Correlation Coefficients of Solute Relative Retentions for Pairs of Modified Cyclodextrins: Evaluation of Selectivity by Differently Responding Gas Chromatographic Stationary Phases

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Abstract

Relative retention times versus the *n*-undecane for ten diverse probe solutes from volatile oils are scatterplotted between pairs of modified cyclodextrin (CD) phases. If the resulting line of best fit has a very high correlation coefficient (*r*), then the two CDs are behaving similarly and will not give different results. A low value of *r* between two CD phases indicates they behave contrastingly enough to give different analyses. Presuming a laboratory wants three differently behaving commercial CDs, twelve are considered in this way to find the optimum three pairings (each showing *r* to be less than 0.800 with an average of less than 0.700). These requirements are met by Chiraldex G-DA (γ-dipentyl) and A-PH (α-hydroxypropyl, dimethyl) with Beta-Dex 225 (β-diacetyl, butyldimethylsilyl) capillaries. Solutes that fall close to the line of fit between two of the phases are undergoing "normal" transient CD molecular interaction with both. They may then show extra retention with the third phase on the other two plots, which suggests close solute-guest/CD-host molecular fit. Another possibility is that this third modified CD may behave merely as a normal non-CD phase to such a solute (shown by the rejection of it) with a lower retention than is normal. Hierarchical cluster analysis seems unreliable to indicate CD-phase relationships.

Introduction

Recently, ten diverse substances that were isolated from volatile oils had their boiling points compared graphically with their gas chromatographic (GC) relative retention times on twelve assorted modified cyclodextrin (CD) commercial stationary phases (1). For a given capillary, the line of best fit and its correlation coefficient (*r*) were calculated. Then, up to five probe solute data points were gradually ignored until *r* had increased to at least 0.990. The linear expression for this line was used to calculate the theoretical relative retentions of the solutes involved in its determination. For each of them, the error should have been no more than ± 7% for the observed versus calculated retention. These two requirements could not be quite obtained for three of the twelve CDs (with the poorest being $r = 0.984$ because of its retention error of ± 9%). Although the selective elimination of data points was wrong statistically, it was believed to be valid chromatographically for identifying solutes involved in "standard" transient interactions with the molecular rings of a particular modified CD. The observed versus calculated relative retentions of the remaining solutes (usually five "ignored") were then used to deduce which of their molecules fitted the particular CD molecular rings very well (seen by extra retention) and which solutes were rejected because they could only respond to the CD as though it were a normal stationary phase (detected by less retention than calculated). Hierarchical cluster analysis was applied to the data by Morgan (1), which is shown here in revised form in Table I. This arrangement needed independent confirmation. It was suggested that the plots of the results for one CD against another could yield interesting information; therefore, this was pursued using the data from the previous study.

Experimental

No new benchwork was conducted, but data from a previous study (1) not represented here was reassessed. The apparatus used and the methods taken have also been described in the previous study, but a summary of the twelve modified CD stationary phases lining the capillaries previously used for GC analysis are as follows: two α-CDs, four γ-CDs, and six β-CDs with their molecular rings made up of 6, 8, and 7 α-glucose

units, respectively. The eight Chiraldex capillaries from Astec (Whippany, NJ) had name suffixes beginning with A-, G-, and B- for α-, γ-, and β-CDs, respectively. Fairly low-polarity (1,2) dipentyl-phase names ended in -DA, and relatively high-polarity hydroxypropyl dimethyl CD names ended in -PH—with all six combinations utilized. The dipentyl Chiraldexes G-BP (butyryl) and G-PN (propionyl) were stable ester γ-CDs with a third ester (diacetyl β-CD Beta-Dex 225). This also had the bulky inert tertiary butyl dimethylsilyl (TBDMS) [-O-Si$(CH_3)_2$-C$(CH_3)_3$] group present as did the dimethyl Beta-Dex 325 (both from Supelco, Bellefonte, PA). Other low-polarity β-CDs were the trimethyls Cydex-B from SGE International (Ringwood, Victoria, Australia) and Cyclodex-B from J&W Scientific (Folsom, CA). All were used at 125°C with a helium gas mobile phase and a flame-ionization detector.

The ten diverse polar probe solutes included two aromatic substances (of which one was an ether) and monoterpenoids that were either bicyclic, monocyclic, or acyclic (being that they were alcohols, aldehydes, or ketones).

Data from the previous study was processed using MicroCal Origin 2.5 with Windows 98. The ten solute relative retention times versus the *n*-undecane for a CD phase were entered against their retentions for another CD with various combinations. A scatterplot was set up, and then a linear fit was plotted to yield the *r* value of each line. For some phases, the boiling points of the five or more solutes that were previously selected to determine linear expressions were plotted against their relative retention times. To these plots, the other solute data points were then added without altering the previous line of best fit (Figure 1).

Table I. Hierarchical Cluster Analysis of the GC Results for Twelve Modified CD Stationary Phases Adapted from the Previous Study

CD phase	*r*		
Chiraldex B-DA	1.000	0.911	0.646
Chiraldex G-PN	0.958	0.953	0.801
Chiraldex G-BP	0.940		
Cydex-B			
Cyclodex-B	0.866	0.921	0.918
Beta-Dex 225	0.646	0.770	1.000
Chiraldex A-DA	0.876	0.943	0.832
Beta-Dex 325	0.829	0.908	0.927
Chiraldex G-DA	0.898	0.759	0.560
Chiraldex B-PH	0.978	0.958	
Chiraldex A-PH	0.911	1.000	0.770
Chiraldex G-PH	0.935	0.986	

* The *r* values for three phases (indicated by 1.000) are compared with those of other phases using the relative retentions of ten diverse solutes.

Results and Discussion

The good relationship on a Chiraldex A-PH (a hydroxypropyl, dimethyl α-CD) between the literature boiling points of six

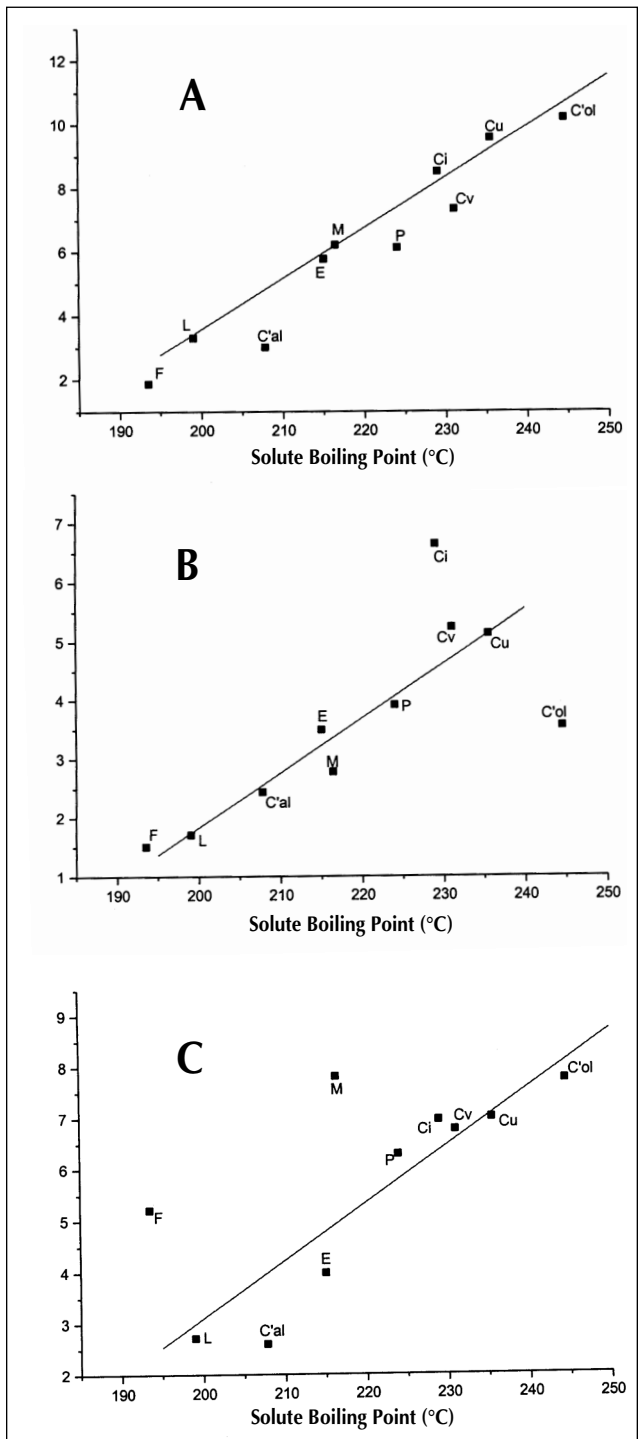


Figure 1. Scatterplots from three different modified CD GC stationary-phase capillaries for relative retentions versus the *n*-undecane of ten solutes at 125°C: Chiraldex A-PH, A; Beta-Dex 225, B; and Chiraldex G-DA, C. Line of best fit is shown for a selected number of solutes (6 used for A and 5 for B and C). Citronellal, C'al; citronellol, C'ol; citral, Ci; cuminal, Cu; carvone, Cv; estragole, E; fenchone, F; linalol, L; menthol, M; and pulegone, P.

probe solutes and their relative retentions versus *n*-undecane is evident in Figure 1A. Their displayed line of best fit had a very good correlation coefficient of 0.993. For all ten solutes, there was still a high value of $r = 0.968$, as the other data points were not far from the line. A similar value of $r = 0.972$ could only be obtained from Beta-Dex 225 (a diacetyl, TBDMS β-CD) by using no more than eight of the solutes—and if citral and citronellol were included, r went down to 0.755. On this phase, selecting just five solutes increased r to 0.992 (line of best fit shown in Figure 1B). These scatterplots suggested that there was a standard transient molecular interaction between the six probes and Chiraldex A-PH and that this may occur for more solutes with Beta-Dex 225. On the latter phase, citral displayed in Figure 1B considerable extra retention over that implied by the line of best fit (presumably because of a close molecular match with its host CD rings) and the similar acyclic monoterpenoid citronellol was firmly rejected as a guest molecule by Beta-Dex 225 and therefore should have responded to it only as if it were a conventional phase. Chiraldex A-PH displayed no extra retention with any solute in Figure 1A but clearly rejected citronellal, pulegone, and carvone. These showed less relative retention than solutes that were near to the line of best fit. Figure 1C indicates that on Chiraldex G-DA (a dipentyl γ-CD), five of the solutes with boiling points above 220°C with linalol were not far from the line of best fit ($r = 0.984$), but the other scatterplot points reduced the overall correlation coefficient to just 0.726. Fenchone and menthol clearly showed strong extra retention for this phase, and citronellal and estragole were rejected by it. The three phases discussed were then decided to be used in further studies.

Table II. Comparison of Correlation Coefficients for Various Pairs of Modified CD Capillary Phases of GC Using Relative Retentions Versus the *n*-Undecane of Ten Diverse Solutes from Volatile Oils

CDs compared	*r*
Beta-Dex 225 and Chiraldex G-DA	0.5597
Beta-Dex 225 and Chiraldex B-DA	0.6463
Beta-Dex 225 and Chiraldex G-PH	0.7334
Chiraldex A-DA and Chiraldex G-DA	0.7406
Chiraldex A-PH and Chiraldex G-DA	0.7588
Chiraldex A-PH and Beta-Dex 225	0.7704
Beta-Dex 225 and Chiraldex G-PN	0.8012
Chiraldex G-DA and Chiraldex G-PH	0.8111
Chiraldex B-DA and Beta-Dex 325	0.8292
Chiraldex A-DA and Beta-Dex 225	0.8323
Chiraldex B-DA and Cyclodex B	0.8664
Chiraldex A-DA and Beta-Dex 325	0.8751*
Chiraldex A-DA and Chiraldex B-DA	0.8757
Chiraldex B-DA and Chiraldex G-DA	0.8975
Chiraldex A-DA and Chiraldex B-PH	0.8976
Beta-Dex 325 and Chiraldex G-PH	0.9021
Chiraldex A-PH and Beta-Dex 325	0.9075
Chiraldex A-PH and Chiraldex B-DA	0.9111
Beta-Dex 225 and Cyclodex B	0.9181
Chiraldex G-PH and Cyclodex B	0.9201
Chiraldex A-PH and Cyclodex B	0.9211
Beta-Dex 225 and Beta-Dex 325	0.9266
Chiraldex B-DA and Chiraldex G-PH	0.9352†
Chiraldex B-DA and Chiraldex G-BP	0.9396
Chiraldex A-DA and Chiraldex A-PH	0.9425
Chiraldex A-PH and Chiraldex G-PN	0.9535
Chiraldex B-DA and Chiraldex G-PN	0.9583
Chiraldex A-PH and Chiraldex B-PH	0.9584‡
Chiraldex B-PH and Chiraldex G-PN	0.9655
Chiraldex B-DA and Chiraldex B-PH	0.9775
Chiraldex A-PH and Chiraldex G-PH	0.9855
Chiraldex G-BP and Chiraldex G-PN	0.9927§
Cydex-B and Cyclodex B	0.99897

* See also Figure 2B.
† See also Figure 2D.
‡ See also Figure 2A.
§ See also Figure 2C.

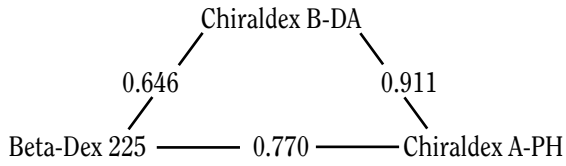
It was realized that the relationship between any two phases in the previously published (1) hierarchical cluster analysis (found in Table I) could be checked by plotting the ten solute relative retentions for one phase against their values on the second CD and obtaining the correlation coefficient between them (several values were calculated and are listed in Table I). The first column of r values was for other CD phases compared with Chiraldex B-DA (a dipentyl β-CD). On scanning this column from top to bottom, correlations at first got worse (as expected) as the phase pairings became more distant in the cluster. However, after a midpoint low with Beta-Dex 225, there followed an unanticipated irregular rise in correlation, including values as high as those observed for some phases placed in the cluster close to Chiraldex B-DA.

The very close pair of Cydex-B versus Cyclodex B had the extremely high correlation coefficient of 0.99897 in Table II. This was almost a perfect correlation, indicating that these two phases were fully interchangeable. This was not surprising because they were both trimethyl β-CDs. Their only difference was that Cydex-B had a diluting polysiloxane, which obviously did not affect its responses. Any correlation coefficient was the same whether a particular CD in a pair was used for the x- or y-axis of the Origin program, although the linear expression constants a and b (not quoted because they were irrelevant) did change. The lower close pairing in the second column of Table I for Chiraldexes B-PH versus A-PH ($r = 0.958$) (which reflected the slightly reduced correlation expected from the cluster levels in Table I) was also seen in the scatterplot in Figure 2A. It appeared that the decreased molecular ring size from β- to α-CD of these two -PH phases was largely irrelevant for all ten probe solutes. An identically good correlation was also seen in the first column of Table I for the superficially nonsimilar Chiraldexes B-DA versus G-PN (propionyl γ-CD). However, the hierarchical arrangement in this table suggested there would be a higher value for the other central-phase pair Chiraldex A-DA versus Beta-Dex 325 (a TBDMS, dimethyl β-CD), but their scatterplot (Figure 2B) did not support this and their r values in Table II were only 0.875. This suggested along with the column of correlations with Chiraldex B-DA discussed previously that hierarchical cluster analysis could not be a reliable indicator of CD-phase relationships.

The pair of monoester γ-CD phases Chiraldexes G-PN versus G-BP (butyryl) exhibited a limited scatterplot (Figure 2C) with an appropriately higher r value of 0.993, but Chiraldex G-PN

versus Beta-Dex 225 (diester) gave a much lower correlation of 0.801 in the third column of Table I. There was also the pair of bulky TBDMS β-CDs Beta-Dexes 225 and 325 (both in a diluting polysiloxane) that had a higher correlation coefficient of 0.927 (more than might have been expected in view of the 225 phase being diacetyl instead of dimethyl). A higher correlation than even this was seen for the two presumably contrasting phases at the opposite ends of the hierarchical cluster in Table I, in which r = 0.935 for Chiraldexes B-DA versus G-PH. This value would have been higher still if it were not for the strong affinity between Chiraldex B-DA and menthol, which can be seen in Figure 2D. Nearly as remote a pairing, Chiraldexes B-DA versus B-PH showed an even better correlation of r = 0.978 in Table II, surprisingly indicating that for these two β-CDs the presence or absence of the polar-PH hydroxypropyl groups did not make much difference to their relative solute retentions. Yet this structural change was important for the corresponding two γ-CDs Chiraldexes G-DA and G-PH. Although these phases were in the same bottom four bracketed cluster of Table I, their r value in Table II was only 0.811. The corresponding α-CD pairing Chiraldexes A-DA versus A-PH had an r value of 0.943, which fell between those of the previous two phase pairs. The correlation coefficients for a number of pairs from the 66 possible combinations of the twelve CDs studied were sequenced, with best correlating phases situated at the bottom of Table II.

There would be no point for a laboratory to purchase both of the CD capillaries showing a high correlation for ten diverse solutes, because the high r value between them indicates they respond so similarly that one phase would be redundant. Conversely, if two capillaries responded so differently to all the solutes that they would give a low correlation coefficient, then they could both be valuable to provide contrasting analyses from any test sample. The previous work has suggested a trio of presumed differently behaving CD phases for the setting up of a chromatographic laboratory. This set can obviously be compared in three pairs, and the resulting three correlation coefficients could be expressed thus:



where the average r value would be 0.776.

Although the correlation coefficients of ≤ 0.770 and less for two of the three pairings were good indications of desirable dif-

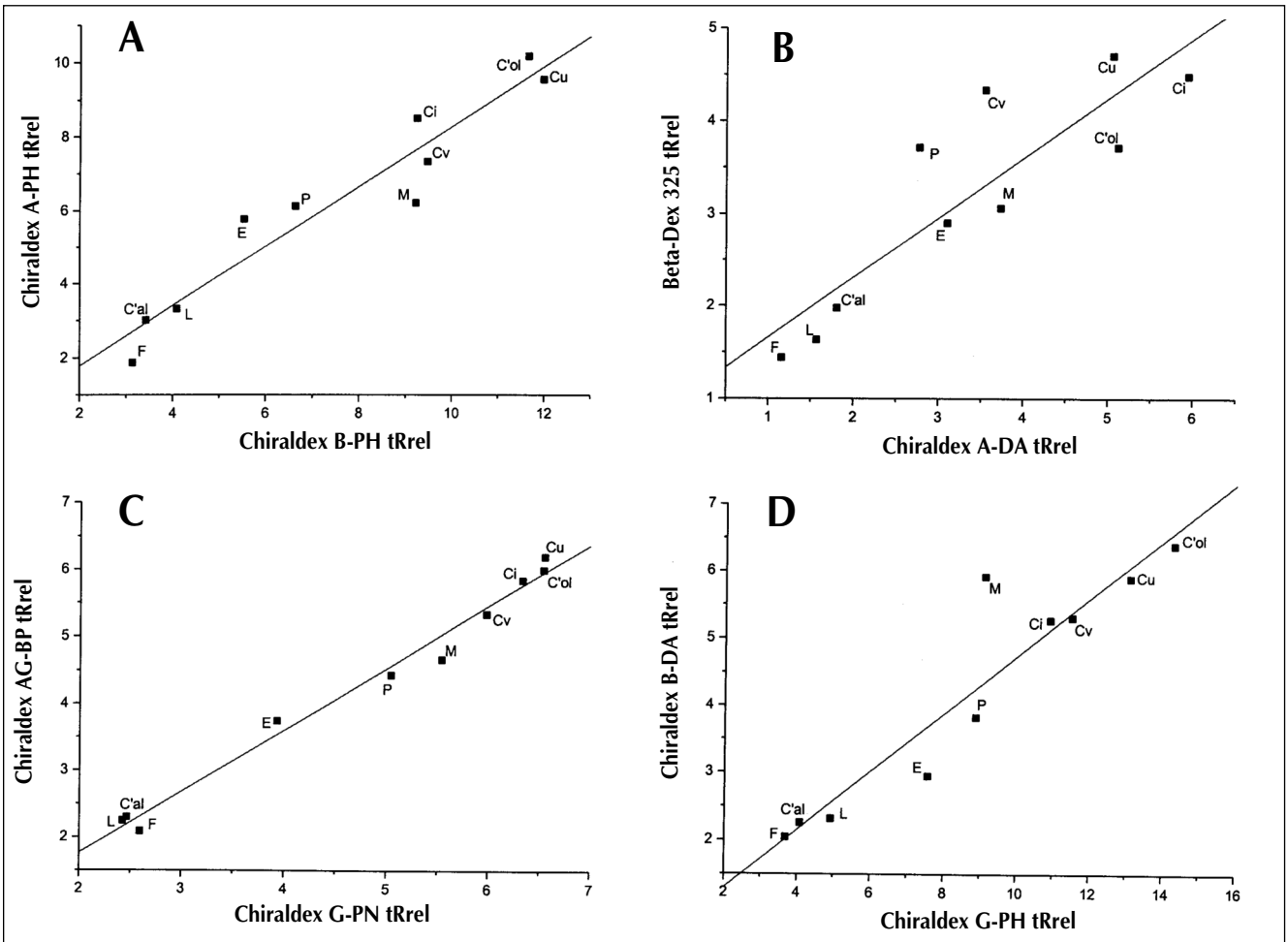
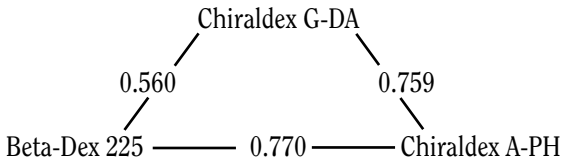


Figure 2. Scatterplots from four different pairs of modified CD capillaries for solute relative retentions (tRrel) versus the *n*-undecane at 125°C with the line of best fit for all 10.

ferently behaving CD phases, the third pair (which gave a value above 0.90) was unsuitable. Chiraldexes A-PH and B-DA had proved surprisingly too similar in solute responses. Yet there would have been nothing much gained by replacing Chiraldex A-PH with a larger CD ring size, because the correlation coefficients in Table I were a high 0.958 for α- versus β-phases and even more for α- versus γ-phases (r = 0.986). However, Chiraldex B-DA versus G-DA had an r value of 0.898, and the correlation of less than 0.911 promised improvement for the trio of phases. A change to Chiraldex G-DA from B-DA in fact gave a satisfactory outcome for the phase pairs of Beta-Dex 225 and Chiraldex G-DA, Chiraldex A-PH and Chiraldex G-DA, and Chiraldex A-PH and Beta-Dex 225 in Table II, which pleasingly included examples of α-, β-, and γ-CDs with three different CD modifications (one being a fairly polar hydroxypropyl-CD):



where the average r value was 0.696.

Figure 3 reveals the desirable widely scattered plots for the three selected CD pairs. This outcome lead to specifications for selecting three usefully different CD phases for a laboratory. These specifications (when considered in three pairs) were that the sets of retentions for a reasonable number of different probe solutes of any type all had to have correlation coefficients below 0.800 and the three values had to have an average less than 0.700. However, this could not be achieved if Chiraldex G-PH replaced A-PH because the consequent two new values of r were 0.733 and 0.811, which gave an average of 0.701. Had the two CD-phase pairs with the poorest correlation coefficients at the top of Table II been chosen (a very reasonable selection), the resultant third pairing would have the undesirably high r value of almost 0.90 and their average would be 0.701 again. This phase trio would also have had the unsatisfactory scientific combinations of two CDs being the same (-DA) dipentyl modification, including two B-CDs.

From the three scatterplots in Figure 3, any of the solutes that fell on or near the line of best fit for just one of the phase pairings could have provided information about its interaction with the remaining phase. In the upper plot, fenchone fell near the best-fit line for Chiraldex A-PH versus Beta-Dex 225 and then confirmed its strong extra retention for the third phase Chiraldex G-DA (as calculated in the previous study) in both the other plots. Estragole and cuminal were on the best-fit line of Figure 3A but appeared to be rejected in the other two plots by Chiraldex G-DA, to which they may have responded as though it were a conventional phase (non-CD). As was also calculated previously, menthol behaved similar to fenchone in Figures 3B and 3C, assuming it was being rejected by Beta-Dex 225 in Figure 3A. Citronellal fell near the line in both Figure 3A and 3B, but the lower plot revealed its previously calculated rejection by Chiraldex G-DA (which can also be seen in Figure 1C).

In Figure 3C, citronellol and citral fell on the line of best fit, with carvone near to it. The two plots above it indicated that citral and carvone showed extra retention on the third phase Beta-Dex 225 as calculated previously, but citronellol was rejected by it (as observed in Figure 1B). Pulegone was on the line in Figure 3B. Chiraldex A-PH may have been rejecting it as was found in the previous study, which is supported by Figure

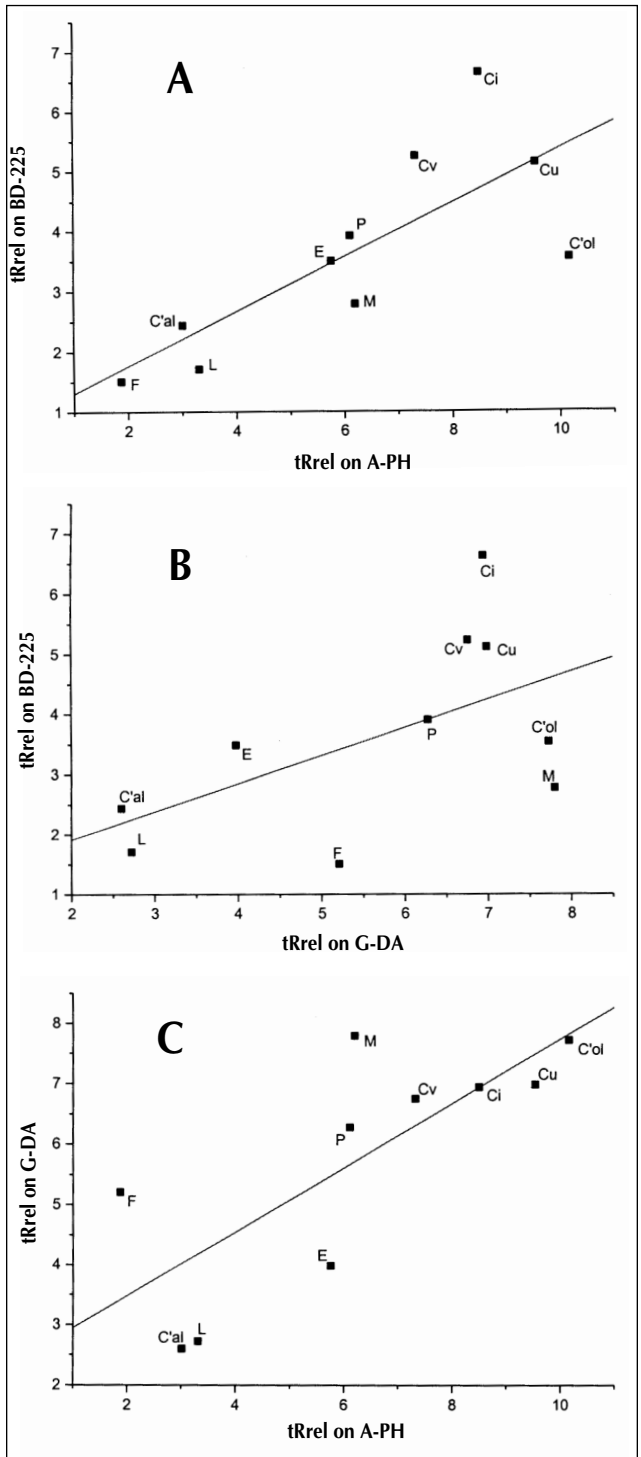


Figure 3. Scatterplots from specially selected pairs of modified CD capillaries for solute relative retentions (tRrel) versus the *n*-undecane at 125°C with the line of best fit for all 10. Solute abbreviations are the same as in Figure 1.

1A. Linalol did not appear close to any of the three lines of best fit in Figure 3; therefore, no deductions were possible. As with cuminal, it was close to all three lines of best fit in Figure 1.

In summary, the following was found. For Chiraldex G-DA, there was extra retention for fenchone (bicyclic monoterpenoid solute type, no hydrocarbon double bonds present, carbonyl polar group) and menthol (monocyclic monoterpenoid solute type, no hydrocarbon double bonds present, alcohol polar group). Chiraldex G-DA showed a rejection of citronellal (noncyclic monoterpenoid, one hydrocarbon double bond present, carbonyl polar group), estragole (aromatic solute type, one hydrocarbon double bond present, ether polar group), and possibly cuminal (aromatic solute type, no hydrocarbon double bonds present, carbonyl polar group). For Beta-Dex 225, there was extra retention for citral (noncyclic monoterpenoid solute type, two hydrocarbon double bonds present, carbonyl polar group) and carvone (monocyclic monoterpenoid solute type, two hydrocarbon double bonds present, carbonyl polar group). Beta-Dex 225 showed a rejection of citronellol (noncyclic monoterpenoid solute type, one hydrocarbon double bond present, alcohol polar group) and possibly menthol (monocyclic monoterpenoid solute type, no hydrocarbon double bond present, alcohol polar group). For Chiraldex A-PH, there was a rejection of possibly pulegone (monocyclic monoterpenoid solute type, one hydrocarbon double bond present, carbonyl polar group).

From this summary, Beta-Dex 225 displayed extra retention of doubly unsaturated carbonyl compounds and rejected alcohols with less than two double bonds. Chiraldex G-DA rejected aromatic solutes and an acyclic carbonyl compound, but favored some rigid molecular cyclic saturated compounds. These sorts of deductions could have been made using various trios of phases providing information about the likely shapes of the molecular ring cavities in many CDs.

References

1. T.J. Betts. The use of linear expressions of solute boiling point versus retention to indicate special interactions with the molecular rings of modified cyclodextrin phases in gas chromatography. *J. Chromatogr. Sci.* **38:** 357–64 (2000).
2. T.J. Betts. Relative polarities of nine modified cyclodextrin commercial stationary phases in gas chromatographic capillaries. *J. Chromatogr. A* **719:** 375–82 (1996).

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Errata

To clarify possible problems for readers who refer back to it, several corrections have been made to reference 1. The missing exponent in the first line of the Abstract is 1×10^{-2}. In the Introduction section on page 358, the temperature range "approximately 50 to 194°C" should be "approximately 194°C to 245°C". On page 361, the sentence "It behaved with the 2 α-CDs and 2 other solutes…" has been changes to "… and 2 other phases…". In the second column of page 363, the segment "… a plot against them), the polarity…" is now "… a plot against them of the polarity…". Finally, in the first sentence of the caption for Figure 1, the segments "by Morgan (17)" and ", 1×10^{-2}" have been omitted.