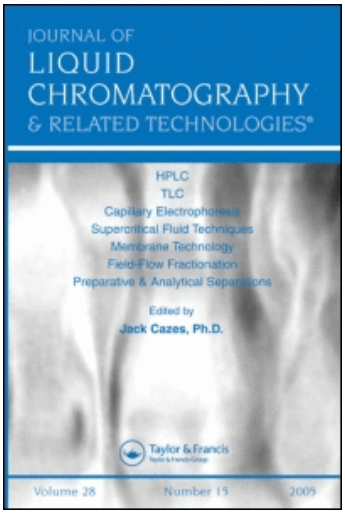

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Low-Cost Liquid Chromatography. I. Weak-Eluent Sample-Loading, A Novel Technique for Injecting Sub-Microliter Samples

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LOW-COST LIQUID CHROMATOGRAPHY. I. WEAK-ELUENT SAMPLE-LOADING, A NOVEL TECHNIQUE FOR INJECTING SUB-MICROLITER SAMPLES

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SUMMARY

A goal of creating a system for very "low-cost liquid-chromatography" "LC-LC", is to eliminate most of the conventional components for gradient LC or to use the components in a more productive manner (e.g. a single autosampler can be used with many LC instrument off-line). This paper describes how an on-line robotic autosampler, required to change samples conventionally, can be used to eliminate the injector. Future work will show how the a slngle robotic autosampler can be used to supply many liquid chromatographs with sample, eliminating injector valves in all of them.

Except for the most polar materials, most compounds in a weak eluent are generally accumulated on a reversed-phase column. Later these "focussed" peaks are eluted by a gradient or by an isocratic eluent of higher organic content. This is the basis for injection by loading sample in "non-eluting solvents", but, still the injection is made with a conventional high-pressure valve. Also, this sample focussing effect is used to evaluate the purity of "LC-grade" water.

This paper shows how an extension of injection via a non-eluting solvent, called "weak-eluent sample-loading", can be used to reproducibly "load" (inject) samples as sharp peaks in the nanoliter and lower range. With weak-eluent sample-loading, no injection valve is used. Rather, sample is dissolved in the weak eluent used for the gradient. The sample size loaded can be changed either (a) by varying the volume (time) of weak eluent that is pumped before beginning the gradient, or (b) by varying the dilution made of the sample in the weak eluent for a fixed time of pumping that preceds the gradient. For the latter method, a robotic autosampler is shown to permit injection of 14 different samples in the nanoliter range in an unattended manner with conventional columns.

Advantages to weak-eluent sample-loading are described, including elimination of the high pressure injection valve, reproducible injection and manipulation of nanoliter samples, and compatibility with water and production stream monitoring. Future work will show the compatibility of weak-eluent sample-loading with new approaches to very low-cost liquid chromatography (LC-LC).

INTRODUCTION

It has long been observed that the impurities in the "weak eluent" (aqueous phase) in reverse phase liquid chromatography (LC) are collected on a column to be eluted later in a gradient when stronger eluent is pumped (1). Indeed, this technique is commonly used to evaluate water to determine if it is suitable for LC. This is a type of weak-eluent sample-loading, however, with no control of what is injected.

A related phenomenon to weak-eluent sample-loading was shown by Berry in 1985 using a conventional reverse phase column operating with a gradient from 12% to 40% acetonitrile in aqueous buffer (2). Pairs of unresolved peaks could be completely separated if a "pulse" of a large plug (500ul) of weak eluent were injected at precise times about one-void volume before the unresolved peak pair elutes. A "secondary effect" was that pulses early in a chromatogram, having no direct effect on late peaks, still improved their resolution. It was suggested that a

"focussing" phenomenon takes place. Possibly the pulse of water, by mass action, displaces acetonitrile sorbed on the silica, which then momentarily dissolves and re-deposits the sample, sharpening the peaks to improve resolution. Later work from Berry (3, 4), showed that a low-sensitivity preparative LC refractive index detector could be used in GRADIENT liquid chromatography if the detector was maintained at the proper "iso-refractive index temperature"; the temperature at which the refractive index of water equals that of acetonitrile (5). Large samples give double inflection peaks by RI detection (which can see most components) but only a single Gaussian peak by UV detection. The initial rise in RI may be due to acetonitrile being displaced by the sample peak and the following dip in RI may be mixed eluent depleted in acetonitrile due to re-coating the surface with acetonitrile from the eluent. The pulsing mechanism may be similar to "programmed multiple development thin layer chromatography" which also produces sharpening of sample zones (6).

Recent work with the concept of "non-eluting solvents" pointed out the use of sample by loading it in a weaker eluent than used to elute the sample (7). For example, if a sample is eluted isocratically by 10% acetonitrile in water, then loading the sample in 5% acetonitrile will lead to a "focussing" effect and very sharp peaks will elute. In preparative chromatography, loading sample in non-eluting solvents put through the high pressure pump is commonly used to load large volumes of sample (8). These non-eluting solvent techniques do require that the sample show sufficient solubility in the non-eluting solvent so enough sample can be fit in the injector loop or in the volume of pumped loading solvent.

All of these earlier methods have the sample and eluent as different entities: first, the sample-containing weak-eluent is "loaded" and, second, this is followed by weak-eluent (with no sample) beginning a gradient to strong-eluent. The work reported here of using weak-eluent sample-loading is novel in that the sample is placed into the eluent, and this single mixture becomes the weak-eluent for the entire run, even the gradient part of the run. To load a new sample, a new weak-eluent mixture, with a new sample, is selected in an automated unattended manner.

Weak-eluent sample-loading has several advantages: (a) a high-pressure injection valve is eliminated by use of a robotic autosampler; (b) samples can be injected reproducibly in the submicroliter (nanoliter) range or lower; (c) peaks are "focussed" into sharp zones for sensitive detection; (d) very small and precious samples (e.g. 1 microliter) can be dissolved in a large volume of weak eluent and manipulated easily and with great precision; (e) enrichment to monitor waste water and production streams is compatible with this sample-loading method, and; (f) weak-eluent sample-loading is compatible with new approaches to very low cost LC for full gradients using only a few of the components used in conventional LC.

MATERIALS

The liquid chromatograph shown in Figure 1 used two Gilson pumps (#302, with #5 heads to 5 ml/min, Gilson Medical Electronics, Middleton, WI, USA) for gradient generation under the control of a Gilson Gradient System Controller using an Apple IIe personal computer and Gilson gradient program (#702). The weak aqueous eluent ("pump aq.") used a pump inlet of Teflon

tubing (2.1 meters of 1/16 inch o.d. X 0.03 inch i.d.) that was press-connected directly into a modified needle probe consisting of a 9 cm length of 18 guage stainless steel syringe tubing (Small Parts Inc., Miami, FL) replacing the needle probe of a robotic autosampler (#213/401 Gilson). The autosampler rack (#24, Gilson) holds two rows of 7 scintillation vials (23 ml filled capacity). The first tube in each row of 7 was filled with acetonitrile to act as an inlet wash solution. The remaining two rows of 6 tubes were filled with weak eluent with different amounts of Stock Solution-B, as described in Figure 4. The weak eluent is 2 mM triethylamine titrated with 1 to 10 diluted phosphoric acid to pH 2.80, using "clean" components, LC grade water (American Lab. Supply, Babson Park, MA), and techniques as described previously (9). The second pump supplied LC-grade acetonitrile through a pressure transducer/pulsation dampener and then to a mixing "T" followed by the LC column, either a C-8 Brownlee column (100 x 4.6 mm, #RP-MO, ABI Analytical, Santa Clara, CA, USA) or a C-18 Ultrasphere column (150 x 4.6 mm, 5 micron, #235330, Berkley, CA, USA). UV detection at 254 nm used either a Model 441 (Waters/Millipore) or Spectromonitor II (LCD/Milton Roy, Riviera Beach, FL, USA). Note that no injection valve was used.

Stock Solution-A of dimethyl-, dibutyl-, diallyl-, dihexyl-, diundecyl-, and ditridecyl-phthalates was made by dissolving ca. 10 microliter (ca. 10 mg) of each into 1 liter of 50% weak eluent in acetonitrile along with 10 mg of phthalic acid. (The phthalic acid eluted as broad peak and the dihexyl- and larger phthalates did not elute with this system; also, diallyl-phthalate, an unsaturated phthalate is not stable for long periods of time.) For weak-eluent sample-loading this was appropriately diluted.

For example, for the plots of peak height vs. loading-time, 50 ml of stock solution was added to 1 liter of weak eluent giving 0.5 mg per liter.

For Stock Solution-B, 250 ml of acetonitrile received dimethyl- (47.6 mg), dibutyl- (30.7 mg), diallyl- (32.4 mg), and dihexyl- (19.5 mg) phthalates. Fifty milliliter of this plus 450 ml of acetonitrile gave a final Stock Solution-B of dimethyl- (9.52 micrograms/ml), dibutyl (6.14 micrograms/ml), diallyl- (6.48 micrograms/ml, and dihexyl- (3.9 micrograms/ml) of the phthalates. For peak height vs. concentration plots, a typical dilution was 200, 400, 600, 800, and 1,000 microliters of Stock Solutions-B per 100 ml of weak eluent, enough to make 4 scintillation vials full of sample plus eluent.

For the dyes, Stock Solution-C (in elution order) was made of methyl red (9.5 mg), tetrabromophenol (22 mg), and alizarin (5.7 mg) dissolved in weak eluent with overnight mixing and then filtering through a 0.45 micron filter. For weak-eluent sample-loading, this was diluted by adding 11 ml of Stock Solution-C to 1-liter of weak eluent giving 0.106 mg per liter of methyl red, 0.245 mg per liter of tetrabromophenol, and 0.064 mg per liter for alizarin.

METHODS

Automated Weak-Eluent Sample-Loading by Varying the Volume (Time) of Sample-Loading Before the Gradient

To investigate techniques for varying the size of a sample injection, the apparatus shown in Figure 1 was used, except the robotic autosampler was not used. The dotted line of Figure 1

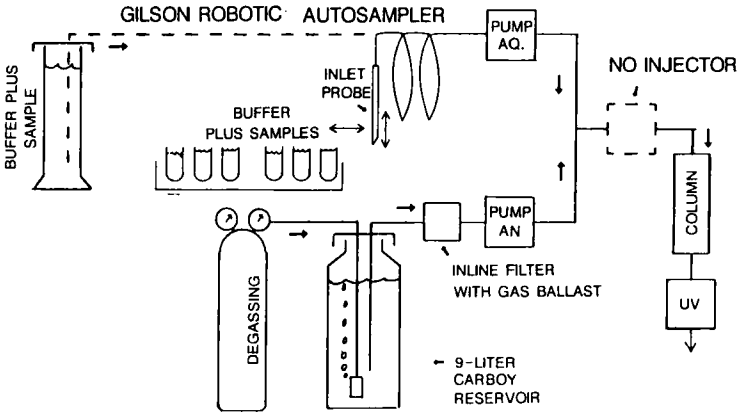


FIGURE 1. Apparatus for weak-eluent sample-loading. For different samples, the autosampler is programmed to insert the pump inlet line for the aqueous weak-eluent pump ("pump aq.", top) into different scintillation vials (23 ml), each containing a different sample dissolved in weak eluent. For calibration by pumping the same sample for different times, the pump inlet line is inserted in a graduated cylinder (upper left). The acetonitrile strong-eluent pump ("pump AN", bottom) is degassed with helium. Note no Injector is used. Other details in text.

shows the eluent-A inlet line to the aqueous pump going to a large (500 ml) volumetric cylinder. This cylinder had mixtures of sample added as indicated in Figures 2 and 3. Table 1 shows the time-programs used to load samples. Note that an "initial-wash" segment of strong eluent begins each run, and this offers the benefit that even the first run becomes useful, without knowing the prior history of the column. Thus, gradients always begin at exactly 15 minutes, and the end of a gradient occurs 10 minutes later at 25 min. This same 25 minute point is also labeled the zero time for the next run. A flag puts a 1.5 volt momentary signal (from a battery) across the detector input of the chromatogram as shown in each figure to mark the beginning of the gradient, and a single mark is put at the end of the run, at

the 25 min/0 min point. (The programming for this is not indicated in the Tables.) For a typical series of runs, the programs are shown in Table 1. Starting with "JOHN-A", runs are repeated two times (by "looping") to check on reproducibility, and then "JOHN-A" is "linked" to the program "JOHN-B", etc. This produces a series of chromatograms in which the "sample-loading" portion of the run varies for a known time, and hence, the volume of eluent varies for a known time.

Automated Weak-Eluent Sample-Loading of Different Samples

Although the autosampler can be readily programmed to make dilutions, in the work reported here, all 12 scintillation vials

TABLE 1

Time programs for establishing a calibration plot. Runs are always 25 minutes long. The strong eluent-wash begins at zero minutes and varies in each run so that the weak-eluent sample-loading times very to achieve different sample loads. Using the Gilson program, each run is "looped" as many times as desired for replicates (generally twice) and each run is "linked" to a the next run for different loads (from left to right).

PROGRAM NAME:	JOHN-A		JOHN-B		JOHN-C		JOHN-D		JOHN-E	
	min	%B	min	%B	min	%B	min	%B	min	%B
	0	95	0	95	0	95	0	95	0	95
Strong-eluent-wash										
	3	95	5.5	95	8	95	10.5	95	13	95
Reverse gradient										
	3.2	0	5.7	0	8.2	0	10.7	0	13.2	0
Weak-eluent sample-loading										
	15	0	15	0	15	0	15	0	15	0
Gradient										
	25	95	25	95	25	95	25	95	25	95

were used to show how samples could be changed when they were manually loaded into different eluent vials. Table 2 shows the gradient program for the Gilson Robotic Autosampler with an explanation of the steps. Note that the "dilutor" part of the autosampler was disconnected (further decreasing the cost with this approach), and the high-pressure valve on the autosampler was not used. The autosampler probe was connected directly to the pump-A inlet. To minimize contamination and evaporation during the 7 hour run of 14 vials, a single sheet of plastic wrap was used over the entire set of vials in the rack. The pump-A inlet probe readily pierced this plastic wrap.

RESULTS AND DISCUSSION

Automated Weak-Eluent Sample-Loading By Varying the Volume (Time) of Sample-Loading Before the Gradient

By increasing the volume (time) of weak-eluent run before a gradient, the amount of sample laid down will also increase. Figure 4 shows such a series of runs for phthalates (left, at high levels) and dyes (right, at low levels) dissolved in eluent. Figure 2 (phthalates) and Figure 3 (dyes and phthalates) shows that plots are linear for peak height vs. the isocratic time ("minutes to onset of gradient") for both phthalates and dyes. The good linearity indicates that this calibration method can be readily used to quantify samples.

Sub-microliter samples can be reproducily loaded using weak-eluent sample loading. Figure 2 shows that the weak-eluent sample-loading for phthalates at low levels: 1 nanoliter

TABLE 2

Program for the gradient, flow, and events for the Gilson System Controller for weak-eluent sample-loading from different 23 ml scintillation vials, each containing a different sample dissoved in weak eluent. Terminals 7 and 8 of the events output on the System Controller are connected to input terminals number 8 (ground) and 6 on the #231 Gilson Autosampler. Flow is stopped between 2.50 and 2.75 minutes and 4.44 and 4.69 minutes so the autosampler probe can move to the next vial without drawing air into pump-A.

PROGRAM NAME:	VERN-X		
	min	%B	ml/min
Strong eluent wash to elute previous sample	0	95	1.3
	2.47	95	1.3
Flow stopped	2.50	95	0
Change pump-A inlet to acetonitrile by pulsing event 4	2.6	10	0
Flow resumed at 10% pump-B to wash pump-A inlet line	2.74	10	0
	2.75	10	1.3
	4.43	10	1.3
Stop flow	4.44	10	0
Change pump-A inlet to next sample by event 4			
Flow resumed to fill pump-A inlet line with new eluent	4.69	10	0
	4.70	10	1.3
Change to high %B	6.81	10	1.3
	6.82	95	1.3
Wash column with high %B			
Reverse the gradient to begin sample loading	8.22	95	1.3
	8.32	0	1.3
Weak-eluent sample-loading			
	16.6	0	1.3
Gradient to elute sample	25	95	1.3

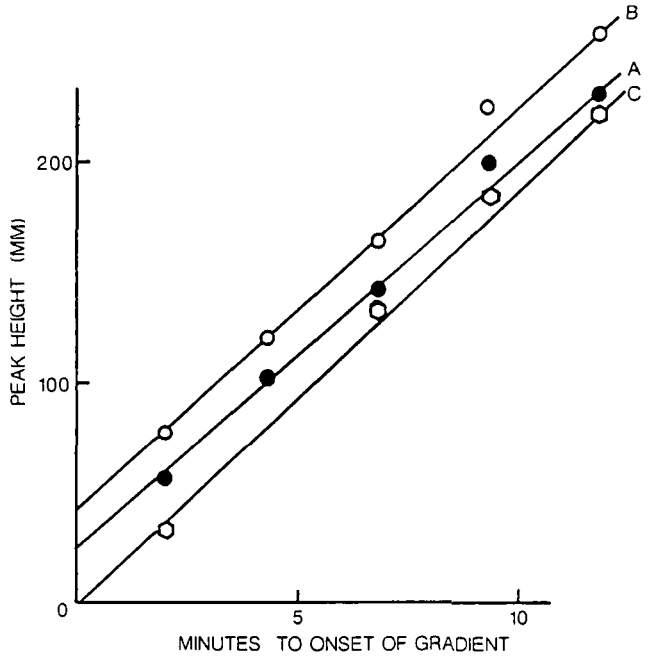


FIGURE 2. Insensitive plot (at 1.0 AUFS) of peak height in millimeters at 254 nm vs. the volume of weak eluent in the isocratic "eluent-load" portion of the run. Samples are the phthalates (each delivered at 0.001 mg/min): dimethyl- (A), diethyl- (B), and diallyl- (C). The Beckman-Altex C-8 column was used (100 X 4.6 mm).

(microgram) per minute of pumping (0.5 mg/liter X 2 ml/min). Figure 3 shows the weak-eluent sample-loading for dyes at even lower levels: 0.212 micrograms per minute for methyl red (0.106 mg/liter X 2 ml/min) and 0.128 micrograms per minute for alizarin (0.064 mg/liter X 2 ml/min). These loading rates as micrograms per minute are reflected in the slopes of the plots in Figures 2 and 3.

Errors in the system are also obvious. Note that mis-pumping of one sample is apparent: the 3 points at 9 minutes isocratic pumping time in Figure 2 all lie above the lines produced by the other points).

TABLE 3

Program for the 231 Gilson robotic autosampler for weak-eluent sample-loading. The robotic arm takes the inlet line to the weak eluent pump (Pump-A) to a sequence of 12 scintillation vials (23 ml each) each containing weak eluent and a different sample. Two vials at the beginning of each row contain acetonitrile as wash solvent to clean between each run the inlet line running from the autosampler to pump-A. The timing and flow control sequence for the Gilson System Controller are shown in Table 2. Note that with weak-eluent sample-loading no injection valve is used or programmed.

1.	RACK CODE 24		Use the 2 X 7 scintillation vial rack
2.	A0	=0	The counting sequence for the 2 X 7 rack
3.	INPUT	A1/0	of 14 scintillation vials.
4.	FOR A	1/7	
5.	FOR B	1/2	
6.	A0	= A0 + 1	
7.	IF	A0 > A1	
8.	HOME		
9.	TUBE	A + 1/B	Go to the bottom of the first vial.
10.	PRINT	A0/0	
11.	WAIT	/6/0	Wait until signal is received from system controller before moving to the next vial, which is the acetonitrile wash
12.	TUBE	1/B	Go to the acetonitrile wash tube, the first tube in the same row
13.	WAIT	/6/0	Wait until a signal is received from the System Controller before moving to the next vial, which is the next sample.
14.	NEXT	B	
15.	NEXT	A	
16.	HOME		

Figures 2 and 3 also show that simple autosampler programming (Table 1) can be used to construct calibration plots.

Reproducibility of data is good, even with samples down to levels of nanoliters. For example, for low levels of the two dyes (Figure 4, right), ranging fom 0.7 to 2 micrograms (0.7 to 2 nanoliters if this were of density 1 g/ml), the average deviation of peak height ranges from 0.2 to 1.3 percent (the average for these deviations is 0.91% for 10 sets of data). For higher levels

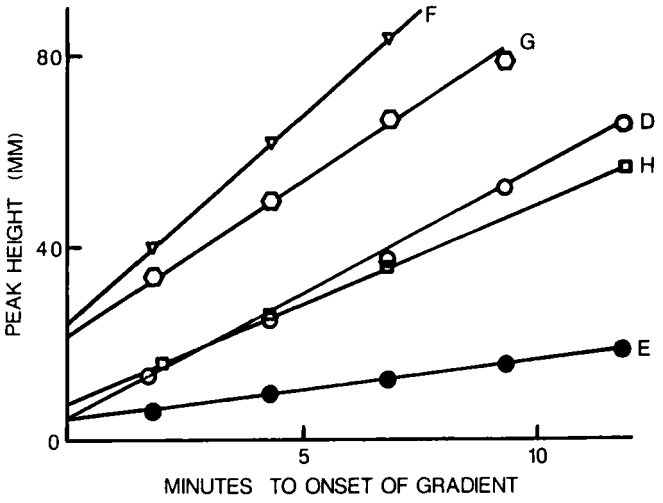
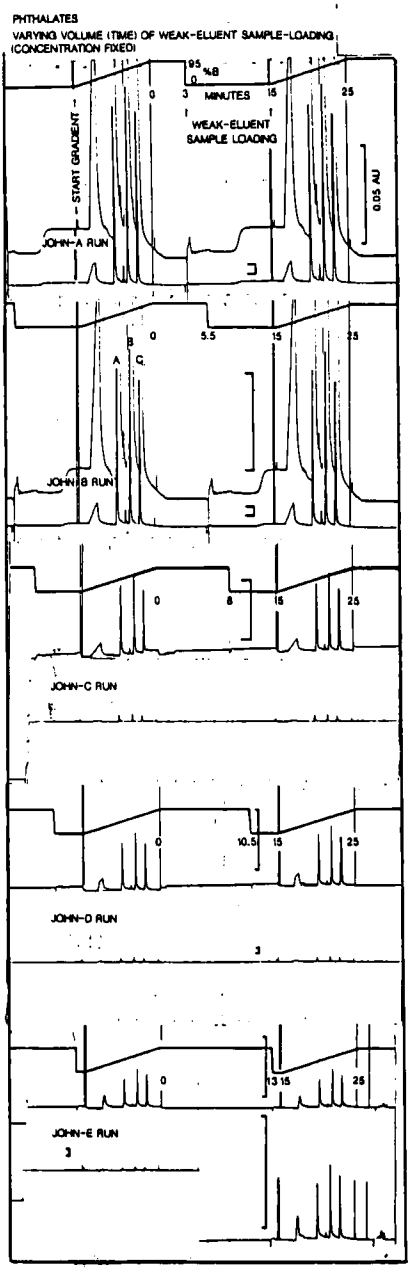
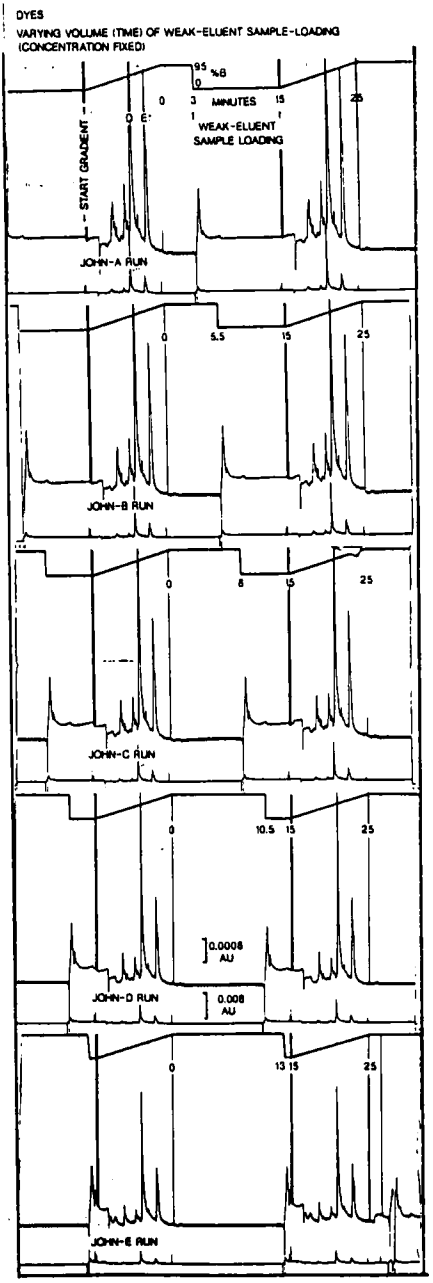


FIGURE 3. Sensitive plot (at 0.1 AUFS) of peak height in millimeters at 254 nm vs. the volume of weak eluent in the isocratic "eluent-load" portion of the run. Samples are the phthalates: dimethyl- (H), diethyl- (F), and diallyl- (G) and the dyes are methyl red (D, delivered at 0.212 mg/min) and alizarin (E, delivered at 0.128 mg/min). The Beckman-Altex C-8 column was used (100 X 4.6 mm).

of sample, the three phthalates, Figure 4, left, and Figure 3, ranging from 5 to 16 nanoliter, the average deviation of peak height ranges from 0.10 to 3.8 percent (the average of these deviations is 1.06% for 15 sets of data). This good reproducility of results by weak-eluent sample-loading is apparent in the sequences of automated duplicate chromatograms in Figure 5.

Sample-loading onto the column takes place both during the isocratic time and gradient time as follows. First, during the isocratic-only portion of the run, sample loads onto the head of the column at a rate in mg/ml indicated in each figure. The slopes of the lines in Figures 2 and 3 are the micrograms loaded





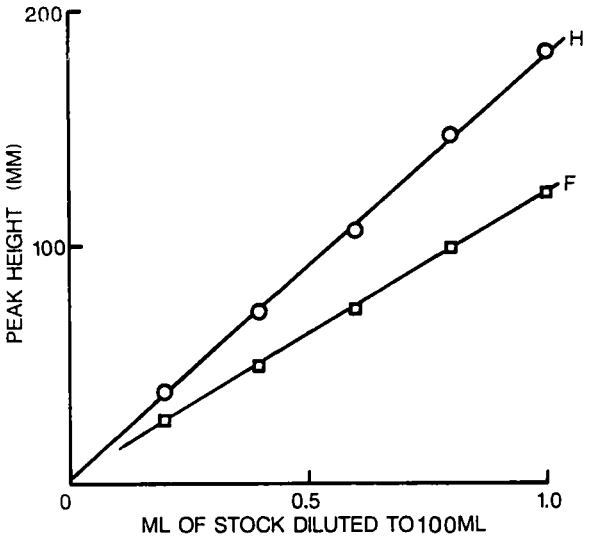


FIGURE 5. Runs of different samples using the same gradient program (Table 2) and autosampler program (Table 3). Samples were dissolved in weak eluent in 23 ml scintillation vials and put in-turn through the weak eluent pump by the Gilson robotic autosampler. Samples are the phthaltes: dimethyl- (H), and diethyl- (F).

FIGURE 4. Peak height reduction as the "weak-eluent sample-loading" time is reduced rom the John-A run (top) to the John-E run (bottom) for sample dissolved in the eluent: phthalates (left) and dyes (right). The sample-loading times decrease from the "JOHN-A" run (top, the time period from 3 to 15 minutes) to "JOHN-E" (bottom, from 13 to 15 mlnutes), see Table 1 for details of the gradient programs. The double mark at 15 minutes signals the beginning of each gradient and the single mark signals the end of each gradient (at 25 min) (this mark is also identical to 0 minutes of the next run). The phthalates (left) are dimethyl- (peak A), diethyl- (peak B), diallyl- (peak C) at 5 mg per liter of eluent. The dyes (right) are methyl red (peak D) and alizarln (peak E) at ca. 0.07 mg per liter of eluent. Runs begin with a wash of strong eluent (acetonitile), followed by weak-eluent, with sample-loading), for various times and then the gradient, always from 15 through 25 minutes at 2 ml/min. A C-8 column (100 X 4.6 mm, 10 micron partices, Brownlee RP-M0) was used with the weak eluent of triethylamine (2 mM) titrated with phosphoric acid to a pH of 2.8. The 254 nm UV detector for the phthalates was a Waters 441 (brackets indicate always 0.05 AU), and the UV detector for the dyes was an LDC/Milton Roy SpectroMonitor II (sensitivities indicated).

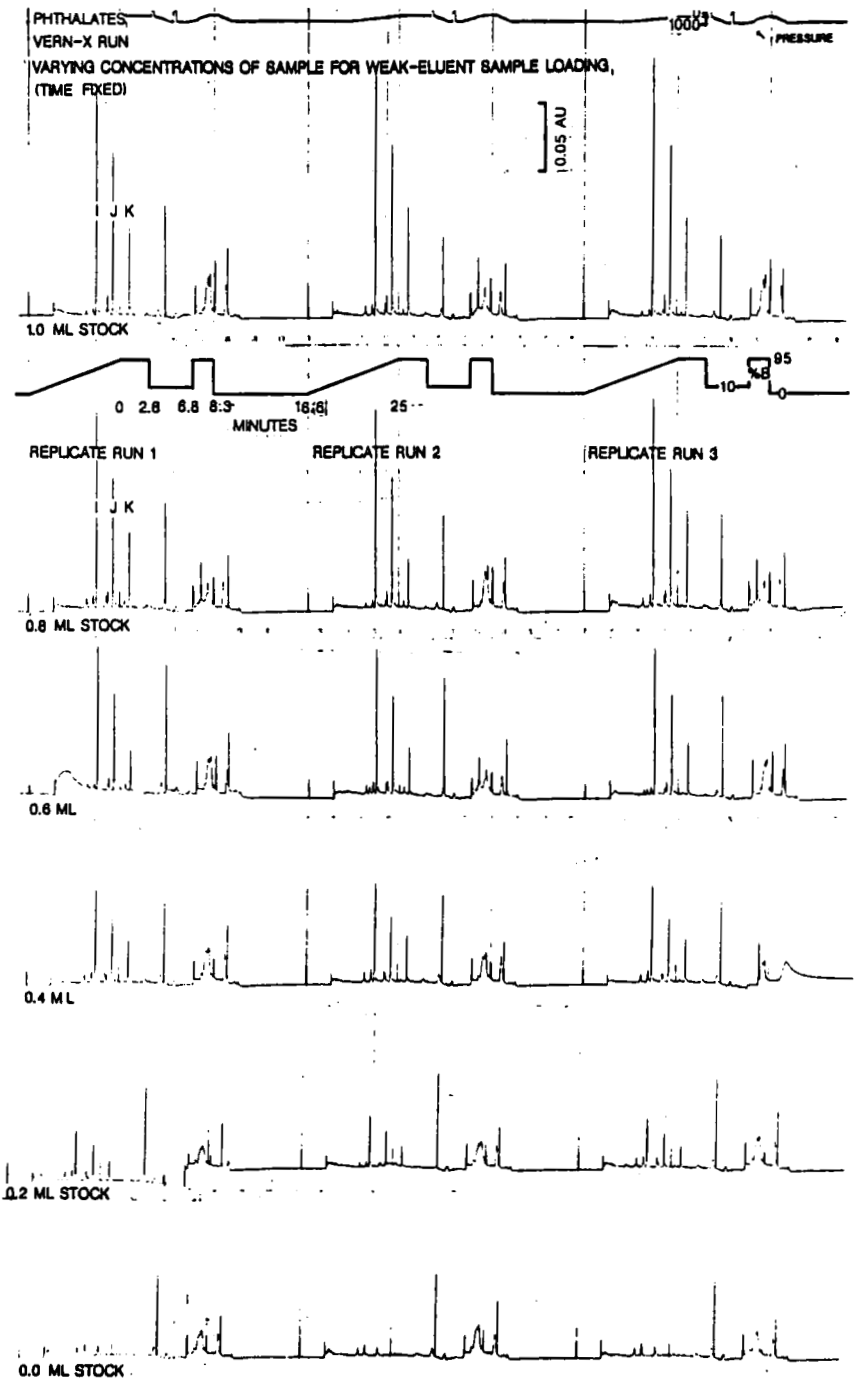
per minute for the isocratic part of the run. Second, during the gradient, up to where the peak begins to elute, sample continues to load onto the column. The first isocratic-only stage provides sample from a volume of eluent equal to the flow rate times the isocratic-time.

Sample provided by the second gradient step, up to where the sample elutes, is more difficult to understand. The volume of aqueous eluent in this portion of the gradient is easiest understood in terms of a gradient from 100% WATER to 0% water (the usual gradient, but referred to as % weak eluent and not as % strong eluent). For example, with a flow of 2 ml/min, a peak eluting exactly half way through a 10 minute gradient from 100% TO 0% WATER, elutes at 50% WATER or after 10 ml (5 min X 2 ml/min) of mixed eluent have passed though the column. Since the gradient started at 100% water, and the peak eluted at 50% water, the average composition of eluent to the point of elution is 75% water. Thus, the 10 ml of gradient eluent of 75% average water composition represents the equivalaent of 7.5 ml of water, from which this particular sample was extracted. Note that this 7.5 ml of water contributed DURING the gradient is an important relative volume, equivalent to 3.75 minutes of isocratic run time at 2 ml/min. Other peaks, eluting later, will have a proportionately larger contribution of sample from the aqueous eluent contributed during the gradient portion of the run. The equation for the milliliter volume of aqueous eluent contributing sample DURING THE GRADIENT, V (aq-grad), is thus:

$$V\,(\text{aq-grad}) = (\text{Flow rate})(\text{retention time into the gradient}) \times [1 - 0.5\,(\text{initial organic \% + organic \% at elution})]$$

A last consideration, is that one void volume (1.80 ml for the 100 X 4.6 mm Brownlee column) is required to move the peak from the head of the column to the end of the column.

Although the plots in Fig. 2 are linear, they do not intercept the zero axes (i.e. when the sample-loading time is zero, sample is still loaded onto the column). The intercept varies with each sample. Indeed, all sample in the aqueous eluent is accumulated by the column until a concentration of acetonitrile in the gradient is reached where that sample is then eluted. A possible unusual background tailing" effect could occur from each peak, after the point of elution. For each peak, after the elution point, that peak is expected to contribute a background UV absorbance to the UV signal. This background UV absorbance is in proportion to: (a) the concentration of that sample in the eluent; (b) the specific absorbancy at the detection wavelength, and (c) the fraction of aqueous eluent in the gradient mix. Concerning this last point, since the gradient fraction of water is continuously decreasing, this background signal is expected to decrease, producing a downsloping tail for the peak. This should produce a steep rise as the peak elutes, followed by a downsloping tail. Experimentally, Figure 4 (left) for high level phthalates (5 - 16 nanoliters per liter) shows that this effect may be visible with the high sensitivity signal (at 0.1 AUFS), but it is difficult to distinguish this downsloping tail from usual peak tailing. For the low sensitivity signal (at 1 AUFS, also in Figure 4), the signal required to keep the peaks on scale, the peaks show good symetry and no apparent downsloping tail.



Automated Weak-Eluent Sample-Loading of Different Samples

Figure 6 shows the chromatograms produced in which scintillation vials in the autosampler contain different concentrations of phthalates. The first two vials in each row in the rack contain acetonitrile wash solution and the remaining 6 vials in each of the two rows contain eluent-A, each with a different sample. The program in Table 2 begins from 2 to 2.47 min with: "Strong eluent wash to elute previous sample" by putting 95% acetonitrile through the column. This is followed by a momentary "Flow stopped" time from 2.50 to 2.74 minutes permits the inlet probe to pump-A to move into the acetonitrile vial at the end of the particular row of tubes. The "Flow resumed" time at 2.74 min is at 10% pump-B operation, but since the pump-A inlet probe is in acetonitrile, and pump-B is in acetonitrile, this period (a) washes the column with 100% acentonitrile, and (b) washes the pump-A inlet probe with acetonitrile. The slight flow from pump-A (10%) is to prevent any wash acetonitrile through the pump-A line from accidentally backing up into the pump-B line, should pump-B fail to close perfectly. Again, there is a momentary period of "Stop flow" from 4.44 to 4.69 minutes to permit the pump-A inlet probe to move to the next scintillation vial, containing eluent-A plus sample. Now flow remains mostly through the pump-A line to fill the pump-A inlet line completely with the new sample. Then, to remove this ambiguous level of

FIGURE 6. Peak height reductions as the concentration of phthalates is reduced from 1 ml to 0 ml of Stock Solution-B using run "VERN-X" program (Table 2). The phthalates are dimethyl- (I), dibutyl- (J), and diallyl- (K). The Beckman-Altex C-18 column of 5 microns particle size was used (150 x 4.6 mm), flow as 1.3 ml/min. Other conditons as in Figure 4.

sample from the column, the step of "Wash column with high %B" from 6.82 to 8.22 min is used. A quick change in composition to zero percent strong eluent for the " Weak-eluent sample-loading" period of the run is found from 8.32 to 16.6 min. The "Gradient to elute sample" terminates a run sequence from 16.6 to 25 min. This wash and timing procedure gives no measureable carry-over from vial-to-vial and eliminates drawing air into the pump. Thus, the autosampler approach permits different samples to be run in sequence both reproducibly and reliably.

CONCLUSIONS

Weak-eluent sample-loading depends on samples being focussed at the head of a column in a "non-eluting" solvent and eluting later in the run by a stonger eluent, such as by a gradient. Conventional valve injection is eliminated by this approach. Weak-eluent sample-loading can reproducibly load very small samples to nanoliters and below (60 nl is the lowest conventional injection valve) (1). This method can also be used to load very dilute samples. Calibration curves can readily be constructed by simply varying the time of the isocratic portion of the run. Different samples can be loaded in an un-attended automated manner by progamming an autosampler to make 12 scintillation vials the source of the weak eluent. This method gives adequate weak eluent, 23 ml, for full gradients with conventional columns (150 X 4.6 mm). Weak-eluent sample-loading should also work very well with low dispersion LC methods requiring very small sample volumes such as fast-LC, micro-column LC, and the short efficient columns using micron-sized non-porous supports for proten

separations. Since these systems use a proportionately smaller volume of weak eluent, then autosampers might readily sample 50 to 100 tubes for weak-eluent sample loading. Weak-eluent sample-loading offers an alternative to conventional valve injection that is reproducible, permits very small sample injections, and has the potential for permitting some new types of low cost approaches to liquid chromatography by eliminating the conventional components of an injection valve, a second pump, a gradient controller, and a gradient mixing chamber.

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