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Preparative chromatographic resolution of racemates on chiral stationary phases on laboratory and production scales by closed-loop recycling chromatography

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ABSTRACT

The time-saving, economical and practical advantages that are obtained when closed-loop recycling chromatography under overload conditions and peak shaving is applied for the preparative chromatographic separations of enantiomers on chiral stationary phases are described. The results of this study indicate that this kind of operation presents an alternative means for the preparation of pure enantiomeric compounds on a large-scale starting from racemic mixtures to the stereoselective synthetic and enzymatic routes currently available. The straightforward scaling up of the procedure from an analytical scale to gram amounts per day in the laboratory or even multi-kilogram separations by automated industrial production chromatography was demonstrated using mixtures of synthetically prepared racemates mainly of pharmaceutical interest.

INTRODUCTION

The economic application of preparative chromatography to the isolation of significant amounts of close structural and steric isomers of natural or synthetic origin is often impossible when difficult separations have to be accomplished and the resolving power of the chromatographic system is insufficient to allow overload conditions for improved throughput. In these cases, an increase in chromatographic resolution can be achieved by multi-dimensional chromatography and/or recycling chromatography.

In 1958, Martin [1] discussed the potential use of elution chromatography in a closed cycle, an approach first realized in practice by Porter and Johnson [2,3], called today closed-loop recycling chromatography. In a different approach, two nearly identical columns are connected by valves in such a way that the sample is pumped alternately through these columns without passing the pump head [4]. Although in this experimental set-up the contribution of the pump head to band spreading by remixing effects is avoided, the economic advantage of a lower solvent consumption is lost. Theoretical treatments on recycling chromatography were given by Chizhkov [5], who described this technique as a special case of the general separation theory, by Martin *et al.* [6], who described closed-loop recycling for analytical purposes and who derived expressions to determine the maximum number of cycles before remixing of eluting bands occurs, by Coq *et al.* [7], who presented a practical and theoretical study of the recycling technique, and by Seidel-Morgenstern and Guiochon [8], who used a mathematical model to design recycling and peak-shaving chromatography under overload

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conditions. Despite the long period during which recycling chromatography has been proposed for preparative chromatographic separations, the number of publications in which this technique has been applied is fairly small [9–42]. Numerous reasons for this have been put forward, mainly of technical origin, as there are insufficiently accurate and low-dead-volume pumps, a lack of adequate packing materials and a risk of damaging detector flow cells, so that despite its inherent advantages recycling chromatography has not become very popular.

Nevertheless, today, owing to the advances in modern HPLC equipment, these objections to the use of recycling peak-shaving technology are no longer valid. In the field of optical isomer separations, Schlögl and co-workers *et al.* [43] and Werner [44] were the first who recognized the time-saving and straightforward scale-up possibilities of recycling chromatography of racemates on chiral stationary phases, particularly microcrystalline cellulose triacetate.

In the last decade, many different chiral stationary phases (CSPs) have been developed for the chromatographic separation of racemates. Starting from simple models to explain chiral recognition phenomena, stationary phase–selectivity relationships for several types of CSP have been defined that allow a more than only empirically based strategy for the evaluation and selection of separation conditions. The use of database systems further eases the survey of the large number of publications dealing with chromatographic enantiomer separations [45].

The fact that almost all national regulatory committees emphasize the use of enantiomerically pure products and recommend the development of only the active enantiomer, whenever possible, has led to increased interest in all kinds of separation techniques in pharmaceutical industrial research and production. Owing to major advances in synthetic organic chemistry and analytical biochemistry in recent years, the tools have become more readily available both to prepare large amounts of individual isomers and to determine them in biological matrices, permitting the investigation of relevant pharmacological, metabolic, toxicological and pharmacokinetic properties of individual stereoisomers. Despite all the merits and the important role that analytical chromatography plays in these areas, preparative chromatographic enantiomer separation is still regarded as difficult to perform, expensive and time consuming. It goes without saying that not all stationary phases designed for analytical purposes are equally well suited for the preparative chromatographic separation of large amounts of a racemate, mainly owing to practical and/or economic reasons (availability, costs, mechanical and chemical stability, loadability, enantioselectivity). The major phases that are suitable for large-scale preparative chromatography are listed in Table I.

Also, in the development or investigation of a new drug, the time constraints are high. It is estimated that in more than 98% of all preparative chiral chromatographic separations to be performed, small amounts of pure enantiomers in the range from a few to several hundred milligrams have to be isolated within a few days or weeks. For the remaining 2% amounts from several grams to kilograms are required within several weeks or months, and only in less than 0.1% of all cases is preparative chromatography chosen as the final production method.

The recommended scale-up procedures in preparative chromatography require a minimum of enantioselectivity. As a general recommendation, an α value of larger than 1.4 allows an efficient and economic separation. Unfortunately, often the solvent–chiral stationary phase system optimized to fulfil this requirement does not allow large amounts to be loaded on the column owing to restrict sample solubility. Efficient peak fractionation is even more compli-

TABLE I

CHIRAL STATIONARY PHASES FOR PREPARATIVE CHROMATOGRAPHIC ENANTIOMER SEPARATION

Microcrystalline cellulose triacetate and tribenzoate
Cellulose ester– or cellulose carbamate–silica composites
Cellulose ester or cellulose carbamate spherical packings
Optically active polyacrylamide–silica composites
Chemically modified silica (Pirkle type)
β-Cyclodextrin-modified silica
Polymeric ligand-exchange packings

cated as extensive tailing of the enantiomer peaks is often observed under these "optimized" conditions. As a result, the throughput and yield of pure fractions are dramatically reduced, especially when both enantiomers have to be isolated. Whereas for synthetic chiral stationary phases one may overcome this problem by using a packing that incorporates the opposite optical conformation of the selector molecule to obtain a reversal of the elution order of the two enantiomers, this is not possible with chiral stationary phases derived from naturally occurring polysaccharides.

The recycling and peak-shaving technique offers a solution to most of the above problems encountered in preparative chromatographic separations of enantiomers, especially when the separation of only partially resolved products is achieved: it is possible to use existing analytical separation protocols for scale-up without further optimization of the separation parameters (regarding solvent composition and operational conditions); both enantiomers can be isolated in an optically pure form in good yields; a higher production rate per time unit is achieved; the solvent consumption is decreased when closed-loop recycling is applied; smaller column dimensions can be used to reach a desired goal; the method is less labour intensive, as there are no mixed fractions that have to be isolated and re-processed; and the recycling and peak-shaving process can be fully automated.

EXPERIMENTAL

Apparatus

Analytical chromatographic separations and scale-up and overload separations were performed on a Merck–Hitachi HPLC system which consisted of an L 6250 pump, a Rheodyne Model 7100 injection valve with sample loops from 20 to 2000 μl, an L-2500 integrator and an L-4250 variable-wavelength UV detector. Polarimetric detection was performed with a Perkin-Elmer Model 4100 polarimeter with a 40-μl flow cell.

Manually controlled preparative separations in the recycling and peak shaving on a laboratory scale were performed on a system consisting of a Shimadzu LC 8A semi-preparative pump, a Knauer variable-wavelength UV detector or a Merck–Hitachi L-4000 variable-wavelength UV detector with a pressure-stable experimental flow cell. Samples were injected on to the column by a syringe via a two-way valve at stopped flow. The solvent lines were arranged as described previously [46]. Selection between the elution or recycling mode was achieved by Valco 1/8-in. (1 in. = 2.54 cm) air-pressure-driven three-way valves. To prevent overpressure in the solvent line section between the detector and the pump in the recycling mode, which can cause destruction of the detector flow cell, a solvent reservoir open to the atmosphere was incorporated in the tubing via a three-way connector.

In all separations shown, one large injection of sample was performed at the beginning of a run. Separated portions of the eluting band were collected after an appropriate cycle number or at each cycle (depending on the individual situation) until the sample was exhausted. The possibility of running the recycling peak-shaving experiment in a semi-continuous mode was not attempted. This operational mode uses the injection of additional new feed at each cycle to increase the mass throughput. The recycling of mixed fractions together with the injection of new feed accompanied by the collection of constant portions of individual enantiomer fractions may result in a steady state after a few cycles.

This and other versions of recycling chromatography, which are not so easy to implement in practice, are described, *e.g.*, in ref. 47.

Automated recycling and peak-shaving experiments were performed with a Novaprep 5000 integrated preparative HPLC unit (Separation Technology). The waste valve in combination with one fraction valve was used to allow closed-loop recycling. Pump A of the two pumps incorporated in this system was used for isocratic elution and for automated sample injection.

For automated recycling and peak-shaving separations on technical and production scales, a Prepbar 200 automated HPLC process chromatographic unit (Merck) was used. A flow chart of this system is shown in Fig. 1.

In combination with technical and production scale HPLC equipment, a fully automated sol-

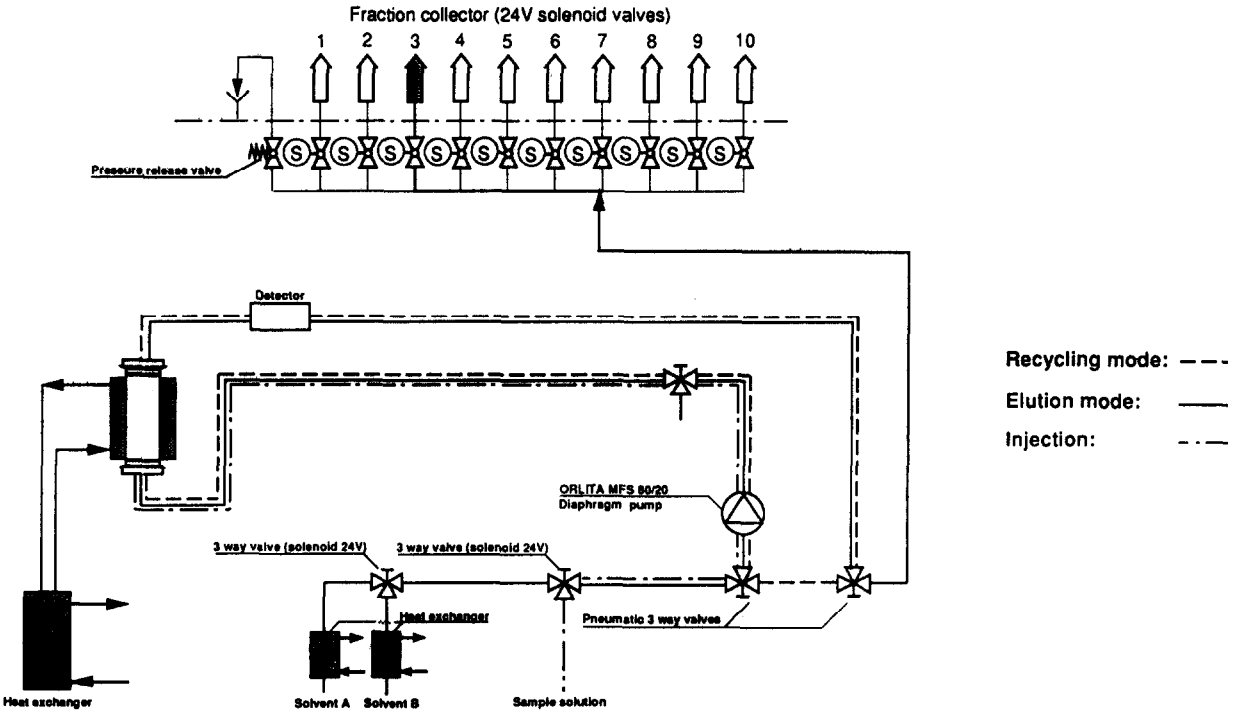

Fig. 1. Flow chart of a process chromatographic unit with closed-loop recycling option.

vent reclamation system, TOM Powervap PV20/50 (Genser–Merck), was examined. This explosion-proof system, based on a rotary evaporator with floating flask (20 or 50 l in volume), heat pump and closed-loop water-jet pump, allowed the unattended automated distillation and recovery of 15 l/h of methanol or 32 l/h of hexane–2-propanol (90:10).

Columns and packings

Cartridges 25 mm in diameter (Merck RT columns, lengths 250 and 125 mm), used in laboratory-scale separations, were slurry packed at 500 bar. The slurry medium was 2-propanol. Microcrystalline cellulose triacetate was packed into Superformance glass columns (300 × 26 mm I.D.) according to described procedures. Separations with columns of 250 × 50 mm I.D. were performed on Prepbar stainless-steel cartridges (Merck) equipped with a compression piston. These columns were packed by the vacuum method with acetone as slurry liquid according to the manufacturer's instructions [48]. Technical-scale separations were performed with either a Prochrom modified dynamic axial compression system (800 × 50 mm I.D.) or Merck Prepbar stainless-steel columns (400 × 100 mm I.D.) with an additional thermostating jacket.

Methods

Reconstruction of partly overlapped chromatographic peaks and determination of fractionation points. During the preparative chromatographic run, samples are taken at regular intervals. Depending on the concentration, the samples are used as such or diluted to a suitable concentration and analysed (preferably a short column is used for this purpose to shorten the analysis time). The measured data (retention times, area counts) are transferred via a network system (Labnet, Spectra-Physics) to a mainframe computer (IBM 3090 series). Thereafter a program allows the elution profiles to be reconstructed by means of cubic splines. The program is written in APL (a program language), essentially a matrix language with powerful functions such as inversion and transposition of a matrix. A cubic spline

function needs at least five data points per peak. Of course, the more data points are available the more accurate the reconstructed peak profiles will be. The accuracy of the reconstructed chromatogram can be easily checked by integration of the peak areas obtained. The ratios of the peak areas of the reconstructed chromatogram are therefore always compared with the initial quantitative analytical data. Experience has shown that for a mixture of two components present in approximately the same concentrations about 15–20 analytical runs are necessary to give a reconstructed chromatogram that differs by less than 1% in the ratios of peak areas from the analytically determined values.

The determination of fractionation points for a stated product quality regarding purity is done by integration of peak areas up to a preset point in the reconstructed chromatogram and calculation of the proportion of these areas. This calculation is repeated until a point is reached which matches the preset quality level within certain limits (about 0.1%).

Integration of cubic spline functions is easily achieved, as these functions are a set of third-order equations, the integrated function of which is of the fourth order. Many different applications to real separation examples have demonstrated that peak collection based on the above principle generally results in a purity of the isolated product that differs by not more than 0.5–1% from the preset value.

Determination of melting point diagrams. A knowledge of melting point diagrams allows the eutectic point (if there is any) of a racemate to be determined. On the right-hand side of the eutectic, the optical purity of a mixture of two enantiomers may be further enhanced by repeated crystallization. Whenever possible, the combination of crystallization and chromatography should be used to improve the overall economy of the preparative separation.

Based on the measured values of the melting temperature and the corresponding enthalpy of fusion of the racemate and the values for one of the pure enantiomers, it is possible by use of the Schröder–Van Laar and the Prigogine–Defaye equations to draw up a melting point diagram [49–51]. The measurements are performed by differential scanning calorimetry (DSC) (Perkin-Elmer DSC-7).

The theoretically calculated melting point curve can be controlled by the determination of the melting point and the enthalpy of fusion of artifically prepared mixtures of known composition. Two approaches are possible in practice. (i) Known mixtures of two enantiomers or a mixture of the racemate and one of the pure enantiomers are transferred into a glass test-tube and dissolved in a suitable solvent. After homogenization, the solvent is removed by a current of dry nitrogen or by distillation. The evaporation residue is thoroughly dried under vacuum at a temperature sufficiently below the melting point of the product. The crystalline residue obtained is subsequently used in the DSC measurements. (ii) Owing to difficulties often observed when the above-described method is applied, it is preferable to use exact weighed mixtures of the crystallized products (about 15-mg amounts weighed on a microbalance). These artificial mixtures are thoroughly ground and mixed in an agate mortar. After the milling and mixing has been completed, the total amount of product is used in the DSC experiments. A 15-mg amount is sufficient to perform three or four measurements. The observed melting temperatures and enthalpies of fusion of the different determinations on the same sample are averaged and this value is used for the comparison with the theoretically calculated point on the melting curve.

RESULTS AND DISCUSSION

Laboratory-scale separations

To demonstrate the time-saving and economic benefits of the recycling and peak-shaving technique in the laboratory and also to show its limitations, the examples given in Table II were selected.

As stated before, most preparative chromatographic enantiomer separations in the laboratory are directed at isolating milligram to gram amounts of the pure isomers. The separation of racemic chlorthalidone, a chiral drug, and the separation of 3-benzoyl-2-(*tert.*-butyl)oxazolidin-5-one, an intermediate in the preparation of

TABLE II

RACEMATES SEPARATED ON DIFFERENT CSPs ON A LABORATORY SCALE

CSP	Separation
Microcrystalline cellulose triacetate	Naringenine 2,3,4,5,6,7-Hexamethyl-9,10-phenanthrenequinone
Poly-N-acryloyl-(*S*)-phenylalanine ethyl ester–diol-silica	Chlorthalidone
Poly-N-acryloyl-(*S*)-phenylalaninediethylamide–diol-silica	1,1′-Dihydroxy-2,2′-binaphthyl
Poly-N-methacryloyl-(*R*)-cyclohexyl-2-ethylamide	Thalidomide
β-Cyclodextrin bonded to silica	Chlorthalidone Propranolol and metaprolol β-blockers 4,5-Dimethyl-3-hydroxy-2-[5*H*]-furanone
3,5-Dinitrobenzoylphenylglycine bonded to aminopropylsilica	3-Benzoyl-2-(*tert.*-butyl)oxazolidin-5-one
L-Proline bonded to polystyrene–silica composite	Proline 3,3,3-Trifluoroalanine
Bovine serum albumin adsorbed on diol-silica	Benzoin

alkylated amino acids, were selected to illustrate the differences between the classical preparative chromatographic approach and the recycling and peak-shaving technique.

The analytical and preparative separation of 3-benzoyl-2-(*tert.*-butyl)oxazolidin-5-one and other derivatives of this class of compounds have been performed by Seebach *et al.* [52] on Chiraspher, an optically active polyacrylamide–silica composite CSP. While this packing exhibited a very large enantioselectivity factor of $\alpha = 2.33$, the separation factors for microcrystalline cellulose triacetate ($\alpha_{D,L} = 1.16$) and 3,5-dinitrobenzoylphenylglycine (DNBPG) bonded to aminopropylsilica (Pirkle phase, $\alpha_{D,L} = 1.27$) were significantly below the recommended value of $\alpha > 1.40$, mentioned before. This indicates that the use of these two systems would require further optimization of the separation conditions before a preparative separation could be attempted with some chance of success. The results for loads of 50 and 120 mg of 3-benzoyl-2-(*tert.*-butyl)oxazolidin-5-one separated on the 3,5-DNBPG–silica are given in Fig. 2. In both instances the complete separation of the two enantiomers was achieved in about 90 min. Peak shaving was performed on the second-eluted enantiomer to prevent remixing of subsequent cycles. As the column dimensions in ref. 52 and in this experiment are the same, the results are comparable despite the large difference in enantioselectivity exhibited by the two CSPs toward this racemate.

Chlorthalidone, a racemic diuretic drug, cannot be prepared by stereoselective synthesis. Conventional enantiomer separation by formation of diastereomers fails owing to the lack of convenient functional groups. By searching the data in the CHIRBASE database, several chromatographic systems [53–57], compiled in Table III, were obtained which describe the separation of chlorthalidone for analytical purposes.

Two of the five CSPs in Table III, α-cyclodextrin bonded to silica and Chiraspher, were available for further experiments. Cyclodextrin-based CSPs are recommended mainly for analytical purposes; their application to preparative separations has not been attempted because of the low mass load capacity. The use of a mobile phase published by Armstrong *et al.* [58], namely methanol–water (40:60, v/v) containing 0.1% triethylamine, adjusted to pH 4.1 with acetic acid, resulted in a nearly baseline separation in the first cycle when 25 mg of sample dissolved in methanol were injected on to the column. To avoid the use of buffered solutions, elution with methanol–water (40:60, v/v) was examined. Only partial separation of chlorthalidone was observed after the first passage through the

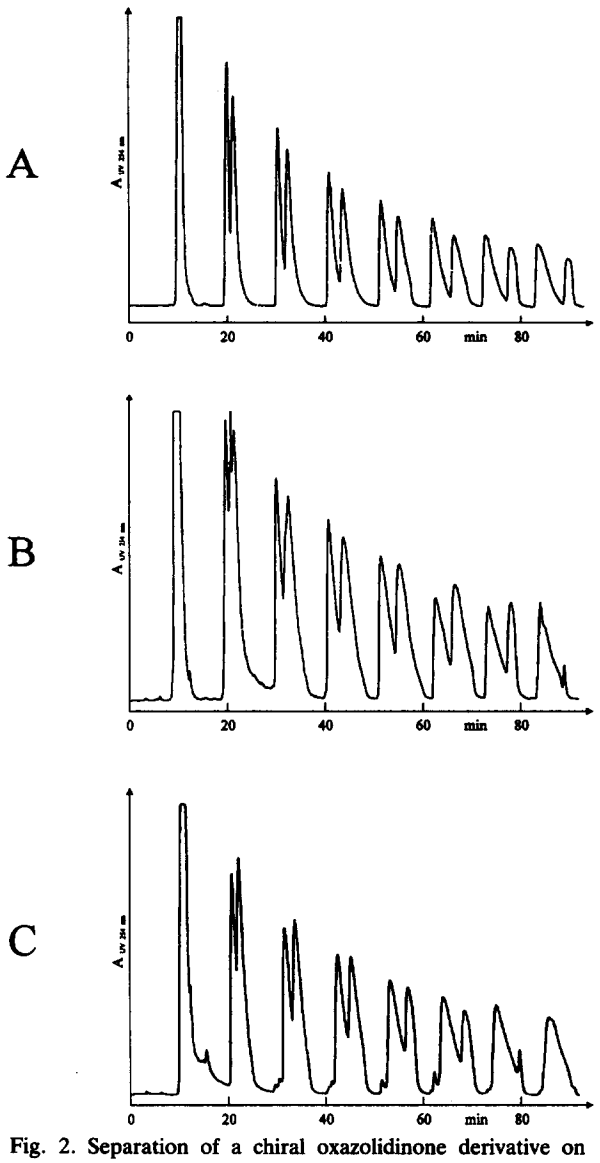

Fig. 2. Separation of a chiral oxazolidinone derivative on (*R*)-(+)-3,5-dinitrobenzoylphenylglycine bonded to aminopropylsilica. Chromatographic conditions: sample, 3-benzoyl-2-(*tert.*-butyl)oxazolidinon-5-one; column, 250 × 25 mm I.D. packed with 3,5-DNBPG bonded to amino-modified silica, 10 μm (Chirasep, Merck); mobile phase, *n*-hexane–2-propanol (90:10, v/v); flow-rate, 40.0 ml/min; detection, UV at 254 nm; sample size, (A) 50 mg dissolved in 1.0 ml of dioxane, (B) 100 mg dissolved in 2.0 ml of dioxane and (C) 100 mg dissolved in 1.0 ml of dioxane.

column. The separation was completely lost in the following cycles 2 to 4 (see Fig. 3). Therefore, the use of a buffered solvent system is essential.

TABLE III

DIRECT CHROMATOGRAPHIC SEPARATION OF THE RACEMIC DIURETIC CHLORTHALIDONE ON DIFFERENT CSPs ACCORDING TO THE ENTRIES IN THE CHIRBASE DATABASE

Type of CSP	Selectivity factor	Ref.
β-Cyclodextrin bonded to diol-silica	1.44	53
Poly-(*S*)-acryloylphenylalanine ethyl ester–diol-silica (Chiraspher)	1.26	54
Bovine serum albumin cross-linked to silica	1.26	55
α-1-Acid glycoprotein bonded to silica	2.45	56
Hydroxypropyl-modified β-cyclodextrin bonded to silica	1.21–1.38	57

Dioxane, a solvent that allows chlorthalidone to dissolve in a much higher concentration than in methanol, could not be used as the sample solvent, as demonstrated in Fig. 4. While an injection volume of 0.5 ml of dioxane resulted in a total loss of separation (Fig. 4A), replacement of dioxane with methanol restored the separation ability of the system towards chlorthalidone (Fig. 4B).

Surprisingly, the separation of chlorthalidone enantiomers did not improve with increasing cycle number despite the nearly baseline separation already observed after the first cycle (see Fig. 5A). We assume that partial racemization of the resolved enantiomers under buffered elution conditions during their passage through the column may be the cause of this effect. To prove it, the separation was repeated with the change that during the second cycle the second-eluting enantiomer was collected by peak shaving. Fig. 5B shows that a small plateau peak evolves after the third cycle. The plateau increases slightly further after the fourth and fifth cycles. The plateau is equivalent to the increasing amount of the second-eluting enantiomer, generated by racemization of enantiomer 1, which remained in the closed-loop system after the first cycle. Collection of this enantiomer 1 after the fifth cycle followed by storage of this fraction for 24 h in the eluent and reanalysis for optical purity

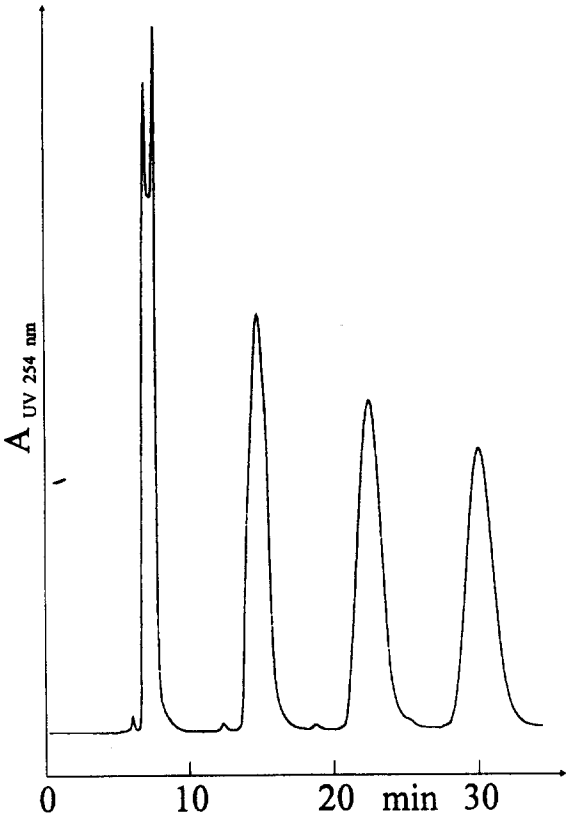

Fig. 3. Recycling chromatography of racemic chlorthalidone on β-cyclodextrin–silica with methanol–water as eluent. Chromatographic conditions: sample, chlorthalidone; column, 250 × 25 mm I.D. packed with β-cyclodextrin bonded to silica, 5 μm (Chiradex, Merck); mobile phase, methanol–water (40:60, v/v); flow-rate, 40.0 ml/min; detection, UV at 254 nm; sample size, 25 mg dissolved in 0.5 ml of methanol.

showed a total loss of enantiomer excess and confirmed our hypothesis.

The maximum load on a column of dimensions 250 × 25 mm I.D. was determined with 70 mg of chlorthalidone with 1 ml of methanol as sample solvent. A further increase in the injection volume led to remixing of peaks of subsequent cycles. The small cycle time period of about 10 min prevented a further increase in sample load to improve the throughput. When an alternative packing to separate chlorthalidone, Chiraspher, was examined, there were no restrictions to using dioxane as the sample solvent. Additionally, it was possible to adjust the capacity factors of the two enantiomers by changing the dioxane content in the *n*-hexane–dioxane eluent. An amount of 240 mg was separated within 90 min without further method optimization. As can be seen in Fig. 6, the limits of sample load are still far from being reached. Larger amounts of chlorthalidone, separated in the described manner, have been used in a study on the long-term stability of enantiomeric pure drugs in pharmaceutical formulations [59].

In a second trial, the question of the time and effort required to transfer published data on analytical separations of enantiomers from an analytical scale to preparative separations by use of the recycling and peak-shaving technique was further examined. The separation conditions for two different chiral β-blockers, propranolol and metaprolol, given in ref. 58 were applied without any further optimization. The separations shown in Fig. 7 were achieved within 4 h.

The application of β-cyclodextrin–silica to the preparative separation of chiral flavour compounds is shown in Fig. 8. A 50-mg amount of 4,5-dimethyl-3-hydroxy-2[5*H*]-furanone was injected as pure liquid without further sample dilution by additional solvent. The separation and collection of the two enantiomers was accomplished in less than 2 h [60].

As a rule of thumb, in the case of liquid samples the administration of undiluted samples is always worth examining. Empirically the results indicate that the possibility of reaching maximized concentration overload conditions together with closed-loop recycling and peak-shaving chromatography is beneficial with regard to economic aspects. The throughput of a separation performed under identical conditions for the same mass load but with diluted sample and therefore a larger injection volume never reached the same level. To obtain the same yield, additional cycles were necessary.

In the next two examples, microcrystalline cellulose triacetate was used as a CSP to separate the atropisomers of 2,3,4,5,6,7-hexamethyl-9,10-phenanthrenequinone [61] (Fig. 9) and the enantiomers of naringenine, the bitter taste in tomatoes [62] (Fig. 10). The solubility of 2,3,4,5,6,7-hexamethyl-9,10-phenanthrenequinone was about 4 mg/ml. The separation of 50 mg of sample dissolved in 11.8 ml of methanol within three cycles is demonstrated in Fig. 9.

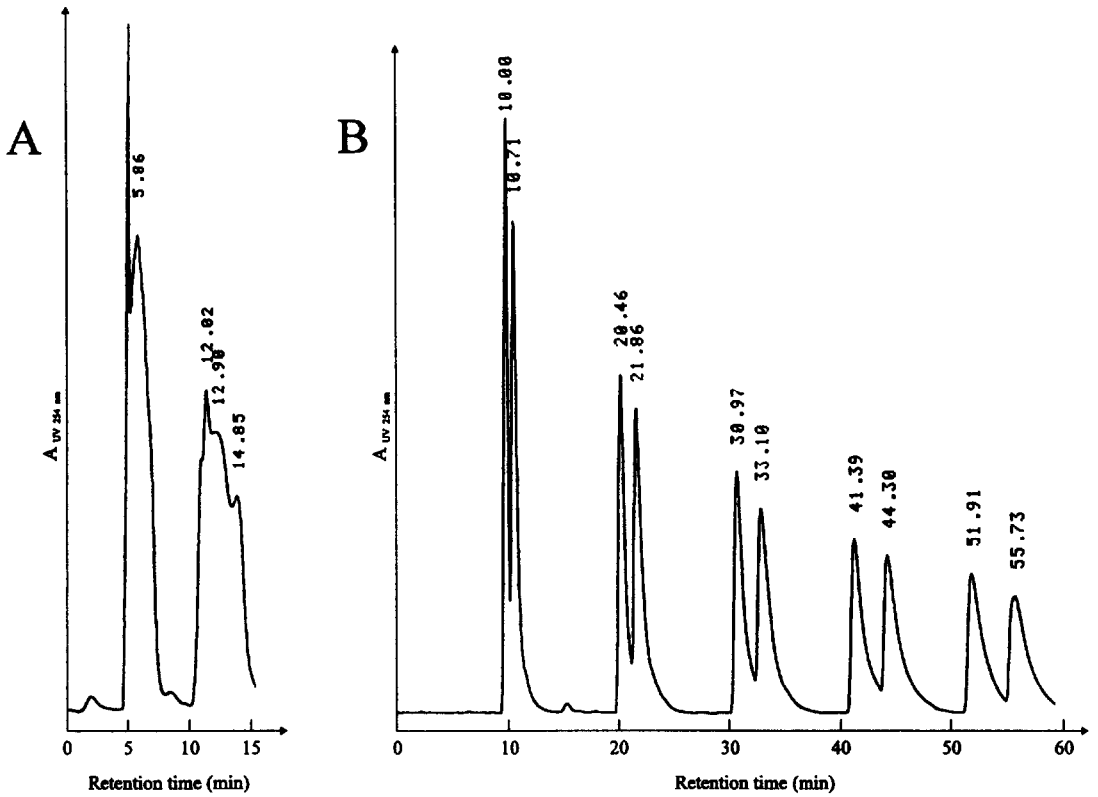

Fig. 4. Deterioration of the separation of chlorthalidone on β-cyclodextrin–silica with dioxane as sample solvent. Chromatographic conditions: sample, chlorthalidone; column, 250 × 25 mm I.D. packed with β-cyclodextrin bonded to silica, 5 μm (Chiradex, Merck); mobile phase, methanol–0.1% triethylamine (40:60, v/v), adjusted to pH 4.1 with acetic acid; flow rate, 40.0 ml/min; detection, UV at 254 nm; sample size, (A) 25 mg dissolved in 0.5 ml of dioxane and (B) 25 mg dissolved in 0.5 ml of methanol.

During the course of this separation, achiral impurities that eluted in front of the first enantiomer were also separated. On the same column and with the same eluent, 5 g of the flavanone naringenine have been separated in twelve runs. Fig. 10 compares the chromatograms of the separation of 100 mg in a single run and the separation of 0.4 g in two cycles.

The increase in efficiency with increasing cycle number for a 300 × 26 mm I.D. column packed with 15–25-μm microcrystalline cellulose triacetate (CTA) is given in Table IV. The test of chromatographic equipment, that is used in recycling and peak-shaving experiments is best performed by the measurement of column efficiency. If no significant increase in plate numbers is achieved by the recycling operation, band broadening due to remixing in the pump heads or by extra-column void volumes may be the cause and has to be reduced.

2,2′-Dihydroxy-1,1′-binaphthyl, whose enantiomers are starting materials for liquid crystals, crown ethers and hydrogenation catalysts, can be separated on a chiral polacrylamide–diol-silica copolymer, prepared from N-acryloyl-(*S*) phenylalanine diethylamide [63]. While the solubility and enantioselectivity of 2,2′-dihydroxy-1,1′-binaphthyl is very high with tetrahydrofuran or tetrahydrofuran–hexane mixtures as eluents, the k' values are extremely low. In pure tetrahydrofuran, the first enantiomer elutes nearly unretained, while the second enantiomer has a retention factor of 0.3. By peak shaving of the first-eluted enantiomer starting in the first cycle, 1.35 g of 2,2′-dihydroxy-1,1′-binaphthyl dissolved in 1.3 ml of eluent were separated within

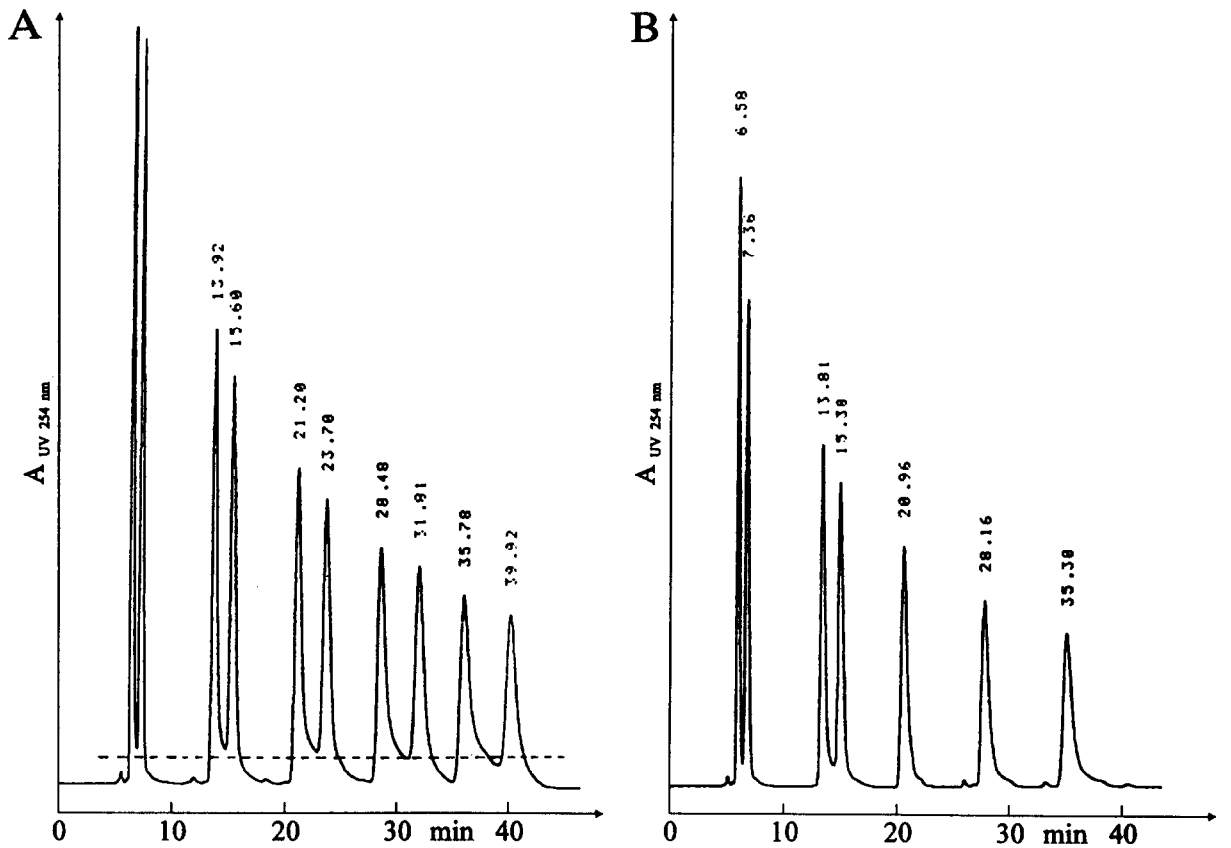

Fig. 5. Partial racemization of chlorthalidone during elution through the column. Chromatographic conditions: sample, chlorthalidone; column, 250 × 25 mm I.D. packed with β-cyclodextrin bonded to silica, 5 μm (Chiradex, Merck); mobile phase, methanol–0.1% triethylamine (40:60, v/v), adjusted to pH 4.1 with acetic acid; flow-rate, 40.0 ml/min; detection, UV at 254 nm; sample size, 25 mg dissolved in 0.5 ml of methanol. (A) No peak fractionation; (B) fractionation of peak 2 during 14.5–16.0 min.

12 min. Enantiomers with high optical purity were recovered from the collected fractions (Fig. 11).

In Table V, the production capacity and solvent consumption for two laboratory-scale column dimensions (250 × 25 mm I.D. and 250 × 50 mm I.D.) and a small technical-scale column are calculated for an automated production running over 24 h. More than 18 kg of racemate can be separated per week on a column that contains about 1 kg of CSP. A comparison of run times, yields for an optical purity of 98% and solvent consumption of the automated unattended separation of 2,2,2-trifluoro-1-(9-anthryl)ethanol on 3,5-DNBPG-silica by the recycling and peak-shaving technique [64] and normal elution chromatography [65] are presented in Table VI. Solvent consumption and yield are in favour for the recycling operation despite the longer run time, especially for the second eluted enantiomer.

The separation of thalidomide was performed on a poly-N-methacryloyl-(*R*)-cyclohexylethylamide–diol-silica (5 μm) column (Fig. 12). Fourteen cycles were necessary to separate 140 mg in a total run time of about 75 min.

In a further study, the capability of CSPs, which are not often used in preparative applications, for use in recycling experiments was checked. In ligand-exchange chromatography, the dependence of the k' values of the enantiomers is very sensitive to mass overload effects [66]. Two typical samples, D,L-proline and 3,3,3-trifluoro-D,L-alanine were applied to a hydroxyproline-modified polystyrene–silica composite packing [67]. To increase the mass transfer

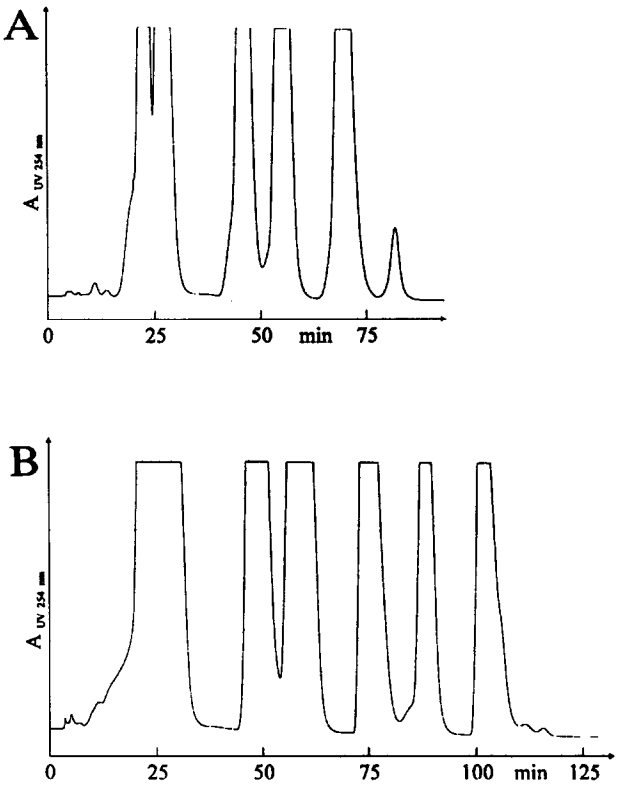

Fig. 6. Preparative separation of chlorthalidone on Chiraspher. Chromatographic conditions: sample, chlorthalidone; column, 250 × 25 mm I.D. packed with poly-N-(acryloyl)-(*S*)-phenylalanine ethyl ester–silica composite based on LiChrospher 100 Diol, 5 μm (Chiraspher, Merck); mobile phase, *n*-hexane–dioxane (60:40, v/v); flow-rate, 40.0 ml/min; detection, UV at 254 nm; sample size, (A) 140 mg dissolved in 1.6 ml of methanol and (B) 240 mg dissolved in 3.0 ml of methanol.

characteristics of this CSP, the separation temperature was set at 65°C and the flow-rate was lowered to one quarter of the normal value. Proline was separated fairly well: 60 mg in 35 min (three cycles) and 250 mg in 65 min (five cycles) (Fig. 13).

For 3,3,3-trifluoro-D,L-alanine, strong tailing of the second-eluting enantiomer prevented an efficient separation of even 12.5 mg with the same column and conditions (Fig. 14). The reduced UV response of the peak of the second enantiomer is caused by a change in the local decrease in the copper ion concentration due to overload effects.

Protein-based chiral stationary phases are among the packing materials used mainly for analytical purposes only. Bovine serum albumin adsorbed on wide-pore diol-silica has been extensively described by Guiochon and Jacobson *et al.* [68]. Therefore, this type of proteinaceous packing was chosen by us for recycling chromatographic evaluation. The strong tailing of eluting enantiomer peaks was found to prevent the beneficial use of this packing for preparative separations in combination with recycling peak shaving. The separation of benzoin is presented in Fig. 15.

In those cases where severe peak tailing may be reduced by mobile phase additives, successful applications of protein phases in recycling peak-shaving operations seem achievable and may extend the use of these CSPs for preparative separations to some extent.

Technical- and production-scale separations

Racemates separated on different CSPs on technical and production scales are listed in Table VII.

The scale-up of preparative separations of enantiomers on microcrystalline cellulose triacetate by elution chromatography has frequently been described. An example, in which kilogram amounts of racemate were injected on to a column packed with this chiral stationary phase, is the separation of a chiral hetrazepine derivative (Table VIII). Owing to a favourable melting curve of the two enantiomers, this specific separation only required a purity of >80%. A further increase in the enantiomeric excess of the product is obtained by crystallization. This circumstance allowed the use of the recycling and peak-shaving technique despite the fact that parts of the second-eluting enantiomer within the first cycle co-eluted with the first enantiomer of the second cycle. Nevertheless, 2 kg of racemate can be loaded on the column; the production rate for the first-eluting enantiomer increases twofold.

To the best of our knowledge, the separation of α-(2,4-dichlorophenyl)-1*H*-imidazole-1-ethanol represents the first example of the use of a CSP based on hydroxypropyl-β-cyclodextrin on a technical scale. One of the major advantages of chemically bonded cyclodextrin or derivatized cyclodextrin–silica packings is their ability to be

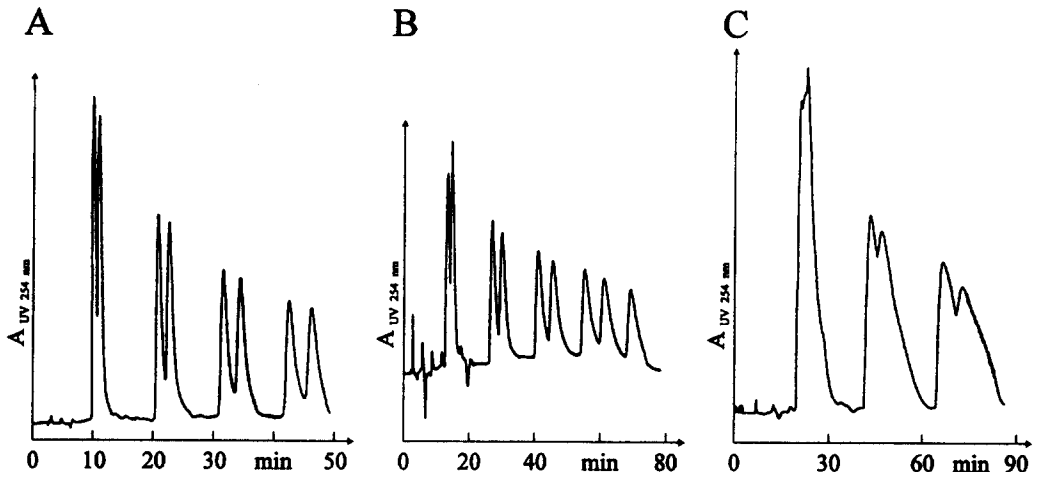

Fig. 7. Preparative separation of the β-adrenalergic drugs metaprolol and propranolol. Chromatographic conditions: sample, (A) propranolol (5 mg), (B) metaprolol (5 mg) and (C) metaprolol (120 mg), dissolved in methanol; column, 250 × 25 mm I.D. packed with β-cyclodextrin bonded to silica, 5 μm (Chiradex, Merck); mobile phase, acetonitrile–methanol–acetic acid–0.1% triethylamine (95:5:0.3:0.2, v/v) according to ref. 58; flow-rate, 40.0 ml/min.

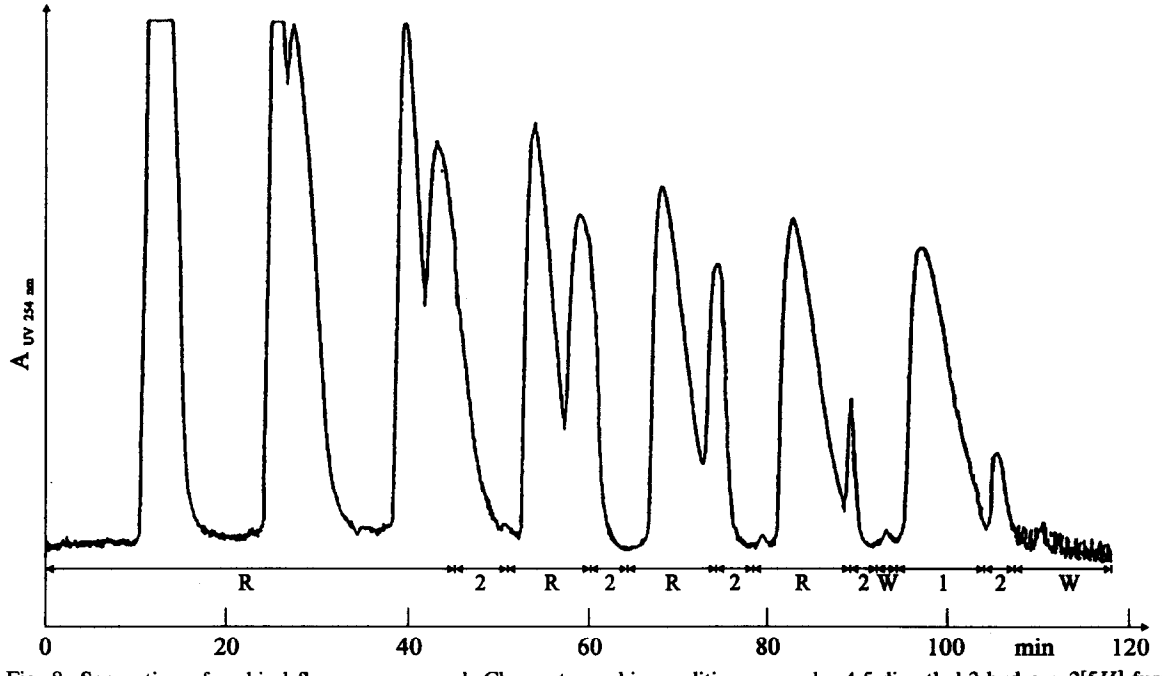

Fig. 8. Separation of a chiral flavour compound. Chromatographic conditions: sample, 4,5-dimethyl-3-hydroxy-2[5*H*]-furanone (sample provided by A. Mosandl, University of Frankfurt/Main); column, 250 × 50 mm I.D. packed with β-cyclodextrin bonded to silica, 5 μm (Chiradex, Merck); mobile phase, methanol–0.1% triethylamine (5:95, v/v), adjusted to pH 4.3 with acetic acid; flow-rate, 80.0 ml/min; detection, UV at 220 nm; sample size, 50 mg of undiluted sample. Here and in further figures: R = recycling mode; W = waste; 1 = enantiomer 1; 2 = enantiomer 2.

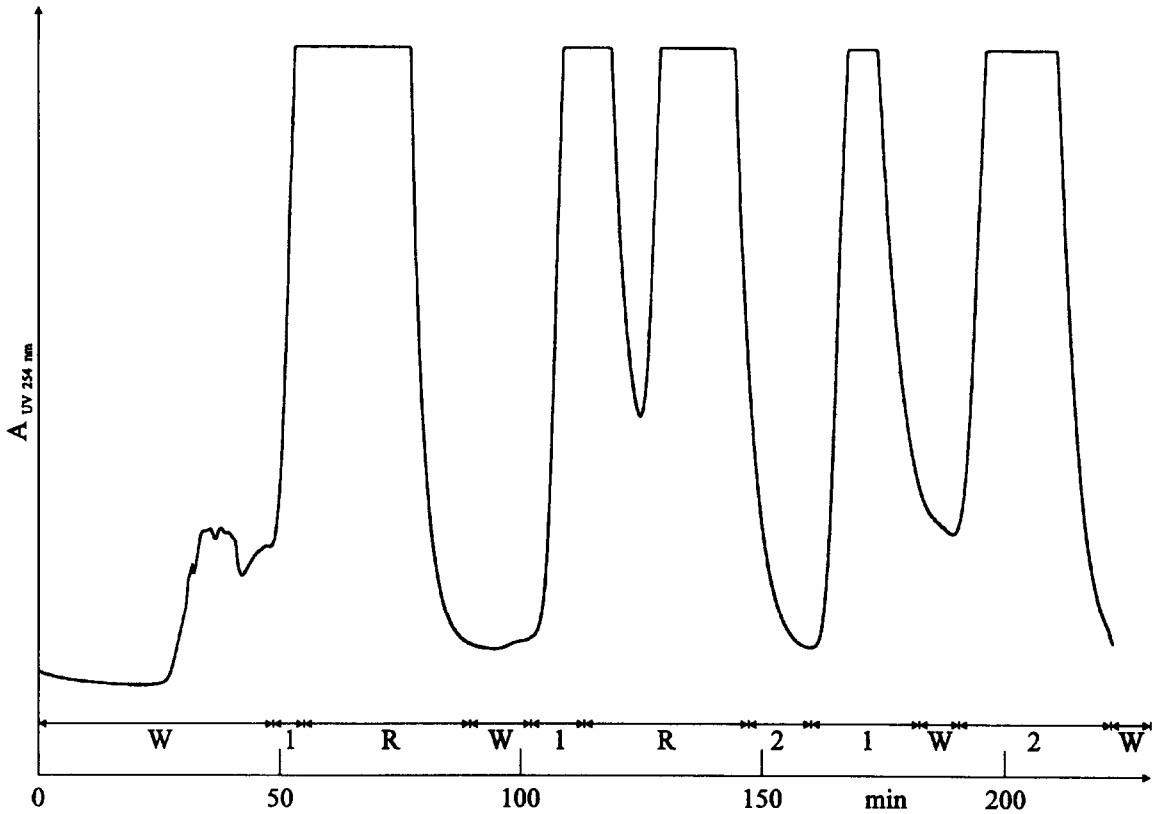

Fig. 9. Separation of 2,3,4,5,6,7-hexamethyl-9,10-phenanthrenequinone on CTA. Chromatographic conditions: sample, 2,3,4,5,6,7-hexamethyl-9,10-phenanthrenequinone (sample provided by A. Mannschreck, University of Regensburg); column, Superformance 300 × 26 mm I.D. packed with microcrystalline cellulose triacetate, 15–25 μm (Merck); mobile phase, methanol; flow-rate, 3.0 ml/min; detection, UV at 278 nm; sample size, 50 mg in 11.8 ml of methanol.

used under reversed-phase elution conditions. At first, it was possible to separate this alcohol, after derivatization with *p*-methylbenzoyl chloride to the respective ester, on a Chiracel OJ type column. Unfortunately, it turned out that after preparative chromatography had been successfully performed it was impossible to liberate the free alcohol from the ester under acidic conditions without racemization. At this stage, the separation of the alcohol was attempted on a hydroxypropyl-β-cyclodextrin–silica column with good success. Elution of the baseline-separated enantiomers was performed in less than 10 min. In order to waste no time with further optimization work, it was decided to separate the total amount of 100 g by means of 1.5-g injections and application of recycling and peak shaving (Fig. 16). Within 2 days of continuous automated operation, the total amount of sample was separated. After evaporation of the methanolic part of the eluent and neutralization with ammonia, the product was easily recovered by extraction with dichloromethane. Based on this and other examples not discussed here, loadabilities of up to 4 mg per gram of packing have been observed. Therefore, in future, cyclodextrin packings should be considered more often as valuable preparative chromatographic tools.

Not in every case is such a favourable situation met. When considerable amounts of a product have to be separated immediately by chromatography, usually a number of parameters that may affect the separation should be examined. By means of a specific case, namely the separation of a benztriazole derivative of pharmaceutical interest and therefore proprietary structure on a

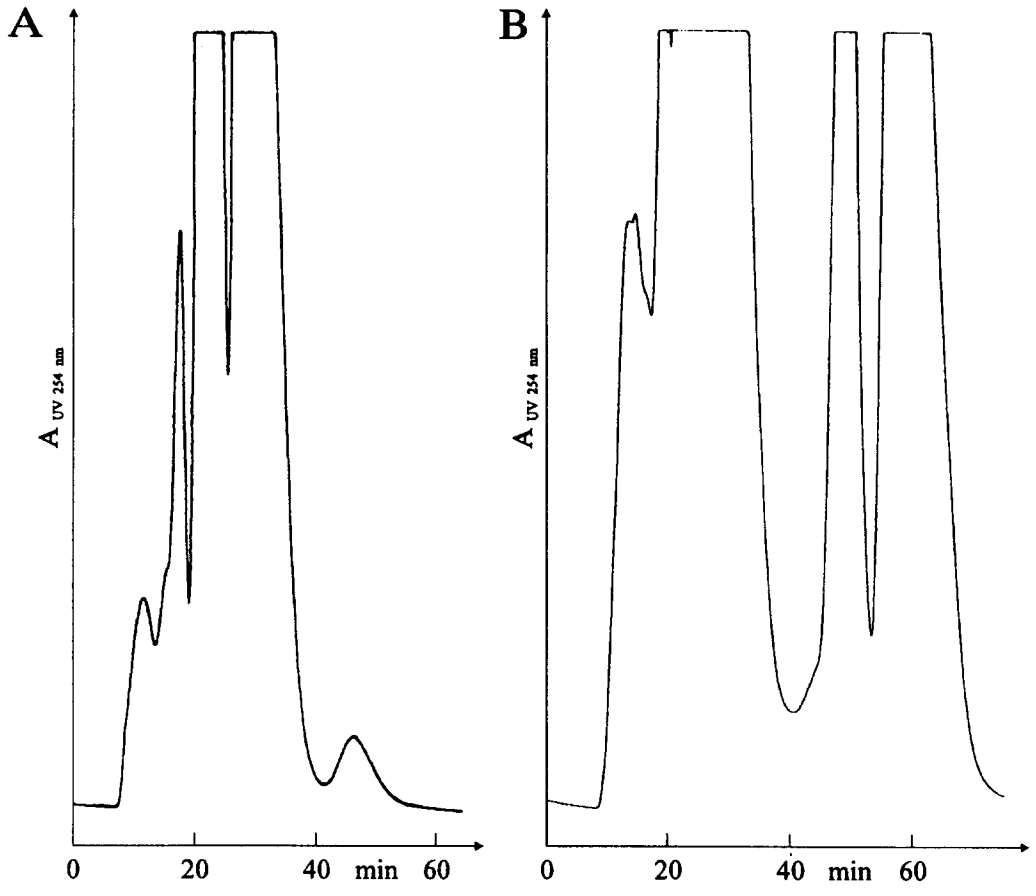

Fig. 10. Separation of naringenine on CTA. Chromatographic conditions: sample, naringenine; column, Superformance 300 × 26 mm I.D. packed with microcrystalline cellulose triacetate, 15–25 μm (Merck); mobile phase, methanol; flow-rate, 8.0 ml/min; detection, UV at 280 nm; sample size, (A) 50 mg dissolved in 2 ml of methanol (elution mode) and (B) 400 mg dissolved in 8 ml of methanol (2 cycles).

TABLE IV

DEPENDENCE OF THE COLUMN EFFICIENCY OF CTA (15–25 μm) ON THE NUMBER OF RECYCLING STEPS

Cycle number	Retention time (min)	Theoretical plate number (plates/m)
1	18.6	4900
2	37.5	8400
3	56.5	11 400
4	75.4	14 300
5	94.4	17 000

Conditions: Column, 300 × 26 mm I.D.; packing, CTA (15–25 μm); eluent, methanol; flow-rate, 6 ml/min; sample, 1,3,5-trihydroxybenzene.

cellulose tri(*p*-methylphenyl ester)–silica material (Chiracel OJ), the influence of several of these factors is illustrated. This example also demonstrates that not in every case is the recycling and peak-shaving technique the best choice in economic terms. This often holds in those instances, where only the first-eluting enantiomer has to be recovered as a pure product and when the racemate is sparingly soluble.

First, the effect of temperature variation on the retention behaviour has to be examined for a few mobile phase compositions. The influence of temperature variation on the k' values of the enantiomers for methanol, ethanol and hexane–ethanol (40:60, v/v) is given in Fig. 17. For the different mobile phase compositions investi-

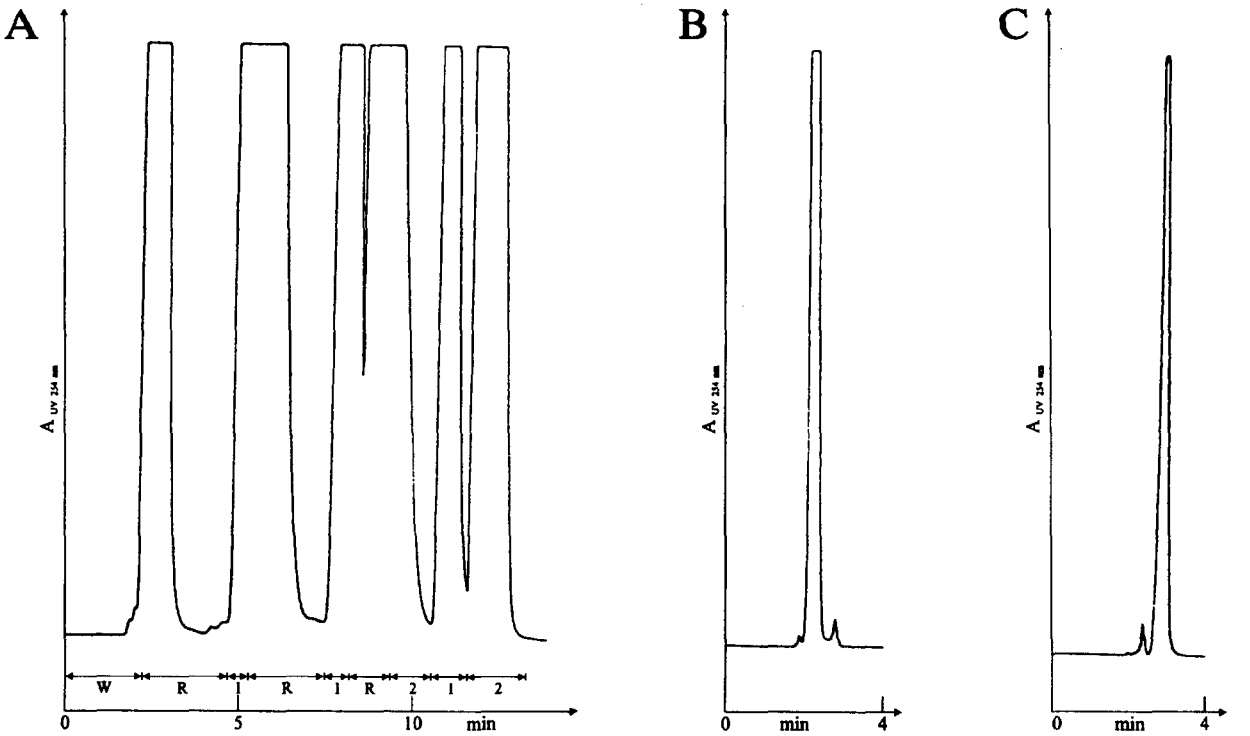

Fig. 11. Separation of 2,2′-dihydroxy-1,1′-binaphthyl on poly-N-acryloyl-(*S*)-phenylalaninediethylamide–diol-silica composite as CSP. Chromatographic conditions: sample, 2,2′-dihydroxy-1,1′-binaphthyl; column, 250 × 25 mm I.D. packed with poly-N-(acryloyl)-(*S*)-phenylalaninediethylamide–silica composite based on LiChrospher 100 Diol, 5 μm (experimental batch, Merck); mobile phase, tetrahydrofuran; flow-rate, 40 ml/min; detection, UV at 254 nm; sample size, 1350 mg dissolved in 1.3 ml of tetrahydrofuran. (A) Separation of sample; (B) and (C) analysis of collected fractions.

gated, a good linear correlation is observed between the logarithm of the capacity factor and the inverse of the temperature. From comparison of the slopes of the different curves, we may conclude that the smallest effect is observed when methanol is used as the eluent. The largest

TABLE V

CALCULATED PRODUCTIVITY RATE OF 1,1′-DIHYDROXY-2,2′-BINAPHTHYL BY THE RECYCLING AND PEAK-SHAVING TECHNIQUE FOR DIFFERENT COLUMN DIMENSIONS

Parameter	Column dimensions		
	250 mm × 25 mm I.D.	250 mm × 50 mm I.D.	250 mm × 100 mm I.D.
Production rate (kg/day)	0.162	0.648	2.59
Solvent consumption (l/day)	34 (40 ml/min)	134 (160 ml/min)	538 (640 ml/min)
Production rate (kg/week)	1.13	4.54	18.14
Solvent consumption (l/week)	235	941	3760

TABLE VI

COMPARISON BETWEEN BATCH AND RECYCLING–PEAK-SHAVING CHROMATOGRAPHY FOR THE SEPARATION OF 2,2,2-TRIFLUORO-1-(9-ANTHRYL)-ETHANOL

Conditions: laboratory scale, preparation of 10 g of both enantiomers with an optical purity of >95%; CSP, Chirasep DNBPG (10 μm) (Merck); column dimensions, 250 × 25 mm I.D.; flow-rate, 20 ml/min; eluent, *n*-heptane–2-propanol (98:2, v/v).

Method	Sample (g)	Run time (min)	Yield (%)		Solvent consumption (l)
			Enantiomer 1	Enantiomer 2	
Batch operation	1.25	41	82	42	32
Recycling–peak shaving (2 cycles)	1.25	77	96	93	24

effect occurs with *n*-hexane–ethanol, although there are only small differences from the results obtained with pure ethanol. The smallest values of the separation factor α, as shown in Fig. 18, are obtained when methanol is used as the eluent. For this solvent the enantioselectivity decreases only slightly with increase in temperature. For ethanol the α-values are much higher, and the highest values are obtained with *n*-hexane–ethanol mixture. As Fig. 18 clearly demonstrates, the temperature dependence of the selectivity factor α is virtually the same for ethanol and *n*-hexane–ethanol as mobile phase.

It was obviously also of interest to assess the effect of mobile phase velocity on the column efficiency at different temperatures. Fig. 19 shows the relationship between the height equivalent to a theoretical plate (HETP) and the flow velocity at different temperatures determined for one of the solvents tested (*n*-hexane–ethanol). It can be concluded that the kinetic circumstances are less favourable at temperatures lower than

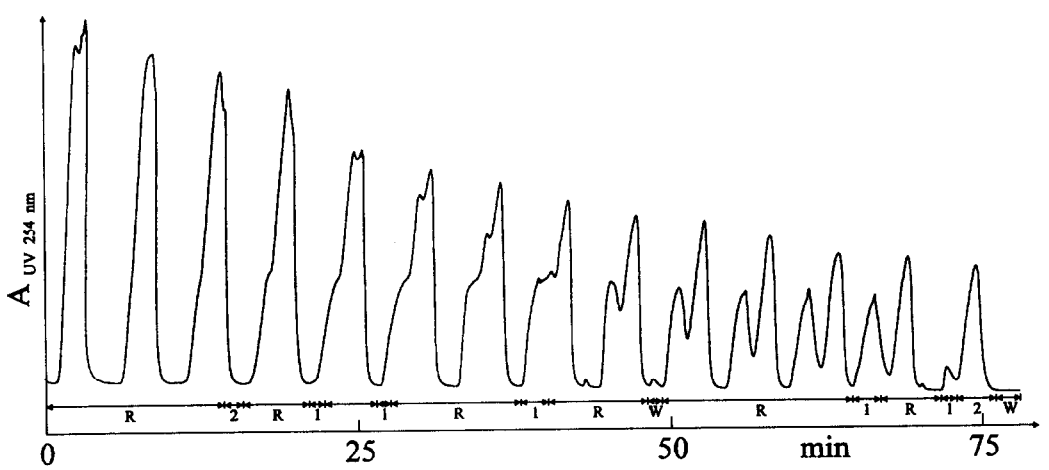

Fig. 12. Separation of thalidomide on poly-N-methacryloylcyclohexylethylamide–diol-silica. Chromatographic conditions: sample, thalidomide; column, 125 × 25 mm I.D. packed with poly-N-(methacryloyl)-(*S*)-(−)-1-cyclohexylethylamide–silica composite based on LiChrospher 100 Diol, 5 μm (experimental batch, Merck); mobile phase, *n*-heptane–dioxane (70:30, v/v); flow-rate, 40 ml/min; detection, UV at 220 nm; sample size, 140 mg dissolved in 2.8 ml of dioxane.

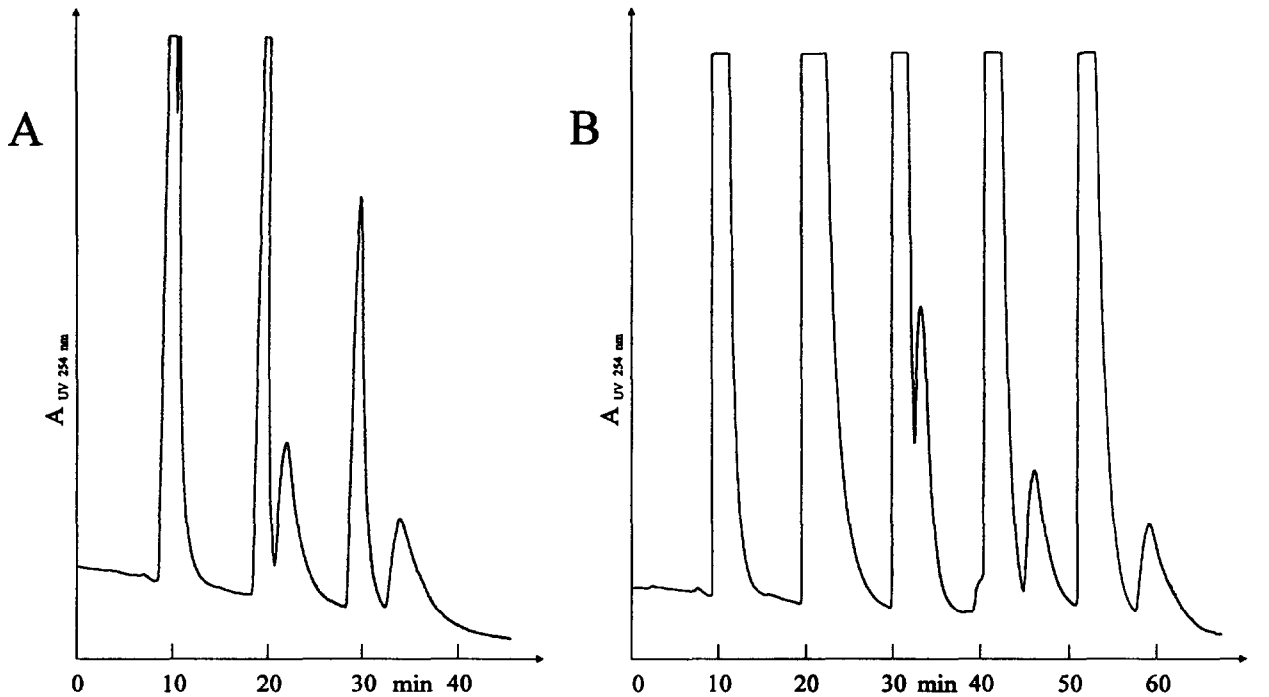

Fig. 13. Separation of proline by ligand-exchange chromatography on an L-hydroxyproline-modified polystyrene–silica composite. Chromatographic conditions: sample, D,L-proline; column, 250 × 25 mm I.D. packed with L-hydroxyproline modified polystyrene–silica composite based on LiChrospher 100, 5 μm (experimental batch, Merck); mobile phase, acetonitrile–water (70:30, v/v) + $Cu(CH_2COO)_2$ (10^{-4} mol/l) + CH_3COOH (10^{-2} mol/l), pH 4.2; flow-rate, 10 ml/min; temperature, 65°C; detection, UV at 220 nm.

20°C. A slow transport and adsorption–desorption process at the available interacting sites in the chromatographic bed determines the band broadening. This effect is the least pronounced with methanol as eluent. The small favourable effect that a decrease in temperature has on the enantioselectivity (Fig. 18) will hence be neutralized by the strongly diminishing column efficiency. If we further analyse the resolution factor, R_s, which is determined both by thermodynamic and kinetic contributions, we may conclude from Fig. 20, which gives the course of the resolution factor as a function of temperature at a given flow velocity, that the lowest resolution is obtained when methanol is used. For this solvent, the resolution factor R_s diminishes to a very limited extent with increase in temperature. The resolution factors are the highest with *n*-hexane–ethanol. For both ethanol and *n*-hexane–ethanol it appears that the resolution is maximized at a certain temperature (25 and 30°C, respectively).

If a larger amount of a racemate has to be separated by means of chromatography, it is advantageous to separate first a small amount of product to obtain both enantiomers in a pure state, before determining the most interesting loadability. These amounts of products are used to draw up a melting point diagram. An example of a phase diagram is given in Fig. 21.

From the location of the eutectic, one is able to determine what the minimum optical purity should be to obtain an enantiomerically pure product by crystallization and therefore the most economical way to set the fractionation in the preparative chromatographic purification. For the determination of the optimum injection amount, different amounts of product are injected consecutively on to the preparative column. During the product elution, samples are

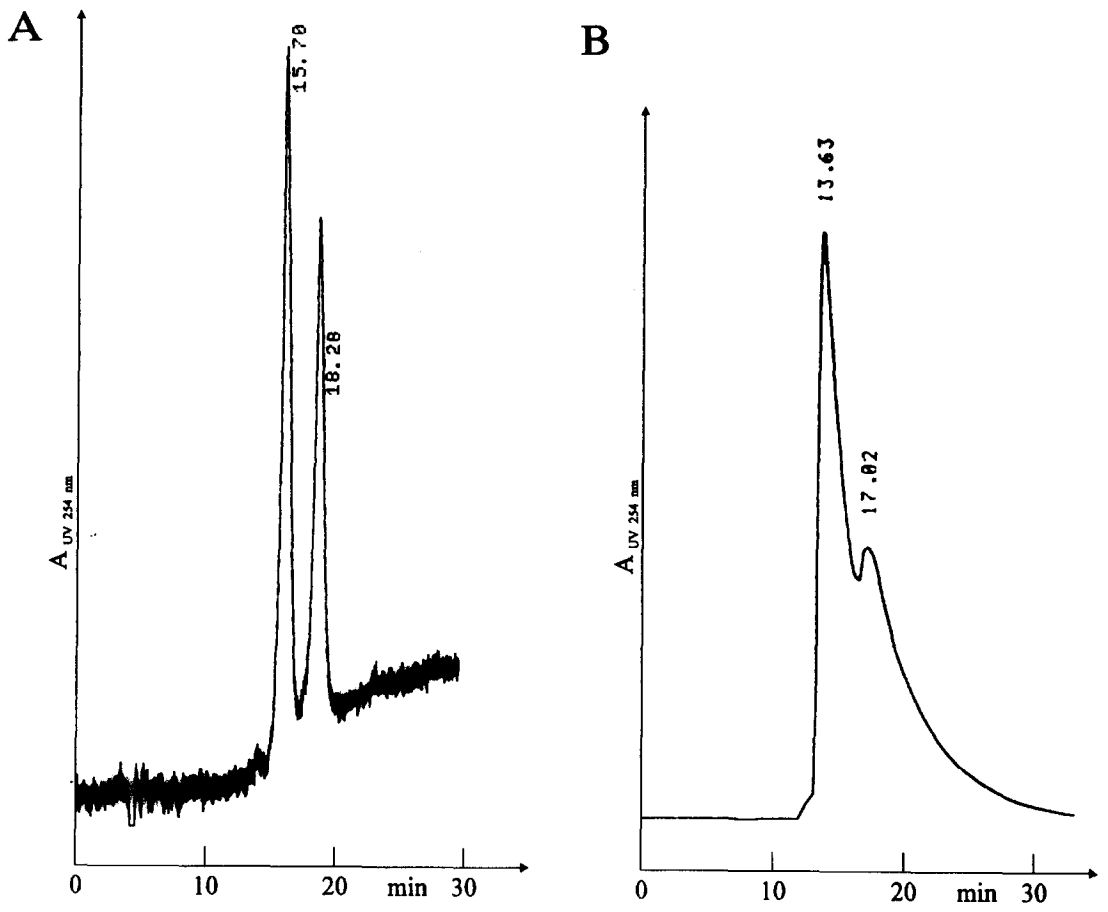

Fig. 14. Separation of 3,3,3-trifluoro-D,L-alanine by ligand-exchange chromatography on an L-hydroxyproline-modified polystyrene–silica composite. Chromatographic conditions: sample, 3,3,3-trifluoro-D,L-alanine; column, 250 × 25 mm I.D. packed with L-hydroxyproline-modified polystyrene–silica composite based on LiChrospher 100, 5 μm (experimental batch, Merck); mobile phase, acetonitrile–water (70:30, v/v) + $Cu(CH_2COO)_2$ (10^{-4} mol/l) + CH_3COOH (10^{-2} mol/l), pH 4.2; flow-rate, 10 ml/min; temperature, 65°C; detection, UV at 220 nm; sample size, (A) 0.0015 mg and (B) 12.5 mg.

taken at regular intervals. After dilution to a suitable concentration, they are analysed and the measured data obtained are plotted against the elution volume. On the basis of these reconstructed chromatograms, one is able to determine the exact fractionation points in order to collect any set product quality.

Table IX shows the share, in terms of percentage peak area, which can be collected at desired product qualities of 90, 95, 98 and 99% optical purity for several injection amounts. It appears that an injection amount of 20 g will yield the largest amount of enantiomer of the required quality per run. For this separation, Fig. 22 shows a preparative chromatogram together with the chromatographic conditions finally applied to separate 14 kg of this racemate. Because of the relatively poor solubility of the product in the mobile phase, the separations were performed at 35°C instead of at the previously determined optimum working temperature of 25°C. Further, it appeared necessary to inject 20 g of racemate dissolved in 600 ml of pure ethanol. To avoid crystallization at the column inlet, this feed solution was constantly stirred at 40°C. This is also the reason why for this particular separation recycling and peak shaving did not result in a more economic way of obtaining the first enantiomer, as the initial load on the column could not be further increased. Finally,

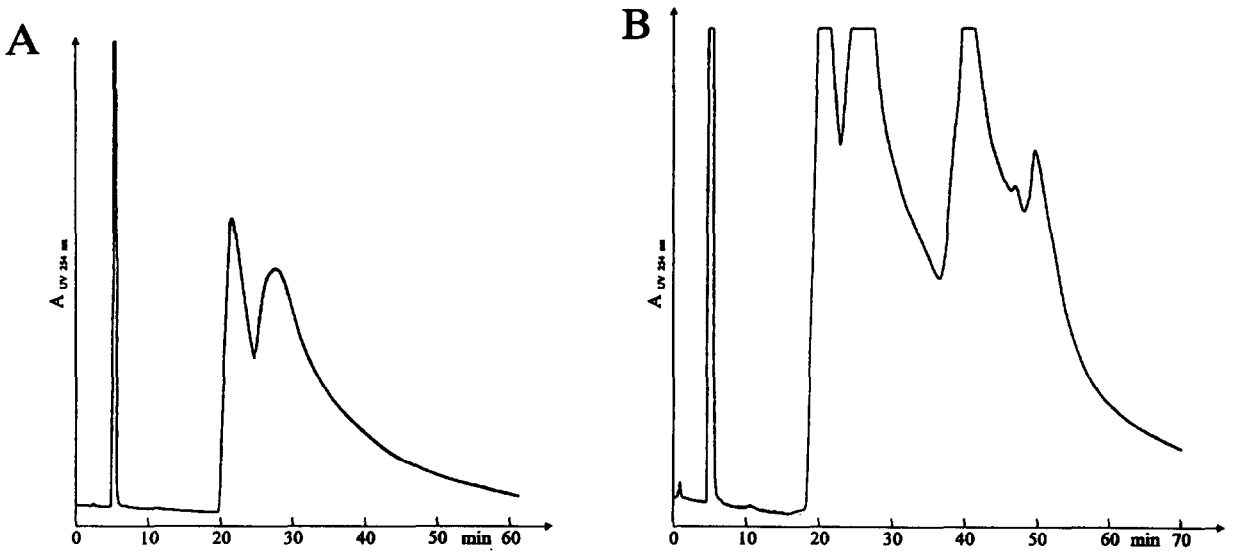

Fig. 15. Separation of benzoin on a bovine serum albumin–wide-pore diol-silica stationary phase. Chromatographic conditions: sample, benzoin; column, 250 × 25 mm I.D. packed with bovine serum albumin adsorbed on 5-μm LiChrospher (mean pore diameter 30 nm) and cross-linked with formaldehyde (experimental batch, Merck); mobile phase, 0.05 *M* phosphate buffer (pH 7.0)–2-propanol (96:4, v/v); flow-rate, 40 ml/min; temperature, 20°C; detection; UV at 254 nm; sample size, 5 mg dissolved in 470 ml of *n*-hexane–1-butanol (80:20, v/v). (A) Elution mode; (B) two cycles.

TABLE VII

RACEMATES SEPARATED ON DIFFERENT CSPs ON TECHNICAL AND PRODUCTION SCALES

CSP	Separation
Microcrystalline cellulose triacetate	Hetrazepine
Hydroxypropyl-β-cyclodextrin–silica	α-(2,4-Dichlorophenyl)-1*H*-imidazole-1-ethanol
Cellulose tri(*p*-methylphenyl ester)–silica	Benztriazole derivative
Cellulose tri(3,5-dimethylcarbamate)–silica	A γ-aryl keto ester
	An alkylated 2-piperazinecarboxamide

TABLE VIII

CONDITIONS FOR LARGE-SCALE PRODUCTION OF A CHIRAL HETRAZEPINE ON MICROCRYSTALLINE CELLULOSE TRIACETATE BY ELUTION CHROMATOGRAPHY AND THE RECYCLING–PEAK-SHAVING TECHNIQUE

Mode	Sample amount (g)	Total run time (h)	Optical purity yield, peak 1 (%)	Yield (g)	Solvent consumption (l) (−)-enantiomer
Elution mode	1000	3.8	>80	340	0.22
Recycling–peak shaving	2500	5.7	>80	1000	0.09

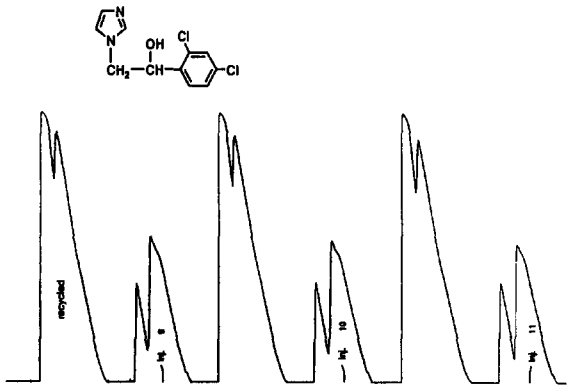

Fig. 16. Continuous automated recycling–peak-shaving chromatographic separation of α-(2,4-dichlorophenyl)-1*H*-imidazole-1-ethanol on a hydroxypropyl-β-cyclodextrin–silica packing. Chromatographic conditions: instrumentation as in Fig. 1; sample, α-(2,4-dichlorphenyl)-1*H*-imidazole-1-ethanol; column, *ca.* 450 mm × 80 mm I.D. packed with 500 g of hydroxypropyl-β-cyclodextrin–silica (experimental packing, Merck); mobile phase, 20% (v/v) methanol–50 m*M* TEA in water + 50 m*M* H_2SO_4 (pH 2.5); flow-rate, 150 ml/min; detection, UV at 220 nm, 2.56 AUFS; sample size, 1.5 g dissolved in 15 ml of water + 0.4 ml of concentrated H_2SO_4.

after recrystallization of the collected first-eluted enantiomer, approximately 6 kg of product with an enantiomeric purity of about 98% were isolated in the normal elution mode (yield 84%). The second-eluted enantiomer was reconverted into the racemate by treatment with a base. In total, more than 30 kg of this product were chromatographed on this column (500 × 100 mm I.D.).

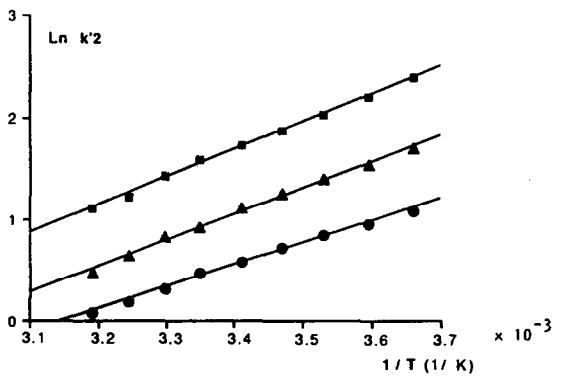

Fig. 17. Effect of temperature variation on the retention of a benztriazole derivative with different mobile phases. Column, Chiracel OJ; mobile phase, ● = methanol; ▲ = ethanol and ◆ = *n*-hexane–ethanol (40:60, v/v).

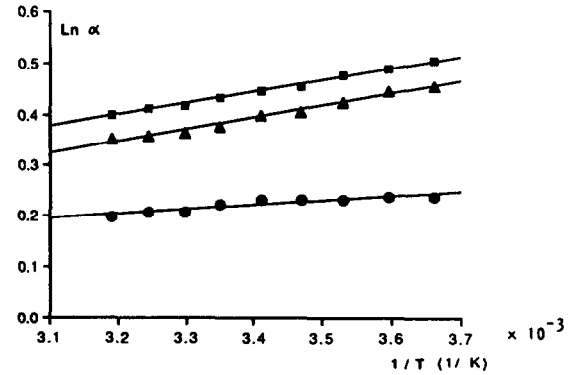

Fig. 18. Logarithm of the selectivity factor α as a function of the inverse of the temperature. Column, Chiracel OJ; mobile phase, ● = methanol, ▲ = ethanol and ◆ = *n*-hexane–ethanol (40:60, v/v).

To demonstrate that continuous automated recycling and peak-shaving chromatography can be performed over large cycle numbers, the next example considers the separation of 80 g of a research compound. In this instance, solubility problems made it necessary to use pure methanol as the eluent and the chiral stationary phase employed was Chiracel OD. To test the recycling system, 0.5 g of sample dissolved in 50 ml of methanol was injected on to the preparative column (100 by 50 mm I.D.). As can be seen from Fig. 23, a fairly adequate separation was obtained after four cycles.

After optimization of the load and reconstruc-

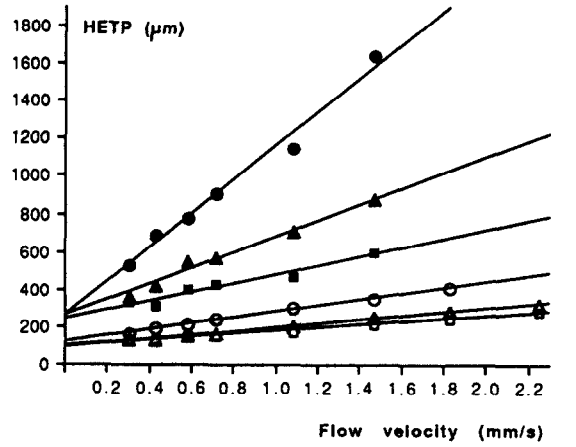

Fig. 19. Dependence of column efficiency on flow velocity at different temperatures: ● = 0; ▲ = 5; ■ = 10; ○ = 20; △ = 30; □ = 40°C.

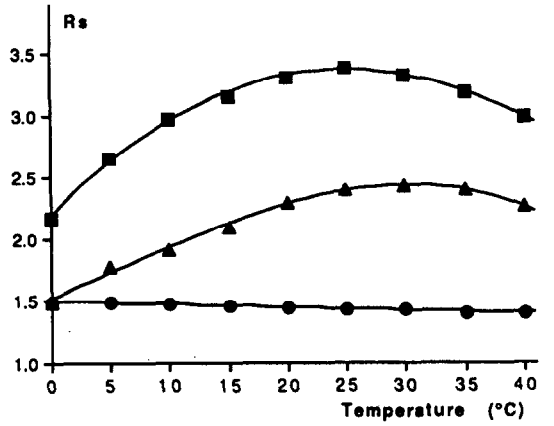

Fig. 20. Resolution as a function of the temperature for different mobile phase compositions. Column, Chiracel OJ; mobile phase, ● = methanol; ▲ = ethanol and ◆ = *n*-hexane–ethanol (40:60, v/v).

tion of the peak profile after two cycles, it proved to be possible to inject 4 g of racemate, dissolved in 100 ml methanol, on a colum that contained approximately 200 g of packing material. In total, nine cycles were required to separate this amount of racemate into the pure enantiomers. By means of this technique, the separation of 80 g (20 injections of 4 g) was performed with automated control in a continuous process over a period of 4 days. The vertical marks in Fig. 24, which represents one of these production runs, indicate the fractionation and recycling points.

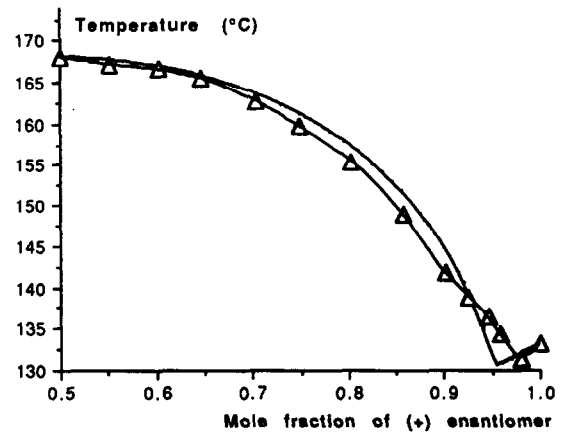

Fig. 21. Melting point diagram (liquidus curves). Line without symbols, calculated by means of Schröder–Van Laar and Prigogine–Defay equations; △ = experimental data.

TABLE IX

RELATIONSHIP BETWEEN PRODUCT PURITY OF ENANTIOMERS AND THE PERCENTAGE OF COLLECTABLE PEAK AREA WITH DIFFERENT AMOUNTS INJECTED

Injected amounts (g)	Percentage of collected peak area of first peak with an optical purity of				
	90%	95%	97%	98%	99%
5	97.6	95.2	93.2	91.8	89.4
10	86.5	79.6	74.9	71.6	66.5
20	67.0	55.8	50.0	45.8	39.7
40	18.5	9.1	6.8	4.1	2.4
50	8.1	3.1	0.9	0.6	0.4
60	5.7	1.7	0.8	0.5	0.3
70	3.6	1.3	0.8	0.3	0.2

In an even more convincing way, the reliability and reproducibility of a recycling run are reflected by the variation of the valve switching times of the first, second and third cycles, summarized for 300 subsequent automated chromatographic runs (equivalent to about 25 days of chromatography) performed in a campaign to separate 4.5 kg of an alkylated 2-piperazinecarboxamide by 7.5-g sample injections on to a 400

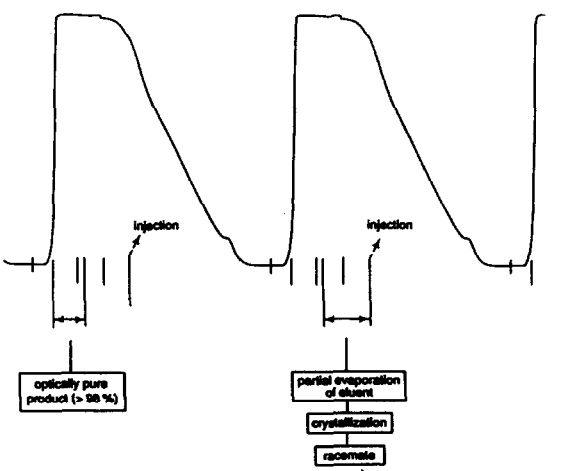

Fig. 22. Continuous automated elution chromatography of a benztriazole derivative on a technical scale. Chromatographic conditions: instrumentation as in Fig. 1; sample, benztriazole derivative; column, 500 × 100 mm I.D. packed with Chiracel OJ, 20 μm; mobile phase, *n*-hexane–ethanol (40:60, v/v); flow-rate, 150 ml/min; temperature, 35°C; detection, UV at 240 nm, 2.56 AUFS; sample size, 20 g dissolved in 600 ml of 100% ethanol at 40°C.

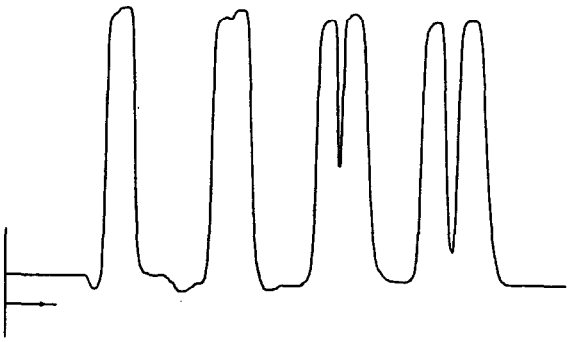

Fig. 23. Preparative separation of a γ-aryl keto ester. Load: 0.5 g. Chromatographic conditions: instrumentation as in Fig. 1; sample, γ-aryl keto ester; column, 100 × 50 mm I.D. packed with Chiracel OD, 10 μm; mobile phase, methanol; flow-rate, 13.5 ml/min; sample size, 0.5 g dissolved in 50 ml of methanol; four cycles per injection.

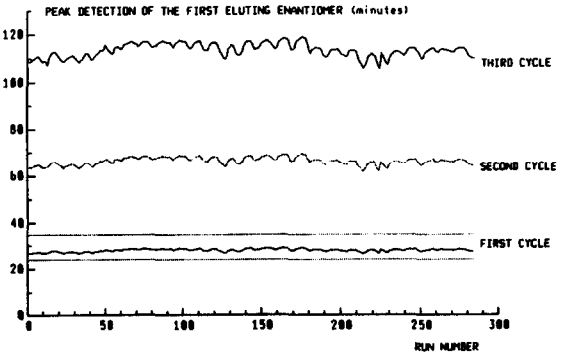

Fig. 25. Deviation of actual switching times for peak recognition of peak 1 in the first and second cycles of a continuous automated recycling–peak-shaving operation. Chromatographic conditions: instrumentation as in Fig. 1; sample, alkylated 2-piperazinecarboxamide; column: 400 × 100 mm I.D. packed with Chiracel OD, 20 μm; mobile phase, *n*-hexane–1-butanol (90:10, v/v); flow-rate, 150 ml/min; temperature, 30°C; detection, UV at 220 nm, 2.56 AUFS; sample size, 7.5 g dissolved in 470 ml of *n*-hexane–1-butanol (80:20, v/v).

× 100 mm I.D. column packed with 1.2 kg of Chiracel OD (20 μm). The two straight lines in Fig. 25 indicate the time window that was established in the steering program for the slope detection of the first peak. The recognition of the first peak in the second cycle is time based. However, the system was programmed in such a way that the window for peak searching in subsequent cycles is related to the time of slope recognition of the first cycle. Although different solvent qualities were used (fresh and reclaimed and readjusted solvent), the peak detection remained perfectly satisfactory within the established time windows. For the 285 injections at an average peak collection time of 27.9 min, the standard deviation was less than 2%, equivalent to 35 s.

CONCLUSIONS

As a general conclusion, we can state that preparative chromatographic separations on optically active stationary phases combined with recycling and peak shaving form an ideal technique for the investigation and development of

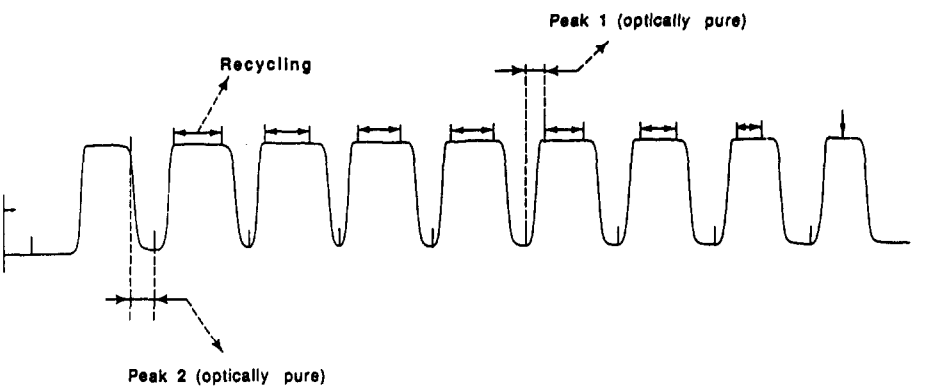

Fig. 24. Preparative separation of a γ-aryl keto ester. Load: 4.0 g. Chromatographic conditions: instrumentation as in Fig. 1; sample, γ-aryl keto ester; column: 100 × 50 mm I.D. packed with Chiracel OD, 10 μm; mobile phase, methanol; flow-rate, 13.5 ml/min; sample size, 4.0 g dissolved in 100 ml of methanol; nine cycles per injection.

new drugs. It is the technique of choice for medicinal chemists to separate, without too much effort, new racemic products into their enantiomerically pure forms, so that their pharmacological characteristics can be tested almost simultaneously with those of the racemate. Further, many possibilities are offered during the course of chemical development of a new drug. In the development stage, preparative chromatographic enantiomer separations provide additional possiblities for producing optically pure intermediates in a way that further reaction steps can be tested, validated and, if necessary, adapted. It further provides the chemist with otherwise unavailable enantiomeric pure starting materials to produce diastereomeric salts or to investigate alternative synthetic pathways. The indisputable advantage of having both enantiomerically pure configurations directly available enables one to synthesize pure reference substances for analytical purposes or to draw up phase diagrams. As with every chromatographic separation, an enantiomer separation can be scaled up to be used as a production method, parallel to or as a substitute for stereospecific synthetic or enzymatic approaches. As has been shown, larger amounts of product can be made readily available. The economic drawbacks of preparative chromatography in general and the use of chiral stationary phases in particular are counterbalanced by large cost savings due to decreased solvent consumption, miniaturized column dimensions with smaller amounts of packing material, increased throughput and yield for both enantiomers, when required, and the possibility of saving labour costs by automated computer-based process control.

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