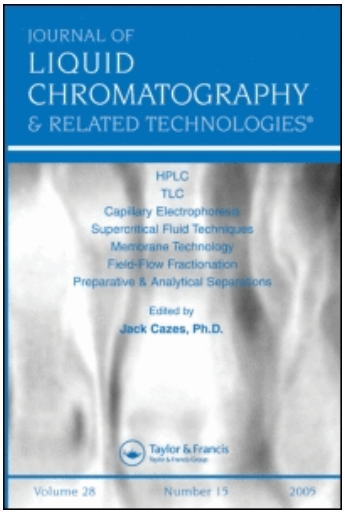

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Effects of Sample Solvent Strength and Injection Volume on Band Broadening and Throughput in Reversed Phase Prep Scale HPLC

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EFFECTS OF SAMPLE SOLVENT STRENGTH AND INJECTION VOLUME ON BAND BROADENING AND THROUGHPUT IN REVERSED PHASE PREP SCALE HPLC

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ABSTRACT

Sample solvents of weaker eluent strength than the mobile phase reduce band broadening induced by simultaneous mass and volume overload; but increase elution times of the sample components. As a result, better throughput is obtained when sample solubility allows for introduction of the sample in a small volume of the chromatographic mobile phase. However, under the constraint that a large injection volume must be employed to introduce the sample (frequently the case in reversed phase separations), the reduced bandwidths obtained in weaker sample solvents may offset the extended run time to provide better throughput.

INTRODUCTION

Prep scale HPLC may be defined as a separation technique carried out for the purpose of isolating purified chemical substances. Practically, this goal dictates maximizing throughput - the amount

of product isolated per unit time at a specified purity. Several recent theoretical studies (1-6) have indicated that this is usually achieved under conditions of considerable mass overload.

To achieve mass overload in Prep scale HPLC without incurring band broadening caused by volume overload, Knox and Pyper (1) have determined that sample volume (V_S) should not exceed one-half the band volume (V_b) of the same sample mass introduced in a small volume. ($V_{S\ max} < 0.5\ V_b$). This constraint can be problematic, particularly in reversed phase HPLC, since many samples display limited solubility in conventional reversed phase solvents.

Several approaches have been taken to overcome solubility problems in reversed phase HPLC. Employing the solubility parameter (P') developed by Snyder (7), it has been suggested that improved solubility may be achieved by using THF/water or ethanol/water mixtures as sample solvents (8). Alternatively, the sample may be introduced in a relatively small volume of a strong solvent such as methanol (9-11). With this method, however, deleterious effects such as crystallization and "oil deposition" of the sample components have been reported .

A third approach, described herein, is to use a relatively large injection volume of a sample solvent with a weaker eluent strength than the chromatographic mobile phase, and thus focus the sample introduced onto the head of the chromatographic column (12-14). This paper addresses the merits of this method in terms of band broadening and throughput as a function of the chemical composition and volume of the sample solvent.

EXPERIMENTAL

Chemicals

HPLC grade methanol and water, obtained from Fisher Scientific (Fairlawn, NJ), were used without further purification. Methyl, ethyl, and propyl paraben (99%), purchased from the Aldrich Chemical Co. (Milwaukee, WI), were used as model solutes.

Chromatographic Conditions

A Hitachi Model 655 Liquid Chromatograph (EM Science, Cherry Hill, NJ) with a variable wavelegth UV detector (set at 307 nm) was used throughout the study. Separations were performed on a Hibar 250 x 10 mm (10 micron) Lichrospher SI 100, RP 18 column (E. M. Science) using 50/50 (v/v) methanol/water, at a flow rate of 5 mL/min., as the mobile phase. Injections were made using a Rheodyne (Cotati, CA) model 7125 injection valve, fitted with 2.40, 3.21, 5.87, 8.27, and 12.21 mL loops, produced in-house from various lengths of 1 mm i. d. stainless steel tubing. Loop volumes were determined from the weight difference of empty and water filled tubing.

Procedure

To study the effect of simultaneous mass and volume overload on band broadening, samples containing: a) 11.50 mg methyl paraben, 12.06 mg ethyl paraben and 12.62 mg propyl paraben per injection volume and b) 23.00 mg methyl paraben, 24.12 mg ethyl paraben and 25.24 mg propyl paraben per injection volume, were prepared in

50/50, 45/55, 40/60 and 30/70 (v/v) methanol/water, and introduced onto the column in the loops described. Previous studies have determined that these samples are sufficient to mass overload the column utilized. When solubility problems were encountered, only methyl and ethyl paraben were introduced. When this also proved unsuccessful only methyl paraben was introduced. Bandwidths of each peak were determined from the resulting chromatograms as the elution volume corresponding to the distance between the points where the baseline is intersected by tangents drawn to the front and tail of each peak. Retention times were measured as the elution times of each peak maxima.

To determine throughput as a function of sample solvent composition and injection volume, samples containing 57.50 mg methyl paraben and 60.30 mg ethyl paraben per injection volume were prepared as above (as permitted by sample solubility). Throughput for ethyl paraben was determined as illustrated in Figure 1. and explained below.

1. Copies were made of each chromatogram.
2. The chromatogram resulting from the injection of the solutes in 2.40 mL of 50/50 methanol/water was cut out and two weighings made.

i. Area X = total weight of peaks 1 and 2 (Figure 1a.). A tangent was drawn to the tail of the second peak to reduce ambiguity with respect to the point at which the peak returned to baseline.

ii. Area Y = weight of peak 2, as determined by drawing tangents to each side of peak 2 (Figure 1b.). Due to slight peak overlap this measurement does give a slightly exaggerated value for the area due to the second peak. However, since Y will be used as a

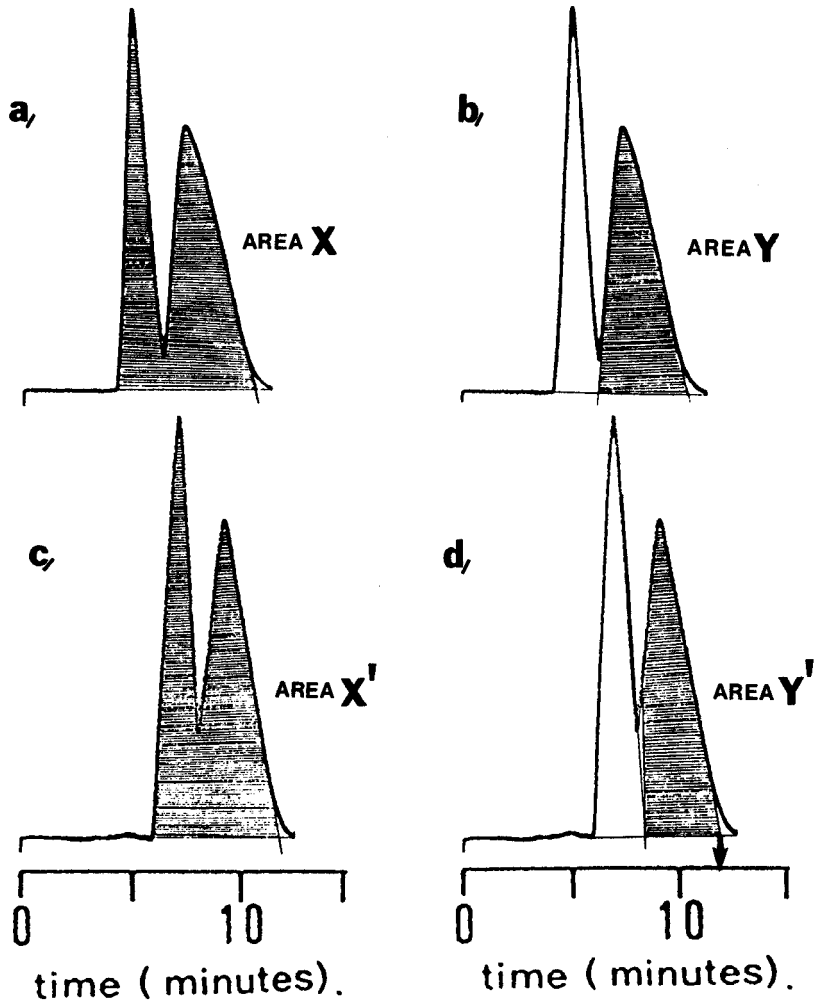

Figure 1. Areas cut and weighed to determine throughput for ethyl paraben. For explanation see text.

reference area for subsequent calculations, this results only in a constant relative error in throughput for each system investigated.

3. The chromatograms resulting from each injection volume/sample solvent system were cut out and two weighings made.

i. Area X' = total weight of peaks 1 and 2 (Figure 1c.)

ii. Area Y' = weight of "pure" peak 2 (Figure 1d.). The weighed peak here is the fraction of peak 2 to the right of a tangent drawn from the tail of peak 1 to baseline. It is assumed that this area represents the fraction of peak 2 with a purity of greater than 99% that could be collected (competitive partition isotherm effects are neglected). As in step 2i. a tangent was, additionally, drawn to the tail of peak 2.

4. From these measurements, throughput of ethyl paraben with a purity greater than 99% is determined for each sample solvent and injection volume as:

$$\text{Throughput} = \frac{\text{mass of ethyl paraben injected} \times (X/X') \times (Y'/Y)}{\text{time}} \quad (1)$$

where time is, as shown in figure 1d., taken to be the point at which a tangent drawn from the tail of peak 2 crosses the baseline. The ratio X/X' is employed in this equation to normalize for detector drift.

RESULTS AND DISCUSSION

Chromatograms of 11.50 mg methyl paraben, 12.06 mg ethyl paraben and 12.62 mg propyl paraben, injected in varying volumes of

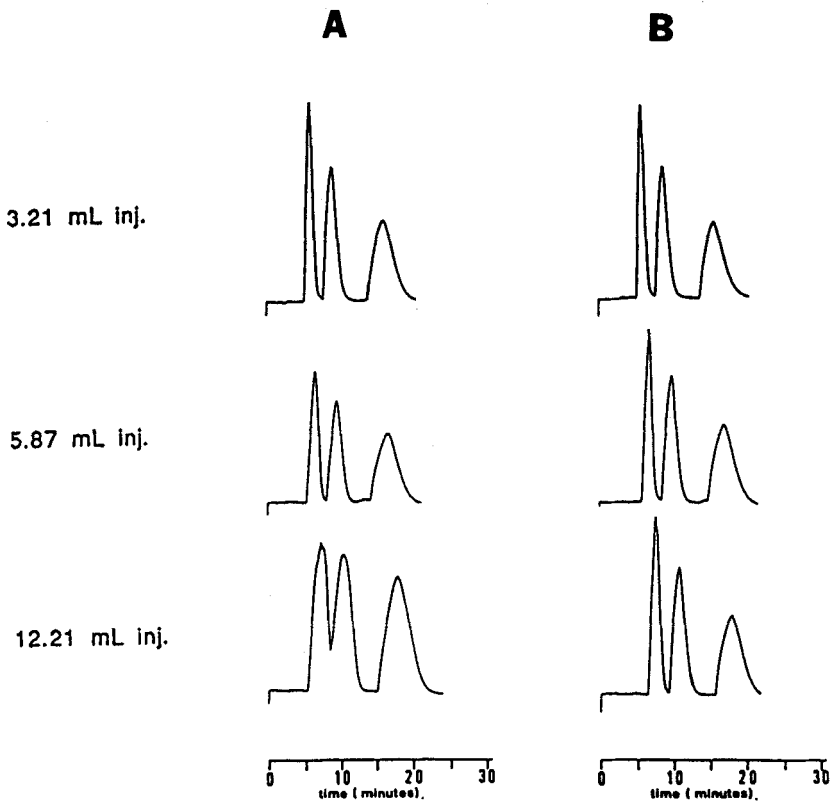

Figure 2. Chromatograms of methyl paraben (11.50 mg), ethyl paraben (12.06 mg) and propyl paraben (12.62 mg), injected in 3.21, 5.87, and 12.21 mL of: A. 50/50 (v/v) methanol/water. B. 40/60 (v/v) methanol/water.

50/50 and 40/60 (v/v) methanol/water, are shown in Figure 2. In 50/50 methanol/water (the chromatographic mobile phase) resolution between sample components decreases, as injection volume is increased, due to increasing bandwidths of the sample components. In 40/60 methanol/water little loss in resolution is observed, but elution time is increased.

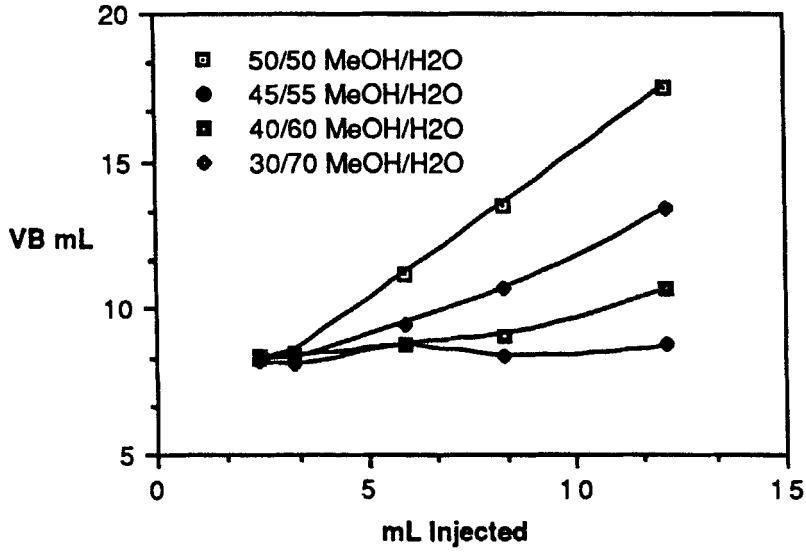

Figure 3. Influence of solvent composition and injection volume on band volume (VB) Data is for methyl paraben (11.50 mg).

The effects of sample solvent composition and injection volume on peak volume are shown quantitatively for 11.50 mg methyl paraben in Figure 3. Similar effects are observed for ethyl and propyl paraben, and for each of the solutes employed at larger mass loadings. However, larger sample masses and later elution results in larger band volumes, even for infinitely small injection volumes. As a result band broadening as a function of injection volume becomes less over the range of injection volumes studied.

The elution times of 11.50 mg methyl paraben, in the various sample solvents and injection volumes employed are listed in Table 1. As noted, retention time increases, for a given injection volume,

Table 1. Retention time (minutes) of 11.50 mg methyl paraben as a function of sample solvent composition and injection volume.

Inj. Volume (mL)	Solvent system (v/v methanol/water) 50/50	45/55	40/60	30/70
2.40	12.04	12.02	12.06	12.23
3.21	12.21	12.34	12.41	12.49
5.87	13.06	13.45	13.66	13.80
8.27	13.57	13.82	14.18	14.47
12.21	14.67	15.00	15.52	16.05

with decreasing solvent strength. It is, consequently, difficult to determine which solvent system and injection volume provides for the best throughput.

To directly determine throughput as a function of injection volume and sample solvent composition, the mass loading on the chromatographic column was increased to 57.50 mg methyl paraben and 60.30 mg ethyl paraben and throughput evaluated as specified in the experimental procedure. The throughput of ethyl paraben, from this mixture, is shown as a function of injection volume and sample solvent composition in Figure 4.

As the result of a more rapid elution time, and little volume induced band broadening, throughput is maximized by using the chromatographic mobile phase as the sample solvent when relatively small injection volumes are employed. When sample solubility prohibits introduction of the sample in a small volume, Figure 4.

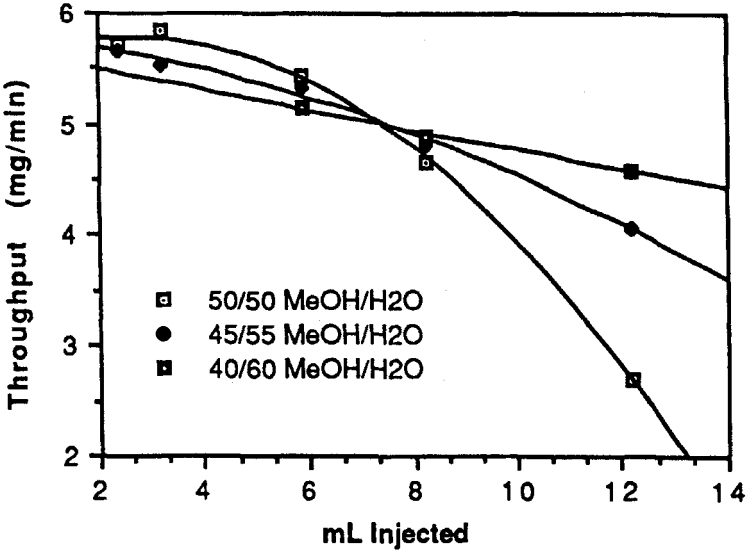

Figure 4. Throughput of ethyl paraben as a function of injection volume and sample solvent composition. Sample: methyl paraben (57.50 mg)/ethyl paraben (60.30 mg).

predicts that increased throughput is obtained in a sample solvent of weaker elution strength than the chromatographic mobile phase. The advantage obtained in the latter case is, however, contingent on the sample being soluble in the weaker solvent to such an extent that the improvement in resolution is not offset by the increase in elution time resulting from a larger injection volume of weaker solvent.

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