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Product purity as a function of elution order[a]

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SUMMARY

Elution order preference in preparative liquid chromatography can be examined if the elution order can be isolated as a variable, as it can with Pirkle-concept chiral stationary phases. With these phases, the elution order can be exactly reversed by replacing a column that contains a given chiral stationary phase by its enantiomeric twin.

This reversal was exhaustively replicated in a set of preparative liquid chromatographic separations (shown herein) of a chiral alcohol.

These data suggested to us that in preparative liquid chromatographic purification of a given major enantiomer, the trace enantiomer should be eluted first.

INTRODUCTION

Should a component to be purified by preparative liquid chromatography be eluted before or after a contaminating trace?

Definitive experimentation on elution order choice[1] can be achieved only by those chiral methods that are based on Pirkle-concept chiral stationary phases (CSPs)[2–7]. In these, the elution order of two enantiomers can be exactly reversed: a first column that contains a given CSP is substituted for a second column, identical to the first, except that the CSP of the second column is the enantiomer of the CSP in the first.

With good success in each of two reported works, the trace component was eluted before the major[8,9]. In the first, an enantiomer was brought to 99.9967% enantiomeric purity[8]; in the second, the methodology was desribed for measuring that enantiomeric purity to that precision, with only conventional equipment[9].

In this paper, we describe the experimentation that underlay chiral purification and analysis, and present chromatographic evidence that caused us to elect trace-first elution as the elution order of choice.

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EXPERIMENTAL

Materials

For this study, trifluoro-1-(9-anthryl)-ethanol was used. The starting material (the racemate and its ketone precursor) had been kindly supplied by Dr. W. Pirkle of the University of Illinois. HPLC-grade solvents were used throughout.

Columns, equipment

The analytical and preparative columns were laboratory-packed and are commercially available from Regis. The columns and the equipment used to pack and test them have been described[8,9].

Procedures

The mobile phase used for both analytical and preparative chromatograms was hexane–isopropanol (90:10, v/v). The concentration of racemate charged to the preparative columns was 5 mg/ml of mobile phase; the concentrations in fractions were as collected.

RESULTS AND DISCUSSION

Our experiments, strictly exploratory, were undertaken in 1984 partly to demonstrate the utility of being able to treat and control elution order as an independent variable, partly to determine the answers to questions about how best to conduct preparative liquid chromatography, and partly to determine whether a contaminating trace (here, the minor enantiomer) should be eluted before or after the main component (here, the major enantiomer). In this paper, we consider only the last aspect.

The alcohol concentrations injected could have been at least 10 times greater. However, they were deliberately restricted enough to allow the initial trace peak to be at least partially resolved from the major. We have not conducted experiments similar to these at concentrations near saturation, wherein the preparative peak would be one undifferentiated mass, and thus our conclusions here do not necessarily apply to such a case.

The chromatograms shown in Figs. 1 and 2 were not informative in choosing a preferred elution order. However, those in Figs. 3 and 4 were more helpful; in these, the first-eluted trace can be seen concentrated in the earlier fractions. Fig. 3 was particularly instructive.

In Fig. 3, the sequence of trace-first analytical chromatograms shows the trace highly concentrated in fraction 4 which, as shown in the corresponding preparative chromatogram, represented nearly the whole of the partly resolved initial enantiomeric peak. Fractions 3 and 5 contained most of the rest of the trace. The presence of the trace in fraction 6, as the major peak was entered, was barely detectable, and the trace was not detected in any later fractions.

On the other hand, in Fig. 3 the sequence of trace-last analytical chromatograms shows not only no purification whatsoever of the major component but also an unexpected and puzzling oscillation of trace concentrations throughout the major peak. (We comment further on this oscillation in the next paragraph.) That the preferred elution order is or should be trace-last[1], conflicts with these data.

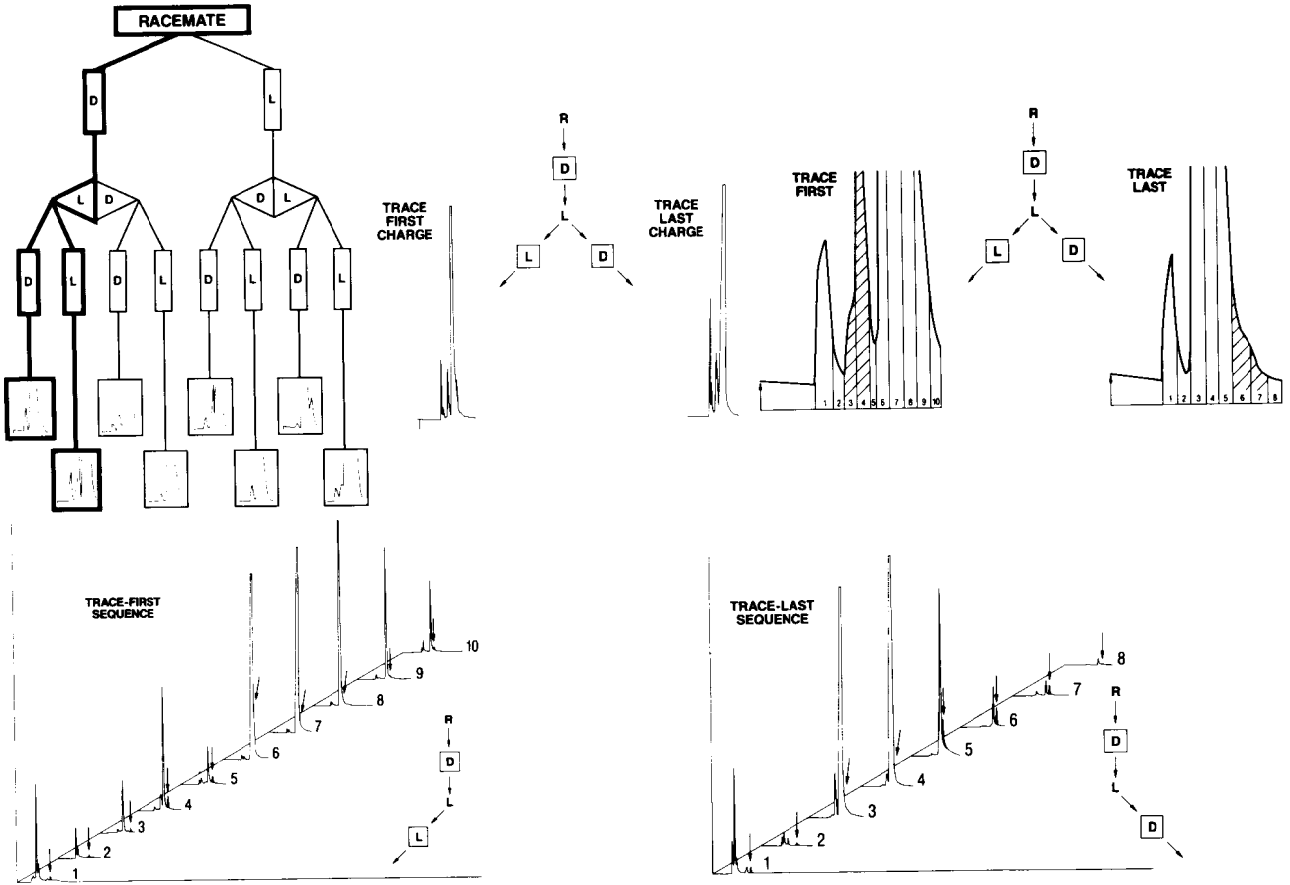

Fig. 1. The diagram at the upper left indicates the pertinent protocol for sample and fraction handling: twice, the racemate was charged to a preparative column that contained the D-enantiomer as CSP, and the L-enriched fractions were separately collected. One L-fraction was then charged to an L-column (giving trace-first elution); the other to a D-column (giving trace-last elution). The analytical chromatograms of the respective charges are shown and identified in the middle of the top row of chromatograms; and the resultant preparative chromatograms, showing the locations and numbering of the fractions, at the right of the top row. Note too the symbolic representations of handling: a boxed D or L indicates a D- or L-column; no box, a D- or L-enriched fraction. The sequences of the analytical chromatograms of the trace-first and trace-last chromatograms are shown and identified in the bottom row. Throughout, the trace peaks are both filled in and marked by an arrow. (In these analytical chromatograms, that the trace peak may have been eluted after the major peak is adventitious, resulting merely from use of a given analytical CSP rather than its enantiomer.)

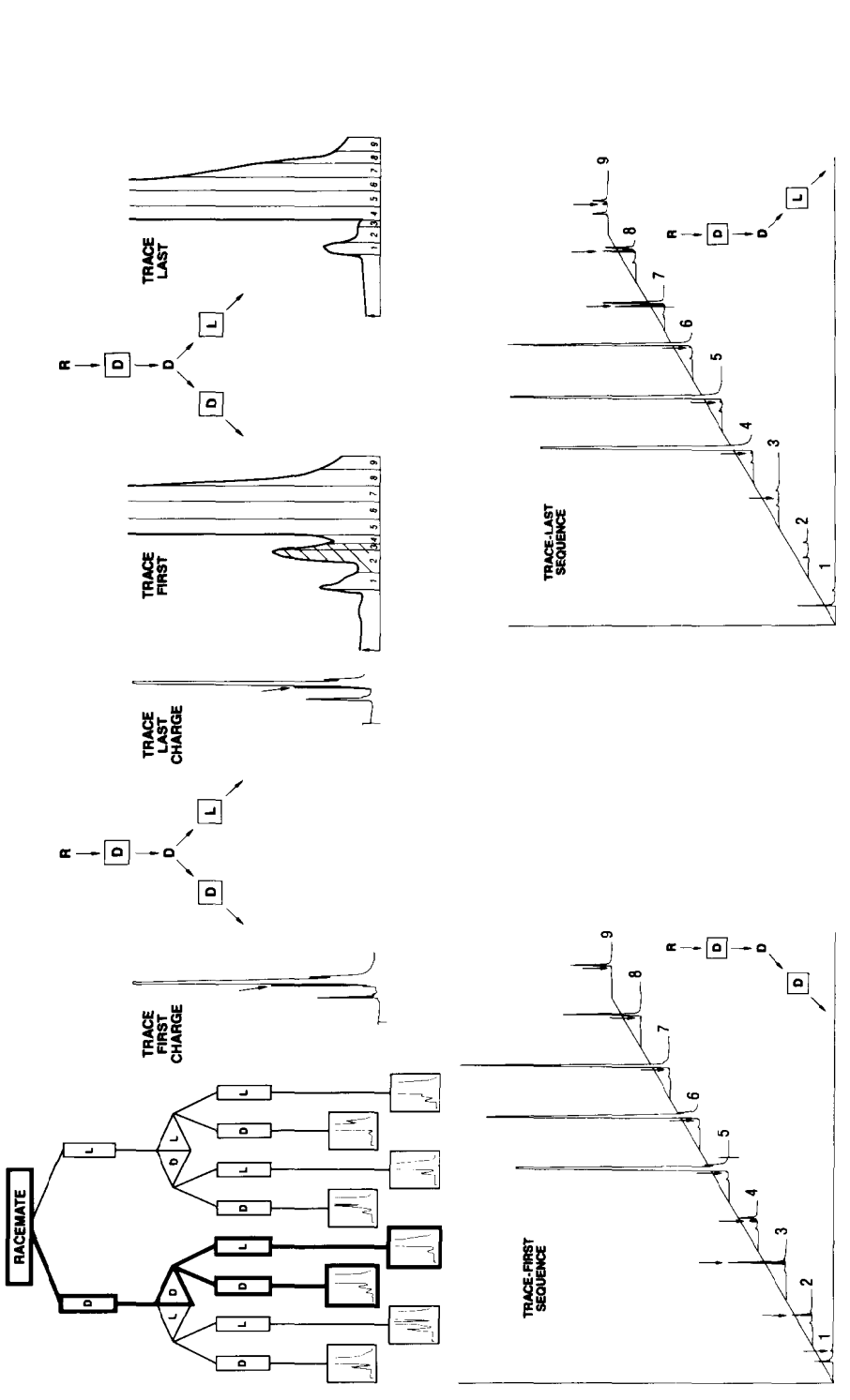

Fig. 2. As in Fig. 1, the diagram at the upper left indicates the pertinent protocol for sample and fraction handling: twice, the racemate was charged to a preparative column that contained the D-enantiomer as CSP. The D-enriched fractions were collected separately and charged separately, one to a D-column (giving trace-first elution); the other to an L-column (giving trace-last elution). The arrangement and identities of the remaining chromatograms are as indicated in Fig. 1 and by the accompanying symbols.

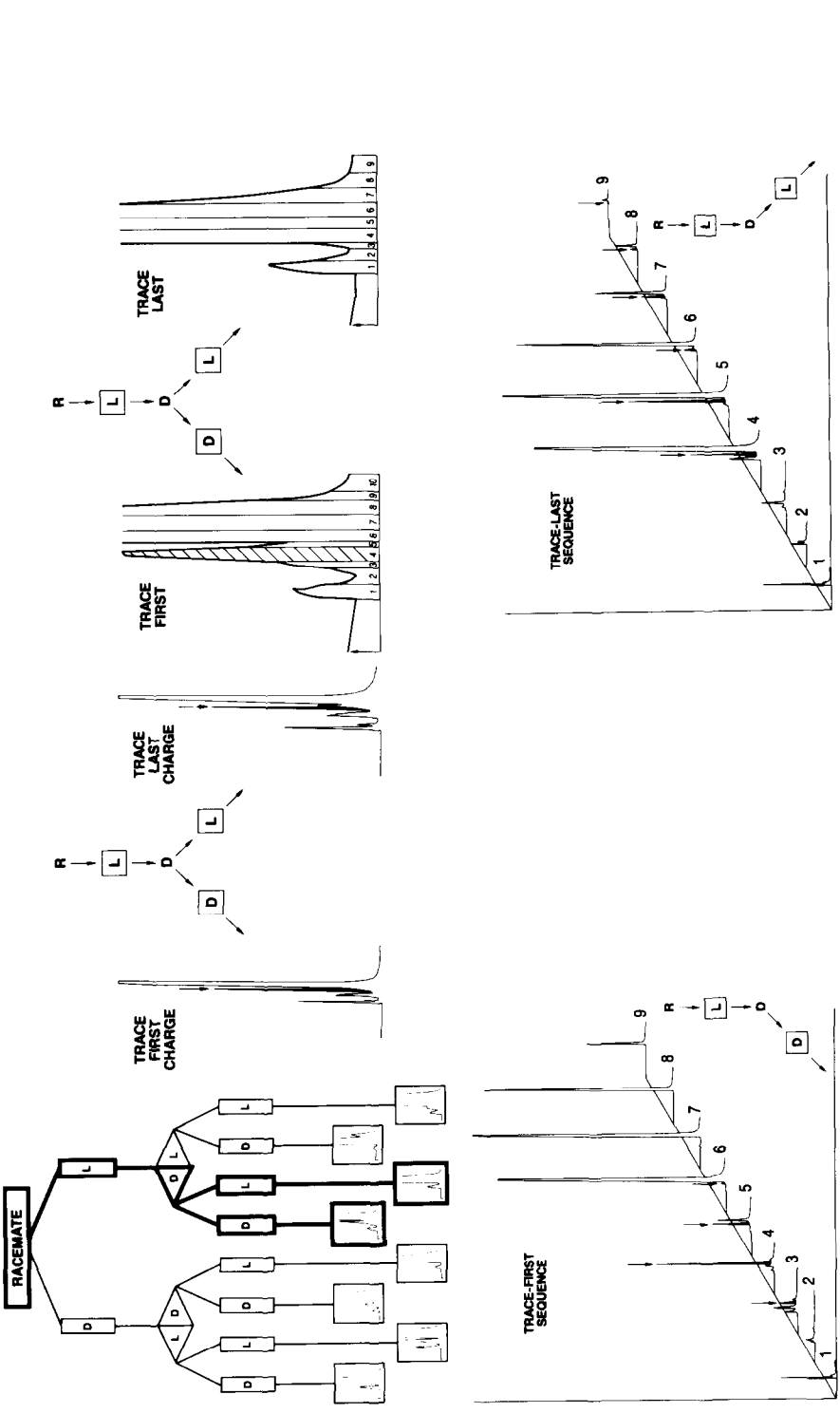

Fig. 3. As in Fig. 1, the diagram at the upper left indicates the pertinent protocol for sample and fraction handling: twice, the racemate was charged to a preparative column that here, however, contained the L-enantiomer as CSP. The D-enriched fractions were collected separately and charged separately, one to a D-column (giving trace-first elution); the other to an L-column (giving trace-last elution). The arrangement and identities of the remaining chromatograms are as indicated in Figs. 1 and 2 and by the accompanying symbols.

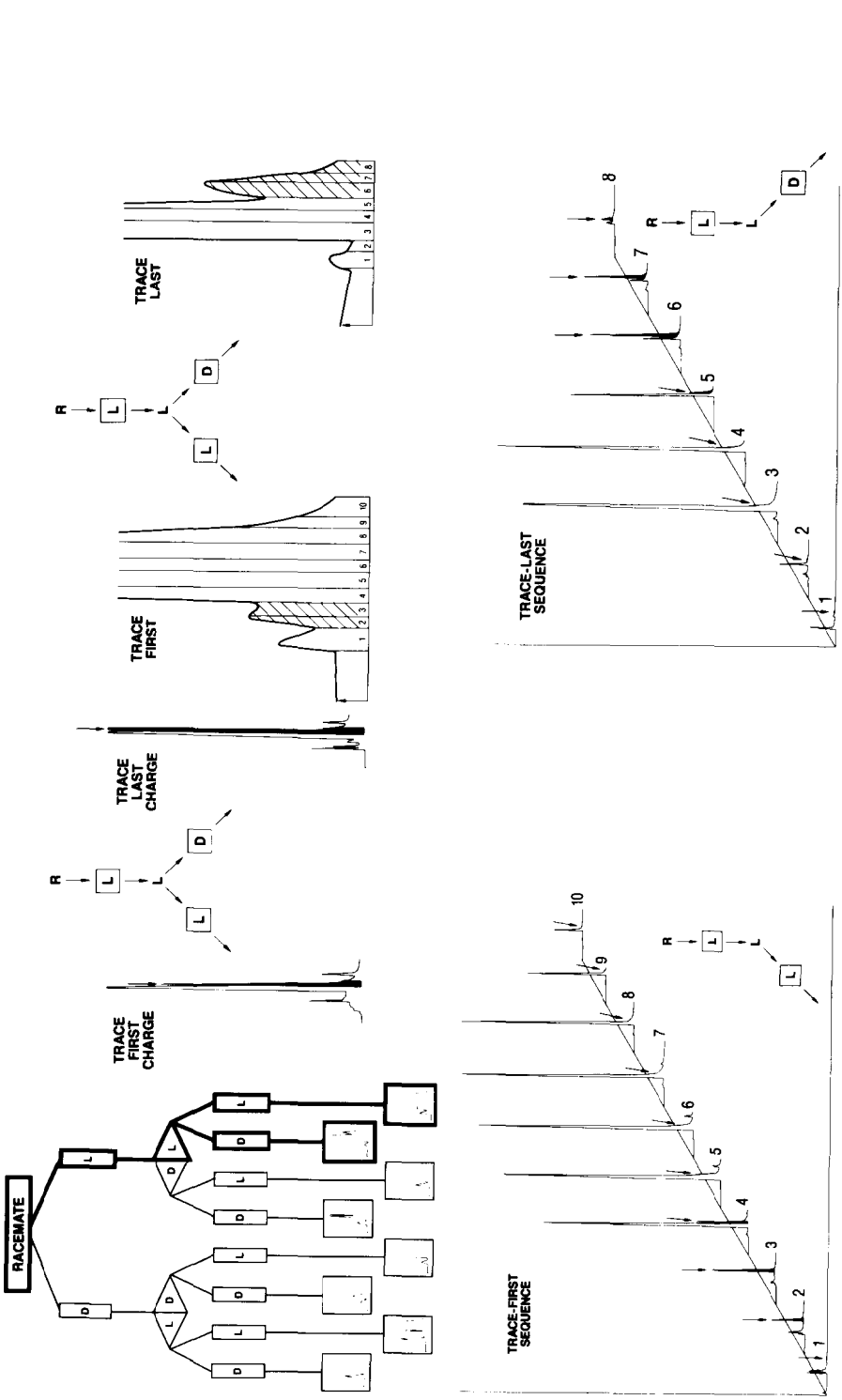

Fig. 4. As in Fig. 3, the diagram at the upper left indicates the pertinent protocol for sample and fraction handling: twice, the racemate was charged to a preparative column that contained the L-enantiomer as CSP. Here, the L-enriched fractions were collected separately and charged separately, one to an L-column (giving trace-first elution); the other to a D-column (giving trace-last elution). The arrangement and identities of the remaining chromatograms are as indicated in Figs. 1, 2 and 3, and by the accompanying symbols.

(Was the Fig. 3, trace-last oscillation of trace concentrations, an artifact? That it was neither an artifact nor merely an unfortunate but at least believable mistake, is suggested by internal evidence within Fig. 3: the trend of major-component concentrations in the successive trace-last chromatograms. In an expectable fashion throughout, these concentrations rise to a maximum in fraction 5 and then diminish gradually.)

Our conclusion from these data was and is that in liquid chromatography, both preparative[8] and high-precision analytical[9], trace components should be eluted before major.

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