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UNAVOIDABLE FLOW-RATE ERRORS IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

Two different types (low-pressure mixing and high-pressure mixing) of high-performance liquid chromatographic (HPLC) solvent delivery systems were evaluated for their flow-rate accuracy using three common hydro-organic mobile phases: methanol–water, acetonitrile–water and tetrahydrofuran–water. Both systems delivered flow-rates 2–12% lower than the desired (set) values, depending on the mobile phase composition. Further investigations revealed that these errors in flow-rate were due in part to non-ideal mobile phase behavior (non-zero volumes of mixing, non-zero compressibilities, pressure dependent viscosities, and deviations from Darcy's law) and non-ideal stationary phase behavior (column expansivity and packing compression). An approach to correct for the systematic errors in flow-rate is described for binary solvent mixtures. The approach utilizes correction factors based on non-ideal mobile and stationary phase behavior and has been experimentally confirmed for the mobile phases and columns we examined. Although approximate in nature, our approach reduces the systematic errors in flow-rate about ten-fold, and provides a means for estimating the instrumental contribution to flow-rate error as well. This leaves only an instrumental bias which can be measured and then factored out for all further experiments with that instrument. The resulting improvements in flow-rate accuracy provided by our approach will be extremely useful to researchers who need very accurate retention data for physicochemical measurements as well as to HPLC manufacturers and users for quality control and troubleshooting of these solvent delivery systems.

INTRODUCTION

Although not commonly appreciated, deviations in the flow-rate from the programmed value are an everyday occurrence in high-performance liquid chromatography (HPLC). Despite claims from numerous HPLC manufacturers who insist that their systems are virtually error free, flow-rate errors can often exceed 5% and may sometimes be a significant source of error in analytical or physicochemical measurements^{1–5}.

The sources of flow-rate errors in HPLC can be broadly classified into four

categories: (1) non-ideal mobile phase behavior, (2) non-ideal stationary phase (packing and column hardware), (3) instrumental imperfections (mechanical limitations of the solvent delivery systems), and (4) operator error. The first category, non-ideal mobile phase behavior, can be described almost entirely in terms of four physical phenomena: (i) the change in volume which occurs when two or more solvents are blended, (ii) the compressibility of any pure solvent or mixtures of solvents, (iii) the pressure dependence of viscosity of any pure solvent or mixtures of solvents, and (iv) deviations in Darcy's law. The second category, "non-ideal stationary phase properties", refers to the very slight compression of the packing and the expansion of the column wall due to the high pressures; this results in a slightly greater than predicted column permeability. The last two categories, instrumental imperfections and operator error, cannot be defined as precisely but certainly include leaks, dissolved gases in the mobile phase, faulty check valves, insufficient pulse dampening, and flow restrictions such as plugged frits.

Whereas flow-rate errors due to category 4 can be identified and eliminated if sufficient attention to detail is given, it is difficult to distinguish experimentally between errors due to categories 1, 2 and 3. Furthermore, although all the underlying phenomena of category 1 are reasonably well understood⁶⁻¹⁰, a unified treatment of their effects on flow-rate in HPLC has yet to be presented. Although some non-ideal mobile phase and stationary phase effects on flow-rate were examined previously⁶, the study was limited by design to theoretical calculations for pure solvents, which are seldom used as mobile phases in HPLC.

The purpose of the present work was the following: (1) to experimentally determine the flow-rate accuracy of modern HPLC systems; (2) to identify and describe quantitatively all the sources of error for the flow-rates and, if possible, to deconvolute the instrumental, mobile phase and stationary phase contributions; and (3) to develop a practical method for eliminating or at least minimizing flow-rate errors and the resultant systematic biases in flow-rate dependent parameters.

Due to both potentially larger flow-rate errors and the greater interest in reversed-phase (RP) liquid chromatography, we decided to investigate typical RP-HPLC solvent systems exclusively in this study, although the theory we present here is applicable to all liquid mobile phases. In future articles we plan to discuss the significance of these flow-rate errors on analytical and physicochemical measurements, including the effect of these flow-rate errors on the measurement of retention time and volume, and the possibility of extending the theory and our practical solution to normal-phase liquid chromatography and supercritical fluid chromatography.

THEORY

The theory for the mobile phase and stationary phase phenomena that give rise to flow-rate errors is described below.

Volume of mixing

When miscible solvents are mixed, the observed volume after mixing (V_{total}) is not always equal to the sum of the individual volumes (ΣV_i) due to attractive (or repulsive) interactions between the different solvent molecules. In general, ΔV_{mix} is negative ($V_{\text{total}} < \Sigma V_i$, attractive interactions) and this results in a negative deviation

in the flow-rate ($F_{\text{observed}} < F_{\text{expected}}$) according to eqn. 1 if the solvents are not pre-mixed.

$$F_{\text{observed}} = \frac{V_{\text{total}}}{\Sigma V_i} F_{\text{expected}} \quad (1)$$

For an ideal solution, $V_{\text{total}} = \Sigma V_i$, and thus we may write

$$\frac{F}{F_{\text{ideal}}} = \frac{V_{\text{total}}}{\Sigma V_i} \quad (2)$$

Because the magnitude of ΔV_{mix} is larger for reversed-phase solvents than for normal-phase chromatography, V_{total} will be smaller and the errors in flow-rate expressed by eqn. 2 will be larger for RP-HPLC. The more negative ΔV_{mix} values in RP-HPLC are attributed to the greater solvent-solvent interactions, *e.g.*, hydrogen bonding.

Katz *et al.*¹⁰ measured the volumes of mixing for the three common RP-HPLC "binary" solvent systems (methanol-water, acetonitrile-water, and tetrahydrofuran-water) over the entire composition range and developed a theory which enables V_{total} to be predicted (with ΣV_i understood to be unity). They showed that these so-called binary solvent systems of methanol-water, acetonitrile-water, and tetrahydrofuran-water are really a more complex ternary system consisting of free organic solvent, M, free water, W, and associated organic solvent-water, MW. The equation for the calculation of V_{total} in eqn. 2 is

$$V_{\text{total}} = \Sigma f_i = f_m + f_w + f_{mw} \quad (3)$$

where Σf_i is the sum of the volume fractions of (free) organic solvent, f_m ; (free) water, f_w ; and associated organic-water, f_{mw} . The individual volume fractions in turn are calculated via eqns. 4-6

$$f_m = [\text{M}]V_m \quad (4)$$

$$f_w = [\text{W}]V_w \quad (5)$$

$$f_{mw} = [\text{MW}]V_{mw} \quad (6)$$

where the square brackets denote molar concentration and V denotes molar volume. The molar concentrations are calculated using eqns. 7-9

$$[\text{W}] = \frac{-\left(K + \frac{\varphi_{\text{org}}}{V_m} + \frac{\varphi_{\text{org}}}{V_w} - \frac{1}{V_w}\right) + \sqrt{\left(K + \frac{\varphi_{\text{org}}}{V_m} + \frac{\varphi_{\text{org}}}{V_w} - \frac{1}{V_w}\right)^2 + 4K\left(\frac{1}{V_w} - \frac{\varphi_{\text{org}}}{V_w}\right)}}{2} \quad (7)$$

$$[\text{MW}] = \frac{1 - \varphi_{\text{org}}}{V_{\text{w}}} - [\text{W}] \quad (8)$$

$$[\text{M}] = \frac{\varphi_{\text{org}}}{V_{\text{m}}} - [\text{MW}] \quad (9)$$

where K is the association constant of the organic solvent and water and φ_{org} is the apparent volume fraction of the organic solvent = $V_{\text{org}}/(V_{\text{org}} + V_{\text{water}})$.

It should be noted here that the volume of mixing phenomenon is essentially independent of pressure, in contrast to solvent compressibility, the pressure coefficient of viscosity and other factors discussed below. Thus for a given temperature, only one calculation is required for each mobile phase composition.

Compressibility, pressure dependence of viscosity, deviations from Darcy's law, and column expansivity/packing compression

A couple of approaches in estimating these effects on the retention time and retention volume in liquid chromatography have already been described by Martin *et al.*⁶. By design their studies were limited to theoretical calculations for *pure solvents*. Moreover, the better approach they described required the use of an equation of state known as the Tait equation¹¹. Unfortunately, an extensive literature search revealed that the parameters needed for the Tait equation are only available for certain pure solvents (including water and methanol, but not acetonitrile and tetrahydrofuran)⁶ and are generally unavailable for mixtures of solvents. Because our study is concerned with typical HPLC mobile phases which are nearly always mixtures, we were thus precluded from using the Tait equation of state. The method we have developed is admittedly somewhat less rigorous than the Tait equation approach, but is much simpler (calculations can be done on a spreadsheet without numerical integration) and can be applied consistently to both pure solvents and binary mixtures. In addition, our study includes experimental data which shows that our approach is equally accurate (see below).

The starting point for our approach is Darcy's law (eqn. 10),

$$u = -\frac{B^0}{\varepsilon\eta} \frac{dP}{dz} \quad (10)$$

which relates the local linear velocity u of an unretained solute to the specific permeability B^0 and external porosity ε of the column, the viscosity η of the mobile phase and the local pressure gradient dP/dz . The negative sign in eqn. 10 indicates the linear velocity (and hence flow-rate) will be toward the region of lower pressure.

In calculating the effects of the various phenomena on the flow-rate, we shall assume that they are independent of one another. Although this is not always strictly true, our assumption results in negligible error because the effects of each of the phenomena are small. Later in this paper we shall demonstrate the accuracy of this approach for two mobile phase phenomena, compressibility and the pressure dependence of viscosity, which could be expected to exhibit a high degree of synergism. Since the assumption holds for this combination, it should hold for any other combination as well.

Since we are starting with Darcy's law, the results obtained for the various phenomena will be expressed in terms of linear velocities (u). These results will be equally applicable to flow-rate due to the relationship $F = uA_c$, where A_c is the cross-sectional area of the column (a constant for all intents and purposes). Thus the final equation obtained for a given phenomenon will frequently be expressed in terms of flow-rate in addition to linear velocity.

Mobile phase compressibility. Compressibility defines how the volume of a sample of matter decreases with increasing pressure. Obviously a gas is much more compressible than a liquid, but liquids may also be compressed. The net effect of mobile phase compressibility is an increase in flow-rate relative to an ideal solvent that is incompressible. This can be shown as follows, beginning with the mathematical definition of compressibility, β (readers only interested in the result should skip to eqn. 16).

$$\beta = -\frac{1}{V} \left(\frac{\partial V}{\partial P} \right)_T \quad (11)$$

(where T = temperature). Since $\partial V/\partial P$ is negative, β will always be positive. In previous treatments, the exact variation of solvent compressibility with pressure has been debated. Although it is generally agreed that the compressibility of a liquid does vary somewhat over a sufficiently wide range of pressure, the assumption of a constant compressibility ($d\beta/dP = 0$) is reasonable and leads to negligible error for pressures commonly encountered in HPLC (≤ 5000 p.s.i.)⁶. With that assumption, integration of eqn. 11 yields

$$V = V_0 e^{-\beta(P-P_0)} \quad (12)$$

where the V and V_0 are equimolar volume elements of the mobile phase at pressures P and P_0 , where P_0 is the outlet pressure and P is the pressure at some point before the outlet ($P > P_0$). Assuming the HPLC column is homogeneous and that its average cross-section A_c is independent of pressure (see ref. 6), a similar equation relates the local linear velocity u to the linear velocity at the column outlet u_0 (eqn. 12a).

$$u = u_0 e^{-\beta(P-P_0)} \quad (12a)$$

Substitution of eqn. 12a into eqn. 10 (Darcy's Law) yields

$$u_0 e^{-\beta(P-P_0)} = -\frac{B^0}{\varepsilon\eta_c} \frac{dP}{dz} \quad (13)$$

where the subscript "c" for the viscosity means we have assumed it is a constant independent of pressure. Rearrangement and integration of eqn. 13 yields

$$u_0 = \frac{B^0}{\varepsilon\eta_c L} \left(\frac{e^{\beta\Delta P} - 1}{\beta} \right) \quad (14)$$

where L = column length and $\Delta P = P_i$ (inlet pressure) $- P_o$ (outlet pressure). Since $\beta = 0$ for an ideal, incompressible liquid, we have

$$u_o(\text{ideal}) = \lim_{\beta \rightarrow 0} (u_o) = \frac{B^0}{\varepsilon \eta_c L} \lim_{\beta \rightarrow 0} \left(\frac{e^{\beta \Delta P} - 1}{\beta} \right) \quad (15)$$

Using L'Hospital's rule, we obtain

$$u_o(\text{ideal}) = \frac{B^0}{\varepsilon \eta_c L} \lim_{\beta \rightarrow 0} \left(\frac{\Delta P e^{\beta \Delta P}}{1} \right) = \frac{B^0 \Delta P}{\varepsilon \eta_c L} \quad (15a)$$

The relative deviation in linear velocity and hence, in flow-rate, is

$$\frac{F_o}{F_o(\text{ideal})} \approx \frac{u_o}{u_o(\text{ideal})} = \frac{1}{\beta \Delta P} (e^{\beta \Delta P} - 1) \quad (16)$$

which, by using $e^x = 1 + x + \frac{x^2}{2} + \frac{x^3}{3!} + \dots$, can be transformed to

$$\frac{F_o}{F_o(\text{ideal})} \approx \frac{u_o}{u_o(\text{ideal})} = \left(1 + \frac{\beta \Delta P}{2} + \frac{(\beta \Delta P)^2}{6} + \frac{(\beta \Delta P)^3}{24} + \dots \right) \quad (16a)$$

Since β and ΔP are always positive, the error in flow-rate due to mobile phase compressibility will always be positive.

Although isothermal compressibilities are known from a variety of pure solvents, they are generally unavailable for the binary mixtures in this study. Therefore it was assumed as before⁹ that the compressibility of a binary mixture can be calculated from the compressibilities of the pure solvents, *i.e.*,

$$\beta_{\text{mixture}} = (1 - \varphi_{\text{org}})\beta_{\text{water}} + \varphi_{\text{org}}\beta_{\text{org}} \quad (17)$$

where φ_{org} represents the volume fraction of the organic strong solvent.

Pressure dependence of mobile phase viscosity. The effect of viscosity on flow-rate (via Darcy's law) and the variations of viscosity with mobile phase composition in HPLC are well known. What is sometimes overlooked by chromatographers is the pressure dependence of mobile phase viscosity. Because of the pressure dependence, a higher inlet pressure is required to reach the same flow-rate than that required by a mobile phase whose viscosity is independent of pressure. The net effect of the pressure dependence of viscosity is a decrease in flow-rate, as we shall now show (readers only interested in the result should skip to eqn. 23).

The pressure dependence of viscosity can be expressed as

$$\eta = \eta_o \exp(\alpha P) \quad (18)$$

where η is the viscosity at pressure P , η_0 is the viscosity at standard pressure, and α is the pressure coefficient for the solvent. Substitution of eqn. 18 into eqn. 10 yields

$$u = - \frac{B^0}{\varepsilon \eta_0 \exp(\alpha P)} \frac{dP}{dz} \quad (19)$$

which upon integration gives

$$u = \frac{B^0}{\varepsilon \eta_0 L} \frac{[\exp(-\alpha P_0) - \exp(-\alpha P_i)]}{\alpha} \quad (20)$$

Since $\alpha = 0$ when the mobile phase viscosity is independent of pressure, we have

$$u(\text{ideal}) = \frac{B^0}{\varepsilon \eta_0 L} \lim_{\alpha \rightarrow 0} \left\{ \frac{[\exp(-\alpha P_0) - \exp(-\alpha P_i)]}{\alpha} \right\} \quad (21)$$

Using L'Hospital's rule, we obtain

$$u(\text{ideal}) = \frac{B^0}{\varepsilon \eta_0 L} \lim_{\alpha \rightarrow 0} \left[\frac{\alpha(P_i - P_0)}{\alpha} \right] = \frac{B^0 \Delta P}{\varepsilon \eta_0 L} \quad (22)$$

The relative deviation in linear velocity (and flow-rate) is then

$$\frac{F}{F_{\text{ideal}}} \approx \frac{u}{u_{\text{ideal}}} = \frac{\exp(-\alpha P_i) - \exp(-\alpha P_0)}{\alpha \Delta P} \quad (23)$$

As before with compressibility the pressure coefficients of viscosity are generally available for pure solvents but not for mixtures. Thus in the absence of any other information we shall assume that the pressure coefficient of viscosity for a binary mixture can be calculated from the pressure coefficients of viscosity of the pure solvents, *i.e.*,

$$\alpha_{\text{mixture}} = (1 - \varphi_{\text{org}})\alpha_{\text{water}} + \varphi_{\text{org}}\alpha_{\text{org}} \quad (24)$$

The apparent validity of this assumption will be discussed later. Note that we are *not* assuming that the viscosity of a mixture varies linearly with pressure (it does not), but only that the *pressure coefficient of viscosity* varies in this manner. The *viscosities* of all mobile phases used in this study were obtained from experimental data (see eqns. 35–37 and related text).

Deviations from Darcy's law. Another potential source of flow-rate error in HPLC is the failure of Darcy's law at the relatively high reduced velocities (compared to gas chromatography) that are generally used. Martin *et al.*⁶ also examined this phenomenon and showed that

$$\frac{F}{F_{\text{ideal}}} \approx \frac{u'}{u'_{\text{ideal}}} = \frac{1}{1 + \frac{1.75 \rho d_p u'}{150 \eta (1 - \varepsilon_c)}} \quad (25)$$

where u' is the superficial velocity, ρ and η are the density and viscosity of the mobile phase, ε_e is the interparticle porosity and d_p is the particle diameter. The superficial velocity u' is the linear velocity of the mobile phase within the column if the column packing was removed. It is given by the product of the linear velocity u and ε_e , where ε_e is the external porosity [$u' = (\varepsilon_e)u$]. Since $\varepsilon_e \approx 0.4$, u' will be *ca.* 40% of u .

Whereas errors due to volume of mixing effects are primarily dependent on the nature of the solvents being mixed and errors due to compressibility and viscosity effects are primarily dependent on the pressure drop (at a given temperature), the errors due to deviations from Darcy's law are directly dependent on several variables (ρ , η , d_p and u') which can have a wide range of values. The values we used for these parameters in our calculation of this effect were chosen so as to be typical for *analytical* (*cf.* preparative) HPLC separations at room temperature (*ca.* 25°C) and reversed-phase mobile phases. Our calculations revealed that for $u \leq 0.5$ cm/s ($u' \leq 0.2$ cm/s), $\varepsilon_e = 0.4 \pm 0.05$, d_p (particle diameter) ≤ 10 μm , a temperature of 25°C, and all possible values of ρ and η for methanol–water, acetonitrile–water and tetrahydrofuran–water mobile phases, the error is always less than 0.12% and is therefore negligible compared to the other effects which are on the order of 1–6%. In the present study we used columns with $d_p \approx 5$ μm (other conditions as before), and the error was less than 0.06%. It should be noted, however, that there may be some separations for which deviations from Darcy's law are not negligible. These include (1) preparative-scale separations that utilize stationary phase particles with diameters in excess of 25 μm ; and (2) high-temperature separations ($> 45^\circ\text{C}$) in which the kinematic viscosity (η/ρ) is very low.

Errors due to compression of the stationary phase and expansion of the column.

Although the compressibility of a solid is less than that of a liquid, and although the elasticity of a solid (as defined by Young's modulus) is generally considered to be small, the effects of stationary phase compressibility and column expansivity are not always negligible. Their combined effect on linear velocity (and hence, flow-rate) was shown by Martin *et al.*⁶ to be

$$\frac{F}{F_{\text{ideal}}} \approx \frac{u}{u_{\text{ideal}}} = 1 + \left[\frac{3\lambda_c}{\varepsilon_e} + \left(\frac{3}{\varepsilon_e} - \frac{5}{3} \right) \frac{\beta}{2} \right] \Delta P \quad (26)$$

where ε_e is the external porosity, β is the compressibility and λ_c is calculated from

$$\lambda_c = \frac{2}{Y_c \left[1 - \left(\frac{r_{\text{int}}}{r_{\text{ext}}} \right)^2 \right]} \quad (27)$$

Y_c is Young's modulus of the column metal, and r_{int} and r_{ext} refers to the internal and external radius of the column, respectively. As shown in eqns. 26 and 27, the effect of column expansivity on flow-rate increases as the ratio of internal to external radius increases. Since the thickness of most stainless-steel analytical HPLC columns is *ca.* 1 mm, the ratio will vary from 0.3 to 0.7 for the typical inner diameter of 1 to 4.6 mm. Thus the column expansivity effect will generally be greater for the larger diameter columns.

Assuming a 4.6 mm I.D. column made of stainless steel ($Y_c \approx 2 \cdot 10^6$ atm) and

a stationary phase of silica or derivatized silica ($\beta = 2.72 \cdot 10^{-6} \text{ atm}^{-1}$), the column expansivity effect ranges from 0.1 to 0.9% over the pressure range 735–5000 p.s.i. (50–340 atm). Thus in precise work with conventional columns this effect may sometimes need to be accounted for.

Total error in flow-rate due to combined effect of all phenomena

Assuming that all of the various non-ideal phenomena which affect the flow-rate are small, they can be treated independently of one another and the overall effect on the flow-rate can be calculated as

$$F_{\text{obs}} = \left(\frac{F}{F_{\text{ideal}}} \right)_{\text{total}} F_{\text{nominal}} \quad (28)$$

where

$$\left(\frac{F}{F_{\text{ideal}}} \right)_{\text{total}} = \left(\frac{F}{F_{\text{ideal}}} \right)_{\Delta V_{\text{mix}}} \left(\frac{F}{F_{\text{ideal}}} \right)_{\beta} \left(\frac{F}{F_{\text{ideal}}} \right)_{\alpha} \left(\frac{F}{F_{\text{ideal}}} \right)_{\text{Darcy}} \left(\frac{F}{F_{\text{ideal}}} \right)_{\text{column}} \quad (29)$$

In eqn. 29, the relative deviations in flow-rate due to (i) volume of mixing, (ii) mobile phase compressibility, (iii) pressure dependence of viscosity, (iv) deviations in Darcy's law, and (v) column expansivity/packing compression are given by eqns. 2, 16, 23, 25 and 26. The data required to calculate these individual effects are given in Table I.

With the exception of the volume of mixing, all the phenomena discussed are interrelated via Darcy's law (eqn. 10), and the accuracy of the independent treatment of these effects may thus be questioned. However, we shall now show by comparing the results of two phenomena treated independently with the results obtained by considering the phenomena together (simultaneously) that the *independent treatment results in negligible error*. The two phenomena we have chosen are (1) the mobile phase compressibility and (2) the pressure dependence of mobile phase viscosity.

TABLE I

VOLUME OF MIXING, COMPRESSIBILITY AND VISCOSITY DATA FOR SOME RP-HPLC SOLVENTS

Solvent	Volume of mixing ^a			Compressibility ^b $\beta \cdot 10^6/\text{atm}$	Viscosity ^c $a \cdot 10^5/\text{atm}$
	K	V_m	V_{mw}		
Methanol	0.0045	40.68	55.46	127[6]	47.6[6]
Acetonitrile	0.206	52.25	53.26	74[15]	48.5(est.)
Tetrahydrofuran	0.0468	81.11	88.80	70[16]	53.0[17]
Water	—	18.00	—	46.3[6]	5.0[6]

^a See eqns. 1–9 and related text. Data obtained from ref. 10.

^b See eqns. 16 and 17. Numbers in brackets indicate references from which data were obtained. The viscosities may be obtained via eqns. 35–37.

^c Pressure coefficient of viscosity. Refer to eqn. 18 and related equations. Numbers in brackets indicate references from which data were obtained.

Simultaneous treatment of compressibility and pressure-dependent viscosity effects on flow-rate. These effects can be treated simultaneously by substitution of eqns. 12a and 19 into eqn. 10. The resulting equation

$$u_o = \frac{B^0 e^{\beta(P-P_o)} dP}{\varepsilon \eta_o e^{\alpha P} dz} \quad (30)$$

can be integrated to yield

$$u_o = \frac{B^0}{\varepsilon \eta_o L} \frac{e^{-\beta P_o}}{\beta - \alpha} [e^{(\beta - \alpha)P_o} - e^{(\beta - \alpha)P_i}] \quad (31)$$

Since $\alpha, \beta = 0$ for an incompressible liquid whose viscosity is independent of pressure, we have

$$u_o(\text{ideal}) = \frac{B^0}{\varepsilon \eta_o L} \lim_{\alpha, \beta \rightarrow 0} \left[\frac{e^{-\beta P_o} (e^{(\beta - \alpha)P_o} - e^{(\beta - \alpha)P_i})}{\beta - \alpha} \right] \quad (32)$$

The first term of the numerator (within brackets) goes to unity. Using L'Hospital's rule for the remaining expression, we obtain

$$u_o(\text{ideal}) = \frac{B^0}{\varepsilon \eta_o L} \lim_{\alpha, \beta \rightarrow 0} \left[\frac{e^{(\beta - \alpha)P_o} - e^{(\beta - \alpha)P_i}}{1} \right] = \frac{B^0}{\varepsilon \eta_o L} (P_o - P_i) \quad (33)$$

The relative deviation in linear velocity (and flow-rate) is then

$$\frac{F}{F_{\text{ideal}}} \approx \frac{u_o}{u_{o\text{ideal}}} = \frac{e^{-\beta P_o} \{e^{(\beta - \alpha)P_o} - e^{(\beta - \alpha)P_i}\}}{(\beta - \alpha) (P_o - P_i)} \quad (34)$$

Results for eqn. 34 are shown in Table II along with results obtained previously by Martin *et al.*⁶ as well as the results obtained when we treated the compressibility and pressure-dependent viscosity effects separately (eqns. 16 and 23) and then simply combined (multiplied) them.

As shown in Table II, the results of our simultaneous treatment of mobile phase compressibility and the pressure-dependence of viscosity (eqn. 34) are in good agreement with those of Martin *et al.* More importantly, virtually the same results are obtained with the independent approach (multiplication of eqns. 16 and 23), indicating that a negligible error results from the independent treatment of (1) mobile phase compressibility and (2) the pressure dependence of viscosity effects. *Given the accuracy of the independent approach for these two highly interrelated phenomena, any errors resulting from the independent treatment of the remaining phenomena will undoubtedly be even smaller (< 0.05%), and thus completely negligible.* Note that for simplicity we used eqn. 34 instead of eqns. 16 and 23 in all subsequent calculations of $\left(\frac{F}{F_{\text{ideal}}} \right)_{\text{total}}$.

TABLE II

COMPARISON OF CALCULATED FLOW-RATE DEVIATIONS BASED ON THE EFFECTS OF MOBILE PHASE COMPRESSIBILITY AND THE PRESSURE DEPENDENCE OF MOBILE PHASE VISCOSITY^a

	<i>Water</i>	<i>Methanol</i>	<i>Blend^b</i>
$\frac{F(\beta)}{F_{ideal}}$ (eqn. 16)	1.0046	1.0124	1.0085
$\frac{F(\alpha)}{F_{ideal}}$ (eqn. 23)	0.9947	0.9553	0.9745
$\frac{F(\beta)}{F_{ideal}} \cdot \frac{F(\alpha)}{F_{ideal}}$	0.9993	0.9672	0.9828
$\frac{F(\beta, \alpha)}{F_{ideal}}$ (eqn. 34)	0.9991	0.9669	0.9826
$\frac{F(\beta, \alpha)}{F_{ideal}}$ (ref. 6)	0.9991	0.9660	—

^a Pressure drop = 200 bar (≈ 2900 p.s.i.). Other conditions and parameters as in ref. 6 to permit comparison. Slightly different conditions and values of parameters were used in subsequent figures and tables.

^b Water-methanol (50:50, v/v). Compressibilities and pressure coefficients of viscosity calculated using eqns. 17 and 24.

EXPERIMENTAL

HPLC systems

Flow-rates were measured on two types of chromatographic systems. System A was a high-pressure mixing ternary HPLC system with three identical single-piston high-pressure reciprocating pumps. Each pump head was of 5-ml capacity with a flow-rate range of 0.005–5 ml/min and a stroke volume of 40 μ l. Flow through the pump cylinder was controlled by two ball check valves which allow flow in only one direction. System B was a low-pressure mixing quaternary HPLC system which employs a set of four solenoid valves to proportion the individual mobile phase components before they enter the single-piston high-pressure pump. The pump head was of 10-ml capacity with a flow-rate range of 0.01–10 ml/min and a stroke volume of 100 μ l. Flow through the pump head was controlled by three check valves. Pump settings on both chromatographs were optimized for minimum pulsation, flow-rate accuracy, solvent compressibility, and/or minimum vapor lock as recommended by the manufacturer for reversed-phased systems.

Reversed-phase C₈ or C₁₈ columns (Rainin, Woburn, MA, U.S.A.) with dimensions 150 mm \times 4.6 mm I.D. and a particle diameter of 5 μ m were used to provide the pressure drops typically encountered. At a flow-rate of 2 ml/min, the pressure ranged from 770 to 4520 p.s.i.

Mobile phase preparation

Mobile phase components acetonitrile (ACN), methanol (MeOH) and tetrahydrofuran (THF) were HPLC grade. Water used was either HPLC grade or triply distilled and doubly deionized. All mobile phase components were filtered through 0.22- μm or 0.45- μm Nylon-66 filters and degassed before use. Mobile phase components were degassed individually either by sparging with helium or by heating in an ultrasonic bath under a partial vacuum.

Flow-rate measurement

Flow-rates of methanol–water, acetonitrile–water and tetrahydrofuran–water mixtures were measured at 25% organic intervals from 0–100% organic. Flow-rates were measured both volumetrically (volume/time) and gravimetrically (mass/time \div density). The volume of a previously calibrated 10-ml graduated cylinder was read to the nearest 0.05 ml and time was measured to the nearest second. Weighings were performed to the nearest 0.1 mg on an analytical balance (Mettler, Hightstown, NJ, U.S.A.) and were made immediately following collection to minimize any errors due to evaporation. Densities of the pure solvents and mixtures were obtained from ref. 12. In general the agreement between volumetric and gravimetric flow-rates was very good ($\Delta < 0.2\%$), and experimental results are shown only in terms of the measured volumetric flow-rates.

RESULTS AND DISCUSSION

As explained in the Experimental section, we have elected to illustrate the errors in flow-rate (theoretically predicted and experimentally measured) on a (nominally) constant flow-rate basis.

Predicted flow-rate errors

As noted earlier, the data required to calculate the individual effects of (i) volume of mixing, (ii) mobile phase compressibility, (iii) pressure dependence of viscosity, (iv) deviations in Darcy's law, and (v) column expansive/packing compression on flow rate for the binary mobile phases of methanol–water, acetonitrile–water or tetrahydrofuran–water are given in Table I. These effects were then calculated using eqns. 2, 16, 23, 25, and 26, and except for negligible deviations in Darcy's law (see discussion in Theory) are illustrated in Fig. 1.

As shown in Fig. 1a, errors in flow-rate due to volume of mixing effects usually exceed -1.5% in RP-HPLC, and in the case of methanol–water mixtures can be as negative as -3.5% at a methanol content of 60–65%. That methanol–water mixtures give the largest flow-rate errors is not surprising, since the attractive forces required for negative volumes of mixing are largest with methanol mixtures because of greater hydrogen bonding. Flow-rate errors are smallest for acetonitrile–water mixtures, for which the error never exceeds -2.0% .

Whereas errors in flow-rate due to volume of mixing effects are independent of the pressure drop ΔP across a column, the errors due to the remaining effects depend greatly on the value of ΔP . Since for a given flow-rate ΔP varies with viscosity according to eqn. 10 and since viscosity varies with mobile phase composition, we assumed for convenience a value of 2000 p.s.i. for ΔP for the 100% aqueous phase (no

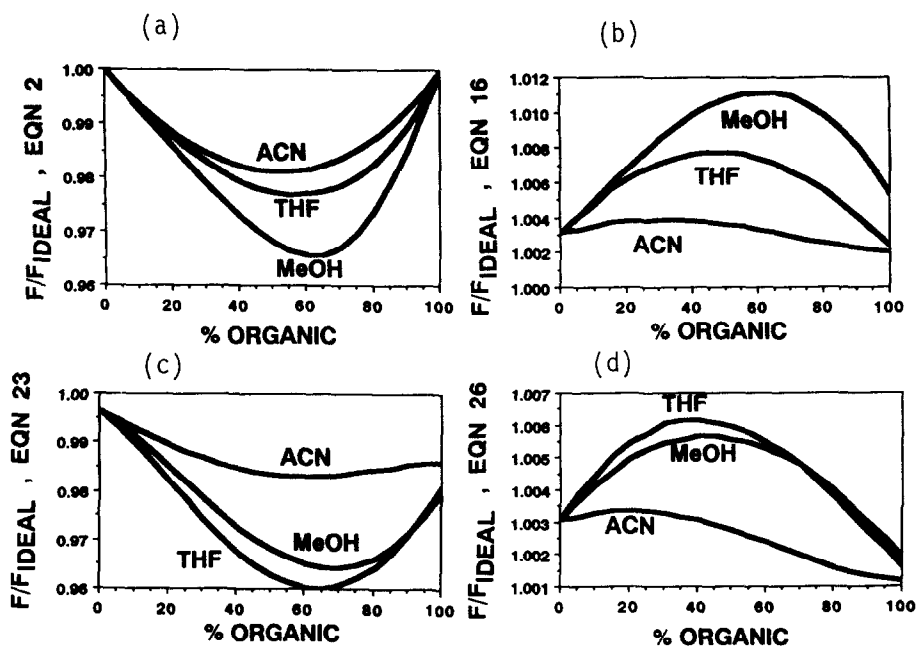


Fig. 1. Effect of (a) volume of mixing (organic solvent + water); (b) mobile phase compressibility; (c) pressure dependence of mobile phase viscosity; and (d) column expansivity/packing compression on flow-rate for three common reversed-phase solvent systems. See eqns. 2, 16, 23 and 26. Comparisons are based on equal nominal flow-rates for all mobile phases and a pressure drop of 2000 p.s.i. for pure water. Pressures at other mobile phase compositions were calculated from viscosity data; see eqns. 35–37 and related text.

organic solvent). All other ΔP values for organic-water mixtures (or pure organic solvents) were calculated according to $\Delta P_{org-water} = \Delta P_{water} (\eta_{org-water}/\eta_{water})$. The viscosities (centipoise, cP) for the mixed mobile phases were calculated from the polynomial expressions below, which were obtained from curve fits of experimentally measured data^{13,14}.

$$\eta_{MeOH-water} = 0.875 + 0.03644 (\%MeOH) - 4.762 \cdot 10^{-4} (\%MeOH)^2 + 7.809 \cdot 10^{-7} (\%MeOH)^3 \quad (35)$$

$$\eta_{ACN-water} = 0.907 + 0.01016 (\%ACN) - 3.218 \cdot 10^{-4} (\%ACN)^2 + 1.665 \cdot 10^{-6} (\%ACN)^3 \quad (36)$$

$$\eta_{THF-water} = 0.846 + 0.04821 (\%THF) - 7.548 \cdot 10^{-4} (\%THF)^2 + 2.313 \cdot 10^{-6} (\%THF)^3 \quad (37)$$

The effect of mobile phase compressibility on flow-rate is illustrated in Fig. 1b. Because the errors resulting from this effect are positive, they compensate somewhat

for the errors due to the volume of mixing effect (Fig. 1a) which are negative. This compensation is incomplete, however, since these errors are typically 2.5 times smaller (usually $< 1\%$). As observed with the volume of mixing effect, the largest errors due to mobile phase compressibility were observed with the methanol-water mobile phases and the smallest with acetonitrile-water. Note that although water is the least compressible solvent (see Table I), the relative error in flow-rate due to compressibility is somewhat larger with pure water than with acetonitrile or tetrahydrofuran, and is comparable to methanol. This apparent anomaly is explained by the greater viscosity of water, which for a given flow-rate requires a higher pressure drop (ΔP). The larger ΔP gives rise to a larger flow-rate error in eqn. 16.

The effect of the pressure dependence of viscosity on flow rate is illustrated in Fig. 1c. Once again, the smallest errors are observed for the acetonitrile-water mobile phases (-0.4 to -1.5%), but in contrast to what is seen previously, however, the largest errors are observed with tetrahydrofuran-water instead of methanol-water, although the difference is slight (-4% vs. -3.5%). The difference in the flow-rate errors for these mixed mobile phases are *not* due to differences in the pressure coefficients of viscosity for the organic solvents (they are all about the same, see Table I), but to differences in the pressures required for the same flow-rate because of the different viscosities of the mixed mobile phases (*cf.* eqns. 35-37). The different ΔP values give rise to different flow-rate errors in eqn. 23.

The effect of column expansion/packing compression on flow-rate is illustrated in Fig. 1d. As expected, the errors are considerably larger for the tetrahydrofuran-water and methanol-water mixtures than for the acetonitrile-water mixtures, since this effect depends strictly on the pressure drop which is considerably smaller for the latter. Note however, that this effect is the smallest of the four illustrated (*cf.* Fig. 1a-c), with the largest error barely exceeding 0.6% for a mobile phase of 35% tetrahydrofuran (at 3800 p.s.i.).

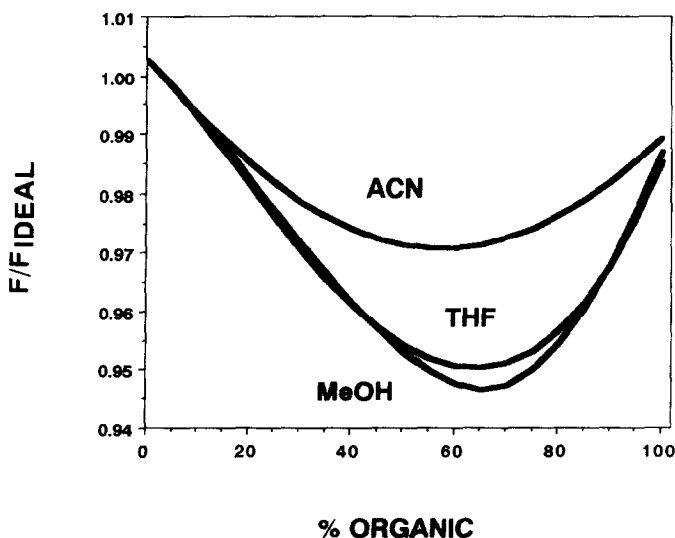


Fig. 2. Total predicted error in flow-rate due to the combined effects of the phenomena in Fig. 1. See also eqn. 29. Conditions as in Fig. 1.

The total error in flow-rate resulting from the combined effects of all phenomena as calculated via eqn. 29 is illustrated in Fig. 2. The lowest errors were obtained for pure water (+0.3%), followed by the pure organic solvents ($\approx -1.5\%$), and then the hydro-organic mixtures (-2 to -5.5%). These results were generally expected, since water is the least compressible of all the solvents employed in this study, and its viscosity is also the least dependent on the pressure. Also, for pure solvents there is no volume of mixing effect.

Given the large compressibilities and pressure coefficients of viscosity of the pure organic solvents relative to those of water and the hydro-organic mixtures, it may first seem surprising that the corresponding errors in flow-rate are not larger for the pure organic solvents than what are shown in Fig. 2. This apparent discrepancy is explained by the much lower viscosities of these pure solvents, and the compensatory effect that the resulting smaller pressure drops has on the various flow-rate effects.

Among the hydro-organic mobile phases, the total errors in flow-rate predicted for the tetrahydrofuran-water and methanol-water phases are nearly twice as large as those predicted for the acetonitrile-water mixtures. This is due primarily to two factors. First, the volume of mixing effect is much larger. Second, the "viscosity hump" observed for the tetrahydrofuran-water or methanol-water mixtures is much larger than for the acetonitrile-water mixture. As noted before, the higher viscosities translate into higher pressure drops, which in turn result in larger flow-rate errors, as seen from eqns. 16, 23 and 26.

Experimentally observed flow-rates

Fig. 3 and 4 show the experimentally measured flow-rates (open symbols) along with the corrected values (closed symbols) for a low-pressure and a high-pressure mixing HPLC system. In general, the uncorrected flow-rates in Figs. 3 and

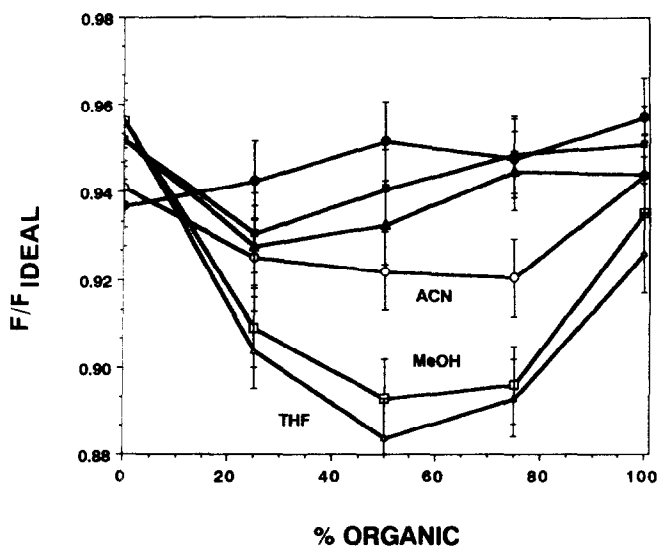


Fig. 3. Comparison of uncorrected (open symbols) and corrected (closed symbols) flow-rates for a low-pressure mixing HPLC system. Error bars represent one standard deviation. Organic solvents: methanol (\blacksquare , \square), acetonitrile (\bullet , \circ), and tetrahydrofuran (\blacktriangle , \triangle).

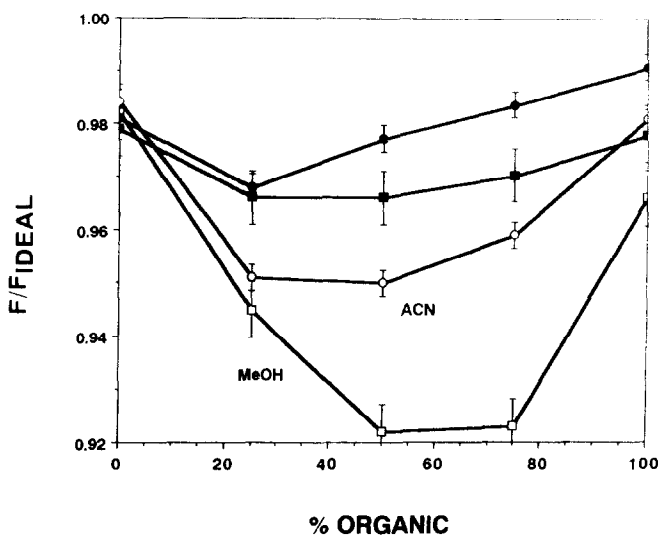


Fig. 4. Comparison of uncorrected (open symbols) and corrected (closed symbols) flow-rates for a high-pressure mixing HPLC system. Symbols as in Fig. 3.

4 correspond very closely to those predicted in Fig. 2, although they are all 2–4% more negative than predicted. Although our uncorrected experimental results in Fig. 3 for tetrahydrofuran–water and methanol–water appear to be reversed from Fig. 2 (larger error for tetrahydrofuran–water instead of slightly smaller), a closer inspection reveals that the differences are within experimental error.

The near-constancy of the corrected flow-rates (within experimental error) shown in Fig. 3 and 4 illustrates the success of our correction factor approach. As shown, the variations in flow-rate have been reduced from 8 to 2% or less, and the systematic negative deviations observed with the hydro-organic mixtures have virtually been eliminated. Unfortunately, the corrected flow-rates are still biased toward low values, indicating that either our theoretical corrections are incomplete or, more likely, that instrumental contributions to errors in flow-rate cannot be assumed to be negligible. Because we obtained different biases with the low-pressure and high-pressure HPLC systems, we are confident that the observed biases are due primarily to instrumental effects.

To date we have been unsuccessful in identifying any specific instrumental contributions to the biases observed in the flow-rates. It is difficult to model instrumental contributions due to the wide variety of pump designs employed in commercial HPLC instrumentation. Two non-instrumental effects which can be disregarded immediately, however, are (1) mobile phase evaporation and (2) inaccurate volumetric glassware. These effects can be discounted simply because they do not explain the results we observed for the corrected flow-rates. If mobile phase evaporation had been a problem, we would have expected to see a skewing of the flow-rate errors in the direction of higher organic solvent content, since all the organic solvents studied evaporate much more quickly than water. However, no such skewing is evident in the data in Fig. 3 and 4. Likewise, if inaccurate volumetric glassware had

been the problem, our gravimetric and volumetric flow-rate measurements would not have been consistent within experimental error (see Experimental section).

As shown in Figs. 3 and 4, the high-pressure mixing HPLC pumping system provided a somewhat higher flow-rate accuracy for all three binary mobile phases over the entire solvent composition range. However, one should not infer from these results that all high-pressure mixing HPLC pumping systems will always be more accurate than all low pressure mixing systems. Our results are based on the comparison of only one model/brand of each type of HPLC solvent delivery system and can only be considered tentative at best. The main point to be appreciated is that both types of HPLC systems may contribute significantly to the total error in flow rate.

Correcting for errors in flow-rate

Since the phenomena described above cannot be eliminated entirely for any combination of known mobile and stationary phases, the best that we can hope to do is to correct for these errors based on what we have predicted theoretically. Thus we propose the use of correction factors based on the theory summarized by eqn. 29. Depending on whether they are being used on a post-run or a pre-run basis, the correction factor is given by the left hand side of eqn. 29 or its reciprocal, respectively. In the *post-run* mode, the correction factor would be used to calculate the *actual-flow-rate* from the current (nominal) setting of the HPLC system, as shown in eqn. 38.

$$F_{\text{actual}} = \left(\frac{F}{F_{\text{ideal}}}_{\text{total}} \right) F_{\text{setting}} \quad (38)$$

In the *pre-run* mode, the correction factor would be used to calculate a *flow-rate setting* for the HPLC so that an accurate desired flow rate is obtained, as shown in eqn. 39. This pre-run correction factor

$$F_{\text{setting}} = \left(\frac{F_{\text{ideal}}}{F} \right)_{\text{total}} F_{\text{desired}} \quad (39)$$

approach amounts to the use of flow-rate programming to compensate for predictable flow-rate errors.

For ease of use, the correction factors can be organized into tables according to experimental conditions (pressure drops, mobile phase composition, stationary phase parameters). If done for a sufficient number of pressure drops and mobile phase compositions, correction factors for pressure/composition combinations not provided in the tables could be obtained by linear interpolation. Since for a given set of conditions the pre-run and post-run correction factors are just reciprocals of each other, only one type needs to be reported. In the tables that follow, we report *pre-run* correction factors for the convenience of those who wish to conduct constant flow-rate experiments in the future.

Since column expansivity/packing compression is independent of mobile phase composition for a given pressure drop, it is convenient to separate the correction factors arising from the stationary phase phenomenon from those resulting from the

mobile phase. This separation permits column-related correction factors to be tabulated in a concise manner for more than one ratio of inner to outer column radius. This is highly desirable since this ratio varies considerably among the plethora of commercially available HPLC columns.

Shown in Table III are the pre-run column expansion/packing compression correction factors for silica-based, stainless-steel RP-HPLC columns. A ratio of internal to external column radius ranging from 0.3 to 0.9 was assumed, corresponding roughly to 1–5 mm I.D. and a column wall thickness of about 1 mm. As shown in Table III, for a constant column wall thickness, the error in flow-rate increases exponentially with the column radius.

TABLE III
FLOW-RATE CORRECTION FACTORS (PRE-RUN) FOR COLUMN EXPANSION/PACKING COMPRESSION^a

$\frac{r_{int}}{r_{ext}}$	Pressure (<i>p.s.i.</i>)						
	100	500	1000	2000	3000	4000	5000
0.30	0.9999	0.9994	0.9989	0.9988	0.9967	0.9956	0.9945
0.50	0.9999	0.9994	0.9988	0.9986	0.9963	0.9951	0.9939
0.70	0.9998	0.9992	0.9985	0.9969	0.9954	0.9938	0.9923
0.80	0.9998	0.9990	0.9980	0.9961	0.9941	0.9922	0.9902
0.85	0.9998	0.9988	0.9976	0.9952	0.9929	0.9905	0.9882
0.90	0.9997	0.9984	0.9968	0.9936	0.9903	0.9873	0.9842

^a Conditions: stainless-steel HPLC column (Young's modulus $\approx 2 \cdot 10^6$ atm) with silica based packing ($\epsilon_c \approx 0.4$, $\beta = 2.72 \cdot 10^{-6}$ atm⁻¹. Refer to eqns. 26 and 27 in text.

Shown in Tables IV–VI are the pre-run mobile phase correction factors for methanol–water, acetonitrile–water and tetrahydrofuran–water. Each correction factor listed is the product of the individual correction factors for the (i) volume of mixing, (ii) mobile phase compressibility, and (iii) pressure-dependence of viscosity, taken from the reciprocals of eqns. 2 and 34. As mentioned earlier, errors resulting from deviations in Darcy's law are generally negligible (<0.06% in our study); for this reason they were not incorporated into our correction factors.

Other discussion

Post-run vs. pre-run correction of flow-rate errors. Whereas good accuracy can be obtained by using either pre- or post-run correction factors, a near constant flow-rate can only be achieved if flow-rate errors are corrected on a pre-run basis. For best results, we recommend the following procedure. First, measure the pressure drop under the desired conditions of mobile phase and nominal (uncorrected) flow-rate. Next, calculate a correction factor based on this observed pressure drop and adjust the flow-rate setting accordingly. If the adjustment of the flow-rate setting results in a change in pressure drop of more than 10–20%, it may be necessary to recalculate the correction factor using the new observed pressure drop and adjust the flow-rate again.

TABLE IV
FLOW-RATE CORRECTION FACTORS (PRE-RUN) FOR METHANOL-WATER^a

Methanol (%)	Pressure (p.s.i.)						
	0	500	1000	2000	3000	4000	5000
0	1.000	1.000	1.000	1.000	1.000	1.001	1.001
10	1.007	1.008	1.009	1.010	1.011	1.013	1.014
20	1.014	1.016	1.017	1.020	1.022	1.025	1.027
25	1.018	1.020	1.021	1.024	1.027	1.031	1.034
30	1.021	1.023	1.025	1.029	1.033	1.036	1.040
40	1.028	1.030	1.033	1.038	1.043	1.048	1.053
50	1.033	1.036	1.039	1.045	1.052	1.058	1.064
60	1.035	1.039	1.043	1.051	1.058	1.066	1.073
70	1.034	1.039	1.043	1.052	1.060	1.069	1.078
75	1.031	1.036	1.041	1.050	1.059	1.069	1.078
80	1.026	1.032	1.037	1.047	1.057	1.067	1.077
90	1.014	1.020	1.026	1.037	1.048	1.059	1.070
100	1.000	1.006	1.012	1.024	1.037	1.049	1.061

^a See eqns. 29 and 39. Temperature assumed to be 25°C, although these correction factors are believed to be valid over the range 20–45°C.

Possible limitations of the correction factor approach. At this time we would like to discuss several questions which may come to mind about potential shortcomings about our approach. As the reader will see, many of these “problems” are either misconceptions about our approach or are easily solved or circumvented.

(1) *Are the data needed to calculate the correction factors available or known precisely?* As mentioned in this report, the data necessary for our approach are

TABLE V
FLOW-RATE CORRECTION FACTORS (PRE-RUN) FOR ACETONITRILE-WATER^a

Acetonitrile (%)	Pressure (p.s.i.)						
	0	500	1000	2000	3000	4000	5000
0	1.000	1.000	1.000	1.000	1.000	1.001	1.001
10	1.006	1.007	1.008	1.009	1.011	1.013	1.014
20	1.012	1.013	1.015	1.018	1.020	1.023	1.026
25	1.014	1.016	1.017	1.021	1.025	1.028	1.032
30	1.015	1.018	1.020	1.024	1.029	1.033	1.038
40	1.018	1.021	1.024	1.030	1.036	1.041	1.047
50	1.019	1.023	1.026	1.034	1.041	1.048	1.056
60	1.019	1.023	1.027	1.036	1.045	1.054	1.062
70	1.016	1.022	1.027	1.037	1.047	1.057	1.068
75	1.015	1.020	1.026	1.037	1.047	1.058	1.069
80	1.013	1.019	1.024	1.036	1.047	1.059	1.071
90	1.007	1.014	1.020	1.033	1.046	1.059	1.072
100	1.000	1.007	1.015	1.029	1.043	1.057	1.072

^a Conditions as in Table IV.

TABLE VI
FLOW-RATE CORRECTION FACTORS (PRE-RUN) FOR THF-WATER^a

THF (%)	Pressure (p.s.i.)						
	0	500	1000	2000	3000	4000	5000
0	1.000	1.000	1.000	1.000	1.000	1.001	1.001
10	1.007	1.008	1.008	1.010	1.012	1.013	1.015
20	1.012	1.014	1.016	1.019	1.022	1.026	1.029
25	1.015	1.017	1.019	1.023	1.027	1.031	1.036
30	1.017	1.020	1.022	1.027	1.032	1.037	1.042
40	1.021	1.024	1.028	1.034	1.041	1.047	1.054
50	1.023	1.027	1.031	1.040	1.048	1.056	1.064
60	1.023	1.029	1.033	1.043	1.053	1.063	1.173
70	1.022	1.028	1.033	1.045	1.056	1.068	1.079
75	1.020	1.026	1.032	1.044	1.057	1.069	1.081
80	1.017	1.024	1.031	1.044	1.057	1.070	1.083
90	1.010	1.018	1.025	1.040	1.054	1.069	1.084
100	1.000	1.008	1.016	1.032	1.048	1.064	1.081

^a Conditions as in Table IV.

generally available in the literature for pure solvents, although not necessarily for mixed solvent systems. Accurate estimates of the pertinent properties of mixed mobile phases can be obtained via equations similar to eqns. 17, 24, and 35–37; alternatively, most of the data are easily measured. Although few reports prior to our study, if any, have confirmed the assumption represented by eqn. 24, our results discussed earlier (the near-constancy of the corrected flow-rates in Figs. 3 and 4) do support this assumption. For the volume of mixing phenomenon, although a well established theory or experimental data are not always available for every possible binary mobile phase, the phenomenon is easily measured as demonstrated by Katz *et al.*¹⁰. In the case of normal-phase solvents, the volume of mixing may be small enough to be neglected.

(2) *Due to the temperature dependence of the physical properties of the phenomena which affect the flow-rate, will the approach require the generation of a set of correction factors at every different temperature of interest?* Over the most widely used range of temperatures in HPLC (20–45°C), the change in the pertinent physical properties (β , α , ρ) of the mobile and stationary phase is actually only slight (generally $\leq 15\%$). Thus even for extremely accurate work, the correction factors presented in Tables III–VI (for $T = 25^\circ\text{C}$) are likely to suffice over the entire 20–45°C range. Note that although mobile phase viscosity is strongly temperature dependent, it does not directly impact any phenomenon except for deviations in Darcy's law, which as discussed earlier is negligible over this temperature interval.

For temperatures exceeding 45°C, errors in flow-rate due to the various pressure-dependent phenomena should be considerably smaller because of the lower mobile phase viscosities and corresponding smaller pressure drops. These errors may or may not be negligible relative to the errors due to non-zero volumes of mixing and deviations from Darcy's law, depending on the specific experimental conditions. As noted earlier, deviations from Darcy's law may no longer be negligible but are easily

estimated from the kinematic viscosities and eqn. 25 and are likely to be reasonably constant for a given mobile phase; volumes of mixing are easily measured.

(3) *Can the approach be used with gradient elution?* Although we have not yet examined errors in flow-rate during gradient elution, it is likely that our pre-run correction factor approach can be used successfully with gradient elution to provide a more constant flow-rate. We envision its application as follows. First, a trial of the desired gradient is run to permit the measurement of the pressure drop across the HPLC column at several intervals (different compositions of mobile phase) during the gradient. Based on the measured pressure drops and the corresponding average mobile phase (mobile phase at the midpoint of the column for a linear gradient), a series of pre-run correction factors is then calculated and incorporated into the flow-rate settings at each of the intervals. An example of this approach is shown in Table VII.

TABLE VII

EXAMPLE OF USE OF PRE-RUN CORRECTION FACTORS TO ACHIEVE A CONSTANT FLOW-RATE OF 1.50 ml/min DURING REVERSED-PHASE GRADIENT ELUTION WITH METHANOL-WATER

Time (min) ^a	Methanol (%) ^b	Pre-run correction factors			
		ΔP (p.s.i.) ^c	Table III	Table IV	Flow-rate setting ^d
0	20	3245	0.9936	1.023	1.560
2	30	3570	0.9930	1.035	1.577
4	40	3705	0.9928	1.047	1.595
6	50	3670	0.9928	1.054	1.606
8	60	3465	0.9932	1.062	1.619
10	70	3110	0.9939	1.061	1.618
12	80	2610	0.9949	1.053	1.608
14	90	1980	0.9961	1.037	1.585
16	100	1620	0.9968	1.019	1.559

^a Retention time for an unretained solute assumed to be 1 min.

^b Arbitrary, but typical gradient of 5%/min.

^c In this example, ΔP was calculated as discussed in the Experimental section, assuming a value of 2000 p.s.i. at 0% methanol. In practice, ΔP would be measured at each solvent composition as discussed in the Results and Discussion section.

^d Product of the pre-run correction factors from Table III, Table IV, and an instrumental correction factor arbitrarily assumed to be 1.023 in this example. Correction factors from Tables III and IV obtained by interpolation.

An operationally simpler approximation to this procedure for correction which may be sufficiently accurate for gradient elution flow-rates is (1) to estimate the changes in pressure drops of the mobile phase gradient from known changes in viscosity (for temperatures $\approx 25^\circ\text{C}$, eqns. 35–37 can be used) and (2) to assume that the average mobile phase experienced by the column at any time is equivalent to the mobile phase composition at the gradient mixer at that same instant. The latter assumption is reasonable provided the gradient delay volume is small and/or the gradient is shallow.

(4) *Can the approach be used with ternary mobile phases?* Although in principle the approach we have developed for binary mobile phases could be adapted to ternary solvent systems, it would admittedly be somewhat less practical, since it would require a matrix of mobile phase correction factor tables (10 or so) organized according to the pressure drop and the percentage of the second and third solvents instead of just one mobile phase correction factor table like those of Tables IV–VI.

(5) *Is the instrumental contribution to flow-rate error accounted for explicitly by our approach?* It is difficult if not impossible to develop a general model for instrumental contributions to flow-rate errors because of the wide variety of pump designs employed in commercial HPLC instrumentation. Presumably, however, the instrumental contribution is the error remaining in the corrected flow-rates, since as shown in Figs. 3 and 4 this error is essentially constant and more or less independent of the organic solvent. Thus although our approach does not allow the instrumental contribution to be predicted from theory, it does provide a means for measuring it experimentally.

(6) *Will similar errors in flow-rate be observed using HPLC equipment other than that of the present study?* Probably. The mobile and stationary phase contributions to flow-rate error are by definition HPLC-independent, and since they account for roughly half of the total error in flow-rate, we would expect at least moderately similar results regardless of the equipment used. A more definitive answer requires knowledge of the instrumental contributions of *all* HPLC systems, which as noted above in (5) is impossible to predict and is clearly beyond the scope of the present study. We recognize that more HPLC systems need to be examined, and we encourage others to report results for their HPLC systems using our approach (we are also planning additional studies). It is conceivable that, for some systems not examined in the present study, the instrumental contribution will not be constant but will depend on the mobile phase composition.

If the instrumental contribution turns out to be similar for all HPLC systems, then of course errors in flow-rate for all HPLC systems will be very similar. If not, flow-rate errors may vary somewhat from instrument to instrument, depending on the relative contributions from the solvent delivery system and the mobile and stationary phases. Certainly a desirable goal for HPLC manufacturers would be the design of new solvent delivery systems or modification of existing ones so that, if used properly, they contribute negligibly to errors in flow-rate.

CONCLUSION

The theory of Katz *et al.*¹⁰ and Martin *et al.*⁶ was adapted to explain the majority of the errors in flow-rate commonly observed (but typically unreported) in RP-HPLC for the three most popular binary mobile phases: methanol–water, acetonitrile–water and tetrahydrofuran–water. Although all three hydro–organic mobile phases give rise to significant errors in flow-rate, the acetonitrile–water mobile phases typically result in distinctly lower errors (2–3%) than the other binary mobile phases (3–5%) and are generally to be preferred if flow-rate accuracy is somewhat important but, for whatever reason, the corrections described here cannot be applied. Instrumental contributions to flow-rate errors cannot be predicted from theory but can be measured experimentally. Our method can, in principle, be applied to any binary mobile phase

(including normal-phase solvent systems) to reduce flow-rate errors during isocratic and gradient elution either before (pre-run) or after (post-run) the experiments are performed. Use of our approach will decrease flow-rate errors by ten-fold or more, particularly if the instrumental contribution are small or are measured as we have suggested and then factored out. If flow-rate settings are adjusted prior to experiments using pre-run correction factors (eqn. 39) interpolated from those we reported in Tables III–VI and any error resulting from instrumental contributions is also factored out, a virtually constant and error-free flow-rate ($\pm 0.5\%$) will be obtained. The resulting improvements in flow-rate will be useful to two groups of people: (1) HPLC manufacturers and users for quality control and troubleshooting of these solvent delivery systems, and (2) researchers who need very accurate retention data for physicochemical measurements.

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