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Applications of simulated moving-bed chromatography to the separation of the enantiomers of chiral drugs

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Abstract

Although most preparative chiral separations have been performed in the conventional batch-mode process, interest in simulated moving-bed (SMB) chromatography is growing, because it permits large amounts of mobile phase to be saved and productivity increased, thus reducing production costs. Based on the examples of three different drugs, the usefulness of this technique for the separation of enantiomers on a preparative scale has been demonstrated. Compared to the batch elution chromatography, a reduction of the mobile phase consumption of respectively 81% and 84% has been achieved for the separation of the enantiomers of the antiasthmatic agent formoterol and of the antitussive agent guaifenesin. Furthermore, a higher throughput could be reached under SMB conditions. The influence of feed rate and extract on the separation has also been investigated. The results show that these two factors considerably affect purity and productivity, and constitute important parameters for finely adjusting the chromatographic conditions depending on the requirements. For all racemates, both enantiomers could be obtained with a purity ranging between 99 and 99.9%.

Keywords: Simulated moving-bed chromatography; Enantiomer separation; Preparative chromatography; Pharmaceutical analysis; Guaifenesin; Aminogluthethimide; Formoterol

1. Introduction

Chiral considerations are now integral parts of drug research and development and of the regulatory process. In this context, chiral chromatography has become the most important analytical tool for determining the optical purity of organic molecules. Thanks to the concomitant development of a wide range of chiral stationary phases (CSPs) and the introduction of new chromatographic instrumentation, the chromatographic separation of enantiomers on a preparative scale is also gaining increasing acceptance as a simple, rapid and generally applicable method for supplying pure enantiomers of bioactive compounds and chiral synthons [1].

Up to now, elution batch chromatography clearly dominates in terms of the number of applications, and various approaches were used for improving throughput, such as close injections, peak-shaving and recycling [1–3], but large-scale separations would require large amounts of CSP and have been considered economically unjustifiable because of the high cost of the CSPs, the high dilution conditions, the consumption of large amounts of mobile phase and the difficulties associated with recycling it. However, with the recent introduction of the simulated moving-bed (SMB) technology in this particular application field [4–19], the technical prerequisites for the performance of such large-scale separations under cost-effective conditions can now

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be met. This chromatographic mode can save up to 90% of the mobile phase and achieves a much higher throughput.

2. Experimental

Guaifenesin was purchased from Aldrich, Orimeten (aminoglutethimide) and Foradil (formoterol) are from Ciba. The feed was prepared by dissolving the racemate in the respective mobile phase. The solvents *n*-heptane (99%) and ethanol were of HPLC grade.

The chromatography was performed at 23°C using a SMB-Unit Sorbex Prep (UOP, Des Plaines, USA) equipped with 16 columns arranged in series. Two sets of columns were used: (a) each column (6×1.6 cm, Knauer, Berlin, Germany) containing 5.5 g of the chiral stationary phase Chiralcel OJ (Daicel, Tokyo, Japan), 20 μm; the total volume and amount of stationary phase was respectively 193 ml and 88 g; (b) each column (6×2.1 cm, Macherey–Nagel, Oensingen, Switzerland) containing 12.56 g of the chiral stationary phase Chiralcel OD (Daicel), 20 μm; the total volume and amount of stationary phase was respectively 333 ml and 201 g. The initial chromatographic data were determined on an analytical HPLC column Chiralcel OJ or Chiralcel OD (25 cm×0.40 cm) filled with the same preparative material (20 μm).

Analytical determinations of the purity of extract and raffinate were performed with analytical HPLC columns (25×0.46 cm) Chiralcel OD and Chiralcel OJ (10 μm) using a HPLC system consisting of a Shimadzu LC-10AD pump, a variable-wavelength Shimadzu SPD-10A UV–visible detector, a SIL-10A auto injector fitted with a 20-μl sample loop and a CBM-10A communication module connected to an Epson personal computer for managing the data.

3. Results and discussion

In the usual batch elution chromatography the sample is injected on the top of the column and the components separate after a certain time by moving through the column under the driving force of the mobile phase. However, this process is not very efficient because during the chromatography only a small part of the whole stationary phase is used for separation. A possibility to improve the packing utilization, is given by the moving bed chromatography principle. This chromatographic process was first introduced in the late 1960s by the Universal Oil Product Company [20–22] and was intensively investigated by Ching and co-workers [6,13,23–26]. According to this concept, it is not only the mobile phase which is moving but the solid-phase is also moving. Now, for a given mobile phase rate, there is a rate of the solid-phase in the opposite direction which should allow that the faster eluting compound (raffinate) continues to move in the liquid direction and the slower eluting compound (extract) elutes in the opposite direction. Under these conditions, the entire mass of the solid-phase contributing to the separation is continuously used, thus improving considerably the productivity of the system. Obviously, this principle is particularly suitable for a binary mixture and racemates are ideal binary mixtures.

However, it is technically difficult to move a solid-phase, and to solve this problem the movement is simulated. In fact, the process is continuous, as it would be in a cyclic column, but in practice the system consists of a number of small columns arranged in series, as shown schematically in Fig. 1. Now, if the injection and collection points are regularly changed, the net result is the same as it would be if the stationary phase were moving.

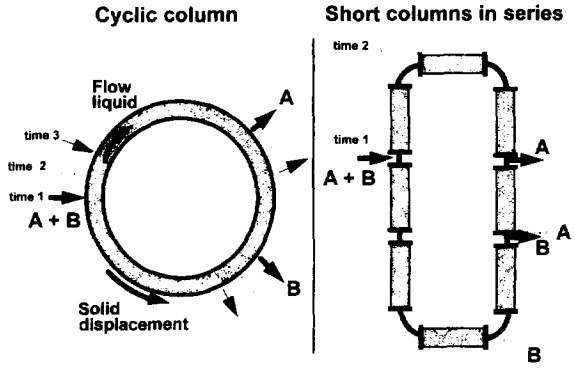
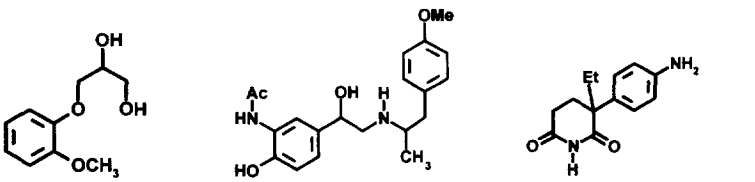


Fig. 1. Column disposition in the simulated moving bed chromatography.



Guaifenesin Foradil® (formoterol) Orimeten® (aminoglutethimide)

Fig. 2. Structure of the racemic drugs.

3.1. Separation of the enantiomers of guaifenesin

3.1.1. Analytical data

The initial chromatographic results obtained for the separation of the enantiomers of the antitussive agent guaifenesin (Fig. 2) on an analytical Chiralcel OD [27] column (10 μm) using heptane–ethanol (80:20) as a mobile phase are summarized in Table 1 and the analytical chromatogram is shown on Fig. 3a. On the analytical column filled with the preparative material (20 μm), as expected the resolution and the selectivity are lower than on the column containing the small particle size material (Table 1 and Fig. 3b). In order to adjust the value of the capacity factors to the recommended ones for performing SMB chromatography, the mobile phase composition has been changed to heptane–ethanol (65:35) (Table 1). Indeed, it is usual that an increase of the content

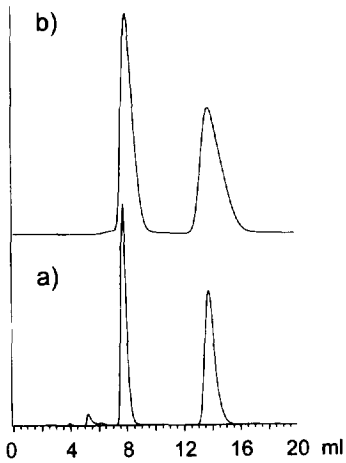


Fig. 3. Chromatographic separation of the enantiomers of guaifenesin on Chiralcel OD; (a) column 25×0.46 cm; flow-rate 1 ml/min; particle size, 10 μm; (b) column 25×0.40 cm; flow-rate 0.7 ml/min; particle size, 20 μm.

Table 1
Chromatographic data of the separation of the enantiomers of guaifenesin, formoterol and aminoglutethimide on cellulose-based CSPs

Racemic drug	CSP	Column[a]	Particle size (μm)	Mobile phase	k_1'[b]	k_2'	α	R_s
Guaifenesin	Chiralcel OD	A	10	Heptane–ethanol (80:20)	0.57 (*R*)(−)	1.72	3.02	5.98
Guaifenesin	Chiralcel OD	B	20	Heptane–ethanol (80:20)	0.63 (*R*)(−)	1.69	2.68	2.29
Guaifenesin	Chiralcel OD	B	20	Heptane–ethanol (65:35)	0.47 (*R*)(−)	1.14	2.43	1.79
Formoterol	Chiralcel OJ	A	10	Heptane–ethanol (70:30)	1.38 (*RR*)(+)	2.00	1.45	1.07
Formoterol	Chiralcel OJ	B	20	Heptane–ethanol (70:30)	0.78 (*RR*)(+)	1.03	1.31	0.39
Formoterol	Chiralcel OJ	B	20	Heptane–ethanol (80:20)	2.25 (*RR*)(+)	3.23	1.43	0.66
Aminoglutethimide	Chiralcel OJ	A	10	Hexane–ethanol (20:80)	2.00 (*R*)(+)	5.14	2.57	5.45
Aminoglutethimide	Chiralcel OJ	B	20	Heptane–ethanol (15:85)	1.00 (*R*)(+)	2.07	2.07	1.73

[a] Column A, 25×0.46 cm; column B, 25×0.40 cm.
[b] Absolute configuration and sign of the optical rotation (λ 365 nm) of the first eluted enantiomer.

of the alcohol modifier causes a reduction of the capacity factors.

Based on these analytical data, the different starting parameters (feed rate, mobile phase rate and extract rate) were calculated using the UOP initialization software which does not necessarily require the previous determination of the adsorption isotherms. The following values were determined: feed rate, 0.2 ml/min; mobile phase rate, 5.71 ml/min; extract rate, 2.5 ml/min.

3.1.2. Results

The feed concentration has been set to 3% which corresponds to a value slightly below the limit of solubility of the racemate in the mobile phase at 23°C. Applying the starting parameters determined above and operating at a cycle time of 120 min, a production of 4.32 g of each enantiomer per day has been obtained with a purity of 99.1% for the extract and 99.9% for the raffinate. By extrapolation, this corresponds to a production of 21.5 g of each enantiomer per kg of CSP per day.

Influence of feed rate on productivity and on purity

In order to increase the productivity, maintaining a high purity, the influence of the feed rate on purity has been determined, operating at constant mobile phase rate (5.71 ml/min) and extract rate (2.5 ml/min). Fig. 4 shows this influence and clearly demonstrates that at the highest values of purity, the productivity is strongly dependent on the desired purity. For the given configuration (cycle time, mobile phase rate, extract rate) we did not observe a dramatic change of the extract purity when varying the feed rate in the range of 0.2 to 0.7 ml/min, which corresponds to a change of productivity of 3.5 times. By contrast, the purity of the raffinate is strongly affected from a feed rate greater than 0.5 ml/min and reaches only 74.8% at a feed rate of 0.7 ml/min.

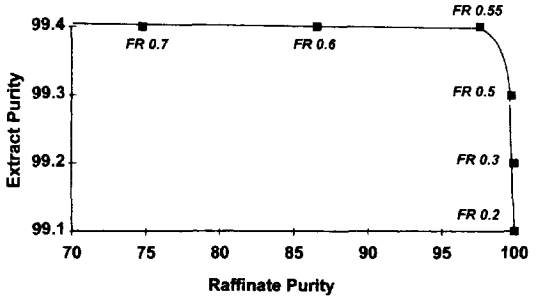


Fig. 4. Separation of the enantiomers of guaifenesin on Chiralcel OD by SMB. Influence of feed rate (*FR* in ml/min) on purity: mobile phase rate 5.71 ml/min, extract rate 2.5 ml/min, cycle time 120 min.

By reducing the mobile phase rate from 5.71 ml/min to 5.3 ml/min it was still possible to reduce the mobile phase consumption by 7% without affecting the purity of the extract (99.4%) and of the raffinate (99.7%).

Reduction of the cycle time also results in an increase of throughput. Cycle time has been reduced to 80 min by respectively increasing the mobile phase rate, the feed rate and the extract rate to 7.85, 0.75 and 3.05 ml/min, maintaining the feed concentration at 3%. Under these conditions, a production of 16.2 g of each enantiomer per day could be reached. The purity of the enantiomers was 99.4% [enantiomeric excess (ee) 98.8%] for the (*S*)(+) enantiomer (extract) and 99.6% (ee 99.2%) for the (*R*)(−) enantiomer (raffinate). By extrapolation, this corresponds to a production of 80.6 g of each enantiomer per kg of CSP per day. The mobile phase consumption was 380 ml per gram of injected racemate and corresponds to a reduction of more than 84% compared to the elution batch chromatography (Table 2).

Influence of extract rate on purity

Furthermore, we determined that the purity of both enantiomers can strongly be influenced by variation of the extract rate (Fig. 5). At a constant feed rate of 0.75 ml/min and a mobile phase rate of 7.55 ml/min (cycle time, 80 min), an increase in the extraction rate shifts the purity of the extract to lower values whereas the purity of the raffinate is increased. For example, an increase of the extraction rate from 3.35 ml to 3.55 ml/min shifts the purity of the raffinate from 99.5% to 99.6% (slight increase of only 0.1%) but that of the extract drops from 90% to 72% (an appreciable decrease) (Fig. 5). A change of the extract rate from 2.75 ml/min to 3.05 ml/min allows the purity of the raffinate to be improved (0.2%) without affecting the purity of the extract. Obviously, change of the extract rate also influences the productivity of extract and of raffinate.

Table 2
Results of the chromatographic separations of the enantiomers of various chiral drugs under batch and SMB conditions

Racemic drug	Formoterol		Guaifenesin		Aminoglutethimide	
	Batch[a]	SMB	Batch[a]	SMB	Batch	SMB
Feed concentration (g/l)	20	2.5	30	30	–	16.3
Amount CSP (g)	1700	88	12.5	201	–	88
Mobile phase rate (ml/min)	150	6.69	3.7	7.55	–	6.00
Feed rate (ml/min)		0.52		0.75		0.45
Injected amount (g/h/kg CSP)	0.196	0.886	3.63	6.72	–	5
Cycle (SMB) resp. run (batch) time (min)	360	90	115	80	–	90
Production of each enantiomer g/day/kg CSP	1.68	10.63	30	80.6	–	60
Mobile phase needed to resolve 1 g of racemate (l)	27	5.15	2.4	0.38	–	0.74
Extract purity (%)	99.7	98.9	99.5	99.4	–	99.8
Raffinate purity (%)	100	100	99.6	99.6	–	99.9

[a] Calculated for 24 h running.

Both Figs. 4 and 5 demonstrate that the productivity is strongly dependent on the desired purity and this can be easily controlled by finely adjusting some parameters such as the feed rate and the extract rate.

3.2. Separation of the enantiomers of formoterol and of aminoglutethimide

Further examples of chiral separations performed in our laboratories under SMB conditions have confirmed the high throughput and the considerable reduction of the mobile phase consumption associated to the SMB technique. The results obtained after about 3 days of optimization for the anticancer drug Orimeten (aminoglutethimide) and the antiasthmatic drug Foradil (formoterol) (Fig. 2) on Chiralcel OJ using a set of sixteen 6×1.6 cm columns are summarized in Table 2. For formoterol the feed concentration was 0.25%. This concentration is rather low, but it corresponds to the saturation concentration of the substance in the mobile phase heptane–ethanol (70:30). This low solubility is also a critical issue in the batch chromatography. Operating at a feed rate of 0.52 ml/min, and a mobile phase rate of 6.69 ml/min, a production of 0.93 g of

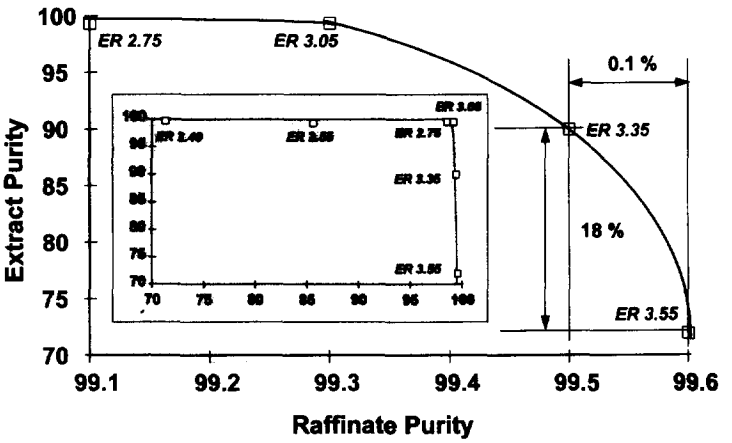


Fig. 5. Separation of the enantiomers of guaifenesin on Chiralcel OD by SMB. Influence of extract rate (*ER* in ml/min) on purity (full scale in the dark frame): mobile phase rate 7.55 ml/min, feed rate 0.75 ml/min, cycle time 80 min.

each enantiomer per day has been reached with a purity of 98.9% for the (*SS*)(−) enantiomer (extract) and 99.9% for the (*RR*)(+) enantiomer (raffinate). By extrapolation, this corresponds to a productivity of 10.6 g of each enantiomer per kg of CSP per day. This is 6.3 times better than the batch-elution, with a reduction of the mobile phase consumption of 81%. In this case also we observed a marked change of the purity of both the extract and the raffinate upon variation of feed rate (Fig. 6). At the highest values of purity, the productivity is strongly dependent on the desired purity. Operating at a constant extract rate of 2.22 ml/min, an increase of the purity of the raffinate from 92.6% to 99.9% (7.3% difference) has to be compensate by a decrease of feed rate from 0.43 ml/min to 0.32 ml/min which corresponds to a decrease of productivity of about 25%. Another critical point concerns the dependence of purity versus extract rate. At a constant feed rate of 0.39 ml/min, a slight increase of the extract rate from 2.22 ml/min to 2.39 ml/min shifts the purity of the raffinate from 94.3% to 97.2% (appreciable increase) but that one of the extract remains unchanged at 99.9% (Fig. 7). By further increase of the extract rate to 2.55 ml/min, the purity of the raffinate still improves close to 99.9% whereas the purity of the extract dramatically drops to 73.2%. These data show again that both feed rate and extract rate can be finely adjusted in order to optimize purity and throughput depending on the requirements.

The enantiomers of aminoglutethimide were also separated using the same set of columns, applying a

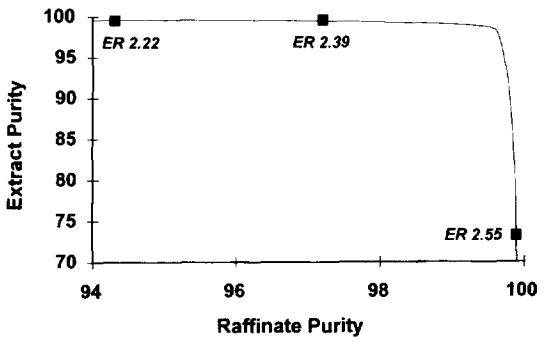


Fig. 7. Separation of the enantiomers of formoterol on Chiralcel OJ by SMB. Influence of extract rate (*ER* in ml/min) on purity: mobile phase rate 4.76 ml/min, feed rate 0.39 ml/min, cycle time 120 min.

feed concentration of 1.63% in a mixture of hexane–ethanol (15:85) used as the mobile phase. For a feed rate of 0.45 ml/min, and a mobile phase rate of 6 ml/min, a production of 5.27 g of each enantiomer per day has been obtained with a purity of 99.8% for the (*S*)(−) enantiomer (extract) and 99.9% for the (*R*)(+) enantiomer (raffinate). By extrapolation, this corresponds to a productivity of 59.9 g of each enantiomer per kg of CSP per day. Similarly to the two previous cases, a considerable change of the purity of extract and raffinate was observed when varying the extract rate from 2.02 ml/min to 2.55 ml/min, maintaining the feed rate at a constant value of 0.34 ml/min (Fig. 8). In this instance also, there is an optimal extract rate between 2.24 ml/min and 2.30 ml/min, allowing both enantiomers to be

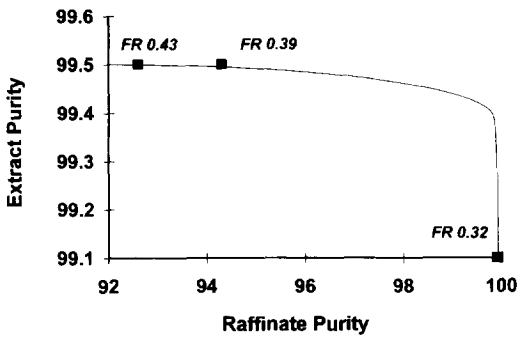


Fig. 6. Separation of the enantiomers of formoterol on Chiralcel OJ by SMB. Influence of feed rate (*FR* in ml/min) on purity: mobile phase rate 4.76 ml/min, extract rate 2.22 ml/min, cycle time 120 min.

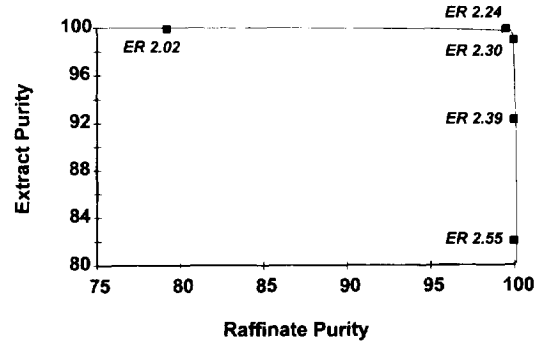


Fig. 8. Separation of the enantiomers of aminoglutethimide on Chiralcel OJ by SMB. Influence of extract rate (*ER* in ml/min) on purity: mobile phase rate 4.5 ml/min, feed rate 0.34 ml/min, cycle time 120 min.

isolated with a high purity. At lower extract rate, the purity of the raffinate greatly decreases while that one of the extract remains practically unchanged. Inversely, at higher extract rate, the purity of the extract considerably decreases and that one of the raffinate further increases only slightly.

4. Conclusion

The high efficiency of the SMB chromatographic mode in terms of throughput and of consumption of mobile phase when applied to the separation of the enantiomers of chiral drugs has been clearly demonstrated. In all cases, more than 80% of the mobile phase could be save compared to the batch elution chromatography. This advantage is particularly valuable for poorly soluble compounds such as formoterol. Moreover different parameters such as feed rate and extract rate could be finely adjusted in order to optimize purity and throughput depending on the requirements and on the desired enantiomer. In all studied cases, it was found that for a constant feed rate there is an optimal value of extract rate yielding both enantiomers with a high purity. Below this value the purity and the productivity of the extract are increasing and inversely for the raffinate. Above this value it is the purity and the productivity of the raffinate which are increased whereas both are decreasing for the extract.

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