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Preparative resolution of praziquantel enantiomers by simulated counter-current chromatography

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ABSTRACT

The continuous chromatographic separation of a racemic anthelmintic drug, praziquantel, was carried out using a simulated counter-current system. The system consists of four identical columns (445 mm x 12.5 mm I.D.) connected in series through solenoid valves. The chiral stationary phase used is microcrystalline cellulose triacetate and the eluent is methanol. Feed at 50 mg/ml was continuously introduced into the system at 0.3 ml/min and 429 mg/h of (+)-praxiquantel and 404 mg/h of (-)-praziquantel were obtained from the extract and raffinate streams, respectively. The optical purity of the products was more than 90%. This method provides an extremely useful technique for preparative-scale enantioseparation. Compared with conventional batch preparative-scale processes, this system offers a higher solute to adsorbent mass ratio.

INTRODUCTION

In recent years, there has been an increasing trend towards restricting the use of chiral drugs as racemates. This has created a demand for preparative-scale techniques for the separation of the enantiomers for pharmaceutical applications. Classical methods of optical resolution, based on recrystallization of diastereomeric salts, are not suitable for industrial scale-up and automation [1]. A more promising technique which has attracted attention recently is the resolution of enantiomers by liquid chromatography, using chiral stationary phases. Several semi-preparative- or preparative-scale batch chromatographic processes based on this direct resolution principle have been developed for the separation of enantiomeric drugs. Although the process is relatively simple and offers operating flexibility, it suffers from the following disadvantages: the whole sorbent bed is not effectively utilized and large amounts of expensive adsorbents are required; a large amount of diluent is consumed, resulting in undesired dilution of the products; and the operation is discontinuous, which makes it difficult to integrate it with other continuous processes.

In this paper, a continuous chromatographic separation of an anthelmintic racemic drug, praziquantel, based on a simulated counter-current system is reported. Praziquantel (Fig. 1)

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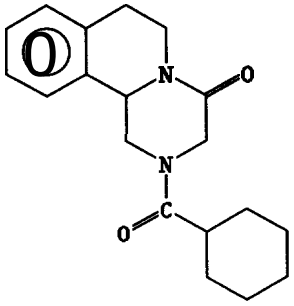

Fig. 1. Structure of praziquantel ($C_{19}H_{24}N_2O_2$).

was recommended as the drug of choice in the treatment of the parasitic disease schistosomiasis in 1977 [2]. Preliminary studies have shown that the primary therapeutic effect of praziquantel resides in its *levo*-isomer. *levo*-Praziquantel has the advantage of high efficacy and low toxicity compared with *rac*-praziquantel [3]. To the best of our knowledge, no preparative-scale chromatographic resolution of praziquantel enantiomers has been reported. In one of the methods reported, the enantiomers were obtained via resolution of an intermediate during the synthesis of praziquantel [4]. However, no details of this method have been elaborated.

A simulated counter-current system retains the main advantage of an equivalent counter-current system where the average driving force is maximized, thus increasing the efficiency with which the adsorbent is utilized. Circulation of the solid adsorbent can be simulated using a multiple column fixed-bed system with an appropriate sequence of column switching, in which effective counter-current operation is achieved by moving sequentially the feed, eluent and draw-off points through the bed in the direction of fluid flow. In this way the adsorbent is seen to be in effect moving counter-current to the fluid flow direction. With sufficiently small elemental beds switched with appropriate frequency such a system indeed becomes a perfect analogue of a counter-current flow system. More detailed information on this separation technique and its design principle can be found in a review by Ruthven and Ching [5].

EXPERIMENTAL

Chemicals

The adsorbent used was microcrystalline cellulose triacetate (MCTA) (Merck, Darmstadt, Germany) with a particle size of 25–40 μm. Prior to packing, the adsorbent was allowed to swell in boiling ethanol [6]. It was packed into the column using the slurry method via a res-

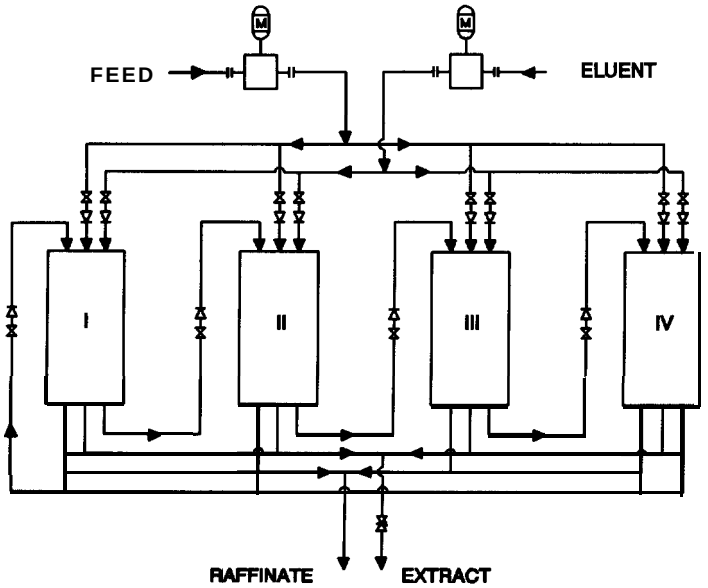

Fig. 2. Schematic diagram of the continuous simulated counter-current system.

ervoir and at a constant flow-rate of 6 ml/min using an HPLC pump (Model 2510, Varian, Palo Alto, CA, USA). The diluent was HPLC-grade methanol (Fisher Scientific, Pittsburgh, PA, USA). The feed was prepared by dissolving *rac*-praziquantel (Sigma, St. Louis, MO, USA) in methanol (50 mg/ml).

Instrumentation

A schematic diagram of the simulated counter-current system which consists of four chromatographic columns is shown in Fig. 2. The columns are made of stainless steel and have dimensions of 445 mm x 12.5 mm I.D. The columns are connected in series through solenoid valves that allow the introduction of feed and withdrawal of products in addition to providing the transfer of the streams. Counter-current contact between the solid and the fluid phases was simulated by switching the feed and eluent inlets and product withdrawal points, *i.e.,* by opening and closing specific groups of valves, at fixed time intervals in the direction of liquid flow. Operation of the solenoid valves was governed by a programmable logic controller. The schematic flow diagram of the four stages in a cycle of the system is shown in Fig. 3.

The flow-rates of the feed and eluent were controlled by two solvent metering pumps (Varian Model 2510). Flow meters were installed at the outlet of extract and raffinate streams to monitor the extract and raffinate flow-rates. A needle valve was also installed at the outlet of extract stream to control the flow-rate further.

The concentrations of the extract and raffinate streams for each stage were analysed using a standard analytical liquid chromatographic system. An analytical Chiralcel OD column [cellulose tris(3,5-dimethylphenylcarbamate) polymer absorbed on 10-μm macroporous silica, 250 mm x 4.6 mm I.D.] (Daicel Chemical Industries, Tokyo, Japan) was used. The mobile phase was a mixture of HPLC-grade hexane and 2-propanol (80:20) (Fisher Scientific). The solvent-delivery system was a high-pressure liquid chromatographic pump (Model LC-9A, Shimadzu, Tokyo, Japan) and sample injection was performed using a syringe loading valve (Model 7125, Rheodyne, Cotati, CA, USA) fitted with a 10-μl sample loop. The eluting enantiomers were monitored with a Varian Model 2070 spectrofluorimeter with excitation and emission wavelengths set at 270 and 300 nm, respectively.

Measurement of the optical rotation of the separated enantiomers showed the more adsorbed component (extract) to be (+)-praziquantel and the less adsorbed component (raffinate) (-)-praziquantel. The eluting sequence of the enantiomers on the analytical Chiralcel OD column was the same as that on MCTA.

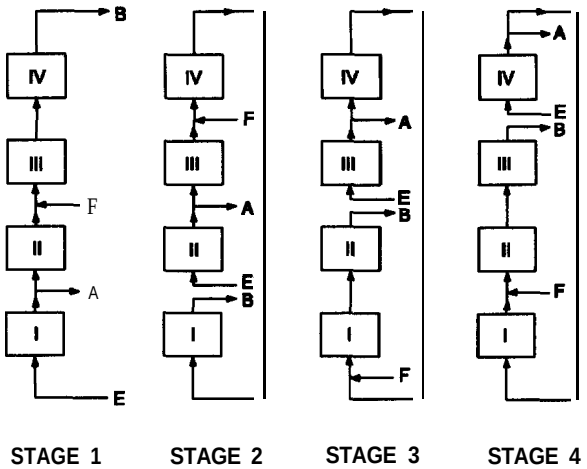

Fig. 3. Schematic flow diagram of the four stages in a cycle of the system. **E = Eluent; F= feed;** A = **extract; B = raffinate.**

RESULTS AND DISCUSSION

The chromatogram for the separation of *rac*-praziquantel (2.5 mg) on a 445 mm x 12.5 mm I.D. column packed with 22 g of MCTA is shown in Fig. 4. The capacity factors were found to be 0.43 and 1.47. Based on these values, the following operating conditions were selected for the subsequent work: eluent flow-rate = 4.10 ml/min; feed flow-rate = 0.30 ml/min; extract flow-rate = 2.60 ml/min; raffinate flow-rate = 1.80 ml/min; and switch time = 45.0 min.

Approximately three complete cycles (9 h) were required for the system to approach the final quasi-steady state. Operation was continued for six complete cycles (18 h). The steady-state concentrations, the recoveries and the optical purities of the extract and raffinate streams were

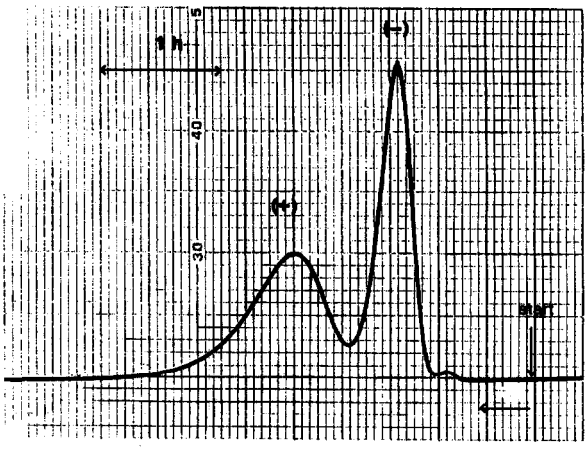

Fig. 4. Separation of *rac*-praziquantel (2.5 mg) on a 445 mm × 12.5 mm I.D. column packed with 22 g of MCTA. Eluent flow-rate, 1.00 ml/min.

as follows: for the extract, (+)-praziquantel 2.710 mg/ml, (-)-praziquantel 0.298 mg/ml, (+)-praziquantel recovery 423 mg/h and optical purity 90.09%; and for the raffinate, (+)-praziquantel 0.252 mg/ml, (-)-praziquantel 3.736 mg/ml, (-)-praziquantel recovery 404 mg/h and optical purity 93.68%. The chromatograms of the feed, extract and raffinate streams are shown in Fig. 5.

In this preliminary study, an optical purity close to 100% has not yet been achieved. However, from the computer simulation result, it is expected that pure enantiomers can be obtained by adding a few more columns (preferably 2-4 columns) to the system. This is currently being investigated.

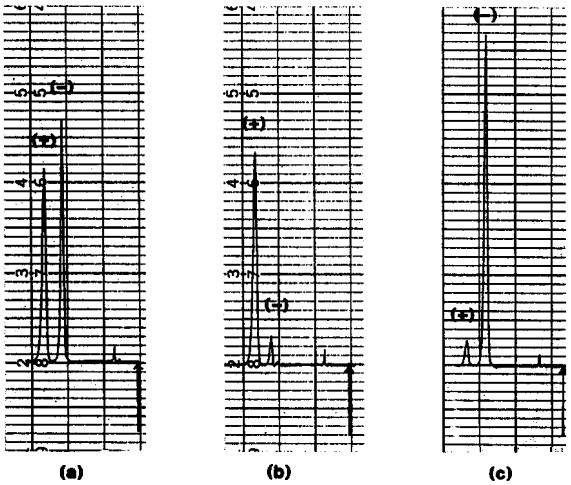

Fig. 5. Chromatograms of (a) feed (tenfold dilution), (b) extract stream and (c) raffinate stream at quasi-steady state on an analytical Chiralcel OD column.

One of the advantages of the continuous simulated counter-current system is its ability to utilize the whole sorbent bed effectively and to achieve a higher mass ratio of solute to sorbent. In our system, cu. 22 g of adsorbent were used for each column. If eight columns were to be used (to achieve 100% purity) for this separation, only 176 g of adsorbent would be required. If the present run conditions were used, 900 mg of *rac*-praziquantel would be separated per hour under steady-state conditions.

As the separation of praziquantel enantiomers by preparative-scale batch chromatography has not been reported, two of the runs reported on oxapadol and methylcyclohexylethylbarbituric acid [7] using the same adsorbent were analysed to compare the performance of the continuous system used in this study. The result reported for oxapadol showed that 2.1 g of racemic compound were separated into enantiomers in 48 h by the use of a 700 mm *x* 38 mm I.D. column packed with 380 g of MCTA. The eluent flow-rate was 90 ml/h. For the separation of *rac*-methylcyclohexylethylbarbituric acid; 205 mg were reported to be separated on 210 g of MCTA (column 85 mm × 25 mm I.D.) in 22 h at an eluent flow-rate of 50 ml/h. If the present result is compared with that reported on oxapadol and methylcyclohexylethylbarbituric acid, on an hourly basis, the grams of solute per gram of adsorbent that our system can handle are much higher than for a batch system. The grams of solute separated per hour are also much higher compared with a batch system. Even if the difference in eluent flow-rate is considered, the difference between these two systems is still significant. Differences in capacity factors and separation factors for the three compounds might have introduced some difficulties, however, in obtaining an accurate comparison between the batch and continuous systems. Nevertheless, a comparison of the general performance could still be made.

CONCLUSIONS

As the cost of most chiral stationary phases is generally very high, the use of a continuous simulated counter-current chromatographic system will provide a better utilization of the whole sorbent bed and a reduction in the cost of the expensive adsorbent. With the other advantages associated with a continuous process, the use of the present system for the resolution of optical isomers for pharmaceutical application is expected to provide an efficient and cost-effective separation method.

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