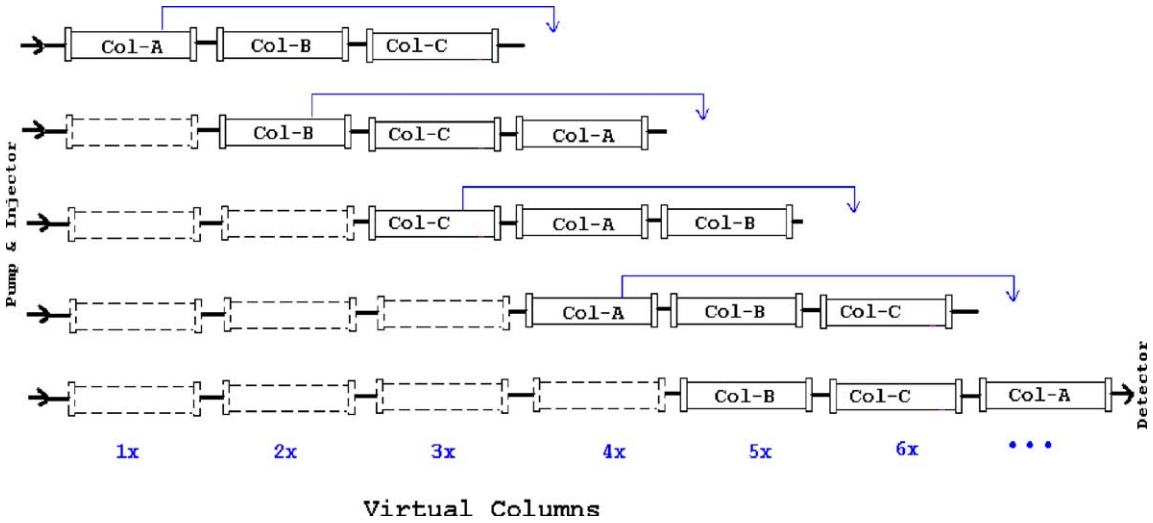

Fig. 2. SMC simulates the movement of the columns at the direction of the mobile phase to keep the enantiomers recycling in the columns. In essence, longer virtual columns were used.

the locations of feed and purified product ports every few minutes. The SMB technique is a continuous binary separation process, in which the sample or feed is continuously injected and two product streams are continuously collected at the two opposite sides of the feed. The new SMC technique discussed in this paper uses HPLC with a novel column switching or recycling scheme. It simulates the movement of the columns in the direction of the mobile phases as well as ahead of the mobile phase. SMC can be used for separation of two or more components. SMC is not continuous, and therefore, it is not applicable for production scale preparative chromatography like SMB. SMC is a technique to help achieve complete chiral resolution at an analytical or semi-preparative scale with columns and conditions that provide only partial separation. In addition, the potential benefit of SMC for achieving high purity and high recovery in binary preparative chiral chromatography by combining with segmented recycling (shaving) will be described. Fundamentally, unlike SMB, complete chiral resolution on a column is not a pre-requirement in SMC chromatography.

Several multicolumn, multiport switching techniques, called moving feed and moving withdrawal chromatography (MFC and MWC, respectively) and a combination approach, i.e. moving port chromatography (MPC), were developed by Wankat and coworkers [13,14], and can be considered as extensions of either SMB techniques or column-switching procedures. MPC is a column-switching technique that can be used to increase throughput by improving chromatographic packing utilization in situations where chromatographic peaks are widely separated. In effect, it splits the normal column into shorter columns connected in series. During separation, the slowest and fastest moving components are withdrawn at intermediate ports as soon as they are separated from other components, and the feed can be introduced at these intermediate ports. As results, injection cycle time is reduced and utilization of the column packing is increased. The technique seeks to reduce or eliminate the gaps between component peaks and thereby improve column utilization to increase throughput. Although SMC as described herein is also a column-switching technique that uses short columns, it has been developed to solve the problem of insufficient resolution in chiral chromatography. As a recycling technique, SMC might be used with MPC to increase column utilization both for closely-eluting components by recycling them through the columns until complete resolution, and for widely separated components by reducing the inefficient utilization of the columns.

2. Experimental

2.1. Chemicals

The commercially available chemicals, ketoprofen and labetalol as well as diethylamine (DEA), trifluoroacetic acid (TFA) and ammonium acetate, were purchased from Sigma–Aldrich (St. Louis, MO). The Wyeth discovery compounds used in the study are small drug molecules (MW: 250–500) and were synthesized in-house. Methanol (MeOH), ethanol (EtOH), acetonitrile (ACN), isopropanol (IPA), dichloroethane (DCE) and hexane are HPLC-grade and obtained from Mallinckrodt-Baker (Muskegon, MI).

2.2. Chiral stationary phases

Chiralpak AD and AS (amylose derivatives), and Chiralcel OD and OJ (cellulose derivatives) columns were

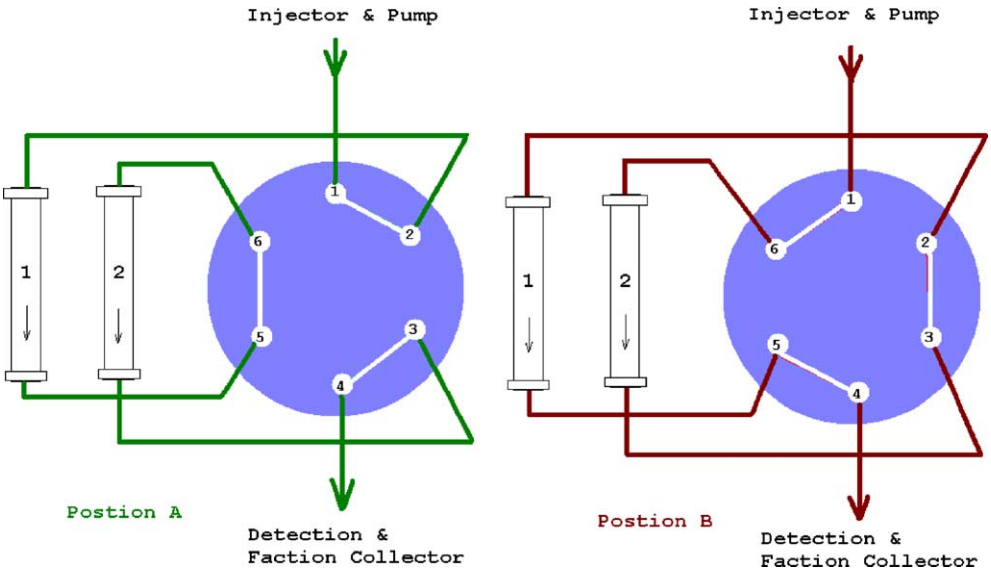

Fig. 3. A two-column SMC configuration: at position A, the enantiomers elute from column 1 to column 2. As they leave column 1, the valve is switched to position B where the enantiomers are forced back onto column 1 from column 2, and so on so forth.

purchased from Chiral Technologies (Exton, PA). The brush-type columns, Chirex 3005, (*R*)-1-naphthylglycine and 3,5-dinitrobenzoic acid with an amide linkage, and Chirex 3022, (*S*)-indoline-2-carboxylic acid and (*R*)-1-(α-naphthyl)ethylamine with an urea linkage, were purchased from Phenomenex (Torrance, CA).

2.3. Instrumentation

The two (2) and three (3) column SMC systems were configured between the injector and detector of a HP1090 HPLC system (Agilent Technologies, Inc.). A six-port two-position valve with a microelectric valve actuator was used in the two-column SMC configuration. For the three-column SMC system, two four-position switching valves mounted on a single multi-position microelectric valve actuator were used. All valves and actuators were purchased from Valco Instruments Co., Inc. (Houston, TX). The valve switching was controlled automatically using ChemStation® Software (Agilent Technologies, Inc.) through time-programmed contact closures. The low volume inline spring-loaded check valves were purchased from Upchurch Scientific (Oak Harbor, WA). A circular dichroism (CD) detector (Jasco CD1595) purchased from Jasco International Co., Ltd. (Maryland, USA) was used after the HP1090 diode array detector. The injection amount is approximately 3 μg unless otherwise noted in the paper.

3. Results and discussion

3.1. Requirement for long columns with low pressure for difficult enantioseparations

Chromatographic resolution can be improved by increasing the selectivity (α) and/or column efficiency (N) at a

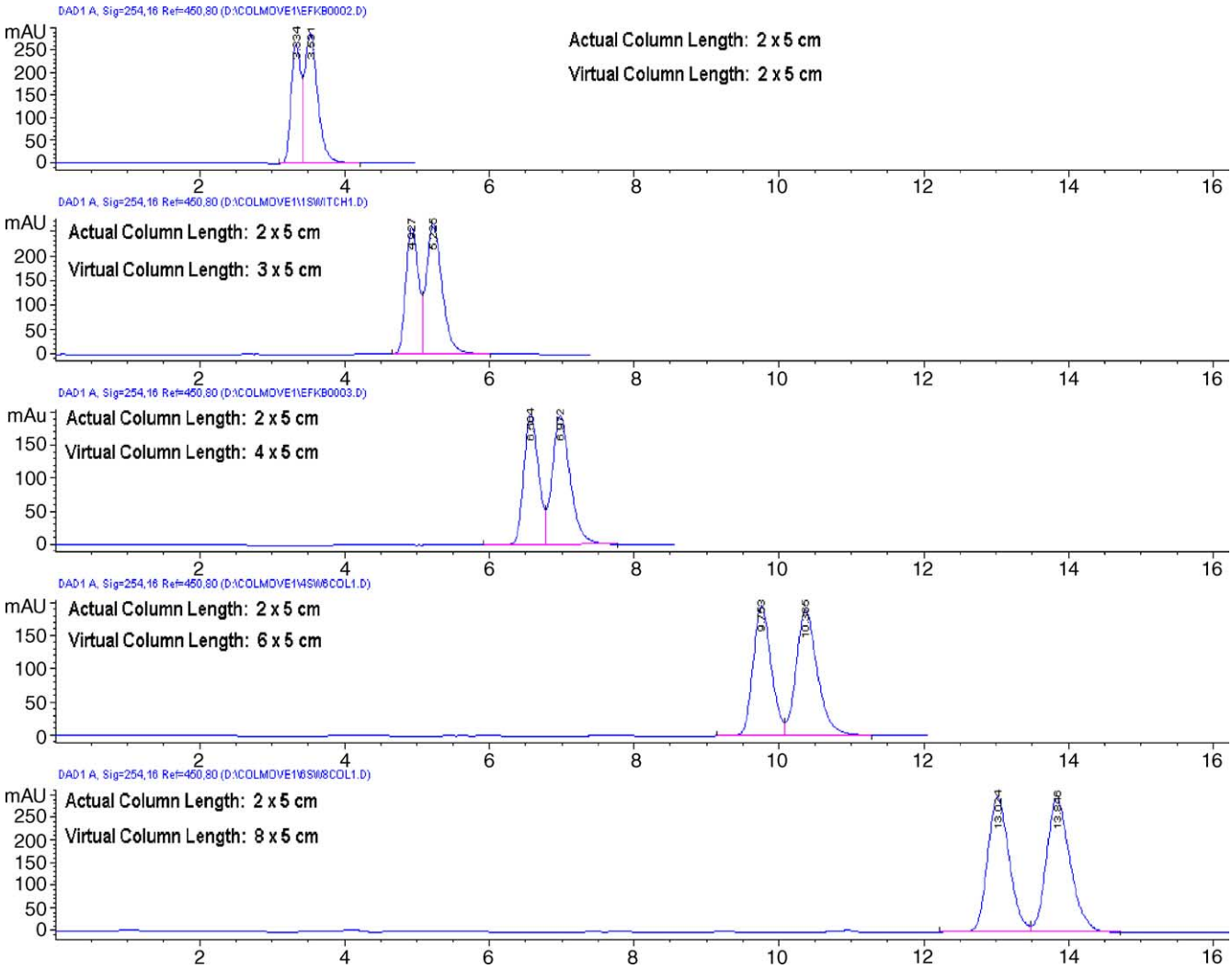

Fig. 4. A chiral separation Wyeth discovery compound (Wy-A) using SMC. The top chromatogram shows the separation without SMC. The chromatograms below are the separation of the same racemate using virtual column length of 15, 20, 30 and 40 cm with SMC. Columns: two Chiralpak AD, 10 μm, 4.6 mm × 50 mm each; mobile phase: 95% ACN, 5% IPA; backpressure 36 kg/cm^2; flow: 1 ml/min; column temperature: 23 °C. The injection amount is 3 μg except the last chromatogram for which 5 μg was injected.

reasonable capacity factor as shown the equation below, where k_2 is the retention factor of the second enantiomer of the enantiomeric pair:

$$Rs = \left(\frac{\sqrt{N}}{4}\right)\left[\frac{\alpha - 1}{\alpha}\right]\left[\frac{k_2}{1 + k_2}\right] \quad (1)$$

For difficult chiral separations, however, enantioselectivity of a chiral selector is often limited, and only partial resolution can be obtained rather quickly after initial column and mobile phase scouting. Fig. 1 shows an example of the best chiral separation of a racemate after an initial screening of five columns and four mobile phases. Clearly, further extensive experimentation to optimize the mobile phase conditions and perhaps testing additional chiral selectors is required to improve selectivity and achieve sufficient resolution. A quicker approach to improve resolution in such a situation is to increase column efficiency by using a longer column. However, according to the Darcy equation [15], the pressure drop is directly proportional to the column length:

$$\Delta P = \frac{\eta u L}{k_0 \mathrm{dp}^2} \quad (2)$$

where k_0 is the specific column permeability (ca. 10^{-3}), η is viscosity, u is linear velocity, dp is particle size. Furthermore, many chiral selectors, especially the popular polysaccharide derivative-based columns, have lower tolerable maximum pressures than regular HPLC columns. Consequently, the column length or percentage of viscous mobile phases that can be used for a separation may be restricted. For example, 100% EtOH at 1 ml/min in a 4.6 cm i.d. × 25 cm L AS-H column packed with 5 μm particle would yield a backpressure around 130 kg/cm^2 (1820 psi) that is more than three times the manufacturer's recommended upper pressure of 50 kg/cm^2 (700 psi) and exceeds the maximum pressure limit of 100 kg/cm^2 (1400 psi) specified by the manufacturer for the column.

To solve the problem where a chiral separation requires additional efficiency and resolution without increasing backpressure, the Simulated-Moving-Columns (SMC) technique was developed.

3.2. Simulated moving columns (SMC) technique

SMC can be envisaged as a movement of two (2) or three (3) columns in the direction of the mobile phase to keep

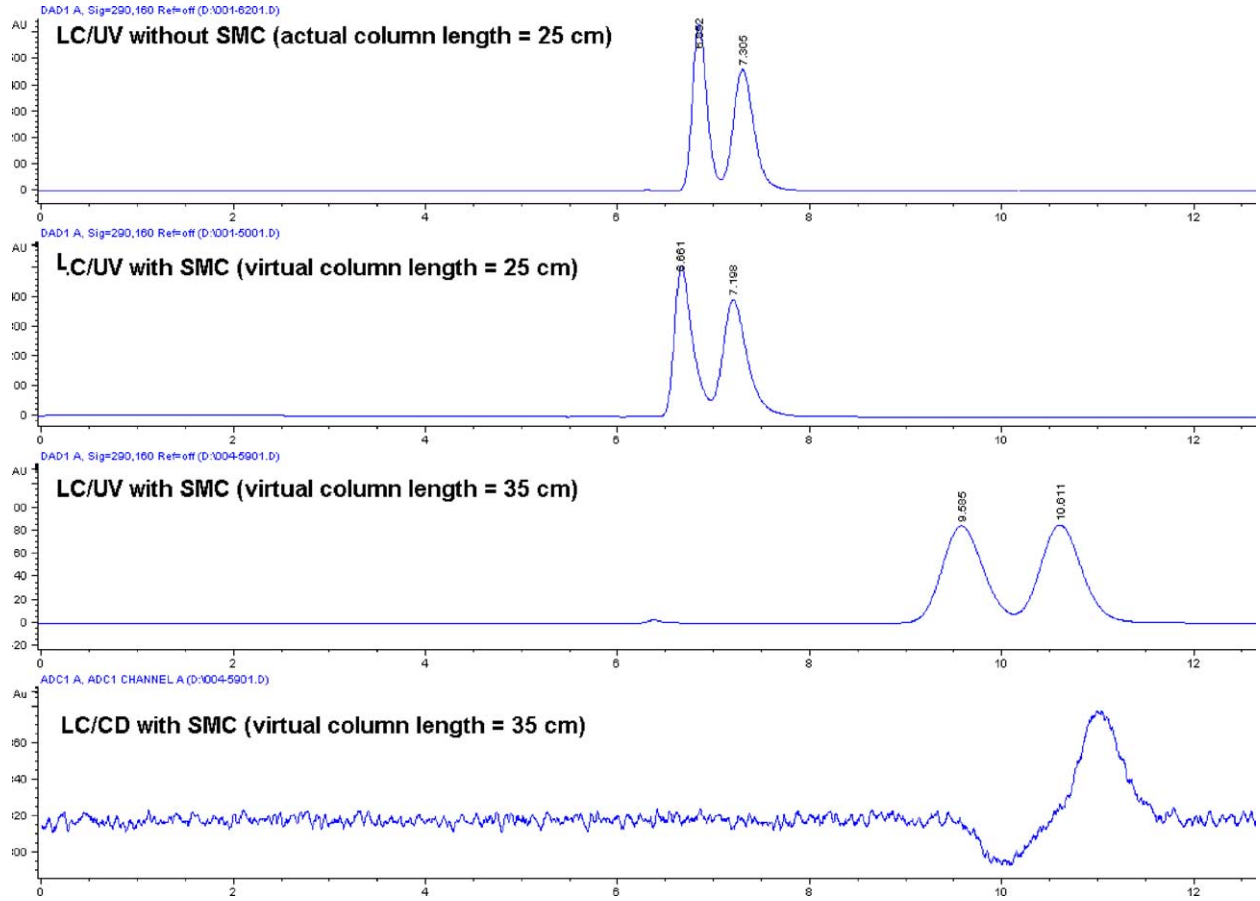

Fig. 5. The top chromatogram is the chiral separation of racemate Wy-B on an actual 25 cm Chiralpak AS, 10 μm column (backpressure 78 kg/cm^2). The second chromatogram from the top shows the separation using two 5 cm actual Chiralpak AS 10 μm columns with SMC that generate 25 cm virtual column length (backpressure 34 kg/cm^2). At the bottom are the LC/UV and LC/CD (circular dichroism) chromatograms on 35 cm virtual column. Mobile phase: 100% EtOH.

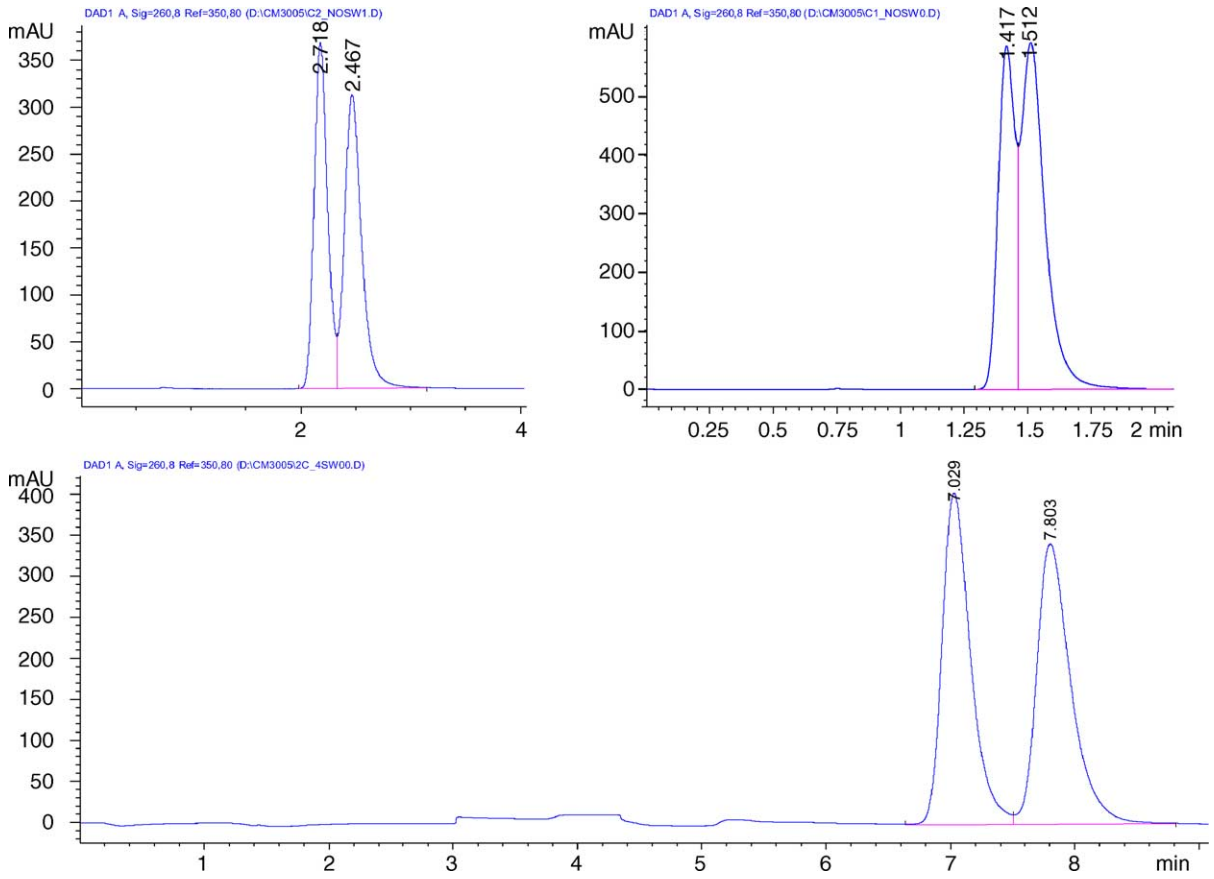

Fig. 6. Chiral separation of Ketoprofen on Chirex 3005 5 μm columns. The top two chromatograms show the separations of Ketoprofen on two individual 5 cm Chirex 3005 5 μm columns. The bottom chromatogram shows the separation on the same columns in series with SMC that generates 20 cm virtual column length. Mobile phase: 30 mM ammonium acetate in methanol.

enantiomers recycling in the columns until sufficient resolution is achieved. As shown in Fig. 2 for a three-column SMC, the enantiomers elute from column A to column B to column C. As they leave column A, the SMC "moves" column A to the end of the column C to facilitate the elution of the enantiomers back onto column A. When the enantiomers leave column B and are on column C and/or column A, the SMC "moves" column B to the end of column A, and so

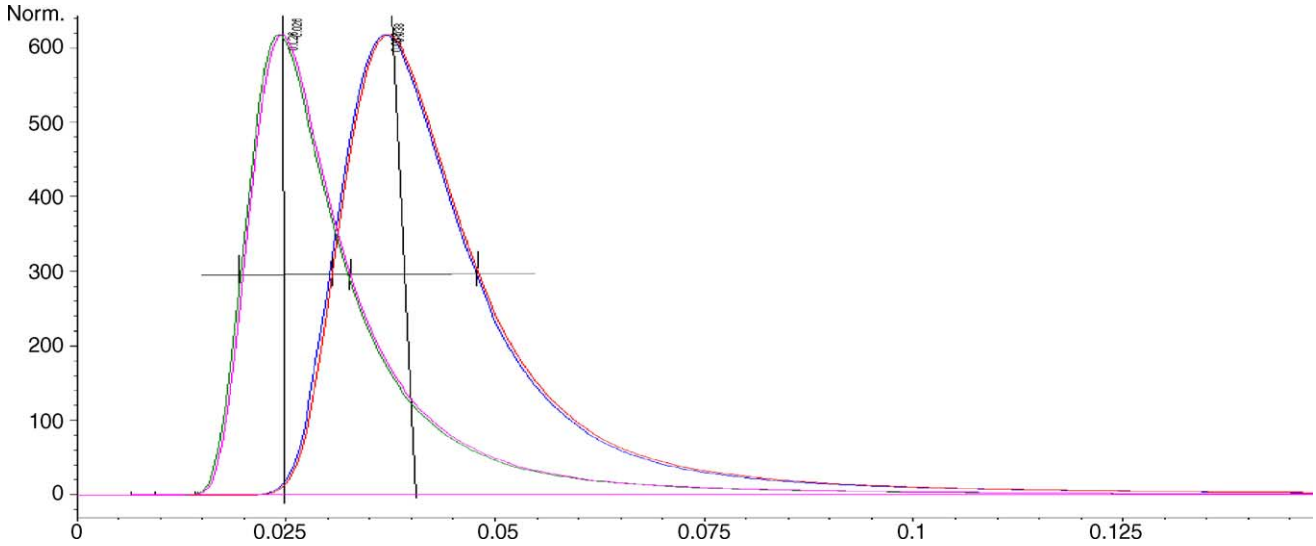

Fig. 7. Injection profile showing the off column band broadening: the narrower peak is the system off column band-broadening without SMC, the broader peak is the system including SMC. The widths at half height were used for the calculation.

on. In effect, the enantiomers elute from column A → B → C → A → B → C → A ... until sufficient resolution is attained. There is no increase on the system backpressure in the process since the actual length of the columns is constant. In fact, shorter columns with lower back pressure enable the use of some of the more viscous solvents. In addition, the enantiomers do not recycle through the pump and detector as they do in other recycling techniques; therefore, they do not suffer from peak dispersion or band broadening.

To perform chiral separation using SMC, the enantiomers are first injected on each individual column. Provided the retention time is proportional to the column length at a constant flow rate in each column, and the change in retention time due to the connecting tubing is negligible, the time when the pair of enantiomeric peaks leave one of the columns connected in series can be easily calculated. The time is then used to program the column switches via contact closures.

3.3. Two-column SMC configuration and application

Two-column SMC can be configured with a six (6)-port or a ten (10)-port two-position switching valve. Fig. 3 shows the flow path with a 6-port valve. At position A, the enantiomers are eluted from column 1 and enter column 2. As they leave column 1, the valve is switched to position B where the enantiomers are forced to move from column 2 back to column 1.

The first application of SMC is shown in Fig. 4 for the chiral separation of a Wyeth discovery compound (Wy-A). Two 5-cm long, 10 μm Chiralpak AD columns were used, and only partial separation was obtained without SMC as shown on the top chromatogram. The following four (4) chromatograms show the improvement of the separation on the same columns using SMC at virtual column lengths from 15, 20, 30 to 40 cm, respectively. Baseline resolution was attained using SMC on a 40 cm virtual column at a backpressure just over 36 kg/cm^2. With a standard HPLC system, a 40 cm column would yield a backpressure around 150 kg/cm^2, which is well above the maximum pressure limit of 100 kg/cm^2 for the Chiralpak AD columns. Another example is shown in Fig. 5 for another Wyeth discovery compound (Wy-B). The top chromatogram is the chiral separation on a 25 cm, 10 μm Chiralpak AS column. The second chromatogram from the top shows the separation using two 5 cm, 10 μm Chiralpak AS columns with SMC that yield a

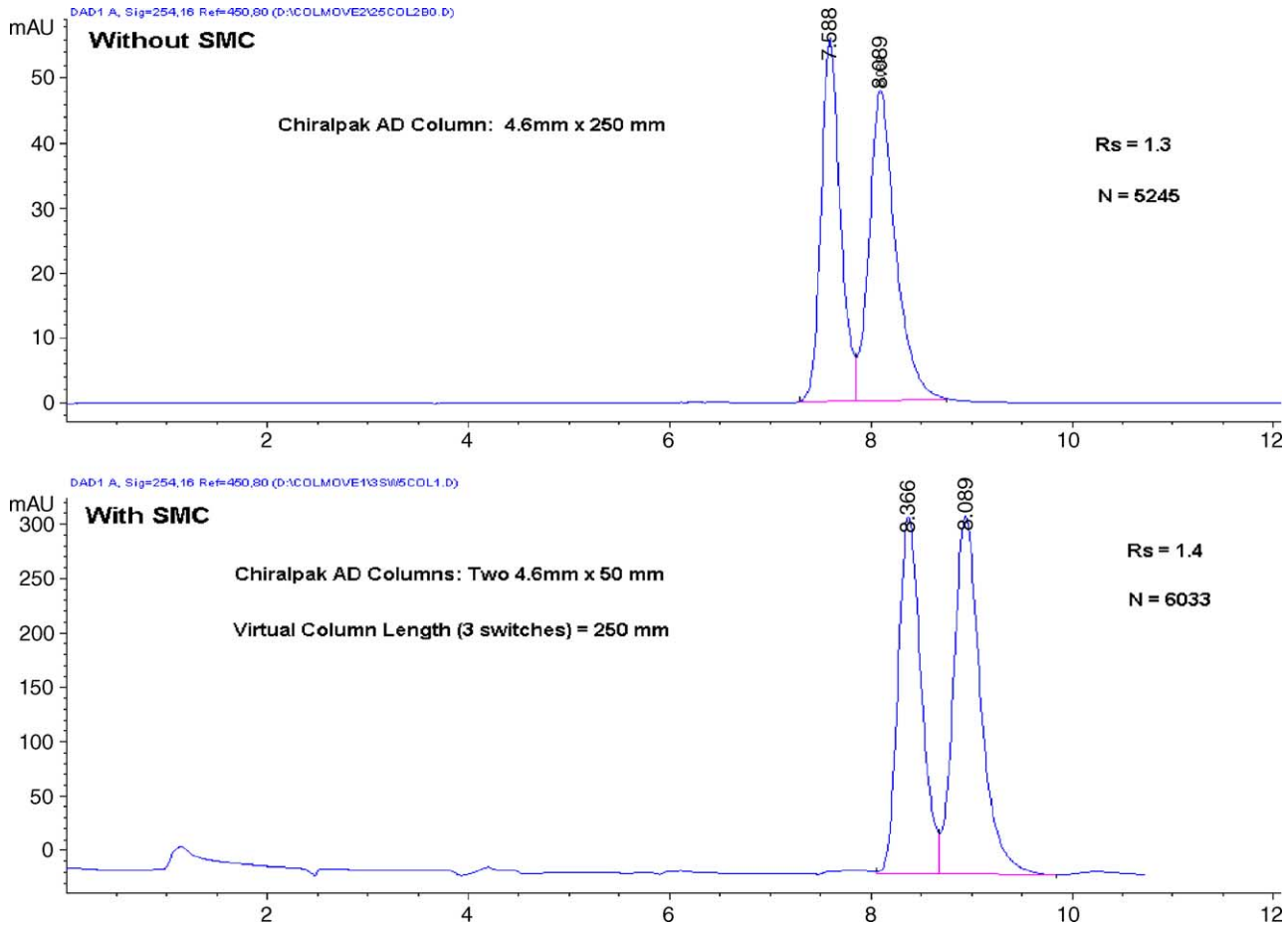

Fig. 8. Comparison of a chiral separation of racemate Wy-C on a 25 cm actual Chiralpak AD 10 μm column (top) and on virtual 25 cm columns generated by two 5 cm Chiralpak AD 10 μm columns.

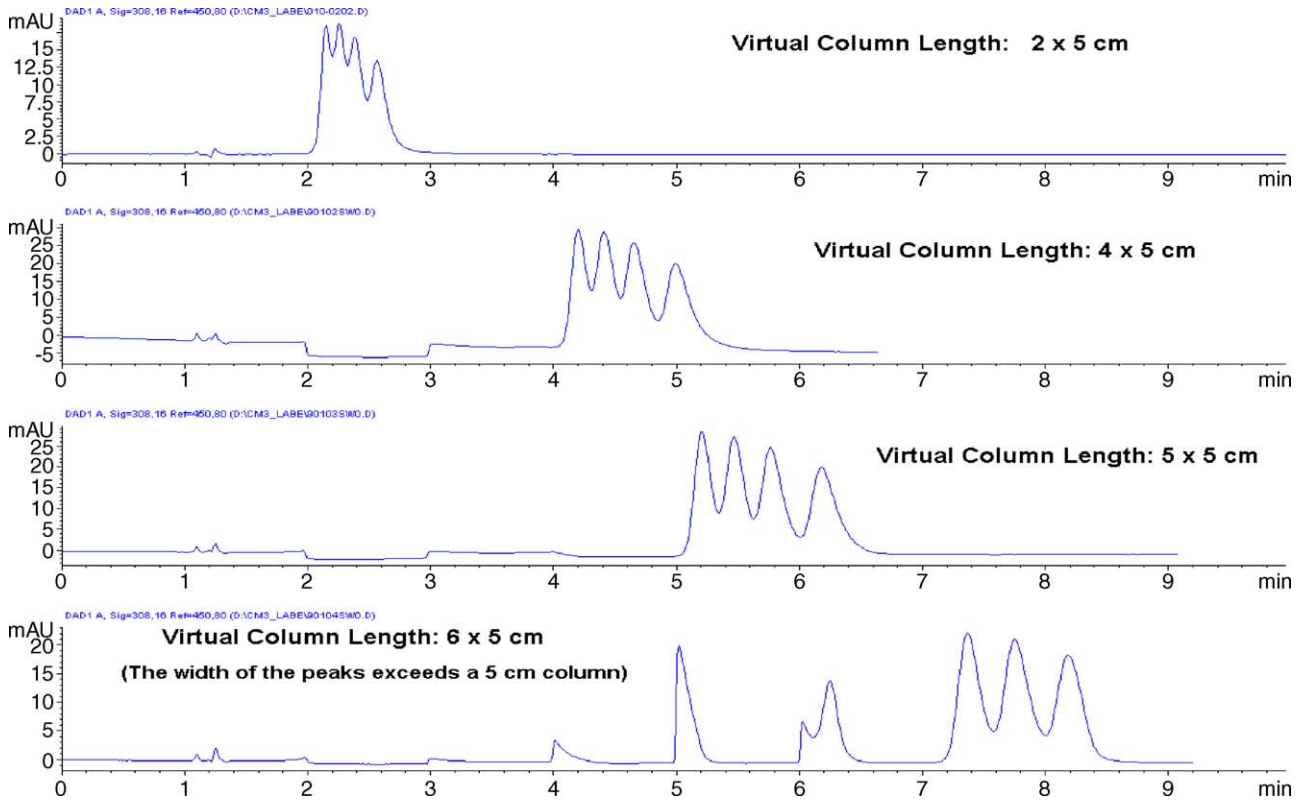

Fig. 9. Chiral separation of labetalol using two 5 cm Chirex 3022 5 μm columns with SMC. Resolution was improved from 10 (2 × 5) to 25 cm (5 × 5) virtual column length. The bandwidth after 25 cm column exceeded the 5 cm actual column length that adverse peak cutting by column moving. Mobile phase: hexane/dichloroethane/EtOH-TFA (20:1): 50%/32%/18%.

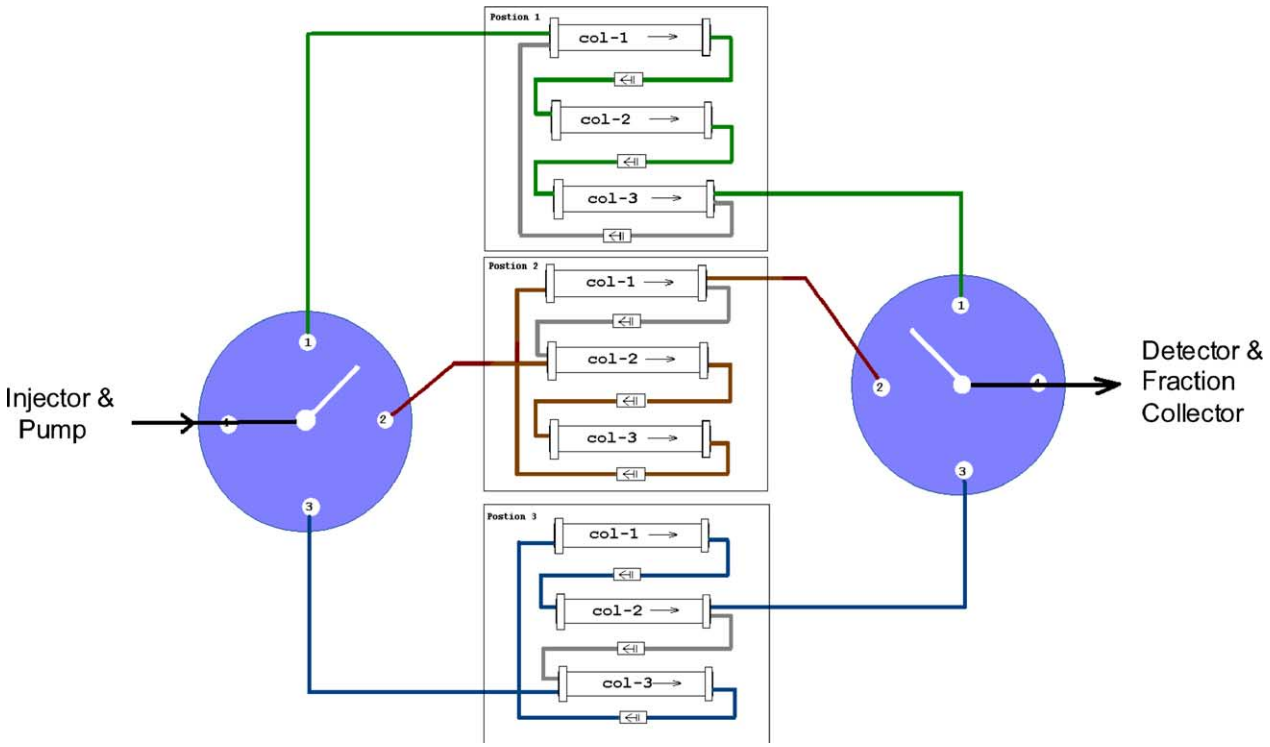

Fig. 10. SMC with three-column configuration. The three boxes in the middle show the flow path under the three valve positions. Three in-line low volume spring-loaded check valves after each column are used to control the flow path. The two valves are controlled simultaneously.

25 cm virtual column length. The two separations are very comparable, however, the backpressure is less than a half on the virtual 25 cm column versus that on the actual 25 cm column (34 versus 78 kg/cm^2). Moreover, using SMC, the chiral separation was further improved to baseline resolution with two additional column switches as shown in the LC/UV and LC/CD (circular dichroism) chromatograms at the bottom of Fig. 5. In fact, the examples illustrate that better resolution can be obtained using SMC with less chiral selector.

The chromatographic efficiency of each column used for SMC need not be identical. The top two chromatograms in Fig. 6 are the separations of Ketoprofen on two individual Chirex 3005 columns, and the column efficiency is significantly different due to a different history of usage. Nonetheless, both columns can be used for SMC as shown on the bottom chromatogram, and baseline resolution was obtained on the two columns with a 20 cm virtual column length.

3.4. Measurement and calculation of off-column band-broadening due to SMC

Off-column band broadening has been the major concern and drawback for the commonly used recycling techniques [9]. To assess the off-column band-broadening due to the SMC, we replaced the column with a zero dead volume connector, and injected a small quantity (1 μl) of a test compound on the HPLC system with and without a two-column SMC. We measured the peak width at half height for the systems with ($w_{1/2}^{w/smc}$) and without SMC ($w_{1/2}^{w/o\ smc}$) (see Fig. 7). The observed variance was calculated for the systems with SMC ($\sigma_{sys}^2 + \sigma_{smc}^2$) and without SMC ($\sigma_{sys}^2$) [16] as follows:

$$\sigma_{sys}^2 = \left(\frac{w_{1/2}^{w/o\ smc}}{2.35} \right)^2 \tag{3}$$

$$\sigma_{sys}^2 + \sigma_{smc}^2 = \left(\frac{w_{1/2}^{w/smc}}{2.35} \right)^2 \tag{4}$$

The variance due to the SMC alone was the difference of the observed variance:

$$\sigma_{smc}^2 = \left(\frac{w_{1/w}^{w/smc}}{2.35} \right)^2 - \left(\frac{w_{1/w}^{w/o\ smc}}{2.35} \right)^2 = (5.08 - 2.78) \times 10^{-5} = 2.3 \times 10^{-5} \tag{5}$$

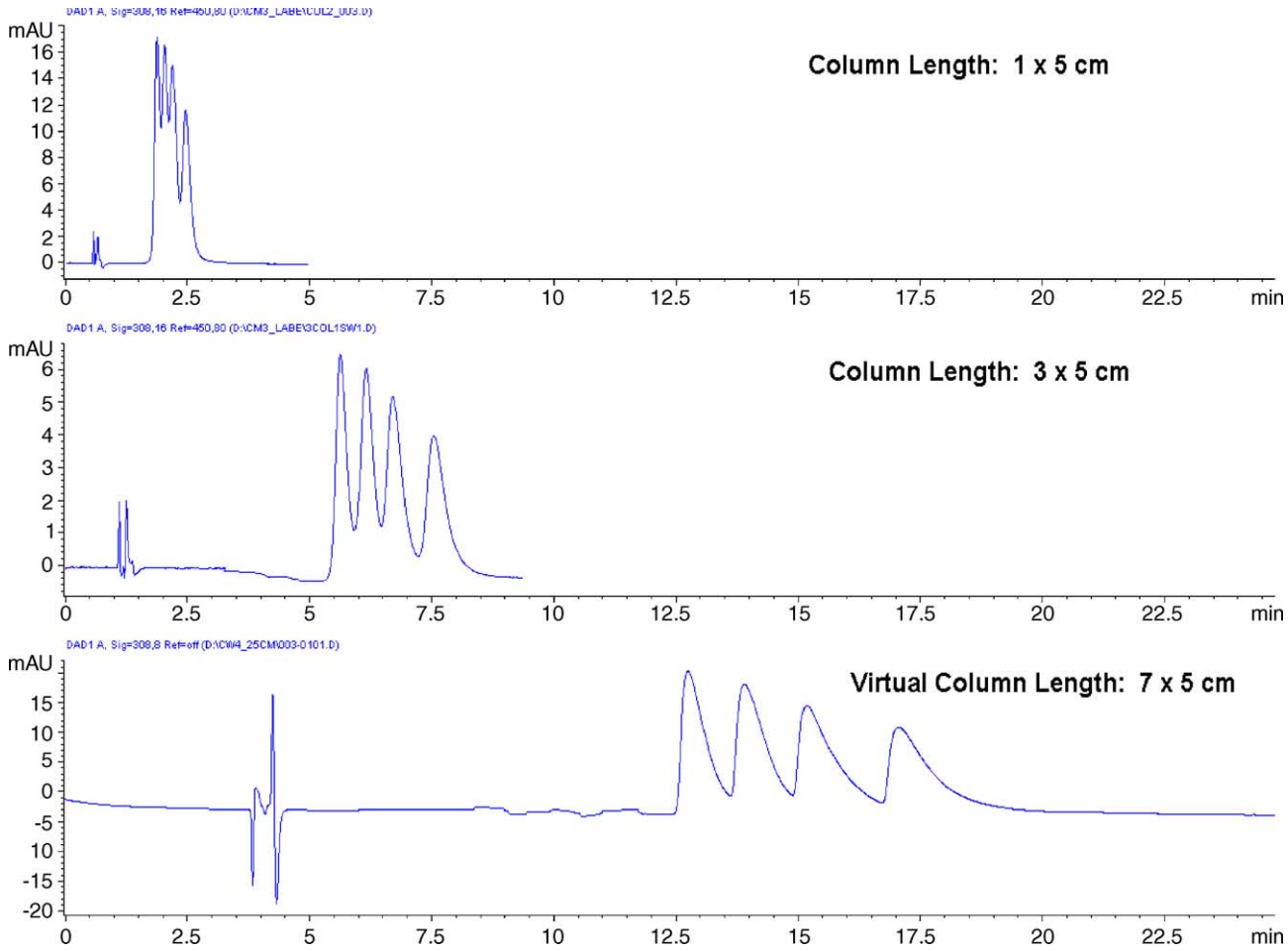

Fig. 11. The chiral separation of labetalol using a three-column SMC (bottom). Columns: three 50 mm × 4.6 mm Chirex 3022 5 μm columns, mobile phase: hexane/DCE/EtOH-TFA (20:1): 55%/35%/10%.

To translate this variance due to SMC to the loss of plate count (N) for a separation on a 50 mm × 4.6 mm column without SMC, we injected the test compound and obtained a Gaussian peak with $k = 2.8$, and plate number $N_{w/o\,smc} = 5.54 \times (t/W_{1/2})^2 = 3570$. By definition, the efficiency for a Gaussian peak is given in the following equations with and without SMC:

$$N_{w/smc} = \frac{V_r^2}{\sigma_{col}^2 + \sigma_{sys}^2 + \sigma_{smc}^2} \quad (6)$$

$$N_{w/o\,smc} = \frac{V_r^2}{\sigma_{col}^2 + \sigma_{sys}^2} \quad (7)$$

V_r, the peak volume, is calculated using Eq. (8), where ε is the column void fraction (~0.6), L and r is the column length and radius.

$$V_r = \pi r^2 L\varepsilon(1+k) = 3.14 \times 0.23^2 \times 5.0 \times 0.6 \times (1+2.8) = 1.89 \quad (8)$$

By rearranging the Eq. (7),

$$\sigma_{col}^2 + \sigma_{sys}^2 = \frac{V_r^2}{N_{w/o\,smc}} \quad (9)$$

Substituting Eq. (9) and the value of σ_{smc}^2 from Eq. (5) into Eq. (6), the plate number with SMC was calculated as:

$$N_{w/smc} = \frac{V_r^2}{(V_r^2/N_{w/o\,smc}) + \sigma_{smc}^2} = \frac{1.89^2}{(1.89^2/3570) + 2.3 \times 10^{-5}} = 3490 \quad (10)$$

The decrease in the plate number (from 3570 to 3490) that reflects the off-column band-broadening due to the SMC is only 2%. Therefore, the calculation indicates that SMC can be configured so that the off-column band-broadening due to SMC is insignificant. A comparison of a chiral separation on a 25 cm actual column and on a virtual 25 cm column generated by a 2–5 cm column SMC configuration is shown in Fig. 8 for Wyeth discovery compound, Wy-C. A slightly higher plate number was observed with the 25 cm virtual column length in this case, which may be attributed to the difference in the column packing between the 25 and 5 cm actual columns used.

3.5. Three-column SMC configuration and application

An attempt was also made to separate labetalol that has two chiral centers and four enantiomers using the two-column SMC system. As shown in Fig. 9, the chiral resolution was improved from the column length of 10 to 25 cm using two 5 cm, 5 μm Chirex 3022 columns with

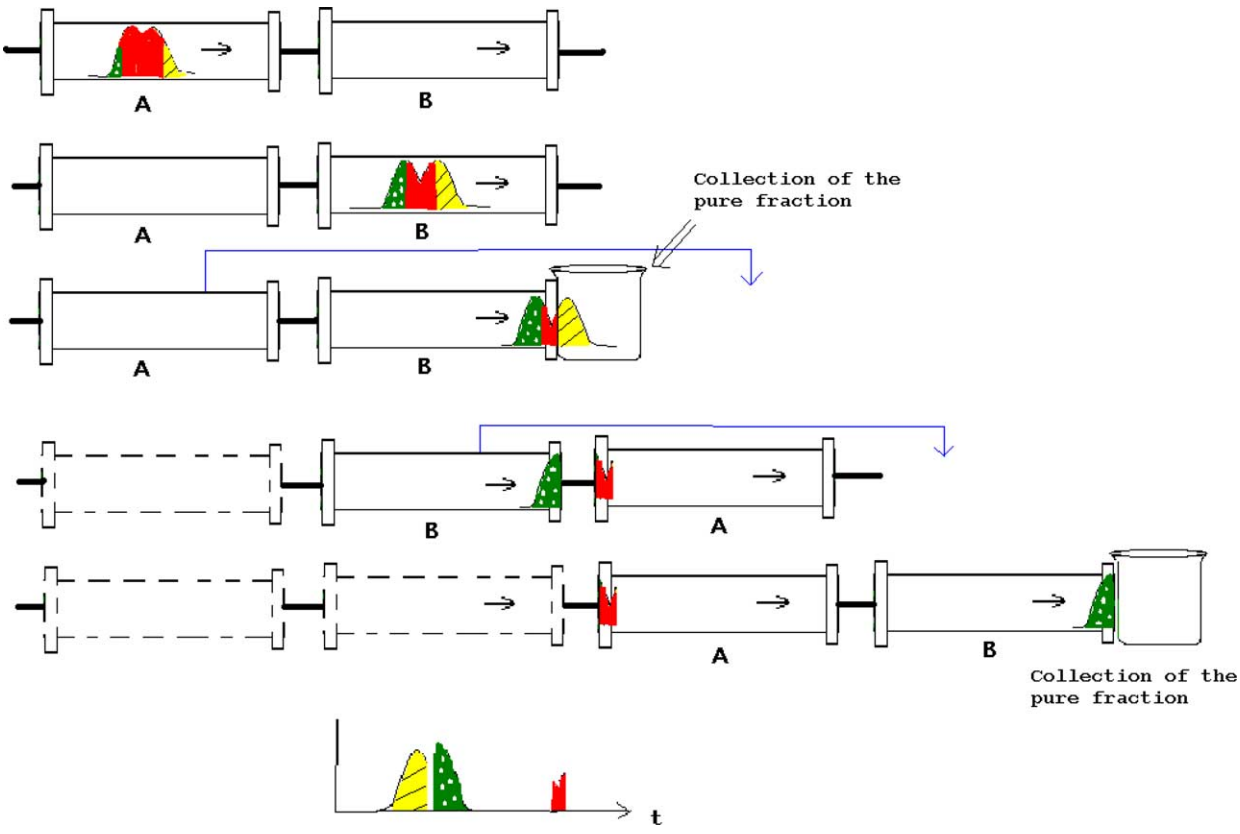

Fig. 12. SMC with peak shaving for preparative chiral HPLC. The pure front peak (lined potion) was collected followed by the column moving that allowed the mixed middle portion of the peaks eluting onto the new column. After the mixed fraction, the column is moved again to allow the pure back peak (doted portion) to elute out and be collected. The mixed fraction remained on the columns for further separation.

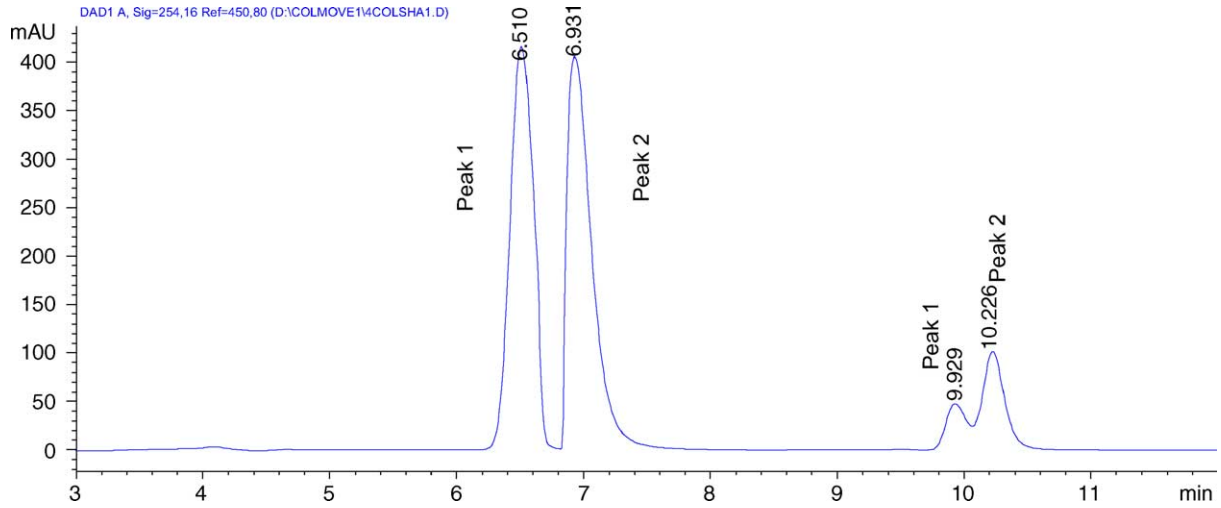

Fig. 13. The chromatogram from a SMC with peak shaving. The baseline between the first peak 1 and peak 2 is resulted from the column switching. The small partially resolved peak 1 and peak 2 are the mixed fraction that has remained on the columns to be further resolved.

SMC. However, additional column-moving resulted in unacceptable slicing of the peaks as shown on the bottom chromatogram of Fig. 9. Due to the on-column dispersion, the width of the four enantiomers exceeded the 5 cm column length after they cycled through the two 5 cm columns 2.5 times. Although using longer columns would obviate this situation, the advantages of the SMC technique (i.e. lower pressure and less costly chiral selector) would be diminished. We developed SMC with a three-column configuration as shown in Fig. 10. Two 4-position switching valves are mounted on a single multi-position microelectric valve actuator, and controlled simultaneously. Three in-line low volume spring-loaded check valves after each column are used to control the flow path. The chiral separation of labetalol was performed on the system. Baseline resolution was attained using the three-column SMC with virtual column length of 35 cm as shown in Fig. 11.

3.6. Application of SMC in preparative chiral HPLC

Not only is SMC useful for enhancing the chiral resolution in analytical and preparative chiral HPLC, but it could also have unique advantages for improving recovery, purity and throughput in preparative chiral HPLC by combining it with segmented recycling (shaving) [13] and steady-state intra-profiling injection techniques [10]. Fig. 12 illustrates a unique application of combining a two-column SMC with the shaving technique. As a partial chiral resolution is achieved for an enantiomeric pair, the peak profile can be divided into three portions: the front portion containing pure enantiomer, a mixed middle portion, and the back portion also containing pure enantiomer. As the partially separated enantiomers elute from column A to column B, the pure portion of the front peak (peak 1) is allowed to elute out of the column and collected under UV monitoring. Column A is then "moved" (switched) to the outlet of column B just before the mixed middle portion follows out of column B. After the mixed middle portion elutes onto column A from column B. Column B is "moved" after column A, and the pure back peak (peak 2) is allowed to elute out of the column and collected immediately after the column switch. In the end, the mixed middle part continues to be separated in the columns while the pure fractions of the enantiomers are collected. The chromatogram in Fig. 13 shows the UV trace of the process. Since the separation and collection of the pure fractions are controlled by the column switching, high purity of the fractions can be assured. High recovery is also guaranteed, as the mixed portion is recycled in the column (and can be combined with new feed by making injections on top of the mixed portion during the column switching).

4. Conclusion

A novel column switching technique called "simulated moving columns (SMC)" with two and three column configurations was developed to increase the effective column length without increasing backpressure. The set-up of SMC, especially two-column SMC, is very simple, and the column switching can be easily automated. SMC was demonstrated to enhance chiral resolution and separation efficiency for analytical chromatography, and has the potential to improve performance in semi-preparative chromatography. Since SMC allows complete chiral resolution of enantiomers that are only partially separated on a column, a partial resolution that is obtained from column and solvent screening can be used without further optimization. Thus, rapid chiral method development followed by immediate scale-up to preparative HPLC is possible with SMC. In addition, since SMC is an effective chromatographic recycling technique that multiplies the column utilization, less chiral selector is required to achieve complete chiral resolution for difficult

enantioseparations. Although cost savings on an analytical scale might be minor, the potential cost benefit for preparative HPLC is significant. Development of the technique continues for semi-preparative chiral HPLC to perfect injection of new feeds on top of the mixed fraction. Using SMC for supercritical fluid chromatography (SFC) is also being studied. Since SFC is fast and provides narrower peaks, more rapid and substantial improvement in chiral resolution can be expected using SMC.

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References

[1] N.M. Maier, et al., J. Chromatogr. A 906 (2001) 3.
[2] E.R. Francotte, J. Chromatogr. A 906 (2001) 379.
[3] T.E. Beesley, R.P.W. Scott, Chiral Chromatography, Wiley, Chichester, 1998.
[4] E. Francotte, in: H.Y. Aboul-Enein, I.W. Wainer (Eds.), The Impact of Sereochemistry On Drug Development and Use, Chemical Analysis Series, vol. 142, Wiley, New York, 1997 (Chapter 23).
[5] C.J. Welch, M.N. Protopopova, G. Bhat, Enantiomer 3 (1998) 471.
[6] Y. Zhao, et al., J. Chromatogr. A 1003 (2003) 157.
[7] M.E. Andersson, et al., J. Chromatogr. A 1005 (2003) 83.
[8] G. Guiochon, S.G. Shirazi, A.M. Katti. Fundamentals of Preparative and Nonlinear Chromagraphy, Academic Press, Boston, MA, 1994.
[9] F. Charton, M. Bailly, G. Guiochon, J. Chromatogr A 687 (1994) 13.
[10] C.M. Gill, J. Chromatogr. A 796 (1998) 101.
[11] I. Quinones, C.M. Grill, L. Miller, G. Guiochon, J. Chromatogr. A 867 (2000) 1.
[12] D.B. Broughton, R.W. Neuzil, J.M. Pharis, C.S. Brearley, Chem. Eng. Prog. 66 (9) (1970) 70.
[13] P.C. Wankat, Ind. Eng. Chem. Fundam. 23 (1984) 256.
[14] M. Agosto, N.L. Wang, P.C. Wankat, Ind. Eng. Chem. Res. 28 (1989) 1358.
[15] L.R. Snyder, in: C. Horvath (Ed.), High-Performance Liquid Chromatography—Advances and Perspectives, vol. 1, Academic Press, New York, 1980, p. 207.
[16] I. Chappell, R.J. Weigand, T.J. Zuzelski, C. Jerslld, LCGC Current Trends and Developments in Drug Discovery, vol. 18, Number 55, May 2000.