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AN EXPERIMENTAL EVALUATION OF COMPRESSIBILITY EFFECTS IN SYRINGE PUMPS FOR LIQUID CHROMATOGRAPHY

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SUMMARY

The performance of a liquid chromatograph consisting of one or more syringe pumps coupled to modern electronic and hydraulic components is shown to be completely satisfactory for qualitative and quantitative work. Previous theoretical treatments of solvent compressibility have discussed problems that may arise when using simplified forms of syringe pumps. The syringe pump described in this paper has been designed to avoid these potential difficulties. Furthermore, it is shown that when the pressure dependence of compressibility is taken into account, theoretical results agree very closely with experimental data.

INTRODUCTION

A recent paper by Martin *et al.*[1] provides a theoretical treatment of the effects of solvent compressibility on the performance of a syringe pump for isocratic elution in liquid chromatography. The theoretical treatment of the paper describes problems associated with the use of simplified models of syringe pumps. However, modern high-performance liquid chromatography (HPLC) systems such as the one described in the present paper, have been designed to avoid these difficulties.

In this paper, we include a refinement of compressibility theory, relevant to application to high pressure operation, and discuss the effects of solvent compressibility on the operation of modern, carefully designed syringe pumps for liquid chromatography. Theoretical predictions are confirmed and corresponding design concepts are reinforced by ample experimental data.

EXPERIMENTAL

The build-up of pressure in a closed cylinder filled with typical HPLC solvents was studied with a Varian Model 8500 syringe pump. A schematic diagram of the system is shown in Fig. 1. The pump was plugged at its outlet, located after the strain gauge pressure transducer. The transducer output was amplified and then displayed on a strip chart recorder. The transducer had been calibrated at pressures of 1 and 540 atm with a reference gauge.

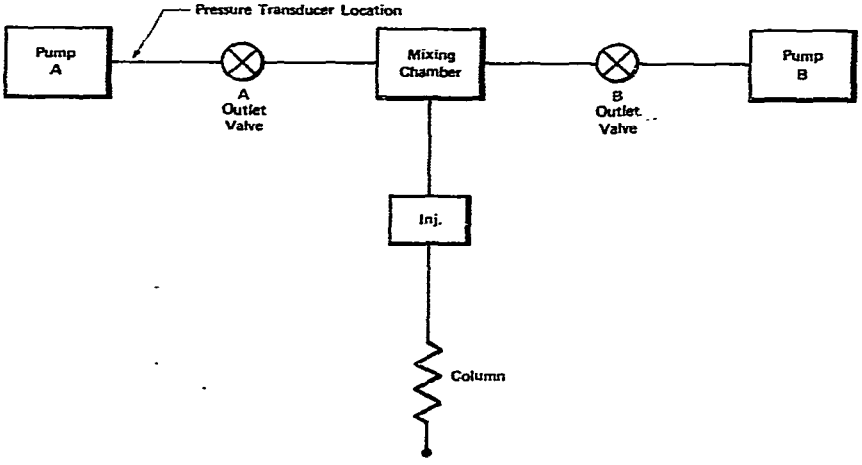

Fig. 1. Schematic of Model 8520 LC system. In isocratic single-pump mode valve B is always closed.

In a subsequent experiment, the outlet plug was removed and the pump cylinder was connected to a chromatographic column. Pressure build-up was monitored. This experiment was run on a 25 × 0.21 cm I.D. column packed with 5-μm silica gel and then on a 25 × 0.21 cm I.D. column packed with a cyanosilane phase bonded onto 10-μm silica gel.

The Model 8500 syringe pump used in these tests has a cylinder volume of 250 ml. Total volume of the associated external plumbing was less than 1 ml, exclusive of the column. A 25 × 0.21 cm I.D. column has a void volume of approximately 0.65 ml.

RESULTS AND DISCUSSION

Isothermal compression of a liquid in a closed cylinder

Previously developed theory[1] predicts the time, t, to reach a given pressure in a closed cylinder of liquid, with a constant velocity piston, to be defined by

$$t = \frac{V_0}{Q_0}\left(1 - e^{-\chi (P - P_0)}\right) \tag{1}$$

where V_0 is the cylinder volume, Q_0 the piston flow-rate, χ the isothermal compressibility, P the final pressure and P_0 the initial pressure.

The validity of eqn. 1 rests on the assumption that the isothermal compressibility is approximately constant over the pressure range P_0 to P. However, this is not the case for typical organic solvents at pressures above 200 atm. As pressure increases, solvent compressibility decreases. This effect significantly reduces the time necessary to attain the high pressures used in HPLC. Thus the use of atmospheric pressure values for solvent compressibility for pressurization to 800 atm[1] results in significant error in the time calculated to reach a given pressure.

TABLE I

ISOTHERMAL COMPRESSIBILITY AT 25° OF SEVERAL LIQUIDS COMMONLY USED IN HPLC

Liquid	*Tait constants*		*Compressibility* $\chi \cdot 10^4$ *(atm*$^{-1}$*)*		
	a	*b (atm)*	*1 atm*	*300 atm*	*600 atm*
n-Hexane	0.0943	580	1.62	1.07	0.80
Dichloromethane	0.1038	1052	0.99	0.77	0.63
Methanol	0.148	1194	1.24	0.99	0.83
Water	0.1368	2957	0.46	0.42	0.38

The decrease in isothermal compressibility with increasing pressure is accurately described in Tait's classical equation[2,3]:

$$\chi = \frac{a}{b + P} \tag{2}$$

where a and b are constants for a given liquid (see Table I).

The isothermal compressibility at 25° of *n*-pentane, calculated as a function of pressure, is plotted in Fig. 2. The Tait's parameters for *n*-pentane given in ref. 3 do not provide an accurate fit of the original data of Bridgman[4]. The latter data were analyzed and the following values were obtained for the Tait's parameters: $a = 0.0923$ and $b = 391.8$ atm. Compressibility decreases by a factor of 2.2 as pressure increases from 1 to 600 atm. Calculated values of compressibility of HPLC solvents at 1, 300 and 600 atm are presented in Table I. A rigorous derivation of the time to reach a given pressure requires substitution of Tait's equation for the compressibility term in the differential equation describing the system:

$$Q_0 - \chi V \frac{dP}{dt} = 0 \text{ (where } V = V_0 - Q_0 t) \tag{3}$$

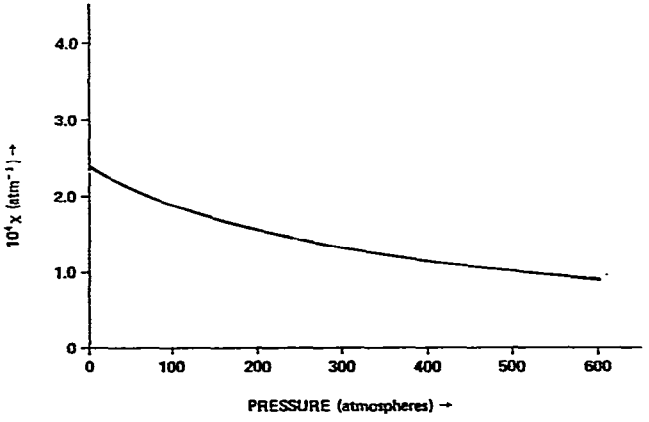

Fig. 2. Isothermal compressibility of *n*-pentane as a function of pressure at 25°. Values calculated from Tait's equation.

Solution of this equation under these conditions yields the expression:

$$t = \frac{V_0}{Q_0}\left[1 - \left(1 + \frac{P - P_0}{b}\right)^{-a}\right] \tag{4}$$

The theoretical predictions of eqn. 1, using the corrected value of $\chi = 2.356 \times 10^{-4}\ \text{atm}^{-1}$ for the compressibility of *n*-pentane at atmospheric pressure, and of eqn. 4 were tested by measuring the build-up of pressure in a closed cylinder of fluid, as described in Experimental. Results are shown in Fig. 3. At a piston flow-rate of 60 ml/h, *n*-pentane was pressurized to 580 atm in 19.9 min. Eqn. 4 predicted 20.1 min, while eqn. 1 predicted 32 min. The large error of eqn. 1 is due to its failure to account for the pressure dependence of compressibility.

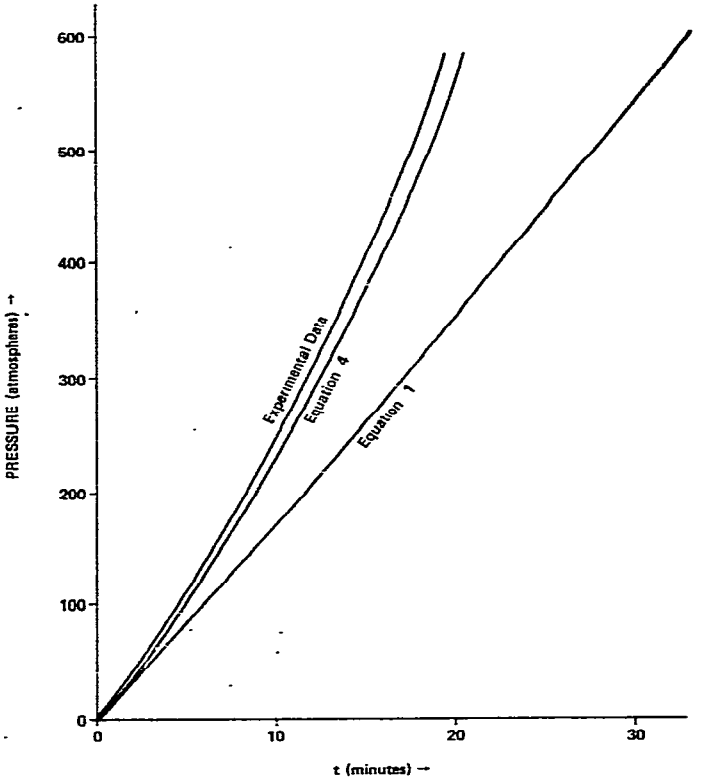

Fig. 3. Compression of *n*-pentane in a closed cylinder. $Q_0 = 60$ ml/h, $V_0 = 250$ ml.

Most solvents used in HPLC are less compressible than *n*-pentane. Experimental data for compression of HPLC solvents are presented in Fig. 4. The data agree with the theoretical predictions of eqn. 4.

In addition, it should be noted that the theoretical values of time to pressurize a closed cylinder of fluid, reported by Martin *et al.*[1] in Table II of their paper, contain a uniform arithmetical error of a factor of 10, in addition to the error due to the pressure dependence of compressibility.

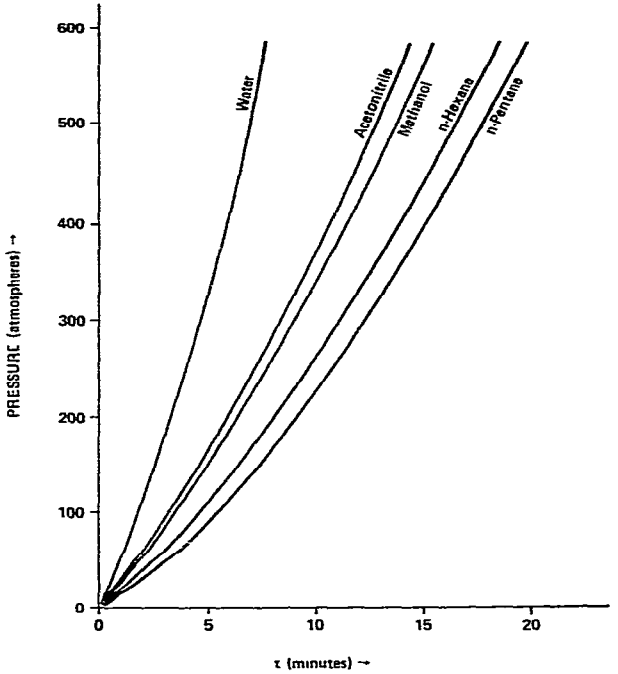

Fig. 4. Compression of HPLC solvents in a closed cylinder. $Q_0 = 60$ ml/h, $V_0 = 250$ ml.

Compression of a liquid in a cylinder with a column attached

Of course, operating chromatographs have columns attached to the outlet of the pump cylinder. In this case, the time required to come up to a given pressure, without accounting for the pressure dependence of compressibility, is

$$t = \frac{V_0}{Q_0}\left[1 - \left(1 - \frac{P}{P_\infty}\right)^{\chi P_\infty}\right] \tag{5}$$

where P is the pressure at time t and P_∞ is the steady-state pressure generated by the resistance of the column to the liquid flow. Eqn. 5 neglects the effect of pressure on compressibility.

When the system includes a column, the governing differential equation is

$$Q_0 - \chi V \frac{\mathrm{d}P}{\mathrm{d}t} = \frac{P}{R} \tag{6}$$

Substitution of Tait's equation for the compressibility term yields eqn. 7 for pressurization of a cylinder with column attached:

$$t = \frac{V_0}{Q_0}\left\{1 - \left[\frac{b\,(P_\infty - P)}{P_\infty\,(b + P)}\right]^{aP_\infty/(b+P)_\infty}\right\} \tag{7}$$

where a and b are Tait's constants. The time required to reach 99% of the final pressure, t_{99}, can be calculated from eqn. 7 as:

$$t_{99} = \frac{V_0}{Q_0}\left\{1 - \left[\frac{0.01\,b}{b + 0.99\,P_\infty}\right]^{aP_\infty/(b+P_\infty)}\right\} \tag{8}$$

n-Hexane is the most compressible solvent commonly used in HPLC. A comparison of t_{99} values predicted by eqns. 5 and 8 with experimental values, is presented in Fig. 5. The experiment was performed under the most extreme pressure requirements encountered by the chromatographer. A 25 × 0.21 cm I.D. column, packed with 5-μm silica gel, was operated at flow-rates of 60–180 ml/h requiring operating pressures of 150–530 atm. One should note that in this region, pressure is not linearly proportional to flow-rate, due to the fact that the viscosity of hexane increases with pressure.

The data of Fig. 5 demonstrate that a significant error arises from the failure of eqn. 5 to account for the pressure dependence of compressibility. Although eqn. 8 yields a better fit to reality, neither equation describes the abnormal shape of the experimental curve. This phenomenon is currently under study.

As the system pressure increases, the flow through the column increases in direct proportion. But the volume is less than would be calculated by simple multiplication of the t_{99} value and the normal flow-rate. For instance, the volume collected before achieving a t_{99} value of 3-ml/min flow of hexane through a column packed with 5 μm silica was 40 ml. Simple multiplication would predict about 63 ml. The pressure corresponding to this steady-state flow was 530 atm. This system was chosen to show that the volume of solvent dissipated during pressurization is small even when conditions are the least favorable, *i.e.*, when the column resistance is high and the solvent is highly compressible. In a later section we will show that with modern instrumentation this pressurization step is required only once, after filling the pumps. Thus the time and eluent waste described by eqn. 5 has been greatly overstated.

Chromatographic practice

The value of a theoretical treatment to the practicing chromatographer lies in

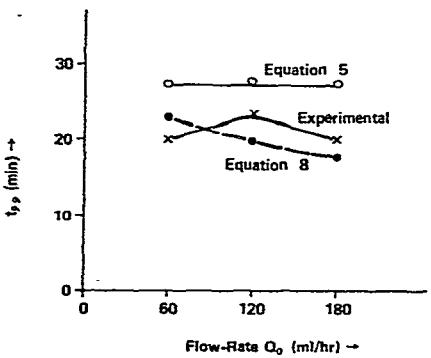

Fig. 5. t_{99} values of Model 8520 liquid chromatograph. V_0 = 250 ml, Q_0 = 60, 120 and 180 ml/h. Solvent, *n*-hexane; column, 25 × 0.21 cm I.D., 5-μm silica gel.

its applicability to experimental practice. Martin *et al.*[1] suggest that the time required for a syringe pump to reach equilibrium pressure is too long and irreproducible to be useful. Unfortunately, this treatment considered only a simplified system, neglecting two common modes of operation that have long been incorporated into the design of commercial syringe pumps in order to avoid these problems. These modes, designed to avoid problems due to compressibility, are referred to as the "fast-pump" mode of pressurization, and the "stop–flow" mode of sample injection.

"Fast-pump" mode of pressurization. To reduce the initial time required to achieve operating pressure, the flow-rate of the piston can be ramped up rapidly to the maximum rate allowed by the stepping motor drive (990 ml/h). In most commercial syringe pumps this is accomplished simply by pushing a button. This rapidly compresses the cylinder liquid. The chromatographer monitors pressure build-up on the pressure read-out and as the pressure approaches the value required by the programmed flow-rate and column permeability, the fast-pump button is released. The pump stepping motor instantaneously switches to the desired piston flow-rate.

Comparison of the pressurization of the pump cylinder with both normal- and fast-pump modes is presented in Fig. 6. In the normal mode, pressurization follows the exponential rise predicted by eqn. 8, requiring 9 min to achieve steady-state pressure. In the fast-pump mode, pressurization to within 95% of the steady-state pressure requires 15 sec, at which point the fast-pump button is released. Steady-state pressure is then achieved within 2.75 min.

It has been the experience of the authors that with the fast-pump mode of operation, the time required to reach system pressure is less than that required to obtain a stable detector baseline, especially at high detector sensitivity. It is definitely shorter than the time required for most columns to come to chemical equilibrium after a change in mobile phase.

In addition, the fast-pump mode of operation is required only when the

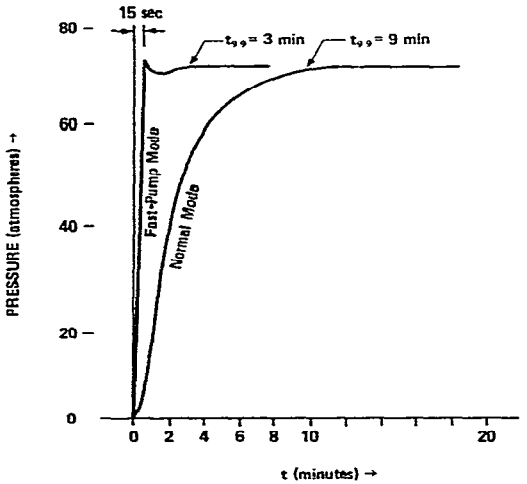

Fig. 6. Comparison of pressurization with Model 8500 syringe pump, in normal- and fast-pump modes. Solvent, *n*-hexane–dichloromethane–isopropanol (80:13.4:6.6). Column, 25 × 0.21 cm I.D., Micropak CN (10 μm): Q_o = 60 ml/h, V_o = 250 ml.

cylinder pressure nas been significantly altered from the steady-state value. It must be noted that it is not necessary to use this mode after each injection. This is due to the incorporation of stop–flow valves into modern syringe pump systems. The design and operation of such a system are discussed below.

Stop–flow mode of sample injection. Obviously, if one chooses to use an on-stream injector, such as a 6-port sample valve, one can inject samples without significantly disturbing the steady-state pressure. However, even in the case of a stop–flow injection, such a situation is achievable, due to modern pump design.

A schematic of a syringe pump system is presented in Fig. 1. Stop–flow injection involves the following commands and associated actions. The response of the system to these demands is shown in the pressure trace of Fig. 7. Pressing the "stop/inject" button of the control module commands the outlet valve to close. The outlet valve is electronically interconnected to the stepping motor drive of the pump. Thus, when the valve shuts, the pump motor stops. The volume V_0 of liquid in the pump is thus stored in the compressed state, at the same pressure P_0 as the column had generated by its flow resistance.

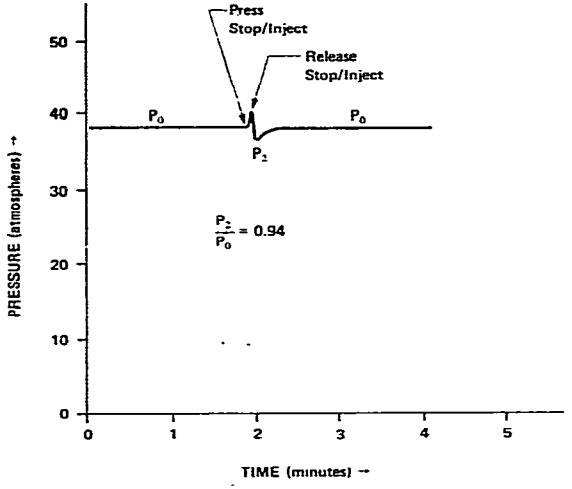

Fig. 7. Stop/inject performance of 8500 syringe pump. Solvent, *n*-hexane, 60 ml/h. $V_0 = 7.8$ ml and $V_1 = 0.5$ ml.

The pressure P_1 of the volume V_1 downstream of the valve decays to ambient within a few seconds (bleeds through column) and the injector is then opened. A syringe injection is made on to the top of the column and the injector is resealed. The "pump" button is then pressed, simultaneously opening the outlet valve and starting the pump stepping motor at the speed determined by the control module setting.

Upon opening the outlet valve, the pressures P_0 and P_1 on each side of the valve will adjust themselves to a new value, P_2, slightly lower than P_0. To minimize the pressure drop, $P_0 - P_2$, the liquid volume downstream of the valve is kept small. This volume, which includes the mixing chamber, connector tubing and injector, as

shown in Fig. 1, is less than 1 ml. In this discussion, the volume of the column was not included in V_1 because the new pressure P_2 establishes itself very rapidly in the pump and connecting tubing which have a very low resistance as compared to that of the column.

The new pressure, P_2, after opening of the valve, is obtained by application of the principle of conservation of energy:

$$P_2 (V_0 + V_1) = P_0V_0 + P_1V_1 \text{ or } P_2 = \frac{P_0V_0 + P_1V_1}{V_0 + V_1} \tag{9}$$

so

$$\frac{P_2}{P_0} \approx \frac{V_0}{V_0 + V_1} \text{ if } P_0 \gg P_1 \text{ and } V_0 > V_1 \tag{10}$$

Because of the small liquid volumes involved and the low resistance of the connecting lines, equilibration to the new pressure, P_2, is almost instantaneous, as can be seen in Fig. 7, which shows the experimental pressure variation during the stop–flow injection. The slight initial pressure rise is due to displacement of liquid (*ca.* 50 μl) by the valve needle towards the pump upon closing. For this experiment, using *n*-hexane as solvent, the initial volumes and pressures were as follows: V_0 = 7.8 ml, V_1 = 0.48 ml, P_0 = 38.42 atm, and P_1 = 1 atm; the new pressure, P_2, was 36.22 atm.

Application of eqn. 9 predicts a value of $P_2 = (38.42 \times 7.81 + 1 \times 0.48)/(7.81 + 0.48) = 36.25$ atm, which is in good agreement with the experimental value. Since the actual drop in pressure was only 5.7%, the time required for pressure recovery should be relatively short. Experimental results in Fig. 7 show the actual time to reach steady-state pressure P_0 was about 30 sec. The loss of flow during the stop–flow pressure transient was obtained by graphical integration of the *P vs. t* curve and was found to be approximately 14 μl, or 2.8% of the flow during the 30-sec time interval.

As seen in eqn. 10 the degree of flow loss will be greatest at low cylinder volume. A full 8500 syringe pump contains 250 ml of liquid. The experimental data discussed above were taken for a V_0 value of 7.8 ml, the lowest cylinder volume used in practice. Thus, the 14-μl loss represents the maximum flow loss to be encountered with use of the stop–flow injection mode.

TABLE II

MAXIMUM ERROR IN MEASUREMENT OF RETENTION VOLUME USING STOP–FLOW INJECTION WITH A SYRINGE PUMP

Q_0 = 60 ml/h, V_0 = 7.8 ml. Column, Micropak CN 10 μm, 25 × 0.21 cm I.D. Column void volume, 0.65 ml.

Peak capacity factor	*Maximum error in retention volume or time (%)*
0	2.2
1	1.1
2	0.7
5	0.4
10	0.2

TABLE III

REPRODUCIBILITY DATA OF ISOCRATIC ELUTION OF STEROID MIXTURE

Column Micropak CN, 10 μm. Mobile phase, hexane–dichloromethane–isopropanol (80:13.4:6.6). Q_o = 60 ml/h. Capacity factors were: testosterone 2.72, Reichstein S 6.48, dexamethasone 19.62.

Injection	*Initial reservoir volume (ml)*	t_R *(min)*		
		Testosterone	*Reichstein S*	*Dexamethasone*
1	198	2.32	4.66	12.80
2	180	2.31	4.65	12.80
3	164	2.31	4.64	12.81
4	↓	2.32	4.64	12.80
5		2.31	4.65	12.81
6		2.32	4.65	12.81
7		2.32	4.65	12.80
8		2.31	4.65	12.81
9		2.32	4.65	12.78
10	47	2.32	4.65	12.77
	Average	2.31	4.65	12.80
	Rel. stand. deviation	0.35%	0.12%	0.11%

The effect of the 14-μl flow loss on the retention volume of a peak is calculated and presented in Table II. Most peaks in HPLC have capacity factor (k') values of 2–10. The maximum absolute error in retention volume due to compressibility effects during a stop–flow injection will thus be less than 1%. The effect on analysis precision of retention volume or time will be less and thus insignificant.

Experimental data and retention time reproducibility

The adequacy of the use of a valve between the pump and injector can best be tested by studying reproducibility of chromatographic retention times, using stop–flow injection. The results of one such experiment, the isocratic separation of a mixture of steroids, are shown in Table III. Ten consecutive injections were performed

TABLE IV

REPRODUCIBILITY OF RETENTION TIMES IN GRADIENT ELUTION SEPARATION OF POLYNUCLEAR AROMATIC HYDROCARBONS

Column, 25 × 0.21 cm I.D., micropak CH-10.

Run	*Cylinder volumes (ml)*		t_R			
	Acetonitrile	*Water*	*Phenanthrene*	*Anthracene*	*Benzanthracene*	*Benz(α)pyrene*
1	250	235	13.13 min	13.43	15.54	17.45
2	230	210	13.13	13.45	15.45	17.34
3	215	185	13.51	13.78	15.58	17.31
4	100	100	13.08	13.44	15.62	17.56
5	50	50	13.24	13.60	15.77	17.67
	Average		13.22	13.54	15.59	17.47
	Stand. deviation		0.17	0.15	0.08	0.15
	Rel. stand. deviation		1.29%	1.11%	0.51%	0.86%

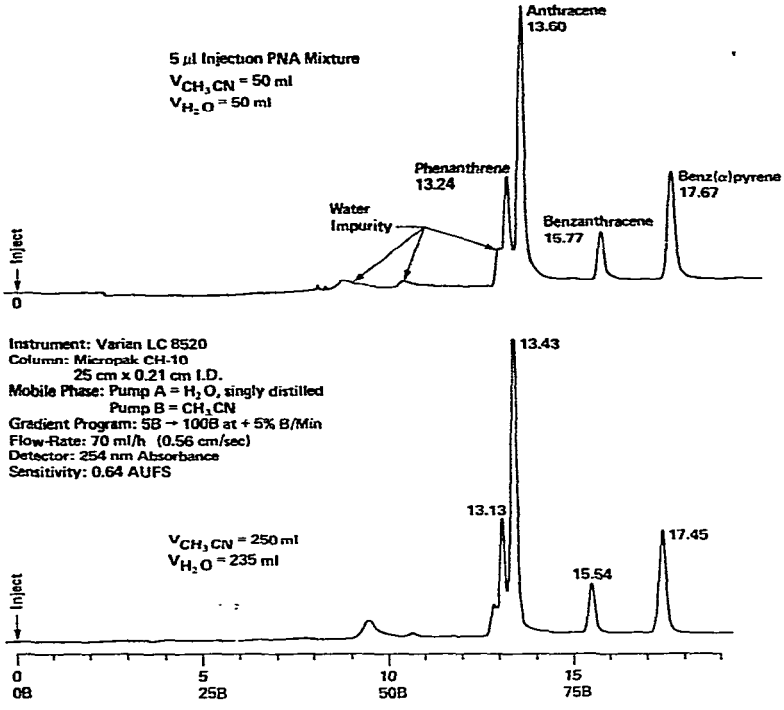

Fig. 8. Gradient elution of polynuclear aromatic hydrocarbons on reversed-phase column. Column back-pressure varies between 3400 and 1500 p.s.i. during gradient. Column regeneration by reverse gradient.

on one cylinder full of the Varian 8500 pump. The reproducibility of retention times is outstanding by any chromatographic criteria. Furthermore, there is no correlation between the measured retention time and the cylinder volume, despite the fact that the volume contained in the cylinder decreased from 198 to about 47 ml.

In a second experiment, a mixture of polynuclear aromatic hydrocarbons was separated on a reversed-phase column with a water to acetonitrile gradient. Five injections were performed over one cylinder full of the same pump. The results are shown in Table IV and Fig. 8. Again, the reproducibility of retention times was excellent.

These experiments demonstrate that properly designed syringe pumps can provide useful, reliable chromatographic data.

CONCLUSIONS

The performance of a syringe pump in a properly designed liquid chromatograph is at the very minimum entirely satisfactory for both quantitative and qualitative work. In our experience, the productivity of a liquid chromatograph is limited by the time required to re-establish a stable detector baseline, or for the column to respond (equilibrate) to a change in solvent composition, especially in gradient elution and not by the characteristics of the syringe pump.

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