Injection Solvent Enhancement of Peak Height in Reversed-Phase Liquid Chromatography

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INJECTION SOLVENT ENHANCEMENT OF PEAK HEIGHT IN REVERSED-PHASE LIQUID CHROMATOGRAPHY

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ABSTRACT

Peak height and plate number were increased under certain conditions when eluites that were dissolved in weak solvents were injected into strong mobile phases. Eluites with a k' of about 3 or greater did not exhibit this phenomenon.

INTRODUCTION

Many workers have observed that the strength of an injection solvent relative to the strength of the mobile phase in reversed-phase high performance liquid chromatography (RP-HPLC) can have marked effects on peak shape and peak height (1-13). The observed result of using an injection solvent that is stronger than the mobile phase is the gradual dimunition of peak height (h_p) and the appearance of more than one peak as the difference in strength is increased (12). Thus, the usual procedure of using the mobile phase as injection solvent has strong experimental justification.

On the other hand, injection of the eluite in a solvent weaker than the mobile phase would not be expected to cause peak distortion because the process is analogous to stepwise gradient elution consisting of one step. However, peak distortion has been reported (2) when

dihydroxybenzenes that were dissolved in water were injected into a methanol mobile phase. To examine peak shape with weak injection solvents, we have systematically studied the injection of elultes into mobile phases that were stronger than the eluite solvent. Both mobile phase and injection solvent strengths were varied. It is the purpose of this report to present and discuss the results of this study.

EXPERIMENTAL

Instrumentation

The chromatograph used consisted of a model 6000 Waters pump, a Rheodyne 7135 injector, a Perkin Elmer LC55 variable wavelength detector and a Fisher Recordall 5000 strip chart recorder. A 25 x 0.46 cm silica saturation column was placed between the pump and the injector. Two 25 x 0.46 cm analytical columns were used: a 10 μm Spherisorb ODS 2 and a 10 μm Econosil C18.

Materials

Eluites were bought from Aldrich Chemical. HPLC grade organic solvents were used for injection and mobile phases. Water was purified with a Barnstead system.

Procedure

Elulte solutions were prepared only in 50 or 100 mL volume and kept at -19°C. The concentration varied from 50 to 390 μg/mL depending on the eluite. The flow rate was 1 mL/min, and the injection volume varied from 30 μL (most of the work) to 120 μL. The detector wavelength was 258 nm. Elulte solutions were injected five times, and the peak heights were averaged and evaluated statistically. Sodium nitrite was used to measure t_o.

RESULTS AND DISCUSSION

Benzyl alcohol was injected into mobile phases of water and methanol differing by 10 vol. %. The benzyl alcohol was dissolved in injection solvents of water and methanol also differing
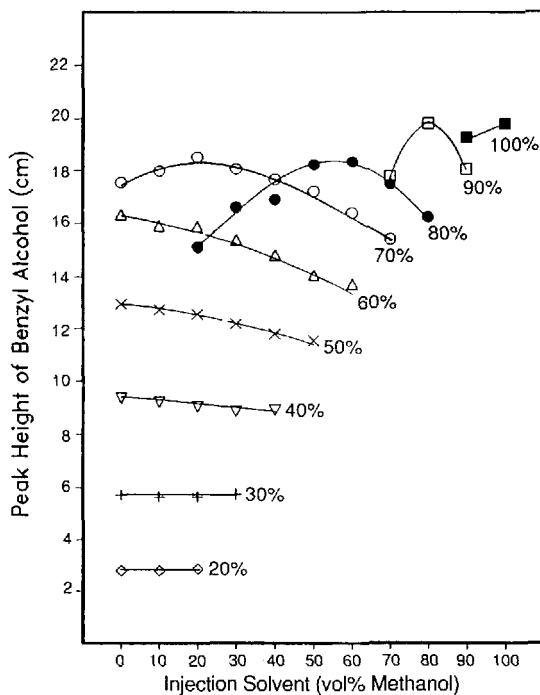
Figure 1.   Relationship between peak height of benzyl alcohol and injection solvent composition. Curves for varying percent methyl alcohol in the mobile phase are shown.

by 10 vol. %. The peak heights obtained are displayed in Fig. 1. Points representing injections in solvents stronger than the mobile phase were not plotted because they were known to give peak distortions. As expected the retention of benzyl alcohol increased with increasing water content of the mobile phase, and the increase caused lower peak heights. Thus, the injection curves in Fig. 1 fall as the mobile phase water content rises.

The results using neat methanol as a mobile phase are probably not meaningful because there was very little retention. Aside from results with neat methanol, the curves can be grouped into three types. From 70 to 90% methanol in the mobile phase each injection curve rises and

then falls. The maximum shifts towards higher water content of the injection solvent as the mobile phase water content increases. Furthermore, the radius of curvature increases as the methanol content of the mobile phase decreases. The decrease in peak height with high water content of the injection solvent was probably related to the observation of peak distortion by Tseng and Rogers(2). They too used a strong solvent as a mobile phase, neat methanol.

Going to weaker mobile phases, 40 to 60% methanol, the peak height rises with increasing water content of the injection solvent and reaches its highest value with neat water. This result is what one might expect if injecting elultes in weaker solvents is viewed as the analog of gradient elution.

The third group of curves is found with mobile phases having 20% to 30% methanol. Here no significant peak height change occurs when the water content of the injection solvent is increased. It is possible that the difference in strengths between the mobile phase and injection solvent was too small to show any effect on peak height. Another explanation is that retention in these mobile phases is too strong (see below).

Two other points should be made about the results shown in Fig. 1. The plate number increased as the peak height increased, and the fractional change in plate number was greater than the fractional change in peak height because of squaring of the width in calculating plate number. If the rising methanol rich sections of the curves for 70 to 90% methanol mobile phases were extrapolated to 0% methanol in the injection solvent, the fractional peak height increase would be very large. In other words, if the phenomenon causing the decrease in peak height were not operative, very great plate numbers could be achieved by injecting the benzyl alcohol in water.

The capacity factors for the runs displayed in Fig. 1 ranged from 0.3 to 7.8. To determine if the constant peak height for mobile phases containing 20 to 30% methanol was the result of large capacity factors, a study was made using constant mobile phase compositions and eluites with varying capacity factors. Table 1 shows the results of injecting phenylalkanols into 70-30 and 50-50) methanol-water(V/V) mobile phases. In the 70-30 methanol-water mobile phase, where capacity factors are smaller, the peak height improvement decreased as k' increased. The k'

TABLE I

Injection of Phenylalkanols Into 70-30 by Volume Methanol-Water Mobile Phase

Phenylalkanol	Mobile Phase[a] vol. CH_3OH: vol. H_2O	% h_p Improvement at Maximum h_p	Injection Solvent at Maximum h_p vol. CH_3OH: vol. H_2O	k'
$C_6H_5CH_2OH$	7:3	24.0	2:8	0.8
$C_6H_5CH_2OH$	5:5	7.5	4:6[b]	1.3
$C_6H_5(CH_2)_3OH$	7:3	18.0	2:8	1.5
$C_6H_5(CH_2)_3OH$	5:5	1.7	4:6[b]	3.8
$C_6H_5(CH_2)_4OH$	7:3	9.0	5:5[b]	2.3
$C_6H_5(CH_2)_4OH$	5:5	1.3	4:6[b]	6.9

[a]Econosil C18 was used. This stationary phase gives capacity factors that are larger than those produced by Spherisorb ODS2.

[b]Peak heights for injections solvents containing more water were not significantly different from the 4:6 or 5:5 solvent heights at the 95% confidence level.

TABLE II

Effect of Injection volume on Peak Height Improvement for a
Mobile Phase of 70-30 by Volume Methanol-Water[a]

Phenylalkanol Eluite	Volume Injected μL	% h_p Improvement[b]	k'
$C_6H_5CH_2OH$	30	23	0.8
$C_6H_5CH_2OH$	90	33	0.8
$C_6H_5(CH_2)_3OH$	30	17	1.5
$C_6H_5(CH_2)_3OH$	90	25	1.5
$C_6H_5(CH_2)_4OH$	30	10	2.3
$C_6H_5(CH_2)_4OH$	90	14	2.3

[a]Injection solvent : 20-80 methanol-water (V/V).

[b]Compared to h_p for injection solvent of the mobile phase.

range was 0.8 to 2.3. For the 50-50 methanol - water phase, no significant peak height improvement was observed for k' = 3.8 or 6.9. The results indicate enhancement of peak height by injection in solvents weaker than the mobile phase applies only when k' is less than 2 to 3.

Our hypothesis is that peak height enhancement is a phenomenon related to the entry of the eluite to the column. When the injection solvent is the mobile phase and the eluite partition coefficient is large, the entry band is narrower than if the coefficient were small. Injecting in a solvent weaker than the mobile phase has no significant effect on narrowing an already narrow band. If the partition coefficient is small, injecting in the mobile phase produces a wider band at the top of the column, and this width is inversely related to the partition coefficient. Injecting in a weak solvent narrows the band because solvent weakness means the partition coefficient is large for the injection solvent.

This hypothesis suggested that there should be a direct relationship between the fractional peak height improvement and the volume injected. Table II shows the injection volume effect at

TABLE III

Injection of Benzyl Alcohol into Mobile Phases of Varying Composition[a]

Mobile Phase		% Improvement at Maximum h_p	k'
Organic Component	Vol. Fraction H_2O		
n-C_3H_7OH	0.59	19	0.9
CH_3CN	0.42	34	0.7
CH_3CN	0.60	17	1.4

[a] Injection volume : 30µL

two volumes. The amount of eluite injected was held constant by adjusting the eluite concentration. For the three phenylalkanols, the fractional h_p improvement was greater for the larger volume. Consistent with other results, there was an increase in h_p as k' became smaller.

The phenomenon of reduced peak heights as the water content of the injection solvent increased when strong mobile phases were used - 70 to 100% methanol - is difficult to interpret. Peak height reduction occurred whenever k' was very small - approximately less than 0.5. Eluites with a larger k' did not exhibit this phenomenon. Thus, it was not the mobile phase strength per se but the small k' that caused the peak height reduction. Of course the rich methanol content of the mobile phase made it possible to have a great difference in strength between the injection solvent and the mobile phase.

A study was made using different organic components in the mobile phase. The injection solvents were methanol-water of varying composition from 0 to 100 vol. % methanol. Table III shows that peak height improvement was again observed. The highest column efficiency was obtained with an injection solvent of neat water in the case of each mobile phase studied.

CONCLUSION

Our results indicate that, when an eluite's k' is less than about 3, the mobile phase is not the best injection solvent for obtaining maximum peak height. A taller peak will be obtained by using a solvent that is weaker than the mobile phase. The improvement of h_p by using a weaker injection solvent is greater with larger injection volumes. This observation should be important in trace analysis. To obtain a tall peak in trace analysis a small k' is desirable, and large injection volumes are frequently necessary. It is exactly under these conditions that using a weak injection solvent is most effective.

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