New hybrid displacement technique for preparative liquid chromatography☆

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Abstract

A new hybrid displacement technique (patent pending) for separating mixtures which incorporates the positive aspects of displacement chromatography and sample self-displacement, has been developed. Immediately after injection of the separation mixture, a relatively large, but finite amount of an inert material is injected onto the column to act as a "displacing compound". The purpose of this displacing compound is to drive the separation by providing additional adsorption site competition, *thus enhancing the displacement effect*. It functions to help maintain the concentrations of the actual separation components so that tailing and/or fronting, yield limiting factors in sample self-displacement, are mitigated. The displacing compound is distinct from a true displacer because it is introduced in a limited quantity at the outset of the separation, not as a continuous additive.

Feasibility has been demonstrated and operational parameters investigated. Using a displacing compound sharpens zone boundaries between mixture components. The practical significance of this effect includes increased loading capability and the ability to achieve high yields when the major component elutes first. The impact of adding a displacing compound is greater when *the separation factors (α) between the mixture components are smaller*.

1. Introduction

The practice of preparative HPLC is undergoing dramatic changes as understanding of column overload phenomena grows. One new method, sample self-displacement [1–3], takes advantage of a displacement effect which occurs when a column is heavily loaded. When component bands overlap in the chromatographic column, their behavior is not independent. It differs markedly from their behavior when they elute individually. For example, in the case of a binary mixture, the second component displaces the first, literally pushing the first component ahead of itself through the column. A sharp delineation between component zones results even though visual resolution is often negligible [4,5]. Modeling studies have shown very good correlation with experimental work [6–8]. Similar separations can be achieved with multi-component mixtures [9]. The practical outcome of this

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☆ Patent pending, US Serial No. 868 075; filed 13 April 1992.

displacement effect is a large increase in the production rate of pure material.

Although the underlying mechanisms are similar, sample self-displacement differs significantly from conventional displacement chromatography in that an external displacing agent is not added to promote displacement. Consequently, a lengthy column re-equilibration between successive cycles is unnecessary, and separation optimization is much less involved. Unfortunately, because there is no constant forward push from a constant concentration displacer, the separation between successive bands remains incomplete. In a binary mixture, for example, as the material elutes and the concentration of the second component decreases, the competitive interaction between the two components is weakened and the first component band tails into the second. Depending on such factors as sample load, mixture ratios, relative retention, or requisite purity, the effect of this tailing on the recovery yield of both components can be negligible or substantial.

By considering and comparing the features of sample self-displacement and displacement chromatography, one solution to the yield loss problem becomes evident. In the former mode, contamination by tailing results from dilution of the mixture material as it elutes down the column. In the latter, the constant addition of an external displacing agent suppresses dilution and, consequently, tailing. In the present work, a new hybrid technique is described which incorporates some positive features of both chromatographic modes. A limited amount of an external compound is introduced onto the column at the start of a run, subsequent to the injection of the feed sample. This "displacing compound" provides competition for adsorption with the last-eluted component of the feed. In this way, it enhances the degree of competition between all component pairs of the feed, increasing the concentration of each of the component bands, and significantly reducing their tailing. Unlike working in the sample self-displacement mode, an external agent is added. However, the hybrid technique differs from displacement chromatography because the addition of the displac-

ing compound is not continuous. Instead, a single, adjustable pulse is injected. Furthermore, the composition of the mobile phase in which the column is initially equilibrated remains constant, so column regeneration is not necessary.

2. Experimental

2.1. Apparatus

Preparative experiments were carried out on a Varex (Burtonsville, MD, USA) VersaPREP preparative liquid chromatograph, equipped with a Kipp and Zonen (Delft, Netherlands) Model BD40 strip-chart recorder.

Component ratios in the regions between bands where these components interfere were determined on an analytical HPLC system consisting of a Perkin-Elmer (Norwalk, CT, USA) Series 4 liquid chromatograph, an Applied Biosystems (Foster City, CA, USA) Model 783A variable-wavelength absorbance detector, a Perkin-Elmer ISS-100 autosampler and a VG (Cheshire, UK) Multichrom data acquisition system.

2.2. Reagents

Solvents were HPLC grade, purchased from J.T. Baker (Phillipsburg, NJ, USA). Diethyl phthalate, dimethyl phthalate, dipropyl phthalate, α-tetralone, β-tetralone and benzosuberone rated 99% + purity were purchased from Aldrich (Milwaukee, WI, USA). The benzyl butyl phthalate, also from Aldrich, was 98% pure.

2.3. Preparative chromatographic conditions

The preparative column consisted of three identical 250×21.4 mm Dynamax 8-μm silica columns (Rainin Instruments, Woburn, MA, USA) connected in series with a minimum length of 0.040-in. (1 in. = 2.54 cm) tubing. Prior to use, the column was washed with the following series of solvents: 1.5 l of tetrahydrofuran (THF)–hexane (30:70), 1.0 l of THF–hexane–ethyl acetate (10:75:15) and 1 l of ethyl acetate–

hexane (15:85). The column was finally washed with 4.0 l of ethyl acetate–hexane (4:96). This procedure was found to be necessary to remove any trace of silica-deactivating solvents (e.g., water) present in the shipping solvent. Failure to wash the columns as indicated resulted in a substantial reduction of the displacement effect.

For all experiments reported, the mobile phase was ethyl acetate–hexane (4:96). The flow-rate was 50 ml/min. Peaks were monitored at a wavelength of 310 nm for experiments separating the benzosuberone/α-tetralone mixture and 290 nm for the dipropyl phthalate/benzyl butyl phthalate mixture.

2.4. Procedures

Compounds were weighed out according to the desired ratio and loadings, added to volumetric flasks, and dissolved in concentrations of 4–8 g/10 ml of 12–14% ethyl acetate in hexane. (Some extra ethyl acetate was added to improve solubility if necessary). Sample mixtures were injected onto the column by filling a 10-ml Rheodyne (Cotati, CA, USA) high-pressure valve injection loop.

The following routine was followed when making injections: (1) the flow-rate was reduced from 50 to 10.5 ml/min; (2) the 10-ml loop was loaded with the separation mixture (12 ml were flushed through to ensure thorough filling) and the injection valve ports opened to the column; (3) after 75 s, the flow was stopped; (4) the 10-ml loop was filled with displacing compound and the valve ports opened to the column; (5) the flow-rate was increased from 0 to 50 ml/min over 20 s; at the same time, the strip-chart recorder was started.

For each experiment, fraction collection began as soon as a slope change was noted in the detector signal. Fraction volumes were monitored indirectly by counting chart paper units. The fraction volumes were approximately 10 ml.

The fractions were analyzed to determine the concentration ratio of the two components, using a Waters Nova-Pak 4 μm silica column, 150 × 3.9 mm. In all studies, a mobile phase of ethyl acetate–hexane (4:96) was used at a flow-rate of 0.9 ml/min monitored at a wavelength of 285 nm. A standard of each component was made up fresh and injected as a single-point calibration standard for every fraction set analysis. Standard curves were run showing that absorbance was linear for the concentration ranges encountered for all compounds used in the experiments.

2.5. Calculations

Recovery yields were determined from each chromatogram by counting the number of chart paper units under the curve for each fraction. This procedure was reproducible within several percents. Because the extinction coefficients of benzosuberone and α-tetralone differ significantly, the "true" chromatographic (i.e., concentration) profile had to be derived first. The elution profiles shown in this paper for these compounds are the concentration profiles, and not the original detector response (i.e., absorbance) profiles. The elution profiles of the dipropyl phthalate and benzyl butyl phthalate mixture are the original chromatograms, not the concentration profiles. To determine the true concentration profiles of benzosuberone and α-tetralone, individual injections of 2, 2.5 and 3 g were made for these two compounds. In both cases, the peak areas increased linearly with increasing sample size, with the average area ratio of benzosuberone to α-tetralone equal to 2.16. Using this factor, the relative composition of each fraction, and the area measured under the curve for each fraction, normalized profiles were derived. From these profiles, the recovery yields were calculated as in previous studies [2,3].

3. Results and discussion

To investigate the potential of displacing compounds for yield enhancement, binary mixtures were selected for study because their behavior in the sample self-displacement mode is well-characterized [1,2]. In choosing a substance to act as a displacing compound, the following factors were considered:

(1) The relative retention of the displacing compound to the feed components should be large enough that these components did not trail significantly into it, but small enough that interaction with them on the column would occur.

(2) The displacing compound should be completely eluted in a reasonable time using the mobile phase selected for the separation.

(3) The increase in separation yield should be sufficient to offset the increase in cycle time in terms of overall production rate.

With these criteria in mind, k' was determined for a number of compounds using a 750×21.4 mm silica column and a mobile phase of ethyl acetate–hexane (4:96). Of these compounds, benzosuberone ($k' = 2.4$) and α-tetralone ($k' = 3.2$) were chosen to make the separation mixtures ($\alpha = 1.33$). Diethyl phthalate ($k' = 5.4$) was chosen to be the displacing compound.

The injection technique was developed to minimize premature elution of the mixture components prior to the addition of the displacing compound. After filling the loop with the separation mixture and triggering the injection valve, flow was continued for a period of 75 s at 10.5 ml/min to completely empty the loop. Flow was then stopped to allow time to fill the injection loop with the displacing compound. Flow was resumed at the elution flow-rate of 50 ml/min immediately after triggering the injection valve.

A possible alternative procedure, injecting the separation mixture and the displacing compound in one solution, simultaneously, was investigated. This method caused severe tailing of the benzosuberone and fronting of the diethyl phthalate with recovery considerably less than for the sample self-displacement yield.

3.1. Effect of the addition of a displacing compound on separations of benzosuberone and α-tetralone (50:50 mixtures)

Fig. 1a shows the chromatogram for a mixture injection of 2.5 g of benzosuberone and 2.5 g of α-tetralone under sample self-displacement conditions. Fig. 1b shows a separation of the same mixture when diethyl phthalate is injected as a displacing compound. Diethyl phthalate does not
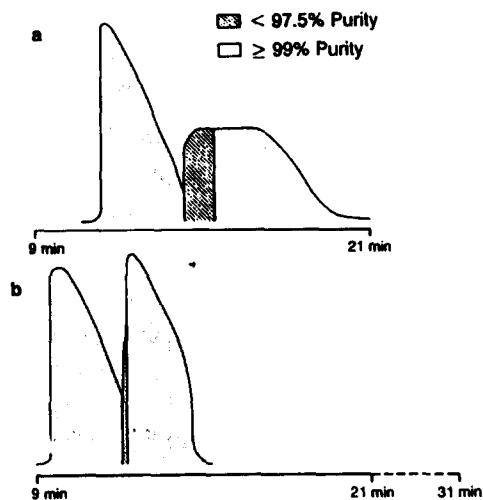

Fig. 1. Comparison of a self-displacement separation and a separation with a displacing compound added. Column: 750 mm × 21.4 mm I.D., packed with 8-μm silica particles. Mobile phase: ethyl acetate–n-hexane (4:96, v/v). Flow-rate: 50 ml/min. Sample: (a) 2.5 g benzosuberone (elutes first) and 2.5 g α-tetralone; (b) 2.5 g benzosuberone and 2.5 g α-tetralone with 7 g diethyl phthalate added.

absorb light at the 310 nm wavelength used for the experiment so its presence is not detected (as determined with refractive index detection, it elutes during the period from 14.5 to 31 min). When Fig. 1a is compared to Fig. 1b, peak compression is noted when a displacing compound is added. Furthermore, there is an important decrease in the amount of impure material found at the component interface. This decrease results from a reduction in tailing of the benzosuberone which occurs when using a displacing compound.

Fig. 2 is a graph which helps visualize the effect of adding a displacing compound on the mixed band interface between the two component bands. The extent of the tailing by the benzosuberone into the α-tetralone band can be expressed by plotting the percentage of benzosuberone present in α-tetralone fractions after the benzosuberone fractions cease to be 99% pure. Each point on the graph represents a collected fraction which has been analyzed to determine the mixture ratio. Points are plotted until the α-tetralone fractions attain a purity of $\geq 99.5\%$. The y-intercept is equal to 99% pure
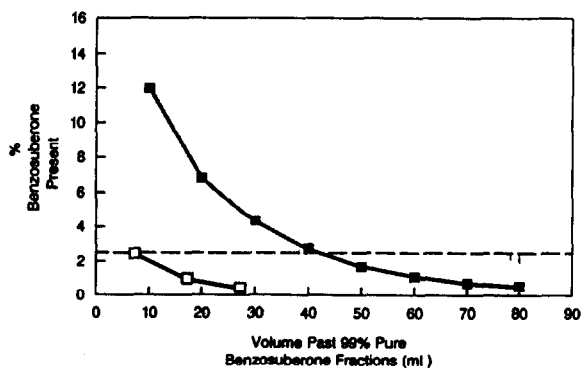
Fig. 2. Effect of the addition of a displacing compound on α-tetralone purity for a 5-g sample of benzosuberone and α-tetralone (50:50). Plot of benzosuberone concentration in collected fractions vs. the volume of solvent eluting past the last 99% pure benzosuberone fraction. Conditions as in Fig. 1. Diethyl phthalate: ■ = 0 g; □ = 7 g.
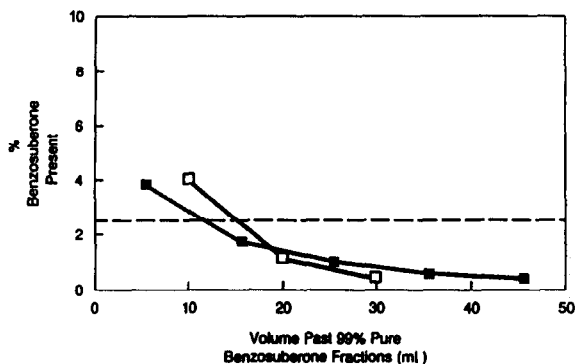

Fig. 4. Effect of the addition of a displacing compound on α-tetralone purity for a 4-g mixture of benzosuberone and α-tetralone (50:50). Plot of benzosuberone concentration in collected fractions vs. the volume of solvent eluting past the last 99% pure benzosuberone fraction. Conditions as in Fig. 1. Diethyl phthalate: ■ = 0 g; □ = 7 g.

benzosuberone. When the hybrid technique is used, the boundary between peaks is sharper and the benzosuberone tailing is greatly reduced.

In sample self-displacement, as loading increases, tailing increases as well. This effect is shown in Fig. 3, a traditional loading study plot for sample self-displacement. As can be seen in Fig. 4, for the 4-g mixture of benzosuberone and α-tetralone (50:50), no significant reduction in tailing is obtained by adding a displacing com-

pound. Therefore, the sample self-displacement technique, which is less complicated and has shorter run times, is a more appropriate technique to use for this injection amount. However, as can be seen in Fig. 5, for 6 g of the 50:50 mixture, the addition of a displacing compound does cause a substantial decrease in tailing. The addition of 8 g of diethyl phthalate gives a better tailing profile than 7 g and is significantly better than adding no displacing compound at all.
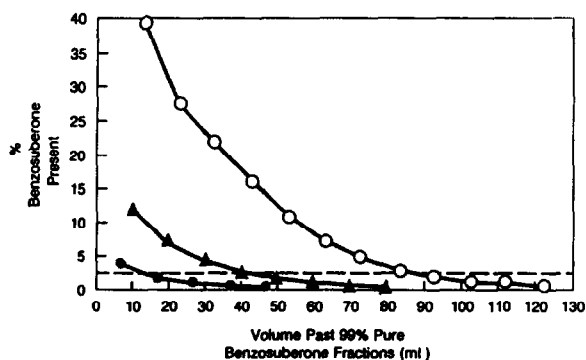

Fig. 3. Effect of loading on α-tetralone purity for a sample self-displacement separation. Plot of benzosuberone concentration in collected fractions vs. the volume of solvent eluting past the last 99% pure benzosuberone fraction. Conditions as in Fig. 1. Sample: 4- (●), 5- (▲) and 6-g (○) mixtures of benzosuberone and α-tetralone (50:50).
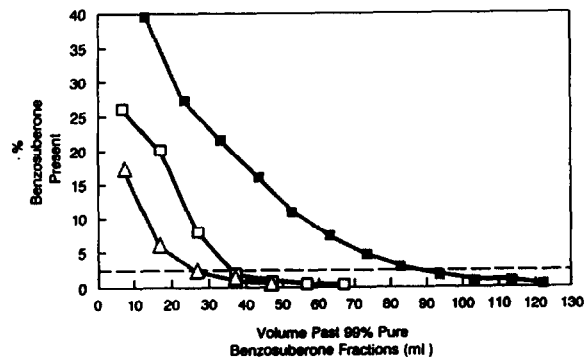

Fig. 5. Effect of the addition of a displacing compound on α-tetralone purity for a 6-g sample of benzosuberone and α-tetralone (50:50). Plot of benzosuberone concentration in the collected fractions vs. the volume of solvent eluting past the last 99% pure benzosuberone fraction. Conditions as in Fig. 1. Diethyl phthalate: ■ = 0 g; □ = 7 g; △ = 8 g.
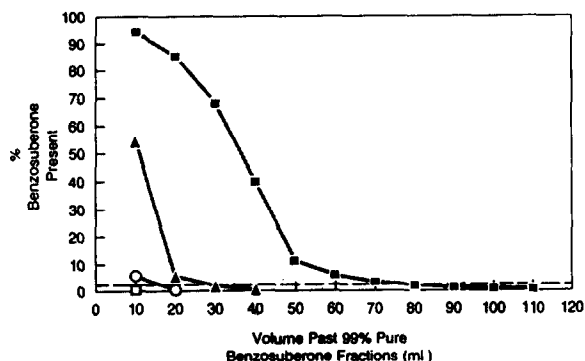
Fig. 6. Effect of the addition of a displacing compound on α-tetralone purity for a 5-g sample of benzosuberone and α-tetralone (75:25). Plot of benzosuberone concentration in collected fractions vs. the volume of solvent eluting past the last 99% pure benzosuberone fraction. Conditions as in Fig. 1. Diethyl phthalate: ■ = 0 g; ▲ = 5 g; ○ = 6 g; □ = 7 g.

3.2. Comparison of separations of benzosuberone and α-tetralone with and without a displacing compound added for different mixture ratios

In sample self-displacement, tailing increases with increasing ratio of the preceding to the subsequent component. In a binary mixture, for example, as the ratio of the first eluting component to the second eluting component in-
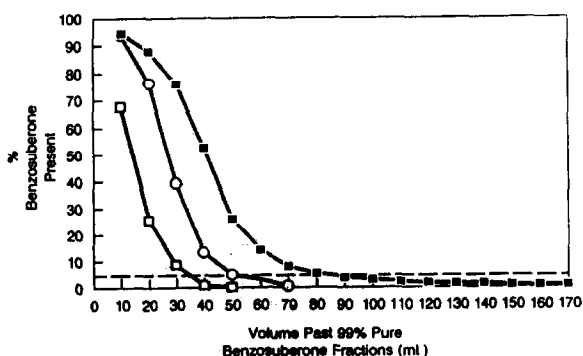
creases from 50:50 (Fig. 2) to 75:25 (Fig. 6) to 90:10 (Fig. 7) and no diethyl phthalate is added, tailing becomes greater. Furthermore, the sharp boundary between the first and second components becomes less defined at higher mixture ratios, with the second component beginning to front, i.e., trail forward, into the first band. This fronting results in contamination of the later eluting benzosuberone fractions as seen in the more gradual change in slope for the initial points plotted in Figs. 6 and 7.

When Figs. 2, 6 and 7 are compared, it can be seen that the effect of adding a displacing compound on both tailing and fronting is substantially more significant at the higher concentration ratios. Thus the derived benefit of using the new hybrid technique is even greater at higher mixture ratios.

The impact of the displacing compound on the band shape of the separation components becomes greater as the mixture ratio of preceding component(s) to subsequent component(s) increases. Comparing the 50:50 mixture (Fig. 1) and the 90:10 mixture (Fig. 8), a dramatic difference is seen when a displacing compound is used.
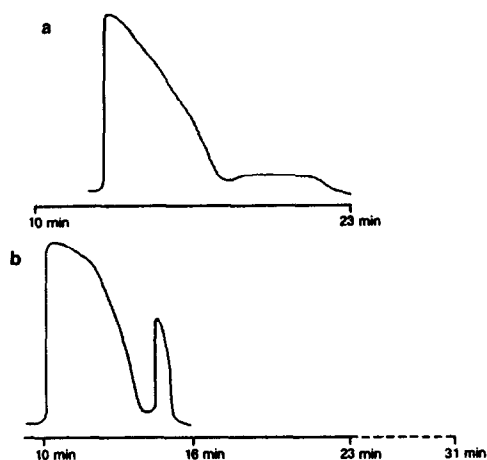

Fig. 7. Effect of the addition of a displacing compound on α-tetralone purity for a 5-g sample of benzosuberone and α-tetralone (90:10). Plot of benzosuberone concentration in the collected fractions vs. the volume of solvent eluting past the last 99% pure benzosuberone fraction. Conditions as in Fig. 1. Diethyl phthalate: ■ = 0 g; ○ = 6 g; □ = 7 g.

Fig. 8. Comparison of a self-displacement separation and a separation with a displacing compound added. Sample: (a) 4.5 g benzosuberone and 0.5 g α-tetralone; (b) 4.5 g benzosuberone and 0.5 g α-tetralone with 7 g diethyl phthalate added. Conditions as in Fig. 1.

3.3. Factors contributing to yield and production when displacing compounds are used

While the purification of the first component of the feed is made easier and its recovery yield is improved for a given sample size, the situation is somewhat different for the second component as it can become polluted by the displacing compound. This auxiliary to the separation must be chosen for its compatibility with the product. The yield of the second component depends on its acceptable degree of purity.

The value of the desired purity for an individual component of the feed depends on the type of separation being performed. For example, if the mixture to be separated is an end product, i.e., a material that will undergo no further chemical processes or purifications before use, a higher percentage of purity, with little or no contamination from the displacing compound, would be preferable. If, however, the recovered material is an intermediate, which will undergo further chemical processes or purifications, removal of the displacing compound may not be critical at this stage, and a lower purity would be acceptable. The determination of the acceptable or desired purity for a particular separation component can be made by the chromatographer. Further, in any separation, the percentage purity deemed acceptable may be a function of the recovery yield required.

From Figs. 5–7, it can be seen that increasing the amount of displacing compound injected leads to a narrower mixed zone at the interface between the two component bands by reduction in the first band's tailing and, when a factor, reduction in the second band's fronting as well. However, tailing and fronting are not the only factors which affect production rate and recovery yield of pure material. The potential formation of a second mixed zone, between the second component and the displacer must be taken into account.

From the plot in Fig. 7 and the chromatograms in Fig. 9 for the 90:10 mixture, we see that the displacement effect is strongest when 7 g of diethyl phthalate are added. With the 7-g addition, however, there is a greater overlap between
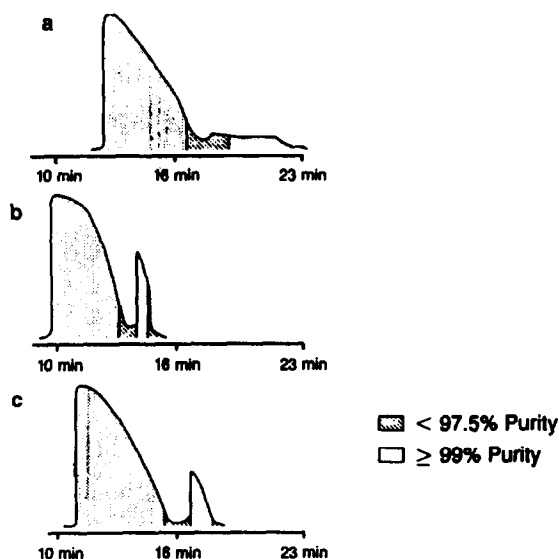

Fig. 9. Effect of the amount of displacing compound added on a 5-g sample of benzosuberone and α-tetralone (90:10). Sample: (a) No diethyl phthalate added; (b) 7 g diethyl phthalate added; (c) 6 g diethyl phthalate added. Conditions as in Fig. 1.

the diethyl phthalate and the α-tetralone than for the 6-g addition, resulting in a larger loss of material due to its contamination with the displacing compound. In both cases, using the new hybrid technique produces significantly higher yields than the sample self-displacement separation. Overall, the addition of 6 g of displacing compound gives the highest yield (Table 1). The choice of how much displacing compound to

Table 1
Influence of the amount of displacing compound on the recovery yield

Amount of diethyl phthalate (g)	Amount of α-tetralone recovered with $\leqslant 1\%$ of each impurity (%)[a]
0	68.7
6	95.7
7	81.7

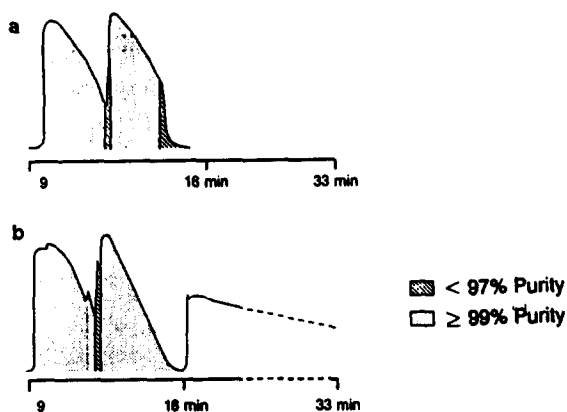[a] Benzosuberone and diethyl phthalate (the latter detected at 285 nm).

Fig. 10. Importance of the relative retention between the separation mixture and the displacing compound. Sample: (a) 2.5 g benzosuberone and 2.5 g α-tetralone with 7 g diethyl phthalate added; (b) 2.5 g benzosuberone and 2.5 g α-tetralone with 7 g β-tetralone added. Conditions as in Fig. 1.

inject is a compromise between minimizing the size of the mixed zone between the two components and minimizing the mixed zone between the last eluted component and the displacing compound. For maximum recovery of α-tetralone, 7 g of the displacing compound would be optimum when displacing compound contamination is not an issue, while 6 g would be optimum for an end product.

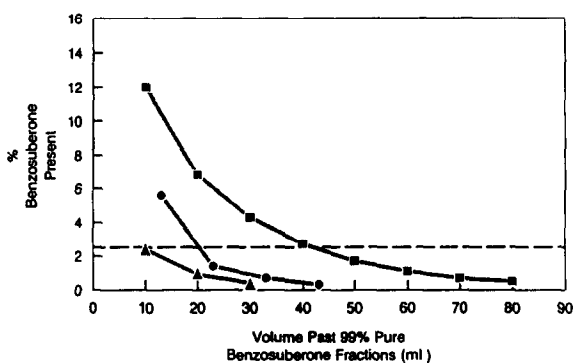Yield enhancement is just one advantage gained by using the new hybrid technique. Al-

Fig. 11. Comparison of the choice of displacing compound on α-tetralone purity for a 5-g sample of benzosuberone and α-tetralone (50:50). Plot of benzosuberone concentrations in the collected fractions vs. the volume of solvent eluting past the last 99% pure benzosuberone fraction. Conditions as in Fig. 1. ■ = No displacing compound; ▲ = diethyl phthalate; ● = β-tetralone.

though the run time increases from 23 to 31 min in Figs. 1 and 8 to 10 because the displacing compound must also elute during a cycle, overall production rates can increase. Zone boundaries are sharper and less ambiguous when the displacing compound is added. Fewer fractions must be checked by analytical HPLC to determine cut points for 99% pure material. Therefore, the total time needed to both collect and process fractions for one injection is less. The production rate can also increase when the displacing compound is added because larger feed samples can be used.

As a separation methodology, the hybrid displacement technique is particularly well suited to high-volume production runs. Both injection and fractionation process are easily amenable to an automated approach and the columns do not require washing between runs, a marked advantage compared to classical displacement chromatography. Follow-up fraction checking is minimal and the displacing compound is easily recovered by solvent stripping. Correspondingly, mobile phase can be recovered and recycled since the process is isocratic. Furthermore, material production is good. For the separation pictured in Fig. 10 (with $\alpha = 1.3$), an hourly throughput of almost 10 g, with 95% recovery, is achieved using a column of only 2.5 cm in diameter. The process was found to be as robust as conventional overloaded elution.

3.4. Importance of the relative retention between the displacing compound and the separation mixture

Different compounds can act as displacing compounds for the same mixture to be separated. The deciding factor will be the relative retention between the last eluting component of the separation mixture and the displacing compound. The relative retentions between α-tetralone and diethyl phthalate and between α-tetralone and β-tetralone are 1.7 and 1.9, respectively. As can be seen in the chromatograms in Fig. 10 and the plot in Fig. 11, when β-tetralone is the displacing compound, the intensity of the displacement effect is reduced. There is more benzosuberone tailing into the α-tetralone band,

Table 2
Comparison between two displacing compounds

Displacing compound (7 g)	α Value	Amount of α-tetralone recovered with $\leqslant 1\%$ of each impurity[a]
Diethyl phthalate	1.7	89.7
β-Tetralone	1.9	95.0

[a] Benzosuberone, diethyl phthalate or β-tetralone.

and the peaks are less compressed. Nevertheless, the α-tetralone recovery yield is slightly higher with β-tetralone, as shown in Table 2, because the displacing compound does not contaminate the α-tetralone.

When the relative retention between the last-eluting mixture component and the displacing compound is too great, there is an adverse effect on the separation. The α value for α-tetralone and dimethyl phthalate is 2.9. As can be seen in Fig. 12, using dimethyl phthalate as the displacing compound causes more tailing than when the sample self-displacement technique is used.

3.5. Effect of using a displacing compound for difficult separations

In sample self-displacement, less material per injection can be separated when the α values
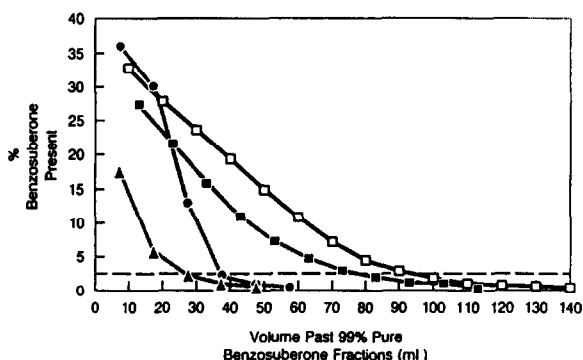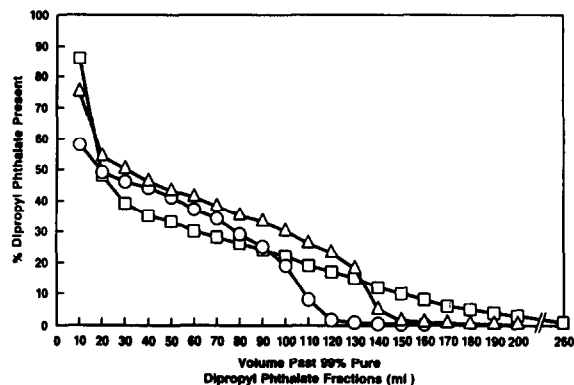
Fig. 13. Effect of the addition of a displacing compound on benzyl butyl phthalate purity for a 2-g mixture of dipropyl phthalate and benzyl butyl phthalate (50:50). Plot of dipropyl phthalate concentration in the collected fractions vs. the volume of solvent eluting past the last 99% pure dipropyl phthalate fraction. Conditions as in Fig. 1. β-Tetralone: $\square$ = 0 g; $\triangle$ = 4 g; $\bigcirc$ = 5 g.

between the separation components are small. For benzosuberone and α-tetralone, the α value is 1.33; for dipropyl phthalate and benzyl butyl phthalate, it is only 1.15. Due to considerable tailing (see Fig. 13), the amount of material collected as mixed fractions during this latter separation is much greater than for the previous separations. Pure material is recovered in a lower yield even though less is injected (2 g in Fig. 13 vs. 5 or 6 g in Figs. 2 and 5, respectively). When β-tetralone is added as a displacing compound, the recovery of benzyl butyl phthalate increases significantly as seen in Figs. 13 and 14. The effect of the β-tetralone on the tailing of the dipropyl phthalate band into the benzyl butyl phthalate fractions is substantial as can be seen in Fig. 13. Many more fractions have $> 99.5\%$ purity when the displacing compound is added. The advantage to using the new hybrid technique instead of sample self-displacement is even greater when α is small.

Fig. 12. Comparison of the choice of displacing compound on α-tetralone purity for a 6-g mixture of benzosuberone and α-tetralone (50:50). Plot of benzosuberone concentrations in the collected fractions vs. the volume of solvent eluting past the last 99% pure benzosuberone fraction. Conditions as in Fig. 1. $\blacksquare$ = No displacing compound; $\blacktriangle$ = diethyl phthalate; $\bullet$ = β-tetralone; $\square$ = dimethyl phthalate.

4. Conclusions

Sample self-displacement offers significant advantages over traditional preparative elution chromatography with touching band separations. However, there is a potential for yield loss with
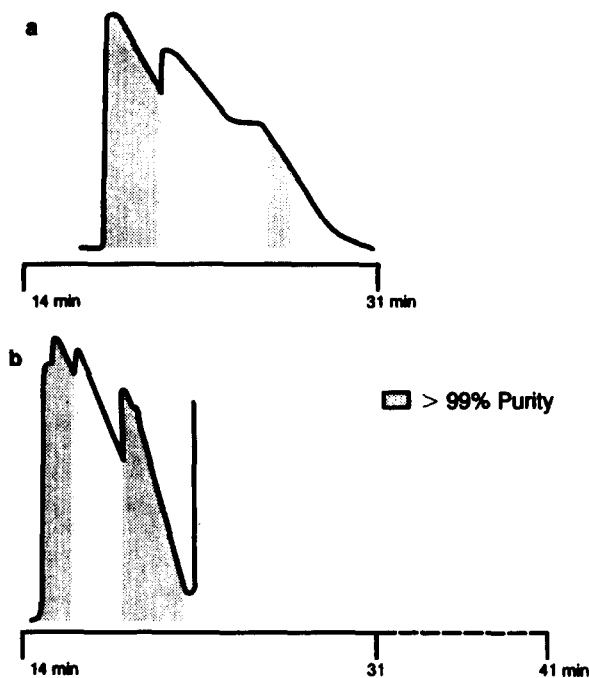
Fig. 14. Comparison of a self-displacement separation and a separation with a displacing compound added when α is small. Sample: (a) 1 g dipropyl phthalate (elutes first) and 1 g benzyl butyl phthalate; (b) 1 g dipropyl phthalate and 1 g benzyl butyl phthalate with 5 g β-tetralone added. Conditions as in Fig. 1.

this technique, depending on sample loading, mixture ratio and the difficulty of the separation. Yield loss, which results from the tailing and fronting of the component bands to form mixed zones at their interface, appears to be less a factor in displacement chromatography. However, the optimization and mechanics of working with displacers is often cumbersome. Chemists avoid it particularly for the rapid separation of batches of limited size where the cost of method development would be too large a component of the total separation cost.

The new hybrid displacement technique described, addition of a displacing compound in a discreet quantity at the outset of a separation subsequent to injection of the mixture, addresses and overcomes the limitations of both techniques. When zone boundaries between separation components are sharp, the sample self-

displacement technique is the easiest preparative method to use. However, when zone boundaries between components are diffuse, especially for the elimination of the impurities which are less retained than the main component, the addition of a displacing compound can result in a substantial reduction in tailing and fronting. The practical outcome of fewer mixed fractions is a higher loading and a greater recovery yield. Like overloaded elution and displacement chromatography, this new hybrid technique is a mode of operating chromatographic separations. As such, it is applicable independently of the retention mechanism used for the separation. Its suitability for large-scale separations depends on economical rather than technical considerations.

In order to realize the optimum benefit from a displacing compound, one must determine which displacing compound to use and how much to inject. The displacing compound which produces the sharpest boundaries between mixture components is not necessarily the best candidate. Similarly, the amount of displacing compound which provides the strongest displacement effect may not be the most appropriate injection quantity. Instead, the choice of a displacing compound and the quantity to use are related to its potential encroachment upon the feed components. The reduction in the number of mixed fractions at the separation component interfaces may be of mere academic interest if an unacceptable amount of useful material is lost through contamination with the displacing compound. The use of the purified materials and the criteria for purity are the controlling factors.

Given these considerations, the practice of this hybrid technique is relatively easy to implement. For the experiments described in this paper, the choice of the displacing compound was made based on analytical retention times. The optimized load, amount of displacing compound, and retention difference between displacing compound and components of the sample were determined with three or four exploratory injections. In all cases, the recovery yields were improved when the hybrid technique was employed. It is a viable approach, without some of the inconvenience of true displacement chroma-

tography, when yield losses from sample self-displacement separations are unacceptable.

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