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Optimization of the preparative separation of a chiral pharmaceutical intermediate by high performance liquid chromatography

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Abstract

The prediction of optimal conditions of the preparative HPLC separation of the enantiomers of a pharmaceutical intermediate was accomplished by employing analytical chromatographic data, i.e. sample injections at low concentrations. Various temperatures and mobile phase conditions were studied. It was assumed that the sample loadability of the stationary phase is constant for a constant value of the separation factor and different mobile phase conditions and temperatures. Using this assumption, possible production rates can be compared for different method conditions. Overloading experiments were carried out to verify that the procedure employed is adequate. It was found that the optimization approach used, changing the mobile phase composition and temperature to achieve the shortest cycle time while keeping the separation factor constant, could be applied to improve the production rate of the separation.

Keywords: HPLC; Preparative chromatography; Scale-up; Optimization; Production rate; Loading factor; Separation factor

1. Introduction

Preparative liquid chromatography is becoming increasingly popular and is often the preferred method for the purification of drug intermediates in the pharmaceutical industry [1–5]. In the early development stages of a new drug candidate where time is the most critical, i.e. in the pre-clinical development phase, the use of preparative chromatography is widely accepted. This is especially true for the purification of chiral compounds because for these the development of non-chromatographic procedures such as crystallization or asymmetrical synthesis is often too time consuming to be considered as first choice [1–3,6–14]. The development of preparative purification processes can be achieved very rapidly, and what is the most important is that procedures can be in operation quickly to produce pure compounds. Preparative chiral chromatography is the most advantageous if small quantities (∼1 kg) of sample need to be purified. The use of preparative chromatography has also the benefit that both enantiomers can be isolated and studied. This is an important advantage to consider when comparing preparative HPLC with alternative purification methods. In many cases, only one enantiomer has the desired pharmacological activity and the other enantiomer might have negative or perhaps harmful effects [1].

In many cases during the early stages in drug development, there is limited sample available for systematic scale-up studies to optimize chromatographic method parameters, such as production rate, recovery and purity. Information about the sample loadability of the column is, therefore, not readily available, and the scientist is often confronted with the optimization of a preparative separation based on only limited analytical data. The production rate depends roughly on two factors, the cycle time and the sample loadability on the column. The loadability itself depends on the separation factor (selectivity) and on the saturation capacity. Information about the cycle time and the separation factor can be obtained from

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analytical scale chromatographic data, but the sample saturation capacity is only accessible by performing overloading experiments or by the measurement of equilibrium adsorption isotherms, although some work has been done to predict adsorption parameters when no reference material is available [1,15]. The scale-up of HPLC methods is often carried out by using trial and error procedures and relies particularly on the experience and skill of the research scientists involved. Many reports use a combined approach of selected analytical chromatographic data and overloading experiments on the laboratory-scale [9,12,16]. Systematic procedures for the optimization of a separation on a chiral stationary phase are presented by, e.g. Cox [1], Küsters [2] and Francotte [3]. First columns are screened to select potential candidates. Once the stationary phase is selected the influence of the eluent composition on the separation is studied. The optimization continues with the investigation of mobile phase additives and the temperature dependence of the separation. The general procedure also requires loading studies for the optimization to be successful. Even if all necessary parameters for the evaluation of optimal conditions of a preparative separation are available, e.g. competitive equilibrium isotherms, band-broadening phenomena such as dispersion and mass transfer effects of the components involved, the optimization procedure is still not trivial [17–19,21]. Adsorption isotherm data are always very useful for loading optimization studies in preparative separations. However, substantial amounts of pure sample are required. Furthermore, the effort and time involved in obtaining isotherm data is only justified if the preparative separation is going to be routinely employed on a large scale, e.g. in the final manufacturing process of a drug.

The choice of the appropriate mode for the preparative process is also very important; these modes include batch chromatography, closed loop steady state recycling or simulated moving bed (SMB) [19,20,22]. In most cases, simple batch chromatography is used because the initial capital investment is much less than for the other options, mostly notably for SMB. All these considerations make it clear that optimization of a preparative HPLC separation is a complex endeavor.

This paper presents an HPLC method development procedure where only analytical scale data are used to predict optimal preparative operation conditions for the separation of a chiral drug intermediate. For the studied separation, the production rate was the most critical parameter, which is the time that is necessary to separate a specified amount of pure compounds. Furthermore, the separation should yield very pure enantiomers and have a high product recovery as well so that there is no need to re-inject mixed fractions. For such a situation, the following operating procedure is most useful, e.g. it is best to inject as much sample as possible so that the apparent resolution is approximately 1.0, i.e. there is little or no overlapping of the chromatographic bands. It will be shown how using only analytical scale chromatographic data can optimize the possible production rate. The optimization procedure is later shown to be suitable by performing overloading experiments on the laboratory-scale.

2. Experimental

2.1. Chemicals and materials

Mobile phase solvents and diluents were HPLC grade *n*-hexane (Fluka, Buchs, Switzerland) and absolute alcohol (Pharmco Products, Brookfield, CT, USA). Separations were carried out on a Chiralpak AD column, 25 cm × 0.46 cm (Daicel Chemical Industries Ltd., Japan). Racemic mixtures and pure enantiomers of the pharmaceutical intermediate were supplied by Merck Frosst, Montreal, Canada. The molecule to be separated is a proprietary intermediate of an active pharmaceutical ingredient that is currently in pre-clinical development at Merck & Co. No structure is presented; however, the approach for the optimization discussed in this paper is generic and can be used as a universal for pharmaceuticals.

2.2. Instrumentation and HPLC method conditions

An Agilent 1100 Series HPLC system (Agilent Technologies, Palo Alto, CA, USA) was used for all experiments. This system is equipped with an auto injector with sample tray cooler, a multi-solvent delivery system and a temperature controlled column compartment. The wavelength of the detector was set to 230 nm. At this wavelength, the response of the detector was linear in the concentration range for all components investigated. The injection volume was 5 μL for analytical size injections and 100 μL for loadability experiments. Samples were dissolved in ethanol for the analytical scale injections. For the loading experiments the sample diluent was the same as the mobile phase. The flow rate used for all experiments was 1.0 mL/min.

2.3. Procedures

The retention times t_R were obtained from the maximum peak height. The peak efficiency was calculated from the peak width at half height as $N = 5.54 t_R^2 / W_{0.5}^2$. The separation factor was calculated from $\alpha = (t_{R,2} - t_0)/(t_{R,1} - t_0)$ where $t_{R,1}$ and $t_{R,2}$ are the retention times of the first and second eluting enantiomer, respectively; and t_0, the column hold-up time. The hold-up time was estimated from the retention time of the first peak disturbance of ethanol injections as 2.0 min. The resolution was calculated as $R_s = 2(t_{R,2} - t_{R,1})/(W_2 + W_1)$ where $t_{R,1}$ and $t_{R,2}$ are the retention times of the enantiomers, respectively; and W_1 and W_2, the peak widths at the baseline. The relationship between the baseline width and the standard deviation of a Gaussian peak is $W = 4\sigma$. Arbitrary definitions were chosen to calculate the time that separates two consecutive injections in a preparative separation experiment, the cycle time Δt_c. Two cases were considered. In case I, the cycle time is based on the difference in retention times of the two components; and in case II, the cycle time is based on the retention time of the second eluting component. The peak width has been added to the cycle times

to account for peak broadening effects. The cycle times for case I is defined as $\Delta t_c = t_{R,2} - t_{R,1} + 6\sigma_1 + 6\sigma_2$. For case II, the definition is $\Delta t_c = t_{R,2} + 6\sigma_2$. Case I can be used for a separation in isocratic elution mode when the sample is relatively pure and only the separation of two peaks is considered. For this case, multiple injections can be performed during a run (overlapping injections), and it is not necessary to wait until all components elute from the column. Case II is applicable when gradient elution is necessary or a cleaning step is required to regenerate the column in-between sample injections. For a given purity requirement, the production rate depends directly on the amount injected and the yield, and it is inversely proportional to the cycle time, as given in the expression: production rate = amount × yield/Δt_c.

3. Results and discussion

3.1. Development of an analytical chiral method

A suitable separation of the pharmaceutical intermediate was achieved on a Chiralpak AD column at a temperature of 20 °C with 20% (v/v) ethanol in hexane as mobile phase. Once the chiral stationary phase and mobile phase are selected the usual optimization procedure is to change the solvent strength of the mobile phase and the temperature [1,2]. This approach was followed in this study. Fig. 1a shows the effect of mobile phase composition and temperature on the chromatographic separation. From this plot, it can be seen that the separation improves with increasing temperature and decreasing solvent strength. According to the manufacturer of the stationary phase, the temperature range of the column is restricted to 0–40 °C. In the current study, 50 °C was attempted, however, due to an unstable baseline, probably due to bleeding of the chiral stationary phase, higher temperatures than 40 °C were not further pursued for the optimization study. The manufacturer also states that a mobile phase containing 15–60% (v/v) ethanol in hexane should be avoided, however, we did not follow this recommendation and did not experience any adverse effects concerning the reproducibility of the separation. From analytical chromatograms as seen in Fig. 1a, many useful parameters can be extracted, e.g. retention time, peak efficiency, separation factor, resolution and the cycle time. Scale-up optimizations are usually conducted by using analytical data in combination with loadability experiments; however, in this study, we attempted to find the best possible preparative separation conditions based on analytical experiments only.

3.2. Solvent strength and temperature experiments

The retention times of both components decrease with increasing ethanol concentration, i.e. increasing solvent strength, and with increasing temperature (see Fig. 1b). The dependence of the column efficiency calculated from the peak width at half height is shown in Fig. 1c. The efficiency increases with increasing temperature but it is not significantly affected by a change in the solvent composition. The dependence of the separation factor is shown in Fig. 1d. Increasing the temperature or decreasing the solvent strength improves the separation factor. The dependence of the resolution is shown in Fig. 1e. The resolution plot can be used for the optimization of the analytical method development. A minimum resolution requirement can be assigned, e.g. $R_s = 2$. Once this assignment is made, the shortest retention time will provide the optimum method parameters provided that the separation is rugged.

The resolution plot is, however, usually not suitable for the optimization of the preparative separation. For the preparative method development, two optimization strategies were considered. The first strategy uses the optimization of α, where α is improved by simultaneously changing the temperature T and solvent strength. For this case, a comparison of relative production rates is not possible without performing overloading experiments. The reason for this is because the exact dependence of sample loading on the separation factor is not known. The second approach is the optimization of the cycle time Δt_c by changing T and solvent strength while keeping the α value constant. A comparison of relative production rates is possible without overloading experiments if this optimization strategy is used. This is true when assuming that a constant α value will result in a constant sample loading factor. This assumption can be made with reasonable accuracy if only the mobile phase composition and temperature are changed [1,15]. However, if the stationary phase is changed, then this assumption cannot be made [1].

3.3. Development of the optimization procedure

Experimental conditions of 20 °C and 20% (v/v) ethanol in hexane (20/20) gave a good separation as mentioned earlier. Fig. 1d can be used to find improved conditions in respect to the preparative separation. At the current conditions, a separation factor of $\alpha = 1.14$ is obtained. The objective is to increase the temperature while keeping α constant, i.e. moving horizontally to the right in Fig. 1d. At 40 °C and 40% (v/v) ethanol in hexane (40/40), the separation factor is the same. Next the cycle times of both experimental conditions can be compared. At 20/20, the cycle time is approximately 9 min and at 40/40, it is below 2 min (see Fig. 1f). Assuming there is the same loadability for both cases, the production rate will improve by a factor of ∼4. For case II, the cycle time will be reduced from 27 to 7 min as shown in Fig. 1g, hence a time reduction of approximately fourfold. Next in order to verify that the procedure used can be employed, overloading studies were conducted. The apparent resolution is shown in Fig. 2a and an overlay of two chromatograms is shown in Fig. 2b. The loadability is slightly better at the initial condition 20/20, however, considering that the retention time and the cycle time is so much improved, the new conditions feature valuable improvement to the separation. The normalized UV traces are very similar and there is only moderate

overlapping of the bands. For this reason, the purities and yields should be the same for both cases.

It makes a significant difference in a preparative separation if it can be finished quickly. This is very evident in early drug development when hundreds of compounds are screened. The time saving approach presented here can be very useful and vital for a continuous success in drug development, especially considering its potential saving in resources and finances. The optimization procedure used in this study that is based solely on analytical chromatographic data predicts an improvement of approximately fourfold. The overloading study shows that at the condition of 40/40, the loadability decreases to 80% of

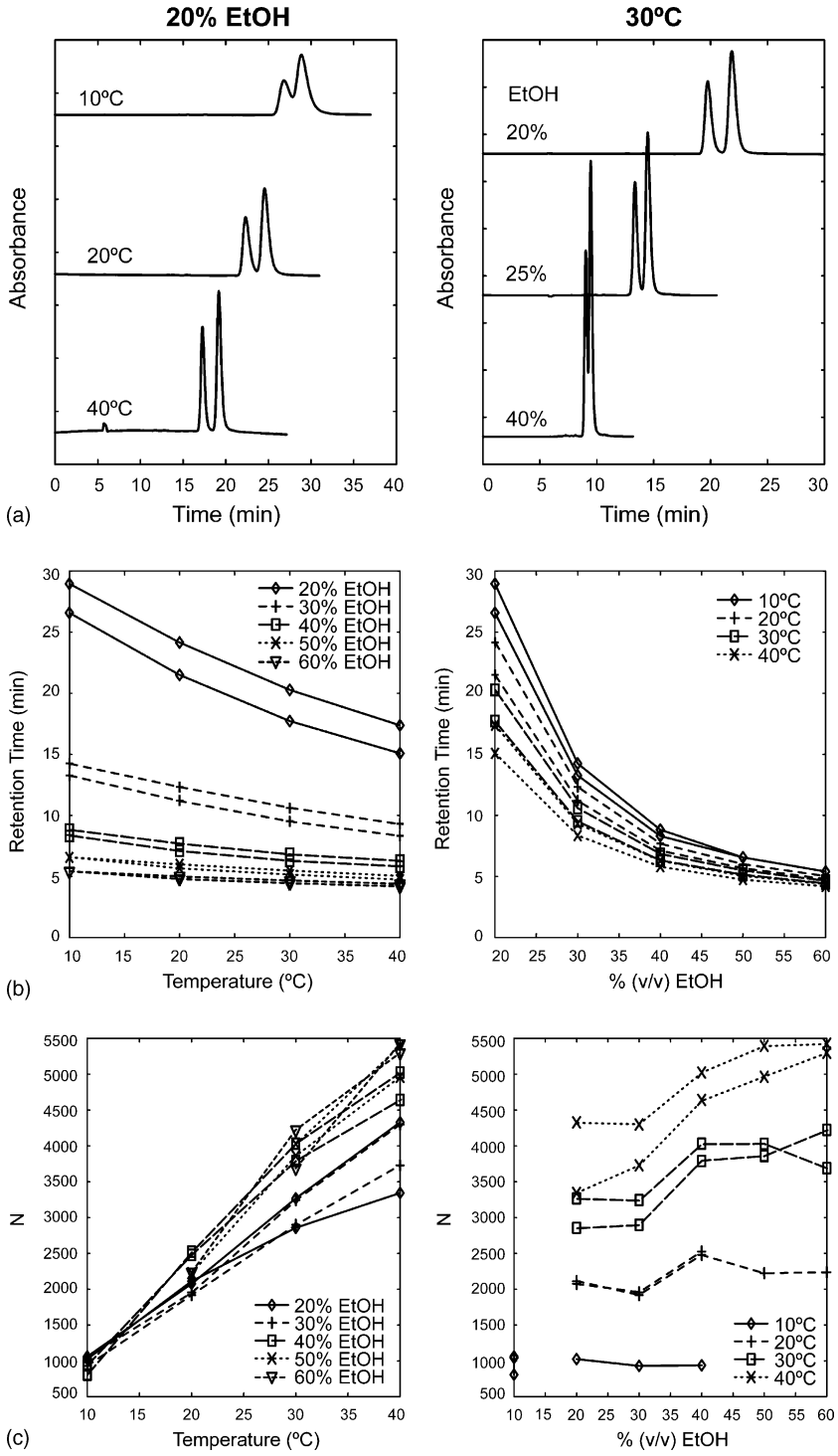

Fig. 1. Dependence of the separation on temperature and solvent strength. (a) Chromatograms; (b) retention time; (c) column efficiency; (d) separation factor; (e) resolution; (f) cycle time (case I); (g) cycle time (case II).

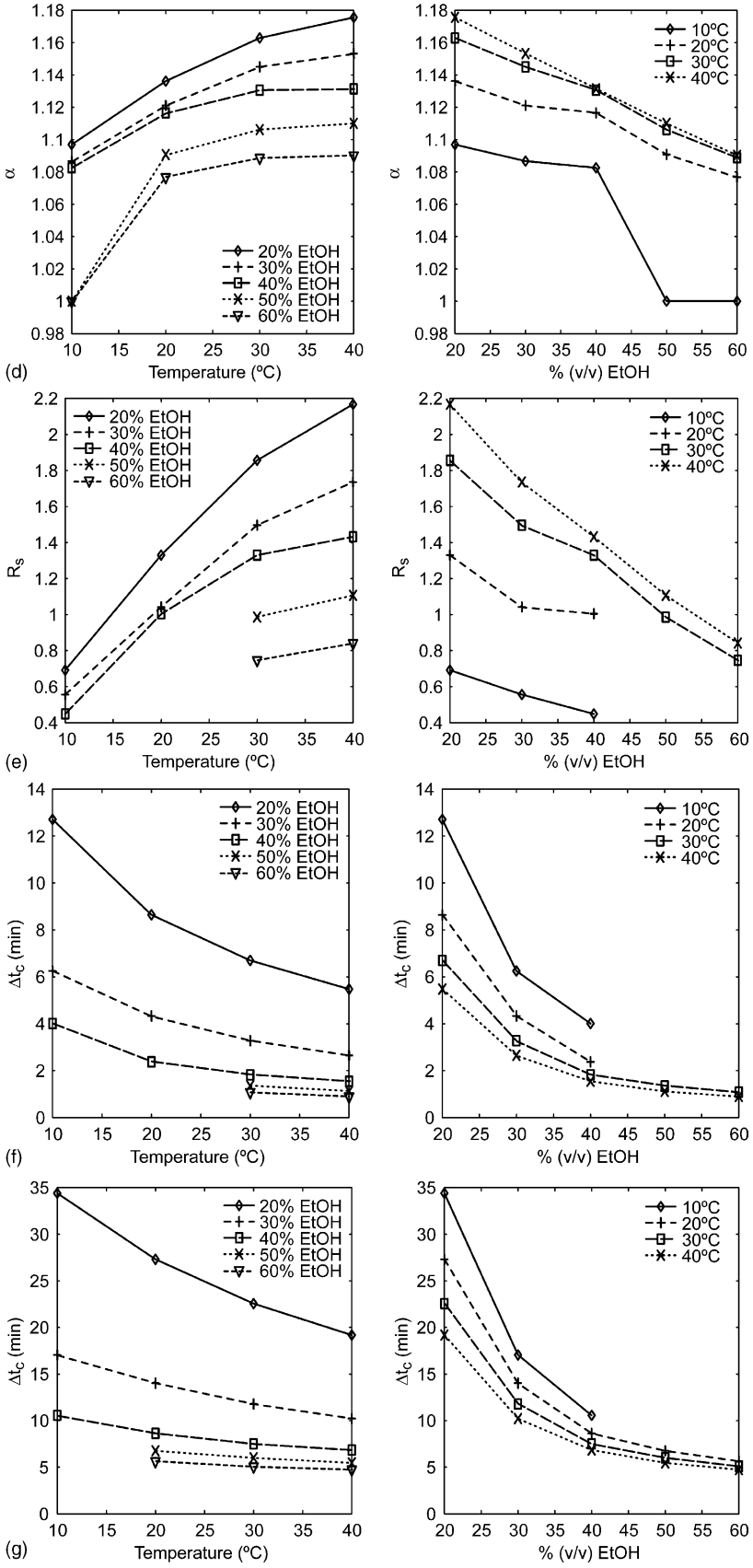

Fig. 1. (*Continued*).

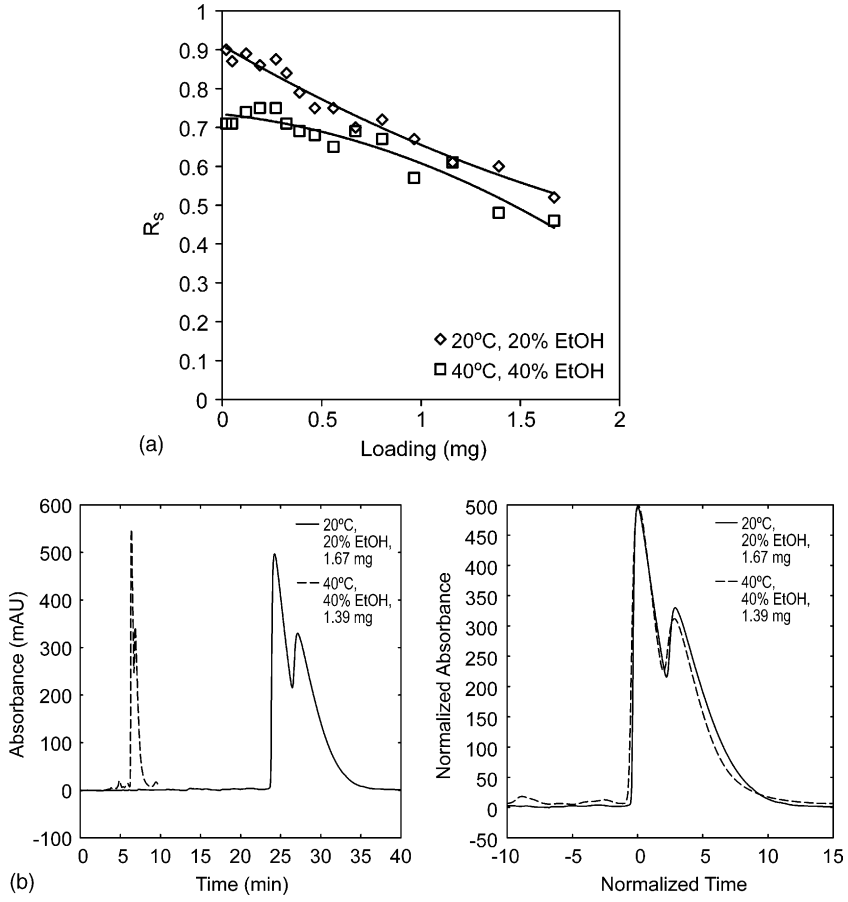

Fig. 2. Comparison of the sample loadability at two different method conditions, i.e. 20% (v/v) ethanol in hexane/20 °C and 40% (v/v) ethanol in hexane/40 °C. The separation factor is the same at both conditions. (a) Apparent resolution of the separation. (b) Overlaid chromatograms at the two method conditions. The chromatograms are shown in regular (left) and in normalized scale (right), respectively.

that at the condition of 20/20. This is not a significant decrease in the loadability considering the time saving because of the reduced cycle time. This result shows that the approach taken in the study is feasible.

It is also important to carefully compare the pressure drop of the column for the two conditions studied. The production rate is directly related to this value because the maximum flow rate that can be achieved on a separation column is limited by the pressure drop. A mobile phase with a higher ethanol concentration in hexane has a higher viscosity, however, this effect is balanced by the temperature increase to 40 °C. Therefore, the pressure drop at both conditions of 20/20 and 40/40 was not significantly higher, it was only ∼10% higher. The pressure drop of the column was 25 and 27 bar for 20/20 and 40/40 conditions, respectively.

Another important point to consider is that when operating a preparative column with a larger inner diameter at elevated temperature, care must be taken to avoid temperature gradients because these can adversely affect the separation. Problems in this respect can be easily avoided by following proper experimental practices, e.g. preheating the mobile phase and using a temperature controlled column jacket.

It is quite possible that a better production rate can be achieved by maximizing the separation factor α while keeping the cycle time constant. This is true, but an estimate of the potential improvement cannot be provided easily for this case. The advantage of keeping α constant and looking for the shortest cycle time as it is suggested in this study is clearly that an estimation can be made of how much the production rate improves when changing operating conditions. If a preparative separation is in operation, it is possible to predict the possible time saving based on limited analytical data using the approach presented in this paper.

There are some other points to consider for the preparative separation. The solubility is important for two reasons. First, a good solubility is necessary to achieve a high sample loading with a reasonably small injection size, thus preventing volume overloading that will limit the productivity. Second, it is important because it is often necessary be able to match the sample diluent with the eluent composition at the initial condition. This prevents a solvent mismatch that can have very negative effects on the loading. The sample diluent is especially important if it contains more organic modifier than the mobile phase. In the present study, the sample is more soluble using the optimized condition at 40/40 (40% (v/v) ethanol in

hexane, 40 °C) than at 20/20 (20% (v/v) ethanol in hexane, 20 °C) because of the higher ethanol content and increased temperature.

Finally, the potential use of sample self displacement is particularly interesting for the present case because the experimental data indicate that it should take place, i.e. the relative retention is near unity and the retention factor k' is moderate [23]. If this effect is present in the separation studied, it furthermore makes the new condition 40/40 more appealing because the displacement effect is usually stronger at lower k' values. Sample self-displacement effects can, however, be neglected for the situation studied because the overloading of the column is only moderate. The degree of overloading is limited to the case of touching bands because the objective is to maximize the recovery yield.

4. Conclusion

The current work shows that analytical chromatographic experiments alone are useful for the prediction of scale-up conditions of preparative HPLC separations. The separation of enantiomers of a pharmaceutical intermediate could be improved by simultaneously changing the solvent strength and temperature while keeping the separation factor constant. The relative production rates could be estimated at different conditions because of the validity of the assumption that the loadability stays constant for a constant separation factor. Overloading experiments verified that this assumption is correct.

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