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Cryogenic Chiral Chromatography for Rapid Resolution of Drug Candidates

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Abstract:

The chromatographic resolution of three racemates is presented at temperature areas extending to the cryogenic area, down to −25 °C. In all examined cases the separation factor between the enantiomers increased with decreasing temperature. The yields and production rates for the enantiomers were calculated from chromatograms to predict optimum conditions for preparative resolutions.

Introduction

Chiral chromatographic methods are increasingly used to speed up drug development at early stages. When up to 100 g of pure enantiomers are needed, the quickest way of obtaining them is often to synthesize a racemate and make the resolution with preparative, chiral chromatography. Low temperatures generally favour enantiomeric selectivity in syntheses as well as in separations. However, the viscosity of the medium increases and molecular diffusivity decreases with decreasing temperature. This puts a lower limit to feasible temperatures when conventional liquid eluents are used in chromatographic separations. Pressure drop over a packed chromatography column may typically not exceed 100 bar. Otherwise there is a risk of damaging the chiral stationary phase. This pressure drop limit is easily reached with conventional liquid eluents, such as hexane and isopropyl alcohol, at lower than room temperature and with conventional flowrates. We have already shown that by using a liquid CO_2-based eluent one can go down to at least −25 °C, without an excessive pressure drop over the column.[1] This is because the viscosity of pressurized, liquid carbon dioxide is much lower than that of ordinary solvents. According to the Stokes−Einstein relation, molecular diffusivity increases linearly with decreasing viscosity at constant temperature. Therefore, the plate number of a chromatography column should be higher with liquid CO_2 than with conventional solvents, because of the viscosity difference at low temperatures.

Wolf and Pirkle reported a considerable and consistent increase of separation factor, enantioselectivity, and resolution with decreasing temperature for eight chiral alcohols and ketones.[2] They used a mobile phase consisting of carbon dioxide modified with different amounts of methanol. Although none of the studied compounds could be completely separated at room temperature, a baseline separation was achieved at cryogenic temperatures. The authors also studied the enantiomeric separation of five axially chiral, arylnaphthalene lignans. Four of them were successfully separated at 0 to −47 °C and one not. The authors attribute the good chromatographic separations at low temperature to the rapid adsorption−desorption kinetics of the brush-type stationary phase which they used.

Stringham and Blackwell showed that for each racemate/chiral stationary phase (CSP)/eluent system there is an isoelution temperature where the enantiomers elute with the same rate and do not separate from each other[3]. Chromatographic selectivity between enantiomers may be related to temperature as

$$\ln(\alpha) = -\delta\Delta H/RT + \delta\Delta S/R \qquad (1)$$

where α is the separation factor between the enantiomers, R is the ideal gas constant, T is absolute temperature, $\delta\Delta H$ is the difference between the enthalpy of the enantiomers' interaction with the stationary phase, and $\delta\Delta S$ is the entropic difference. At isoelution temperature, $\ln(\alpha) = 0$ and the enthalpy and entropy terms are equal.

Thermodynamics predicts that when moving away from the isoelution temperature the logarithm of the separation factor ($\ln(\alpha)$) increases linearly with the reciprocal of temperature in Kelvin ($1/T$). Stringham and Blackwell showed experimentally that the relationship was indeed linear at below the isoelution temperature with a carbon dioxide/2-propanol eluent. They covered a temperature range from +200 °C down to −20 °C using a brush-type chiral stationary phase.

So it has already been shown that low temperatures may increase the resolution and enantioselectivity of chiral separations. This is immediately useful in analytical work. However, lowering column temperature increases retention and prolongs cycle time. Cycle time is the minimum time interval between repeated injections made so that peaks from adjacent injections do not overlap when leaving the column. Lowering the temperature also decreases column efficiency expressed as plate number. For preparative, production-scale separations it would be important to know if the favourable resolution and enantioselectivity at cryogenic temperatures translate into increased throughput despite the longer cycle time and decreased plate number. It is also of interest to know how other types of CSPs respond to cryogenic temperatures.

We have previously reported a successful resolution of the enantiomers of a drug candidate, Finrozole, at cryogenic

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(1) Alkio, M. In *Proceedings of the 8th Meeting on Supercritical Fluids*; April 14−17, 2002, Bordeaux; pp 754−759.

(2) Wolf, C.; Pirkle, W. H. *J. Chromatogr., A* **1997**, *785*, 173.

(3) Stringham, R. W.; Blackwell, J. A. *Anal. Chem.* **1997**, *69*, 1414.

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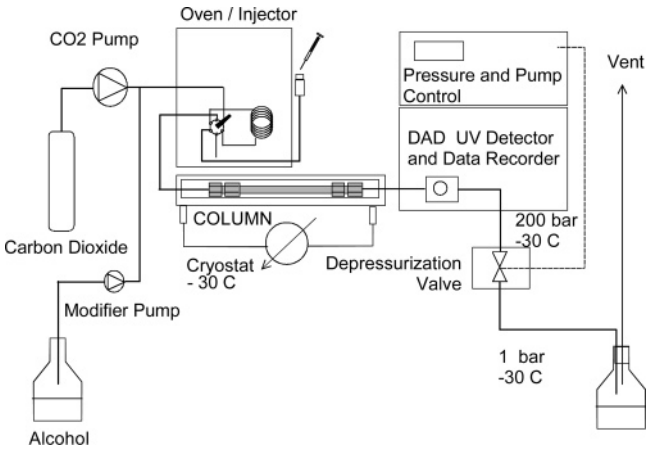

Figure 1. **Chromatographic setup for studying cryogenic chiral separations.**

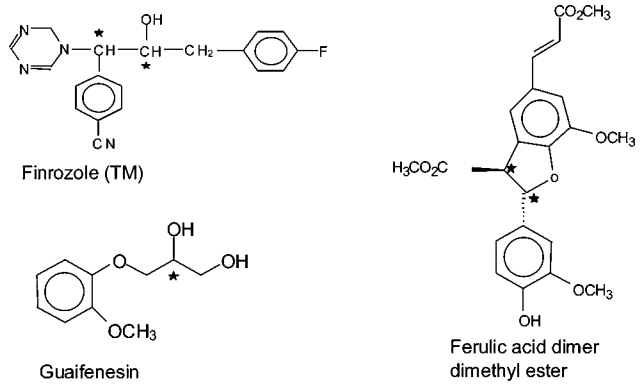

Figure 2. **Structures of the chiral racemates.**

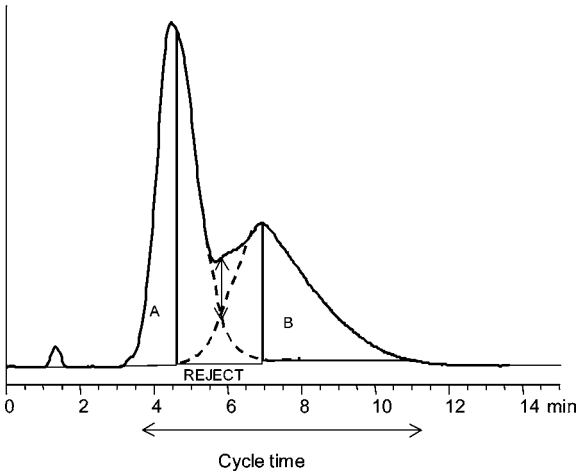

Figure 3. **Method of estimating the yields of each enantiomer in the case where enantiomer peaks overlap.**

temperatures with an L-tartar diamide CSP.[1] The results were verified with preparative resolutions in pilot-plant scale. The linear ln(α) vs 1/*T* correlation was also confirmed in the studied case. The results from Finrozole resolution are summarized here in Table 5. In this paper we present further examples of low-temperature chiral chromatography and also a systematic approach to find the optimum conditions for maximum throughput. Finrozole and ferulic acid dimer dimethyl ester were chosen for cryogenic resolutions because they were subjects of contract work at VTT and unsatisfactory results were previously obtained using other methods.

Table 1. **Variables and their levels selected for the orthogonal design of chromatography experiments for guaifenesin[a]**

variable		levels		
guaifenesin		minimum	middle	maximum
temperature	°C	−25	0	+25
load ratio	g/kg of CSP	5.0	9.0	12.5
modifier concentration	% EtOH	15.0	20.0	30.0
eluent linear velocity	mm/s	4.0	4.5	5.5

[a] Ethanol was selected as the injection solvent for the racemate.

Table 2. **Variables and their levels selected for the orthogonal design of chromatography experiments for ferulic acid dimer dimethyl ester[a]**

variable		levels		
ferulic acid dimer dimethyl ester		minimum	middle	maximum
temperature	°C	−25	0	+25
load ratio	g/kg of CSP	1.25	1.88	2.50
modifier concentration	% EtOH	15	22.4	30
eluent linear velocity	mm/s	3.0	4.5	6.0

[a] Dichloromethane was selected as the best injection solvent for the racemate.

Guaifenesin was chosen as an example of an easy resolution and because of existing literature data for comparisons.

Experimental Methods

The chromatograph setup is depicted in Figure 1. The equipment was a Hewlett-Packard G1205A Laboratory SFC unit, with a diode array UV detector.

Two chromatography columns were screened. Chiralcel OD CSP, 4,6 mm × 250 mm, from Daicel Chemical Industries, Ltd., Japan was packed at Cultor Oy, Finland. The chiral stationary phase (CSP) of Chiralcel OD is cellulose tris(3,5-dimethylphenylcarbamate) coated on a silica support. The Kromasil CHI-TBB colums were from Eka Chemicals, Sweden. The CSP of the CHI-TBB column is *O*,*O*′-bis(4-*tert*-butylbenzoyl)-*N*,*N*′-diallyl-L-tartar diamide covalently bonded on silica.

Methanol, tetrahydrofuran (THF), and toluene were HPLC-grade from Rathburn, UK. Dichloromethane was from Fluka. Ethanol was absoluted, technical (Ba) grade from Altia Oy, Finland (min purity 99.5%). Carbon dioxide was food grade from Oy AGA Ab, Finland (min purity 99,7%). Guaifenesin (min 98%, GC) was purchased from Sigma-Aldrich Chemicals (Figure 2). Ferulic acid dimer dimethyl ester was synthesized and purified at VTT using a previously described procedure.[4] Finrozole was obtained from Hormos Medical Ltd., Turku, Finland.

The target function to be maximized was the daily production rate (PR) of both enantiomers per kg of CSP. It was calculated from chromatograms as follows: From each run the chromatographic bandwidth, i.e., the cycle time (ct) was measured. For nonoverlapping peaks the yields of pure

(4) Chioccara, F.; Poli, S.; Rindone, B.; Pilati, T.; Brunow, G.; Pietikäinen, P.; Setälä, H. *Acta Chem. Scand.* **1993**, *47*, 610.

Table 3. **Results from the chiral separation chromatography of guaifenesin enantiomers[a]**

T, °C	LR, g/kg of CSP	modifier EtOH, %	linear velocity, mm/s	estimated yield, %	cycle time, min	PR, g/kg of CSP/24 h
25	5.0	15	4.0	100.0	2.4	3000
25	12.5	15	4.0	100.0	3.0	6000
25	5.0	30	4.0	100.0	1.5	4800
25	12.5	30	4.0	33.0	1.6	3713
25	5.0	15	5.5	100.0	1.8	4000
25	12.5	15	5.5	100.0	2.2	8182
25	5.0	30	5.5	100.0	1.5	4800
25	12.5	30	5.5	25.0	1.0	4500
0	9.0	20	4.5	100.0	3.0	4320
0	9.0	30	4.5	100.0	2.0	6480
0	9.0	30	5.5	100.0	1.6	8100
−25	5.0	15	4.0	100.0	9.5	758
−25	12.5	15	4.0	100.0	8.8	2045
−25	5.0	30	4.0	100.0	4.0	1800
−25	12.5	30	4.0	100.0	4.0	4500
−25	5.0	15	5.5	100.0	6.5	1108
−25	12.5	15	5.5	100.0	6.4	2813
−25	5.0	30	5.5	100.0	2.9	2483
−25	12.5	30	5.5	100.0	2.7	6667

[a] Column: Chiralcel OD. Eluent: CO_2 + ethanol.

Table 4. **Results from the chiral chromatography of ferulic acid dimer dimethyl ester enantiomers[a]**

T, °C	LR, g/kg of CSP	modifier EtOH, %	linear velocity, mm/s	estimated yield, %	cycle time, min	PR, g/kg of CSP/24 h
−25	1.25	15.0	3.0	100.0	20.0	90
−25	1.25	30.0	3.0	100.0	10.0	180
−25	1.25	15.0	6.0	100.0	8.0	225
−25	1.25	30.0	6.0	85.0	5.0	306
25	1.25	15.0	3.0	100.0	6.0	300
25	1.25	30.0	6.0	0.0	2.0	0
25	1.25	30.0	3.0	10.0	2.5	72
25	1.25	15.0	6.0	10.0	3.0	60
0	1.88	22.4	4.5	100.0	4.0	675
0	1.88	22.4	6.0	100.0	2.8	982
0	1.88	30.0	4.5	95.0	3.0	855
25	2.50	15.0	3.0	50.0	6.5	277
25	2.50	15.0	3.0	10.0	3.5	103
25	2.50	15.0	6.0	20.0	3.2	225
25	2.50	30.0	6.0	0.0	2.0	0
−25	2.50	15.0	3.0	80.0	16.0	180
−25	2.50	15.0	6.0	60.0	12.0	180
−25	2.50	30.0	6.0	12.0	7.0	62
−25	2.50	30.0	3.0	40.0	8.0	180
−10	2.50	15.0	6.0	50.0	6.5	277
−10	2.50	30.0	3.0	55.0	7.5	264
−10	2.50	15.0	3.0	90.0	10.0	324
0	2.50	22.5	6.0	72.0	10.0	259
0	2.50	30.0	3.0	68.0	10.0	245

[a] Column: Chiralcel OD. Eluent: CO_2 + ethanol.

enantiomers were estimated to be 100%. For overlapping peaks, the net yield was estimated from the measured resolution. Figure 3 illustrates the method of estimating the net yield from a chromatogram where peaks overlap.

Column load ratio (LR) was calculated as

$$\mathrm{LR} = (c * V_{\mathrm{inj}})/M_{\mathrm{CSP}} \qquad (2)$$

where:

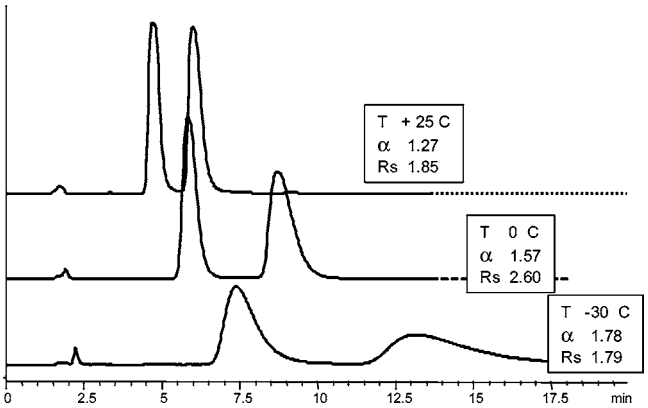

Figure 4. **Effect of temperature on the resolution of ferulic acid dimer ester enantiomers.**

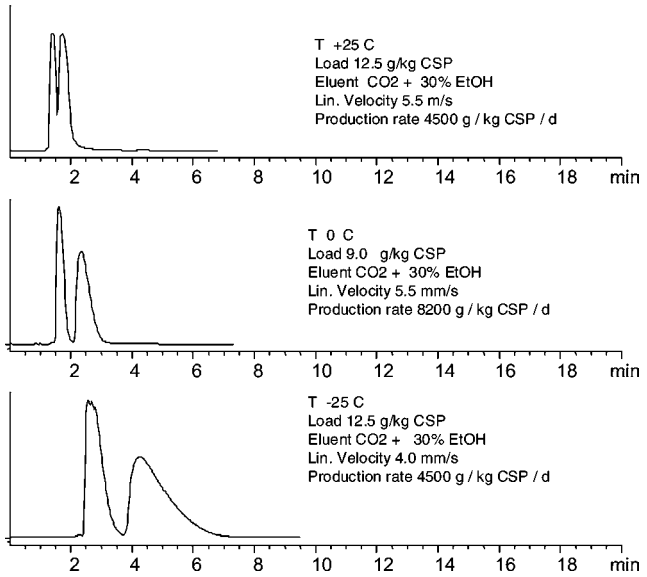

Figure 5. **Effect of temperature on the resolution of guaifenesin enantiomers.**

c is concentration of racemate in feed solution (g/mL).

V_{inj} is injection volume (mL).

M_{CSP} is mass of CSP in the column (kg).

The daily production rate of both enantiomers per kg of CSP was calculated:

$$\mathrm{PR} = \mathrm{LR} * (60/\mathrm{ct}) * Y * 24 \qquad (3)$$

where:

LR is load ratio (g racemate/kg CSP.

ct is cycle time (min).

Y is estimated, combined yield of both pure enantiomers (a fraction of the mass of injected racemate).

Results

Before designing the experiment matrix a few chromatographic runs were carried out to locate a feasible parameter area for each racemate. Methanol and ethanol were tested as modifiers in carbon dioxide eluent, and ethanol, tetrahydrofuran (THF), toluene, and dichloromethane were tested as injection solvents for the racemates. Chiralcel OD was superior to Kromasil CHI-TBB for the guaifenesine and ferulic acid dimer dimethyl ester cases, and therefore it was

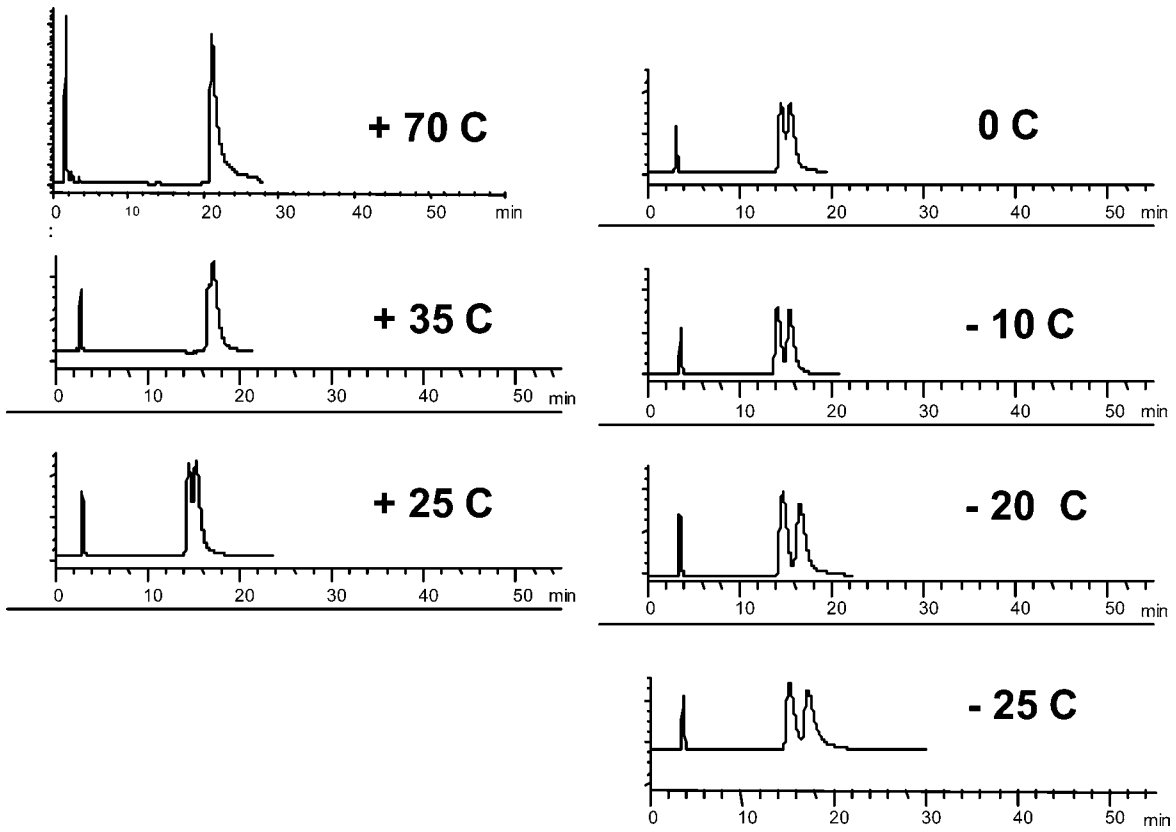

Figure 6. **Effect of temperature on the resolution of Finrozole enantiomers.[1]**

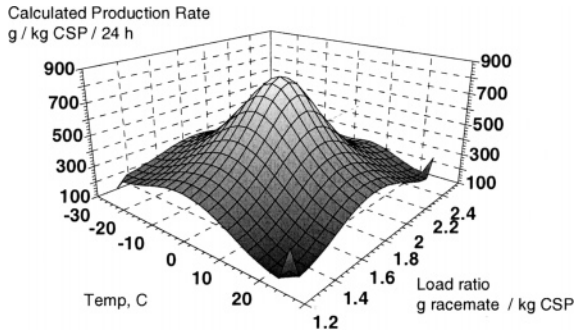

Figure 7. **An interpolated-surface plot of the effect of column temperature and load ratio on the production rate of ferulic acid dimer dimethyl ester enantiomers.**

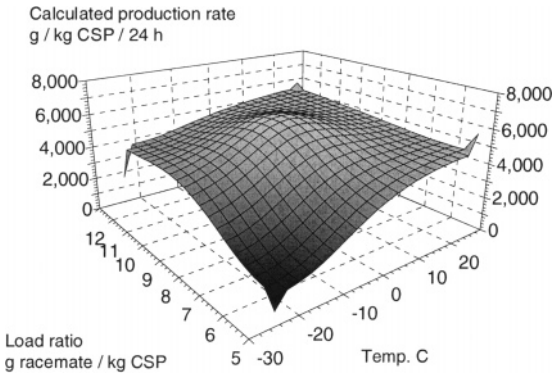

Figure 8. **An interpolated-surface plot of the effect of column temperature and load ratio on the production rate of guaifenesine enantiomers.**

selected for the systematic experiments. For finrozole, enantiomer separation was achived only with the Kromasil CSP.

Based of these screening runs the orthogonal experiment matrices of Tables 1 and 2 were designed for the racemates. Modde 5.0 software (Umetrics AB, Umeå, Sweden) was used to design experiment matrices for the minimum number of chromatography runs and with an orthogonal location of experiments in the parameter space.

The results for guaifenesin and for ferulic acid dimer ester are shown in Tables 3 and 4, respectively. Estimated yield is the yield of both pure enantiomers based on the amount of injected racemate.

Parameter analysis with Modde 5.0 software revealed that temperature and load ratio were most significant in determining the PR. Linear velocity and modifier concentration were less important.

Chromatograms showing the effect of temperature are presented in Figures 4−6.

The production rates of pure enantiomers for guaifenesine and for ferulic acid dimer dimethyl ester are shown in Figures 7 and 8, respectively.

The optimum conditions and maximum productivity for guaifenesin, ferulic acid dimer dimethyl ester, and Finrozole are collected in Table 5 where the PR results are based on the surface plots of Figures 7 and 8. The fitted surface plots are smoothed and therefore do not necessarily coincide with all the experimental points in Tables 3 and 4. The fitted plots average the scattering of the measured data. Therefore the results in Table 5 should give a more realistic view of the achievable production rates than the results from individual experiments shown in Tables 3 and 4.

Table 5. **A comparison of optimum parameter values and maximum productivities of pure enantiomers obtained for the three studied chiral chromatography cases at lower than ambient temperatures**

racemate	chiral stationary phase	column size, mm	CO_2 modification	temp, °C	load ratio (LR), g/kg of CSP	productivity (PR), g/kg of CSP/d
guaifenesin	Chiralcel OD	4.6 × 250	30% EtOH	0	9.0	6400
ferulic acid dimer ester	Chiralcel OD	4.6 × 250	20% EtOH	0	1.9	840
Finrozole[1]	Kromasil CHI-TBB	10 × 250	5% MeOH	−30	0.4	250

Conclusion

The results show that the productivity of chiral chromatography may be increased by lowering the operation temperature below ambient. Temperature should be included in the set of parameters which are optimized when developing a preparative method for chiral separation. Complementing previous findings with brush-type chiral stationary phases we have shown that the positive temperature lowering effect on productivity may apply for cellulose-based and for L-tartar diamide-based CSPs as well. The effect of lowering the temperature appears to be specific to each racemate/eluant/CSP system. The optimum temperature and other optimum conditions need to be found experimentally. We have shown that, by systematic experimental planning, one may find an estimate of optimum conditions in a couple of days.

The enantiomer needs at an early stage of drug development may be in the order of 100 g. Chiral chromatography with a 50 mm diameter column and 1 kg of CSP can produce the needed batch of pure enantiomers in about 4−100 h, depending on the separation task, when the operating conditions are optimized. The basic scale-up of preparative chromatography is fairly straightforward. The capacity is linearly related to the cross-sectional area of the stationary phase bed, provided that the linear flowrate of the eluent and the load ratio (LR) are kept constant. Dynamic axial compression (DAC) columns are used for keeping the CSP under constant compression. They are commercially available for preparative enantiomer resolutions and can be modified for cryogenic operation.

For large installations the authors would like to stress the following safety considerations. Carbon dioxide is heavier than air. In the case of carbon dioxide leakage from the chromatography system it may replace air in confined spaces. Dizziness, fatigue, increased heart rate, and other symptoms may arise when the concentration of carbon dioxide rises to 2−10%. Exposure to higher concentrations may lead to unconsciousness or death. Rooms where carbon dioxide is used in large amounts shall be well ventilated and equipped with carbon dioxide sensors and alarms.

A comparison with HPLC is available for the resolution of guaifenesin. Jusforgues[5] et al. report a maximum productivity of 2892 g of injected guaifenesin/kg of CSP/day with HPLC. Our results with cryogenic carbon dioxide eluent are more than two times higher (Table 5). Liquid solvent consumption in the HPLC runs was 160 g of hexane/ethanol per g of guaifenesin racemate, while in the cryogenic carbon dioxide system it was 72 g of ethanol per g of racemate.

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