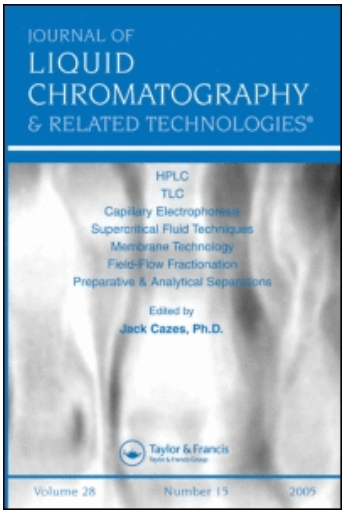

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PREPARATIVE CHROMATOGRAPHY AND PURIFICATION

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ABSTRACT

The chiral stationary phases (CSP's) that have proceeded from the work of Dr. Pirkle of the University of Illinois have characteristics especially useful to preparative liquid chromatography: Packings made with such CSP's can be highly efficient, lose efficiency only gradually when overloaded and regain it immediately when overload is removed, and allow the elution order of enantiomers to be reversed. These have been used at Regis in both preparative and analytical chromatography: in demonstration, to purify an enantiomer to 99.9967% optical purity and to determine that degree of optical purity with adequate precision and sensitivity. Both the relevant theory and the experimental work suggest that preparative trace-first chromatography is eminently suited to purification to high degree, and becoming the better suited as the degree of purity increases.

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INTRODUCTION

The work of Dr. William H. Pirkle has led and continues to lead to a series of rational, direct, efficient, overload-tolerant, enantiomeric chiral stationary phases (CSP's)(1). We comment briefly on these characteristics, which make these CSP's peculiarly suited to both analytical and preparative liquid chromatography, especially high pressure liquid chromatography (HPLC).

The Pirkle CSP's are rational, extensible, and convenient: They allow separations to be understood and predicted, new phases to be sought in a logical fashion, and enantiomers to be separated rather than diastereomers.

More pertinent to this report are the efficiency, overload tolerance, and enantiomeric nature of these CSP's: They can be chromatographically highly efficient (55,000 plates per meter are commercially and routinely available from Pirkle CSP's that have been covalently bound to 5-micron spherical porous silica). They are robust, decreasing in efficiency only gradually under increasing load and returning promptly to original efficiency on full elution. And they are singular in easily reversing the elution order of injected enantiomers when a column that contains a given CSP is replaced by its enantiomeric twin.

Because trace-first elution is so easily attained with Pirkle CSP's, we have investigated its use for producing (2) and analyzing (3) enantiomers of high optical purity. We have also considered a theoretical aspect of the chromatographic production of such materials.

An aspect of theory

The concentration of a component in a Gaussian chromatographic peak is described (4) in equation (1):

$$c = \frac{w\sqrt{n}}{V_R\sqrt{2\pi}} \exp\left\{-n'\left(1-\frac{V}{V_R}\right)^2\right\} \qquad (1)$$

In equ. (1), c is the component concentration entering the detector; w, the component quantity injected; V_R and V, the retention volumes at the peak apex and any location of choice, respectively; n, the number of theoretical plates measured from injection; and n', n/2 (for convenience).

Consider two such Gaussian peaks, as shown in Figure 1. Let equation (1) be expressed separately for components 1 and 2, the equation for the concentration of component 2 be divided by that for component 1, the separation factor SF (5) be the ratio of the retention volumes of these peaks, and the quantity SF be substituted in the quotient. The result, equation (2), expresses the major-to-trace concentration ratio at the apex of the second peak--that of the major component:

$$\frac{c_2}{c_1} = \frac{w_2}{w_1}\sqrt{\frac{n_2}{n_1}}\;\frac{\exp\left\{n_1'\left(1 - SF\right)^2\right\}}{SF} \qquad (2)$$

We defer numerical evaluation of equation (2) until the experimental results have been presented.

Theory and practice in preparative trace-first purification

A chromatographic column can show within a given chromatogram a high plate number n_1 for a trace and a low plate number n_2 for a major component present in great excess.

The combination of theory and practice in preparative trace-first purification is synergistic: In theory, as presented in equation (2), the major-to-trace concentration ratio is positively and primarily determined by the large and exponentially effective plate number n_1 of the trace peak. In addition, in

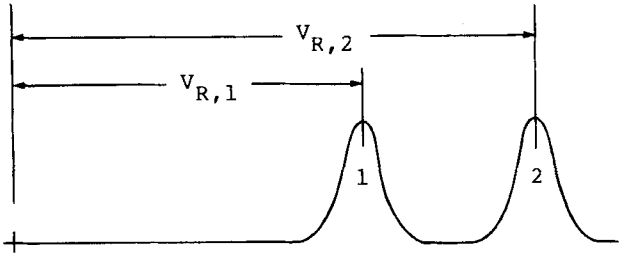

Figure 1. As the basis of equation (2), we assume two Gaussian peaks of retentions from injection $V_{R,1}$ and $V_{R,2}$.

practice the trace impurity is cleanly and easily separated from the major component by trace-first elution--a phenomenon that can be extremely useful in both analytical and preparative chromatography.

In this paper we present theory and review experiment in a demonstrative chromatographic preparation and analysis of an isomer of high optical purity.

MATERIALS

The chiral Pirkle columns were obtained from the Regis Chemical Company, Morton Grove, Illinois. All were 25 cm long. All chiral stationary phases were either D- or L-dinitrophenylglycine. Those 4.6 mm in inner diameter contained packing particles 5 microns in diameter, and were used for analytical determinations: D-, Code 731021; L-, Code 731024. Those 21.1 mm in inner diameter contained packing particles 10 microns in diameter, and were used for preparative separations: D-, Code 711121; L-, Code 711221.

Analyses were performed on modular equipment: A Beckman Model 110A pump (Beckman Instruments, Berkeley, California); a Rheodyne Model 7010 injection valve and sample loops (Rheodyne,

Cotati, California); and an Altex Model 153 UV detector (Beckman Instruments).

Preparative separations were also performed on modular equipment: a Fluid Metering Model RP-SY-2 pump (Fluid Metering Co., Oyster Bay, New York); an Omnifit injection valve, catalog number 1106 (Omnifit, Atlantic Beach, NY, NY), fitted with a 23-ml injection loop; and an Altex Model 153 detector fitted with a 2-microliter preparative flow cell, catalog number 235242.

Mobile phase components were purchased from Burdick and Jackson, Muskegon, MI.

The racemate of the 1-(9-anthryl)-trifluoroethanol was kindly furnished by Dr. William Pirkle, Department of Chemistry, the University of Illinois, Urbana, IL.

METHODS

The figures reveal the course of the experimentation. Preparative runs were monitored by analyses of the products.

The crude material, the composition of which is shown in Figure 2, contained a large proportion of ketone precursor. This was responsible for the first large peak. In preparation for the later preparative runs, the proportion of ketone was first reduced by recrystallization from hexane.

The determinations of optical purity were based on synthetic mixtures. The components of the synthetics were obtained from previous preparative runs. The composition of each of the last three increasingly pure products (Figs. 3-6) was approximated by that of a synthetic mixture of known composition. The impurity content was then determined from the analytical peak area ratio, product to synthetic.

The conditions for the analytical and preparative chromatography are stated in the figure captions.

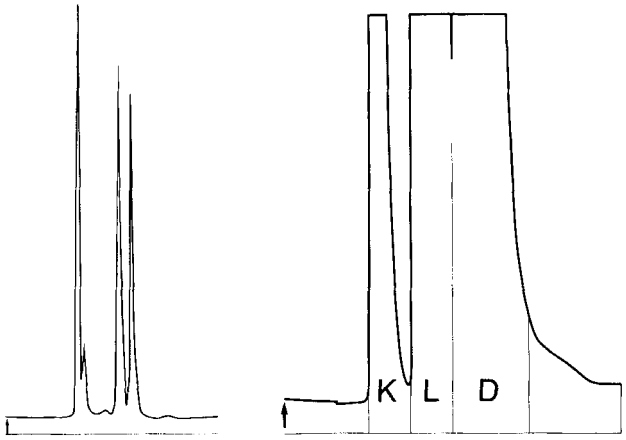

Figure 2. Analytical (left) and preparative chromatograms of the original, crude fluoroalcohol.

Analytical conditions: CSP: D. Mobile phase flow rate: 0.6 ml/min. Mobile phase composition: hexane/isopropanol 80/20. Injected: 10 microliters, at 2.5 mg/ml.

Preparative conditions: CSP: D. Mobile phase flow rate: 3.1 ml/min. Mobile phase composition: hexane/isopropanol 90/10. Injected volume, concentration, and quantity: 23 ml at 4.6 mg/ml, thus, 106 mg. Two repetitions; products combined.

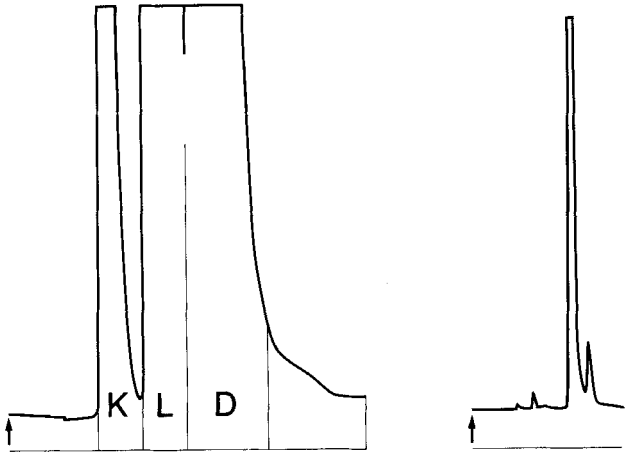

Figure 3. Left (as shown in Fig. 2), the preparative chromatogram of the material to be purified; and right, the analytical chromatogram of the product from that run.

Preparative conditions: as stated for Figure 2.

Analytical conditions: Same as stated above, Figure 2, except that the mobile phase composition was hexane/isopropanol 90/10. Injected: 10 microliters of straight collected fraction of the L isomer, concentration not measured.

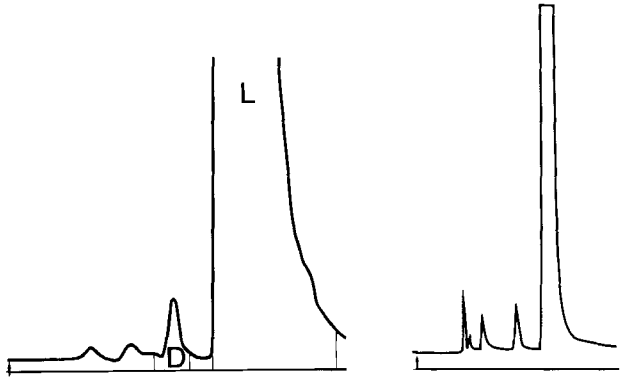

Figure 4. Left, the preparative chromatogram of the material that was then found to contain 0.27% minor enantiomer; right, the analytical chromatogram of that material.

Preparative conditions: CSP: L. Injected volume: 23 ml; concentration: 2.38 mg/ml; and quantity: 54.7 mg. Otherwise, as stated for Figure 2.

Analytical conditions: CSP: L. Otherwise, as stated for Figure 3.

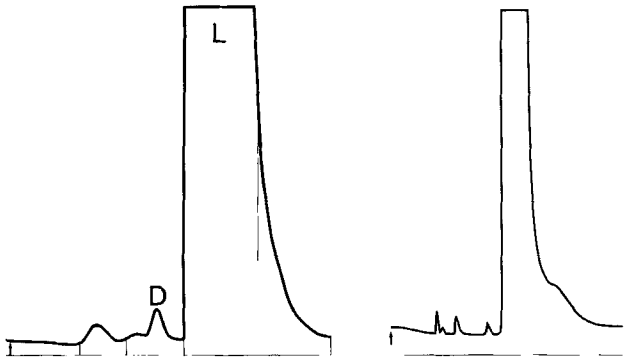

Figure 5. Left, the preparative chromatogram of the material shown analyzed in Figure 4; right, the analytical chromatogram of that product, showing it to contain 0.024% minor enantiomer.

Preparative conditions: Injected volume: 23 ml; concentration: 4.8 mg/ml; and quantity: 110 mg. No repetition. Otherwise, as stated for Figure 4.

Analytical conditions: Injected volume: 100 microliters; concentration: 3.5 mg/ml. Otherwise, as stated in Figure 4.

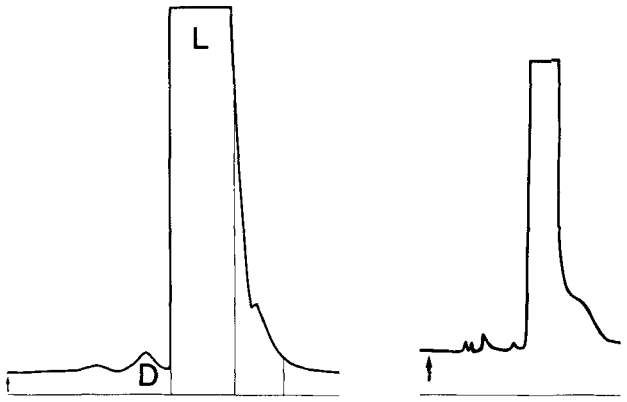

Figure 6. Left, the preparative chromatogram of the material shown analyzed in Figure 5; right, the analytical chromatogram of that product, showing it to contain 0.0033% minor enantiomer.

Preparative conditions: Injected volume: 23 ml; concentration: 3.56 mg/ml; and quantity: 71.5 mg. Otherwise, as stated for Figure 5.

Analytical conditions: Injected concentration: 1.6 mg/ml. Otherwise, as stated in Figure 5.

RESULTS

The results are simply stated: successive applications of preparative HPLC produced an increasingly pure product. Each of the last three stages reduced the impurity level by an additional order of magnitude. In each case, and with only conventional equipment being used, the presence of the impurity was unequivocally revealed and its level, quantitatively established (6). In the product that contained 0.0033% impurity, the determination was made on 160 micrograms of material, and 5 nanograms of impurity were detected.

DISCUSSION

The Experimentation

The experimentation reported here has apparently demonstrated that enantiomers can be purified by preparative HPLC to virtually any desired level, determinations of purity can be made to keep pace, and conventional apparatus can be made adequate to these purposes.

Theory

Equation (2) is expressed graphically in Figures 7 and 8.

In these figures, the magnitudes for the separation factor SF and plate number n are conservative; in the derivation of equation (2), the assumed cut point is realistic.

The values of separation factor SF and plate number n treated in Figures 7 and 8 are taken from the preparative runs described here. The separation factor SF was around 1.30 or 1.40, depending on the mobile phase used; and the plate number n ranged from very low values for the major peak up to over 5,000 for the trace impurity. In the figures the maximum value assumed for the plate number n is 1,000, and thus is conservative.

In the derivation of equation (2), the apex of the second major peak was chosen as cut point. Such a cut point, reported for a preparative isolation at this meeting in 1985 (6), is realistic. (Beyond this cut-point, the product would become even less contaminated by the earlier-arriving impurity).

Also, in Figures 7 and 8 the first two terms of equ. (2), both favorable, are neglected. The second term, for the square root of the plate number ratio, never becomes large, and may as well be neglected. The weight ratio term, however, can be sizeable. For the purest product injected here, for instance, that

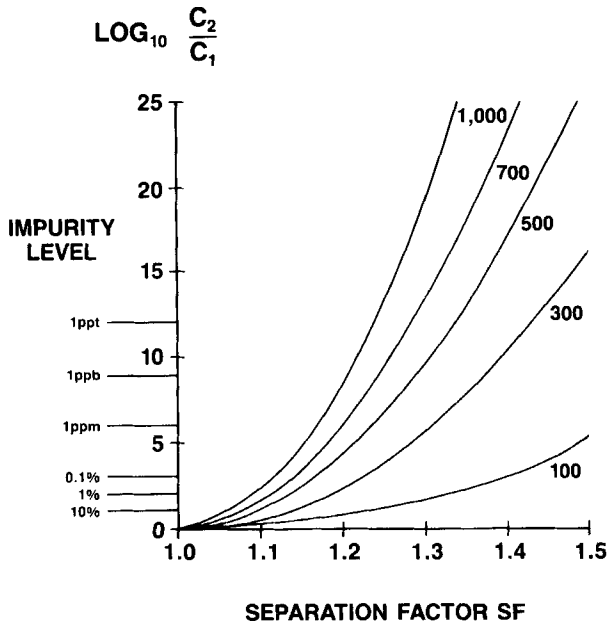

Figure 7. Based on equation (2), the logarithm of the major/minor concentration ratio is shown plotted against the separation factor SF, for values of the plate number n up to 1,000. Also marked on the concentration ratio axis are corresponding values of impurity concentration, from 10% to one part per trillion (ppt).

ratio was (1/0.00026), or almost 4,000. With increasing purity of product, the weight ratio term becomes increasingly favorable and effective. However, it is difficult to evaluate on any general basis, so to be conservative it was neglected.

Figure 7 shows the logarithm of the c_2/c_1 product/impurity concentration ratio plotted against the separation factor SF. The plots are promising, but pale against those in Figure 8.

In Figure 8, log concentration ratio is plotted against plate number. The plots are a set of increasingly straight lines.

(The straight lines are expectable: In equ. (2), log concentration ratio is a function of exp n. The lines are

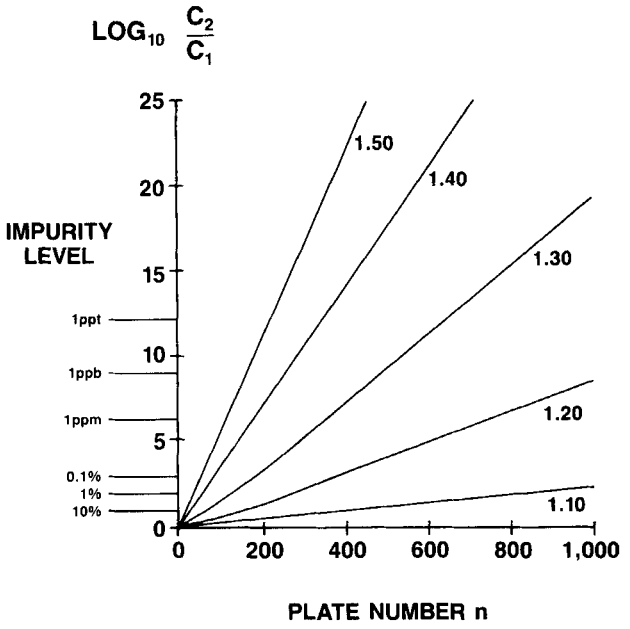

Figure 8. Without overload-caused peak distortion, 1,000 plates could provide more than enough purification with a separation factor equal to that used here: 1.40.

Here, again based on equation (2), the logarithm of the major/minor concentration ratio is shown plotted against the plate number n, for values of the separation factor up to 1.50. Also marked on the concentration ratio axis are corresponding values of impurity concentration, from 10% to one part per trillion (ppt).

increasingly straight as the exponential numerator increasingly dominates the linear denominator, which remains near unity. The slope coefficient is the (1 - SF)-squared term.)

In Figures 7 and 8, a yardstick for the corresponding impurity levels has been plotted alongside the log concentration ratio axis, to make the concentration ratio values more comprehensible. As can be seen, the illustrative impurity levels include 1%, ppm, ppb, and ppt. However, as can also be seen, these levels are low on the vertical axis, and are quickly left behind.

The purities predicted are many orders of magnitude higher.

The Suggestions of Theory

Theory suggests on the one hand that more than enough plates have been provided, but on the other hand that those plates should have been far more effective than was demonstrated here.

Theory assumes Gaussian peaks, but preparative peaks are certainly not Gaussian. How might they become more Gaussian?

Most obviously: reduce overloading. But we do not wish to reduce the utility of the column, which is to say, the quantity charged to it. What might be done, therefore, is to increase the inner diameter of the column, so as to reduce the load per unit cross-sectional area.

Further, there are in theory quite enough plates. Therefore, reduce the column length (which was only 25 cm) to perhaps 10 cm.

Consider a column of large inner cross-section, with no more than a thousand plates:

Such a solumn would show shorter retention times in the ratio of its present to its former length. Therefore it could yield the same throughput by allowing correspondingly more injections of correspondingly reduced quantity--and in this way reduce even further the loading per unit area.

Such a column could also contain smaller particles because with short length and wider area its backpressure would be low; so it could be extremely efficient.

The column that theory suggests would look like a thick pancake. Perhaps that is the future shape of preparative liquid chromatographic columns.

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