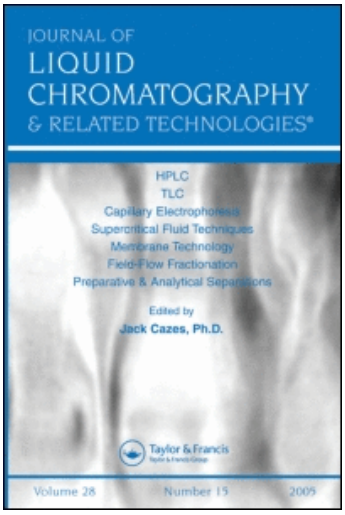

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HIGH-PERFORMANCE LIQUID DISPLACEMENT CHROMATOGRAPHY OF ENANTIOMERS ON A CHIRAL POLY-L-VALYL-BASED STATIONARY PHASE

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ABSTRACT

A direct enantiomer separation according to the displacement mode of liquid chromatography has been developed on a silica-based stationary phase bearing as chiral selectors poly-L-valyl groups. Milligram amount resolution of DL-methionine ß naphthylamide was accomplished on two analytical size columns using methanol-containing acetate buffer as the carrier and DL mandelic acid dissolved in the carrier as the displacer.

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INTRODUCTION

Separation of optical isomers is one of the more difficult purification process owing to the similarity of the compounds to be separated. Great improvement in the enantiomer resolution has been achieved in the last few years by the modern liquid chromatography, due to the use of microparticulate silica gel in the range of 3-10 μm as packing material, the well established bonding chemistry of its surface and the selection of effective chiral agents (1-5). To date most of the classes of racemates are resoved on rationally designed chiral stationary phases (CSPs) using elution chromatography. However, this mode of chromatography, when is extented to scaling-up separations, is handicapped by relatively poor utilization of the sorbent and mobile phase (6), and it requires the employment of larger column volumes in order to guarantee satisfactory levels of sample load and product yield (7). This generally leads to prohibitive operating costs of purified product in terms of packings and solvent consumption.

There are two modes to carry out a preparative separation by chromatography: elution and displacement. In elution chromatography the bands of the sample components migrate at a rate depending on the equilibrium isotherms of the species between mobile and stationary phases, and therefore scaling-up applications are car-

ried out using the same chromatographic conditions set_ tled with analytical columns. A successful separation is related to the selectivity, efficiency and cost of the preparative column (8).In displacement chromatogra_ phy the separation process operates in conditions of non linearity of the isotherms. The column is first e_ quilibrated with the carrier solution, the sample is injected on to the column, followed by the displacer, which is a solution containing a compound with stronger affinity for the stationary phase than any other com_ pounds to be separed. As the displacer front moves down the column, the feed components are displaced and sepa_ rated as adjacent bands according to their adsorption strength. The potential of this technique has been re_ viewed by Horvath (9,10) and Cramer (11,12). During the displacement run the products are concentrated and, taking advantage of the non linearity of the isotherms, the displacement process provides high throughout and purity, if highly efficient columns are used. Several examples of milligram amount separations on analytical columns are reported (13,14).

Despite its distinct advantages and the growing interest of pure optically active compounds in several fields of the life sciences, displacement chromatogra_ phy has remained a relatively unknown technique for the preparative-scale enantioseparations (15).

In connection with our previous studies concer_ ning the direct resolution of optically active isomers on CSPs (16-18), we have modified silica gel with L-va_ lyl-L-valyl-L-valine as resolving agent and an approach to use this support for the liquid displacement chroma_ tographic resolution of a methionine derivative is re_ ported.

EXPERIMENTAL

Instrumentation

Experiments were carried out with a multifunc_ tional apparatus assembled from commercially available components that operated for both displacement and ana_ lytical separations. The instrument consisted of a sol_ vent deliver pump Model 2B (Perkin Elmer, Norwalk, CT, USA) and a Rheodyne Model 7125 injection valve,equipped with one ml or 20 μl loops, that were connected to a Model 401 rifractive index (RI) detector (Waters, Milford, MA, USA) and to a Model 201 fraction collector (Gilson, France) for the displacement mode, or to a UV detector Model Uvidec-100-V (Jasco, Tokyo, Japam), for the analysis of the collected fractions.

Detector signals were recorded by a Model 3390 integrator (Hewlett Packard, Palo Alto, CA, USA).

Materials

Si 300 silica gel and 3-glycidoxypropyltrimethoxysilane were from Serva, Heidelberg, Germany). The tripeptide L-valyl-L-valyl-L-valine was from Bachem (Buberdorf, Switzerland). L- and DL methionine ß naphthylamide (Met ß NA) and DL mandelic acid were obtained from Sigma (St. Louis, MO, USA). Methanol and water of HPLC grade were from Carlo Erba (Milan, Italy). All other chemicals used for the preparation of the stationary phase and the eluents were of analytical grade and were purchased from Merck (Darmstadt, Germany).

The carrier was methanol-0.05 M potassium acetate buffer (pH 5.1) (10:90 v/v). The displacer solution was prepared by dissolving mandelic acid in the carrier to obtain a corrisponding concentration of 50 mM.The regenerant was methanol-50 mM potassium acetate buffer (pH 5.1) (80:20 v/v).

Preparation of the chiral stationary phase

3-glycidoxypropyltrimethoxysilanized silica gel was prepared by heating a mixture of vacuum dried Si 300 silica gel (average particle size 10 μm, average pore diameter 300 Å) (15 g), 3-glycidoxypropyltrimethoxysilane (5 ml) and anhydrous toluene (200 ml) at 110°C under dry nitrogen according to the method previously described (19).

The material was filtered and repeatedly washed with toluene, n-hexane, 2-propanol and diethyl ether, and vacuum dried. Surface coverage, 0.54 mmole/g, was determined by %C analysis.

To 10 g of glycydoxypropylated silica suspended in 30 ml of methanol was added 1.0 g (3.2 mmol) of L-valyl-L-valyl-L-valine. The mixture was gently stirred at room temperature for 48 hours. The material was then filtered, washed successively with methanol and diethyl ether, and dried under vacuum. Elemental analysis gave N= 0.98%, C= 6.84%, H= 1.07%, corresponding to a sur_ face coverage of the ligand of approxymately 0.21 mmole per gram of silica gel.

The sorbent, slurried in 2-propanol, was packed in different size stainless-steel tubes 0.46 cm I.D., using n-hexane as pressurizing solvent.

Chromatographic procedures

The reservoir of the chromatograph was filled with the carrier and two chiral columns were conditio_ ned with the eluent. Then the sample was injected by pumping the carrier at 0.1ml/min. The pump was stopped, the columns disconnected in order to wash the chromato_ graph with the displacer, then the columns were con_ nected again and the system was flushed with the dis_ placer at 0.2 ml/min. Fractions of 200 μl were collec_ ted recording the displacement chromatogram at 254 nm.

Once all sample was emerged from the columns, the pump was stopped and the system was flushed with the carrier and successively with the regenerant solution.

The enantiomeric composition of each collected fraction was controlled following the method reported by Armstrong (20), using a commercially available ß-cyclodextrin-silica column (ß-Cyclobond, 5 μm, 25x0.46 cm I.D.) (Astec, Whippany, NJ, USA) and water-methanol (1:1 v/v) as the eluent.

RESULTS AND DISCUSSION

In order to develope a suitable chromatographic displacement system, the parameters that influence the elution mode retention of methionine ß naphthylamide enantiomers and the displacer on tri-L-valyl stationary phase were investigate, followed by the measurement of their adsorption isotherms. Then the displacement chro_ matographic separation of the solutes was carried out to examine the feasibility of the preparative separa_ tion method.

Figure 1 shows the possible structure of the li_ gands anchored to the silica surface, those the average coverage is reported in the experimental section. The resolution of DL Met ß NA on this chiral sorbent using methanol-0.05 M potassium acetate buffer (pH 5.1) (10: 90 v/v) as the eluent is shown in figure 2, where the

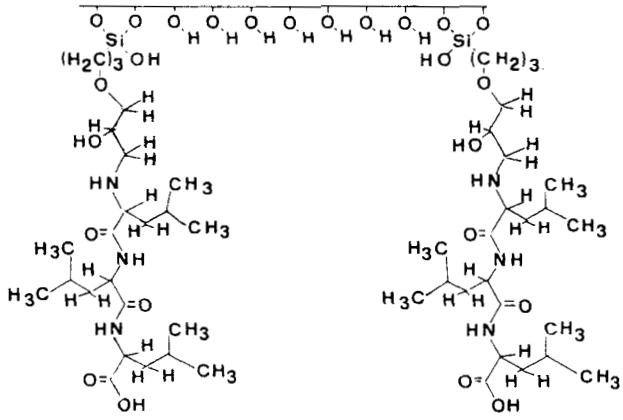

Figure 1: Structure of the chiral ligand anchored to the silica gel.

elution sequence of the two enantiomers was L-form less retained than D-form. Maximal enantioseparation was observed to occur in a restrict range of pH (4.5-6.0), where the tripeptide and the solutes are supposed to exist as ionic species and to form diastereomeric ion-pair complexes involving the amino group present in the poly-L-valine and the carboxylic group of Met ß NA. Additional stereoselective secondary interactions have to be ascribed to the presence in the two adducts of peptide bonds, hydrophobic side chains and to the sterically hyndering naphthyl group.

An analogous example of "pH window", determinant for the resolution of dipeptides has been reported by Knox et al. (21), who used L-leucyl-L-leucyl-L-leucine

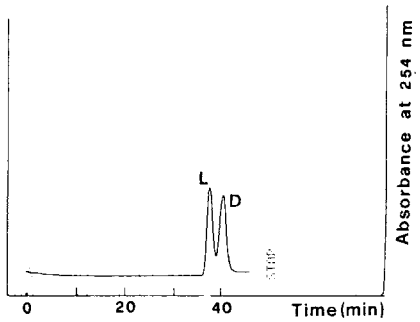

Figure 2: Resolution of DL-methionine ß naphthylamide on a column (30 x 0.46 cm I.D.) packed with L-valyl-L-valyl-L-valine silica gel. Mobile phase: methanol-0.05 M potassium acetate buffer (pH 5.1) (10:90 v/v). Flow-rate: 0.5 ml/min. Temperature: 25 °C. Detector: UV at 254 nm. Sample: 23 ng in 1 μl of eluent.

as zwitterionic pairing agent, added to the eluent. To explain the chiral recognition these authors postulated the formation of quadrupolar ion pairs as due to the two strong electrostatic interactions between solute and eluent, plus one further interaction through hydro_ gen bonding or Van der Waals repulsions.

Besides, the influence of the organic modifier on the retention mechanism of the two optical isomers and the displacer was studied, both because the solutes have to be strongly retained in the carrier solution, and to improve their selectivity factors in the chroma_ tographic system.

Fig. 3 shows the log k' vs. methanol concentra_ tion relationship for the Met ß NA enantiomers and man_

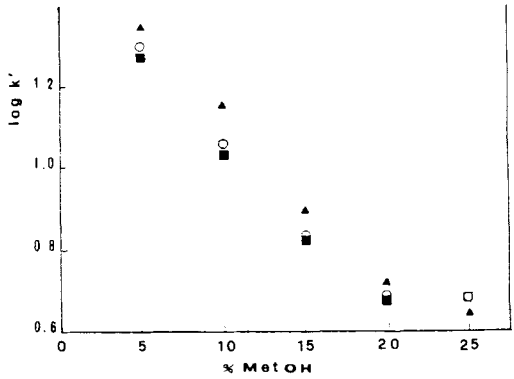

Figure 3: Retention of the D- and L-Met ß NA and DL mandelic acid as a function of the methanol concentration of the eluent. Other chroma_ tographic conditions as in fig. 2. (■) L- and (○) D-Met ß NA; (▲) DL mandelic acid.

delic acid, that was selected as the displacer. It can be seen that at high organic modifier concentration the two enantiomers are more retained than DL mandelic acid probably owing to the decrease of medium polarity, that promotes ion-pairing formation (22). Therefore, in this range of methanol percentages, mandelate cannot be used as the displacer. Below 15% the k' of all the three so_ lutes exceed 10, that is an optimal retention value for displacement runs (10). Table 1 shows respectively the capacity and selectivity factors of D- and L-Met ß NA at three methanol concentrations in the eluent. At 5% and 10% methanol there are no significative differences in enantioselectivity, but at the later percentage the

TABLE 1

Capacity factors (k') and enantioselectivity factors (α) of DL methionine ß naphthylamide at different methanol concentrations (v/v) in the eluent (buffer, 50 mM potas_ sium acetate, pH 5.1).

Enantiomer	5 %		10 %		15 %	
	k'	α	k'	α	k'	α
L	18.3	1.07	10.6	1.08	6.71	1.02
D	19.6		11.0		6.83	

mandelate adsorbs much more strongly on the stationary phase than the solutes.

Based on such results 10% methanol in the buffer was selected as the carrier solvent.

In addition to the elution data,further informa_ tions for optimizing the composition of the displacer solution were obtained by measuring the adsorption iso_ therms of DL mandelic acid and L-Met ß NA. The two iso_ therms were determined using frontal chromatographic measurements. Regular Langmuirian adsorption behavior was observed for concentrations of the solutes in the carrier below 10 mmol/l, as shown in fig 4.

Using the pattern of the adsorption isotherms, the displacement chromatography of 0.92 mg sample of racemic Met ß NA was obtained with 50 mM solution of

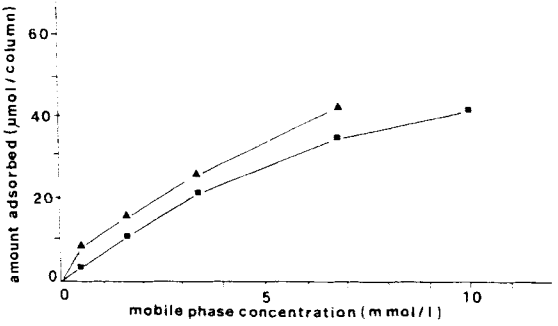

Figure 4: Adsorption isotherms of (■) L-Met ß NA and (▲) DL mandelic acid from methanol-0.05 M K acetate buffer (pH 5.1)(10:90 v/v).

potassium mandelate in the carrier solvent as displacer. The displacement chromatogram is shown in fig. 5.

Because the isotherms of the enantiomer (in figure 4 only the isotherm of the L-form is reported) and mandelic acid were very close to each others, a steep operational line was selected. This seems to be the main reason for the peak shape of the first step. However, good enantiomer separation was achieved even though the selectivity factor determined by elution mode is small as 1.08 (Table I).

By collecting fractions of 200 μl during the displacement run, and by analysing the enantiomeric content, the reconstructed chromatogram was obtained. Figure 6 shows that to the first (L-form) and second (D-form) band corresponds respectively a yield of 93.0 % and 78.5 %.

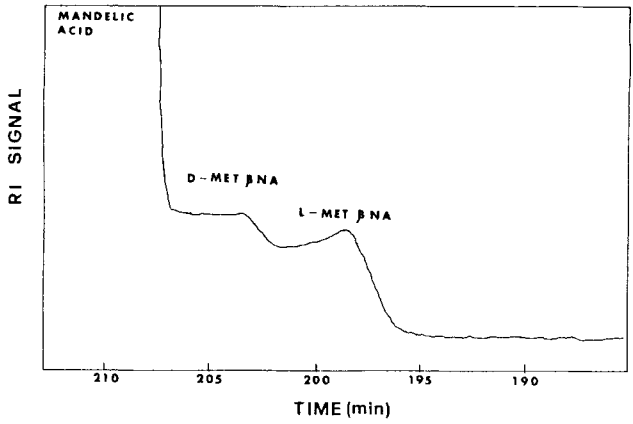

Figure 5: Displacement chromatogram of 0.92 mg racemic Met ß NA on two 25 x 0.46 cm columns packed with CSP. Displacer: 50 mM mandelic acid in methanol-0.05 M potassium acetate buffer (pH 5.1) (10:90 v/v). Flow-rate: 0.2 ml/min. Room temperature.

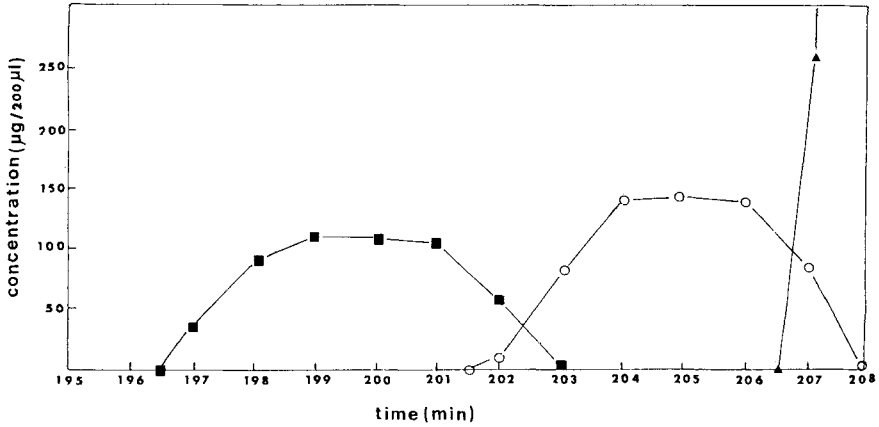

Figure 6: Reconstructed displacement chromatogram. Fraction volume: 200 µl.(■) L- and (○) D-isomers; (▲) mandelic acid.

CONCLUSIONS

The potential of the displacement chromatography for the preparative-scale separation of optical isomers from a racemic mixture has been shown. The method,based on the retention study by the elution mode and on the adsorption isotherm measurements, was developed for the resolution of DL-methionine ß naphthylamide on a chiral sorbent bearing poly-L-valyl groups. 3.4 µmol racemic mixture purification , otherwise impractical by elution chromatography, was accomplished in a reasonable time on two connected analytical size columns. Higher and good yield of pure enantiomers could be achieved by us_ ing structurally related compounds as displacers, and in this line further work is now in progress.

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