ELSEVIER

Journal of Chromatography A, 731 (1996) 322–326

JOURNAL OF CHROMATOGRAPHY A

Short communication

Some thoughts on the coupling of dissimilar chiral columns or the mixing of chiral stationary phases for the separation of enantiomers

William H. Pirkle[a,*], Christopher J. Welch[b]

[a] *University of Illinois at Urbana-Champaign, Department of Chemistry, 1209 West California Street, School of Chemical Sciences, Urbana, IL 61801, USA*

[b] *Regis Technologies, Inc., 8210 Austin Avenue, Morton Grove, IL 60053, USA*

Received 14 February 1995; revised 24 October 1995; accepted 31 October 1995

Abstract

Consideration of some basic aspects of the separation of enantiomers on chiral stationary phases suggests that the coupling of dissimilar chiral columns to afford a broad-spectrum screen for the separation of otherwise pure enantiomers cannot be recommended. However, this arrangement can sometimes be useful for the separation of enantiomers in complex mixtures. An analysis of various arrangements is discussed, including tandem column arrangements containing dissimilar chiral packings, columns containing mixed packings or mixed selectors, and the use of columns containing selectors of varying degrees of enantiopurity.

Keywords: Enantiomer separation; Chiral stationary phases, LC

1. Introduction

Development of chiral stationary phases (CSPs) which are capable of resolving the enantiomers of a wide variety of structurally diverse racemates is the goal of a number of researchers, including ourselves. A number of times, we have been asked if, by mixing several different chiral packings, one might obtain a column having a broader scope than any single packing. The frequency with which this idea has been suggested over the years is testimony to its seductive allure. Such mixed-packing experiments have been reported [1] and more recently, the separation of enantiomers using two or more dissimilar chiral columns, coupled in a tandem arrangement, has been described [2,3].

The use of coupled columns and mixed-bed packings is a well known and useful strategy when separating mixtures of rather different compounds, as has been discussed quite recently [4]. However, the separation of enantiomers presents a somewhat different problem. Consideration of some chromatographic fundamentals allows one to anticipate the outcome of coupled-column experiments as well as that of the conceptually equivalent use of mixed-bed stationary phases. Suspecting that many of those engaged in the chromatographic separation of enantiomers may not fully appreciate the implications of coupled chiral column/mixed chiral packing experiments, we examine several possible situations and ask the question, "When, if ever, is the use of

*Corresponding author.

0021-9673/96/$15.00
SSDI 0021-9673(95)01240-0

tandem dissimilar chiral columns the optimal means for the separation of enantiomers?''

2. Discussion

Since the mechanistic details of how a given CSP actually differentiates between enantiomers are not always well understood, a great deal of 'trial and error' experimentation is often involved in finding a CSP–mobile phase combination which satisfactorily separates the enantiomers of a given compound. Presumably, the idea behind using an assembly of different chiral columns is the hope that at least one of the columns in the assembly will separate the enantiomers of interest. Indeed, the view that one might thus ''reduce the number of analyses to be carried out on individual CSP columns before acceptable separation of racemates is achieved'' has been expressed [2].

In considering possible outcomes of attempts to separate enantiomers using coupled columns containing dissimilar CSPs, we specifically address the use of two columns, although the arguments can be extended to apply to any number. In all cases, we assume isocratic elution on brush-type CSPs in columns of equal dimensions and phase ratio unless otherwise stated. We also assume operation in an analytical mode where retention is not influenced by sample size. This is done to simplify the presentation and is not essential to the arguments themselves.

One key thing to remember is that the retention time observed for an analyte on a tandem column arrangement is simply the sum of the retention times afforded by each of the columns in the series, independent of the order of the columns. For example, the coupling of identical chiral columns will double the retention times of each enantiomer and will not change retention factors (k') or enantioselectivity (α) but will increase resolution (R_s) owing to the increased number of theoretical plates (N). Clearly, this can be advantageous at times; it is simply equivalent to using a longer column.

Consider the case where the analyte enantiomers are separated on neither of two chiral columns. There will, of course, be no separation with the tandem column arrangement. In the case where only one of the columns (column A) provides separation, the degree of separation provided by the tandem arrangement will *always* be less than that afforded by column A. This diminution in enantioselectivity is further exacerbated when the retention afforded by column B is large (Table 1). In situations where column A is operating near its limit of ability to provide a useful separation, the efficiency of the tandem column arrangement is important. What will be the effect of tandem column B on the resolution, R_s, afforded by column A? Column B affords no separation of the enantiomers but it does contribute to the broadening of the chromatographic bands. Thus, column B does not alter the distance between the bands but does broaden them. Clearly, resolution will be reduced and the tandem arrangement is inferior to the use of column A alone. Since enantiomer separations are often marginal under the best of conditions, many small but useful separations afforded by a single column will fall below the limit of resolution of the tandem column arrangement.

When column A and B both separate the analyte enantiomers, the separation factor provided by the tandem arrangement will *always* be less than that provided by the better of the two columns *were it used alone*. This result is intuitively expected in the case where the two columns have opposite elution orders, but it also obtains in the case when both columns have the same elution order (Table 2). As in the previous case, when the separation factor afforded by column B is less than that afforded by column A, the enantioselectivity of the tandem arrangement is attenuated, particularly when the retention afforded by B is large relative to that of A. In most instances, resolution, R_s, will be intermediate between that achievable on each of the individual columns. Thus, at the cost of increased analysis time, the coupled dissimilar column approach will afford

Table 1
Comparison of chromatographic parameters for the separation of enantiomers on two separate columns and on the tandem column arrangement when one of the columns affords no enantioselectivity

Column A		Column B		Tandem A+B	
k'_1	α	k'_1	α	k'_1	α
1.00	1.06	1.00	1.00	1.00	1.03
1.00	1.06	5.00	1.00	3.00	1.01
1.00	1.50	9.00	1.00	5.00	1.05

Table 2
Comparison of chromatographic parameters for the separation of enantiomers on two separate columns and on the tandem column arrangement when both columns separate enantiomers with the same elution order

Column A		Column B		Tandem A+B	
k'_1	α	k'_1	α	k'_1	α
1.00	1.06	1.00	1.02	1.00	1.04
1.00	1.06	5.00	1.02	3.00	1.03
1.00	1.50	9.00	1.20	5.00	1.23

results inferior to those obtainable using the better of the two columns alone. Only when the two dissimilar CSPs afford similar α values, R_s values, and elution orders will the coupled-column arrangement outperform the better of the two columns used alone. In this event, one has approximated the coupling of two identical columns.

In the case where columns A and B both separate the enantiomers, but with opposite elution orders, the diminution of enantioselectivity observed for the tandem column arrangement is more severe (Table 3). How does one avoid this undesired result?[1] Without prior knowledge, one is just as likely to couple columns affording unlike as like elution orders. Consequently, separations which would be satisfactory using either column alone might be missed if one used only the tandem arrangement. Thus, the use of coupled chiral columns of different types to search for a separation of enantiomers is clearly a risky business.

Table 3
Comparison of chromatographic parameters for the separation of enantiomers on two separate columns and on the tandem column arrangement when the columns separate enantiomers with opposite elution orders

Column A		Column B		Tandem A+B	
k'_1	α	k'_1	α	k'_1	α
1.00	1.06	1.00	1.02	1.01	1.02
1.00	1.06	5.00	1.02	3.03	1.01
1.00	1.50	2.50	1.20	2.00	1.00

[1] Undesired if one is endeavoring to separate enantiomers. Actually, this is a useful means of determining whether two CSPs afford the same elution order. This approach can be used with minute quantities of racemic sample in the absence of chiroptic detectors or enriched samples. One compares the sums of the retentions on the individual columns with the values observed for the tandem arrangement.

In our view, one should not couple chiral columns when searching for a means to chromatographically separate the enantiomers of an otherwise pure substance but should examine columns singly. This is not to say that enantiomers cannot be separated using tandem column arrangements, for clearly they can and have been [2]. We are simply saying that this is not the approach which maximizes enantioselectivity, resolution, and productivity.

In response to repeated suggestions that we mix different chiral sorbents in the same 'mixed-bed' column, we have always maintained that this is precisely what one does not want to do. Since almost all of the surface of modern-day silica-based adsorbents is inside pores, each particle acts independently of its neighbors. Thus, a series of coupled columns containing different adsorbents is equivalent to a longer column containing these same sorbents mixed together [5]. However, once mixed, the particles are not easily separated, where as a series of columns can be disconnected and either reconnected in a different combination or used individually (Fig. 1).

The mixing of different chiral selectors so as to produce a heterogeneous population of selectors on the surface of silica particles might appear to be equivalent to the 'mixed-bed' approach [1]. This is not necessarily the case. To whatever extent an analyte molecule can interact simultaneously with two or more immobilized chiral selectors (Fig. 2b) or to whatever extent two or more nonidentical chiral selectors interact with each other (Fig. 2c) the situation may differ. When these interactions occur,

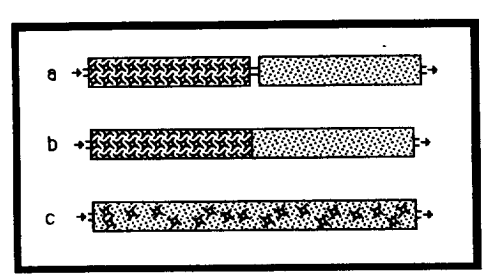

Fig. 1. Tandem column arrangement (a) is equivalent to a single column containing the two stationary phases in either separate (b) or intermingled (c) regions.

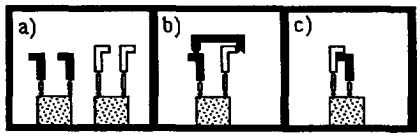

Fig. 2. Mixed-bed chiral stationary phase (a) differs from mixed-selector CSPs which can allow simultaneous interaction of an analyte with both selectors (b) or interaction of the two selectors (c).

retention mechanisms become available on the heterogeneous surface which are not available on the homogeneous surfaces. These multiple interactions are known to occur [5–7] and are doubtless more common than is generally realized. Simultaneous interaction of an analyte molecule with two or more immobilized selectors is most apt to occur when the analyte is large and/or polyfunctional. Rigorously, one cannot anticipate just how the 'mixed-selector' approach will differ from the 'mixed-bed'/'coupled-column' approach, but, to the extent that nonidentical selectors interact with each other, it seems likely that both retention and enantioselectivity will be reduced.

An interesting special case of the 'mixed-selector' approach involves CSPs prepared from a selector which is not enantiopure. It is useful to model this arrangement as a pair of tandem columns of opposite elution order and of varying length (a model which assumes independent selector action). For example, suppose a column containing an enantiopure selector affords a separation factor of 100 for a given racemate. If the enantiopurity of the selector is reduced to 98% ee (i.e. a 99:1 ratio) the performance of the resulting column can be modeled as a tandem arrangement of a column containing 99 units of enantiopure CSP and a column containing 1 unit of the antipodal (but otherwise identical) CSP. Such an arrangement reduces the separation factor from 100 to 50. Reduction of the enantiopurity to 90% ee (i.e. a 95:5 ratio) results in a separation factor of 16. High selector enantiopurity is much less important when enantioselectivity is low, but, in order to achieve high levels of enantioselectivity, selector enantiopurity is critical (Table 4) [9].

From the preceding discussions the reader might form the opinion that we advise against any use of coupled chiral columns. This is not the case. We are merely pointing out some of the considerations

Table 4
Effect of CSP enantiopurity on separation factor

CSP %ee	Separation factor (α)				
100	100	10.0	2.00	1.50	1.05
98	49.8	9.09	1.97	1.49	1.05
90	16.0	6.58	1.86	1.44	1.04
50	2.92	2.38	1.40	1.22	1.02
20	1.50	1.39	1.14	1.08	1.01

involved in these experiments and noting that, potentially, there is a better approach to the separation. In fact, there are times when it is advantageous to couple dissimilar chiral columns.

Suppose that one has a mixture of two racemic diastereomers and hopes to perform a separation of all four stereoisomers. Further, suppose that only three peaks are present in the chromatogram (Fig. 1). Two explanations are possible: (i) the enantiomers of one diastereomer may have been separated and do not coelute with the nonseparated enantiomers of the other diastereomer; (ii) the column may have separated the enantiomers of each diastereomer, but there may be accidental coelution of one enantiomer of each diastereomer. In the first case, one knows that an identical CSP *of the other absolute configuration* will separate the enantiomers of the previously unresolvable diastereomer but not those of the previously resolvable diastereomer. Finding a chiral column capable of separating the enantiomers of both diastereomers will require a further search. However, a simple remedy for the case (ii) situation can be suggested (Fig. 3).

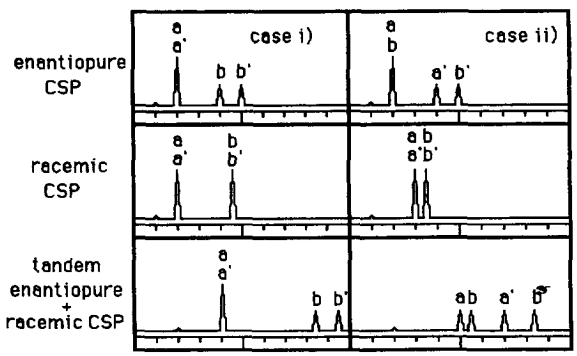

Fig. 3. Use of tandem column arrangement containing an enantiopure and a racemic CSP can be useful in the analysis of diastereomers or other complex mixtures.

To a first approximation, a racemic version of a chiral column will perform a separation just as does its enantiopure counterpart, *except* that it does not separate enantiomers [10]. In principle, other achiral (or even chiral) columns could be similarly employed. However, these would have to be found by trial and error experimentation and would not necessarily afford the desired chromatographic behavior using the mobile phase required by the chiral column. The racemic column does separate diastereomers by essentially the same mechanism(s) as its enantiopure counterpart, therefore the two columns may be coupled and, *using the same mobile phase*, the time interval between the elution of the diastereomers can be changed while keeping the time interval between the elution of enantiomers unchanged. One should now separate all four stereoisomers unless accidental coelution of a different pair of stereoisomers has occurred. In this case, the intervals between the peaks can be predictably adjusted by altering the length of the racemic column segment(s). Several short columns containing the racemic version of the chiral phase are useful if one is separating complex mixtures of stereoisomers. In effect, one is using a longer column of lower enantiomeric purity (or a mixed bed of the enantiomeric CSPs). We have found that for a particular series of repetitive assays, a mixed bed column containing a 2:1 ratio of enantiomeric CSPs was helpful. The least retained enantiomer was caused to be retained longer than early-eluting impurities and the more retained enantiomer was caused to elute earlier, reducing analysis time [8].

The coupling of chiral and achiral columns to alter the dispersion of peaks is not a new idea and has been practiced in a number of laboratories. However, despite earlier advocacy [10], it is not widely appreciated that one can use the racemic analog in series with a chiral column to accomplish the same end in a predictable manner.

3. Conclusion

Consideration of some basic aspects of the separation of enantiomers on chiral stationary phases suggests that the use of coupled dissimilar chiral columns will never afford the best separations (as judged by R_s and separation factor) of otherwise pure samples of enantiomers. Moreover, an unfortunate combination could cause one to overlook the fact that one or both of the columns would be satisfactory if used alone. Similar arguments are advanced for 'mixed beds' of different chiral adsorbents and for 'mixed-chiral selector' stationary phases. However, the coupled-column approach can be advantageous when more complex mixtures are encountered. The greater dispersion of the various components of the mixture afforded by the coupled columns may be necessary to cause the enantiomers of interest to stand 'free and clear' and, even though the quality of the separation of the enantiomers themselves may be somewhat reduced, the separation may still be adequate for the purpose at hand.

References

[1] L. Oliveros, C. Minguillon, B. Desmazieres and P. Desbene, J. Chromatogr., 543 (1991) 277.
[2] P. Sandra, A. Kot and F. David, Chim. Oggi, 12(9) (1994) 33.
[3] A. Kot, P. Sandra and A. Venema, J. Chromatogr. Sci., 10 (1994) 439.
[4] M.E.P. McNally and A. Barefoot III, Am. Lab., December (1994) 28N–28W.
[5] W.H. Pirkle and C.J. Welch, J. Chromatogr., 589 (1992) 45.
[6] W.H. Pirkle and T.C. Pochapsky, Chromatographia, 25 (1988) 652.
[7] W.H. Pirkle and R. Readnour, Anal. Chem., 63 (1989) 16.
[8] W.E. Bowen, Ph.D. Thesis, University of Illinois, 1993.
[9] E.L. Eliel, S.H. Wilen and L.N. Mander, Stereochemistry of Organic Compounds, John Wiley and Sons, New York, 1994, Ch. 6, eq. 6.17, p. 253.
[10] W.H. Pirkle, R. Dappen and D.S. Reno, J. Chromatogr., 407 (1987) 211.