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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC THEORY FOR THE PRACTITIONER

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1 INTRODUCTION

The theory of high-performance liquid chromatography (HPLC) was developed before and during the development of the method. Although concise descriptions of this theory can be found in all HPLC books, *e.g.*, refs. 1–4, it seems that laboratory technicians and in some cases even analytical chemists have little knowledge of it other than the formula for calculation of the plate number. This is very regrettable because careful application of the practical aspects of HPLC theory in routine laboratory work could be fruitful and satisfying. As will be shown in this text, the calculations needed are quite simple.

The question to be answered in this paper is: What kind of useful information can be calculated beginning with the geometrical properties of the HPLC column at our disposal? As the basis of the calculations, no more than three parameters, namely the length of the column, its inner diameter and the particle diameter of the stationary phase, will be employed. To illustrate the formulae given, values for these parameters were chosen to be 25 cm, 4.6 mm and 5 μm, respectively, corresponding to a very common type of HPLC column. The key to the calculations is the concept of reduced (dimensionless) parameters as introduced by Bristow and Knox in 1977[5]. Two of the four reduced parameters are of fundamental importance in this connection. They are the reduced plate height and the reduced velocity.

The reduced plate height is defined as $h = H/d_p$ where H is the height of a theoretical plate and d_p is the particle diameter of the stationary phase. The value of h indicates how many layers of particles of the stationary phase are needed to yield one theoretical plate. The smaller this value the better is the column. For excellently packed columns the reduced plate height reaches a value of 2. Smaller values seem not to be possible. On the other hand, liquid chromatography columns with $h = 10$ do not merit the term "high performance".

The reduced velocity is defined as $v = ud_p/D_m$, where u is the linear flow velocity of the mobile phase, d_p the particle diameter and D_m the diffusion coefficient of the solute of interest in the mobile phase. The great importance of v lies in the fact that the optimum velocity of the mobile phase in liquid chromatography is known to occur at $v = 3$. If the molecular mass and the density of the solute are known or estimable, its diffusion coefficient in a given mobile phase can be calculated by use of the Wilke–Chang equation[6]. Therefore, with all necessary parameters known, the optimum velocity of the eluent can easily be calculated. (Of course, the optimum velocity can also be determined experimentally.) At this velocity a liquid chromatography column performs at its best, *i.e.*, the plate height has the minimum value possible with the given stationary phase.

Any discussion on chromatographic performance gains clarity and simplicity if reduced parameters are used because the considerations are set free from external factors as there are the dimensions of the column and stationary phase. On the other hand, only with reduced parameters is it possible to predict the performance of a chromatographic column and to estimate such interesting parameters as the maximum allowed injection volume and many others. This paper will show how to do this.

In the course of the calculations we have to choose other parameters in addition to those mentioned above. They are the porosity of the column packing, the

diffusion coefficient of the solute, the capacity factor of the solute, the fraction of the allowed increase in peak width, the inner diameter of the connecting capillaries, the concentration of the solute injected, the molar mass and the molar absorption coefficient of the solute, the path length of the detector, the viscosity of the mobile phase and the flow resistance of the column. All these parameters will be accorded values typically found in liquid chromatography.

All the equations are given without derivations, which can be found in the literature cited. However, the reader should be familiar with the basic theory of liquid chromatography

2 CALCULATIONS

2.1. Plate number

Consider an HPLC column of length, l_c = 25 cm, inner diameter, d_c = 4.6 mm and particle diameter of the stationary phase, d_p = 5 μm. The number of theoretical plates, N, can be calculated by assuming that the column is very well (not excellently) packed, *i.e.*, h = 2.5:

$$N = \frac{l_c}{h d_p} = \frac{250\,000}{2.5 \times 5} = 20\,000 \tag{1}$$

2.2 Dead volume

The total porosity, ε_{tot}, is about 0.8 for all column packings of totally porous particles. Thus, the dead volume, V_0, can be obtained from:

$$V_0 = \frac{d_c^2 \pi}{4} \cdot l_c \varepsilon_{tot} = \frac{4.6^2 \pi}{4} \cdot 250 \times 0.8 \text{ mm}^3 = 3320 \text{ mm}^3 = 3.3 \text{ ml} \tag{2}$$

2.3. Flow velocity and flow-rate

The column is regarded as being used at its optimum flow which corresponds to v_{opt} = 3. For the calculation of the matching flow velocity of the mobile phase, a mean diffusion coefficient, D_m, of the solute molecules in the mobile phase of 1×10^{-9} m^2 s^{-1} is assumed Thus the linear flow velocity, u, and the flow-rate, v', of the mobile phase are given by·

$$u = \frac{v D_m}{d_p} = \frac{3 \times 1 \times 10^{-9}}{5 \times 10^{-6}} \text{ m s}^{-1} = 0.6 \times 10^{-3} \text{ m s}^{-1} = 0.6 \text{ mm s}^{-1} \tag{3}$$

$$v' = \frac{u d_c^2 \pi \varepsilon_{tot}}{4} = \frac{0.6 \times 4.6^2 \times \pi \times 0.8}{4} \text{ mm}^3 \text{ s}^{-1} = 8.0 \text{ mm}^3 \text{ s}^{-1}$$

$$= 0.48 \text{ ml min}^{-1} \tag{4}$$

2.4. Retention time

The retention time of an unretained solute, t_0, is:

$$t_0 = \frac{l_c}{u} = \frac{250}{0.6} \text{ s} = 417 \text{ s} \approx 7 \text{ min} \tag{5}$$

For two retained solutes having capacity factors, $k' = 1$ and $k' = 10$:

$$t_R = k' \times t_0 + t_0 = 1 \times 7 + 7 \text{ min} = 14 \text{ min}$$
$$t_R = 10 \times 7 + 7 \text{ min} = 77 \text{ min} \quad (6)$$

2.5. Retention volume

The volumes of mobile phase, V_R, necessary to elute peaks of either $k' = 1$ or $k' = 10$ are:

$$V_R = V' \times t_R = 0.48 \times 14 \text{ ml} = 6.7 \text{ ml}$$
$$V_R = 0.48 \times 77 \text{ ml} = 37 \text{ ml} \quad (7)$$

2.6. Peak capacity

The peak capacity, n, of the column if $k' = 10$ is not exceeded is:

$$n = 1 + \frac{\sqrt{N}}{4} \cdot \ln (1 + k'_{max}) = 1 + \frac{\sqrt{20\,000}}{4} \ln (1 + 10) = 86 \quad (8)$$

2.7. Peak width and peak volume

If the first peak has $k' = 1$, its base width, 4σ, and its volume, V_p, are:

$$4\sigma = 4 \frac{t_R}{\sqrt{N}} = 4 \frac{14 \times 60}{\sqrt{20\,000}} \text{ s} = 23.8 \text{ s} \quad (9)$$

$$V_p = 4\sigma \times V' = 23.8 \times 8 \ \mu\text{l} = 190 \ \mu\text{l} \quad (9a)$$

2.8. Injection volume

The maximum allowed injection volume, V_i, to avoid an excessive broadening of the first peak is defined as:

$$V_i = \theta V_R \cdot \frac{K}{\sqrt{N}} \quad (10)$$

where K is a parameter characteristic of the quality of injection and is assumed to be equal to 2 and θ^2 defines the fraction of peak broadening. Thus at 1% peak broadening, *i.e.*, $\theta^2 = 0.01$ and $\theta = 0.1$

$$V_i = 0.1 \times 6700 \cdot \frac{2}{\sqrt{20\,000}} \ \mu\text{l} = 9.5 \ \mu\text{l}$$

At 9% peak broadening, *i.e.*, $\theta^2 = 0.09$ and $\theta = 0.3$:

$$V_i = 0.3 \times 6700 \ \frac{2}{\sqrt{20\,000}} \ \mu\text{l} = 28 \ \mu\text{l}$$

2.9. Detector volume

The maximum allowed volume of the detector cell, V_d, is defined as:

$$V_d = \frac{\theta V_R}{\sqrt{N}} = \frac{V_1}{2} \tag{11}$$

Thus, at 1% peak broadening, $V_d = 9\,5/2\ \mu l \approx 5\ \mu l$ and at 9% peak broadening, $V_d = 28/2\ \mu l = 14\ \mu l$.

2.10. Detector time constant

The maximum allowed time constant of the detector, τ, is defined as:

$$\tau = \theta \cdot \frac{t_R}{\sqrt{N}} \tag{12}$$

At 1% peak broadening:

$$\tau = 0.1\ \frac{14 \times 60}{\sqrt{20\,000}}\ s = 0.6\ s$$

At 9% peak broadening:

$$\tau = 0.3 \cdot \frac{14 \times 60}{\sqrt{20\,000}}\ s = 1.8\ s$$

2.11. Capillary tube length

In most HPLC instruments, capillary tubing is used to connect the injector, column and detector. An inner diameter of the capillary, d_{cap}, of 0.25 mm is usual. The maximum allowed length, l_{cap}, of such a capillary is defined as.

$$l_{cap} = \frac{384\ \theta^2 D_m V_R^2}{\pi N V' d_{cap}^4} \tag{13}$$

At 1% peak broadening:

$$l_{cap} = \frac{384 \times 0.01 \times 10^{-5} \times 6.7^2}{\pi \times 20\,000 \times 8 \times 10^{-3} \times 0.025^4}\ cm = 8.8\ cm$$

At 9% peak broadening:

$$l_{cap} = \frac{384 \times 0.09 \times 10^{-5} \times 6.7^2}{\pi \times 20\,000 \times 8 \times 10^{-3} \times 0.025^4}\ cm = 79\ cm$$

2.12. Dilution factor

Due to the chromatographic process, the injected solutes become diluted, *i.e.*,

the concentration of the solute in the peak maximum, c_p, is lower than that in the injected solution, c_i. The dilution factor is defined as:

$$\frac{c_i}{c_p} = \frac{V_R}{V_i}\sqrt{\frac{2\pi}{N}} \tag{14}$$

Thus, if 9% peak broadening is allowed, for $k' = 1$

$$\frac{c_i}{c_p} = \frac{6700}{28}\sqrt{\frac{2\pi}{20\,000}} = 4.2$$

and for $k' = 10$

$$\frac{c_i}{c_p} = \frac{37\,000}{28}\sqrt{\frac{2\pi}{20\,000}} = 23$$

2.13. Concentration at peak maximum

If the concentration of each of the solutes in the sample is 1 ppm (10^{-6} g ml^{-1}), their concentrations at the respective peak maxima are, for $k' = 1$

$$c_p = c_i \cdot \frac{c_p}{c_i} = 10^{-6} \cdot \frac{1}{4.2}\ \text{g ml}^{-1} = 0.24 \times 10^{-6}\ \text{g ml}^{-1} \tag{15}$$

and for $k' = 10$

$$c_p = 10^{-6}\ \frac{1}{23}\ \text{g ml}^{-1} = 0.043 \times 10^{-6}\ \text{g ml}^{-1}$$

2.14. UV detector signal

The second component is nitrobenzene with a molar absorption coefficient, $\varepsilon = 10^4$ (254 nm), and a molar mass of 123 g mol^{-1}. The signal, E, which will result in the UV detector can be calculated with the Lambert–Beer law, $E = \varepsilon cd$, where c is the concentration in mol l^{-1} and d the path length in cm. Thus, if $c_p = 0.043 \times 10^{-6}$ g $ml^{-1} = 0.35 \times 10^{-6}$ mol l^{-1} and $d = 1$ cm (as is the case for many UV detectors), we obtain:

$$E = 10^4 \times 0.35 \times 10^{-6} \times 1 = 3.5 \times 10^{-3}\ \text{absorption units (a.u.)} \tag{16}$$

2.15. Detection limit

A common definition of the detection limit is a signal of four times (sometimes also twice) the noise level of the detector. The typical noise level of a UV detector is

10^{-5} a.u. This means a detection limit of 4×10^{-4} a.u. which is about one order of magnitude lower than the signal calculated in eqn. 16. Since the injected solution had a concentration of 1 ppm, the detection limit is about 0.1 ppm or, when related to the injection volume of 28 μl, 2.8 ng

2.16. Pressure drop

What pressure drop, Δp, will result if the given column is used at v_{opt} which corresponds to a mobile phase flow-rate of $V' = 0.48$ ml min^{-1}? Of course, the pressure depends on the viscosity, η, of the mobile phase used. Let us assume $\eta = 1$ mPa s, the value for water or mixtures of water and acetonitrile. The pressure drop can be calculated by use of the dimensionless flow resistance parameter, φ:

$$\Delta p = \frac{\varphi l_c \eta u}{d_p^2} = \frac{\varphi \times l_c \text{ (mm)} \times \eta \text{ (mPa s)} \times u \text{ (mm s}^{-1}\text{)}}{100 \times d_p^2 \text{ } (\mu\text{m}^2)} \text{ bar} \quad (17)$$

Assuming $\varphi = 500$ for slurry-packed spherical totally porous stationary phases used in HPLC:

$$\Delta p = \frac{500 \times 250 \times 1 \times 0.6}{100 \times 5^2} \text{ bar} = 30 \text{ bar}$$

3 DISCUSSION

3.1. Eqn. 1

The number of theoretical plates is the first parameter out of three in the calculations described which results from the application of reduced parameters[5]. The other two are the linear flow velocity in eqn. 3 and the pressure drop in eqn. 17. The value for N of 20 000 is rather high and in fact is seldom reached by 25-cm HPLC columns ($d_p = 5$ μm) used routinely. A value of 10 000 or 15 000 is, unfortunately, more realistic.

3.2. Eqn. 2

The so-called dead volume is the elution volume of an unretained solute. It should be mentioned that the value of V_0 would be reduced by half if a column of 3.2 mm I.D. were to be used instead of one of 4.6 mm I.D. since V_0 is proportional to d_c^2. From this point of view it is not clear why the 4.6 column is so popular; one of 3.2 mm would enable a saving in solvent consumption of 50%!

3 3 Eqns. 3 and 4

The linear flow velocity is obtained by rearrangement of the equation which defines the reduced velocity[5]. It allows the calculation of the flow-rate by simple geometrical considerations. However, in all calculations of flow-rates from velocities or *vice versa* it must not be forgotten that the chromatographic column is not empty but a rather small fraction of it is occupied by the framework of the stationary phase; this fact is represented in eqn. 4 (as well as in eqn. 2) by the term ε_{tot}.

The diffusion coefficient, which needs to be known for the calculation of the linear flow velocity, can be obtained from the Wilke–Chang equation[6]

$$D_m\ (m^2\ s^{-1}) = \frac{7.4 \times 10^{-12} \sqrt{\psi M} T}{\eta \times V_s^{0.6}} \tag{18}$$

where ψ = a solvent constant (2.6 for water, 1.9 for methanol, 1.5 for ethanol, 1.0 for other solvents), M = the molecular mass of the solvent, T = the absolute temperature, η = the solvent viscosity in mPa s and V_S = the molar volume of the solute in $cm^3\ mol^{-1}$. In eqn. 3 the diffusion coefficient was assumed to be $1 \times 10^{-9}\ m^2\ s^{-1}$. The value is often higher in adsorption chromatography due to the use of non-viscous organic solvents, *i e*, the optimum flow velocity is higher. On the other hand, the diffusion coefficients of solutes in the methanol–water mixtures often used for reversed-phase chromatography are smaller due to the rather high viscosity of these eluents[4,5]. Although this effect is of minor practical relevance it should be kept in mind. Moreover, it is of great importance in the chromatography of macromolecules which have very small diffusion coefficients which result from their large molar volumes This means that large molecules must be chromatographed at low flow velocities[4]

Chromatography at higher flow-rates than the optimum one results in a decrease in the separation ability of the column. For well packed microparticulate HPLC columns this effect is nearly negligible if the reduced velocity is less than 10.

3.4. Eqns. 5 and 6

The retention times which result from chromatography at the optimum flow-rate are rather high Note that all retention times are independent of the inner diameter of the column if the analysis is performed at a given reduced velocity. At a higher flow-rate the peak widths of all peaks, as calculated in eqn. 9, will diminish, a fact which influences the value obtained from eqn. 12. if the flow-rate of the mobile phase is increased, the time constant of the detector has to be decreased. All values obtained by use of the following equations, except 9 and 12, are independent of the flow-rate and the retention time.

3.5. Eqn. 7

As already mentioned in the discussion of eqn. 2, the retention volume, V_R, of a peak is proportional to the square of the column diameter! If the first eluted peak has $k' = 0$, *i.e.*, it is a non-retained solute, its retention volume is half the value calculated for $k' = 1$: only 3.3 ml (the dead volume) In this case the values obtained from eqns. 10, 11, 13 and 14 would be decreased!

3.6. Eqn. 8

The peak capacity indicates the number of peaks, all eluted with the same plate number, *i.e.*, by isocratic elution, which could be placed in the chromatogram side by side (with resolution 1) from $k' = 1$ to the given capacity factor; in the example shown, to $k' = 10$. The equation was first published in this form by Grushka[7].

3 7. Eqns. 9 and 9a

Eqn. 9 is a rearrangement of the well known relationship which allows calculation of the plate number of a column if the peak width and retention time are known: $N = 16(t_R/4\sigma)^2$. As already mentioned above, the peak width in seconds (eqn. 9) is influenced by the mobile phase flow-rate, whilst the peak width in microlitres (the peak volume, eqn. 9a) is not if we assume that the decrease in plate number by a flow-rate other than the optimum one is negligible.

3.8. Eqn 10

This relationship was first derived by Martin *et al.*[8] in 1975. Whilst the retention volume, V_R, and the plate number, N, are known and the fraction of peak broadening, θ, can be assumed, the quality parameter of the injection, K, depends on the particular circumstances. It was claimed to be about 2 by Karger *et al.*[9] who also gave a method for its determination. Clearly, the better the injection, the higher is K and the allowed injection volume Moreover, the maximum injection volume depends on the retention volume of the solute; therefore, for capillary columns with their extremely small retention volumes (since V_R decreases with the square of the capillary diameter), the allowed injection volume has values in the nanolitre range, making trace analysis impossible[10].

There is one way to avoid the limitations in injection volume. If the sample solvent is markedly weaker than the mobile phase for the HPLC analysis, *i.e.*, if its elution strength is low the solutes are concentrated at the top of the column. In this case the injection volume may be unusually high, in the range of millilitres or even litres[11,12]. However, a prerequisite is that the amount of solute is small enough to prevent the adsorption isotherm from becoming non-linear

For the calculation of the injection volume, a rule of thumb was proposed by Huber[13]: the maximum injection volume should be equal to the volume standard deviation, σ, of the peak of interest, *i.e.*, to 1/4 of its volume as calculated by eqn. 9a Since V_p was calculated to be 190 μl, the injection volume should be 47 μl. This is markedly higher than the 28 μl calculated with eqn. 10 and a peak broadening fraction of 9% Thus Huber's recommendation has to be applied with caution.

At this point the attention of the reader is drawn to a fact whose importance will be apparent later in the discussion of the detection limit (eqns. 14–16). If for the calculation of the injection volume in eqn. 10 the retention volume (37 ml) of the second peak with $k' = 10$ is used, V_i is found to be much higher· 157 μl may be injected without broadening of the second peak by more than 9%. Of course the first peak would be broadened dramatically but one can imagine situations where the early eluted peaks are not of interest.

3 9. Eqn. 11

This equation was also reported by Martin *et al.*[8]. The relationship $V_d = V_i/2$ is only true if K is assumed to be 2. It is obvious that a standard 8-μl detector cell causes a peak broadening of more than 1% for the early eluted peaks on a 25 cm $\times$ 4.6 mm column.

3 10. Eqn. 12

This equation was also reported by Martin *et al.*[8]. The time constant of a

detector should not exceed 0.5 s if used with columns of 25 cm in length, a requirement which is satisfied by all modern HPLC detectors. However, if the columns are as short as 10 or 5 cm, a time constant of 0.5 s is too high. As already mentioned at the discussion of eqns. 5 and 6, the time constant must be decreased if the reduced velocity of the mobile phase is increased.

3.11. Eqn. 13

The maximum allowed capillary length may be calculated by this equation of Martin *et al* [8] or by use of a similar equation of Scott and Kucera[14]. It is important to realize that the capillary length depends on the square of the retention volume of the peak of interest and on the inverse fourth power of the inner diameter of the capillary. In practice, the inner diameter of connecting tubes should not exceed 0.25 mm; they should be as short as possible.

3.12. Eqns. 14 and 15

The important eqn 14 was derived by Karger *et al.*[9]. Of course the later eluted peak is diluted more than the early eluted one. However, if only the peak with $k' = 10$ is important, the injection volume can be increased to 157 μl as shown in the discussion of eqn. 10 In this case the dilution factor of this second peak is as small as that for the first peak with an injection volume of 28 μl, namely 4.2. Therefore, the peak maximum concentrations will be the same in both cases, 0.24×10^{-6} g ml^{-1}. Note that this is only true if the respective maximum allowed injection volume is used for both peaks

The validity of these conclusions can also be shown by combination of eqns. 10 and 14:

$$c_p = c_i \cdot \frac{\theta K}{\sqrt{2\pi}} \tag{19}$$

Eqn. 19 can also be found in the paper by Karger *et al.*[9]. It means that, contrary to what is often claimed, the peak maximum concentration (and thus the minimum detectable concentration) does not depend on the column inner diameter, the capacity factor of the peak, *i e* , its retention volume or the plate number of the column if, as already mentioned, the injection volume is as high as allowed by the peak of interest. Besides the two concentrations c_p and c_i, eqn. 19 only involves constants. Therefore, if $\theta = 0.1$ (1% peak broadening), $c_p = 0.08\ c_i$, and if $\theta = 0.3$ (9% peak broadening), $c_p = 0\ 24\ c_i$ (assuming $K = 2$).

3.13. Eqn. 16 and the detection limit

In the case of the detection limit we have to distinguish between the minimum detectable concentration and the minimum detectable mass. The minimum detectable concentration at the peak maximum of a given solute only depends on the properties of the detector (for concentration-dependent detectors such as UV detectors) as can easily be shown. Rearrangement of eqn. 16, the Lambert–Beer law, gives:

$$c_p = E/\varepsilon d$$

With a typical UV detector noise of 1×10^{-4} a.u., the minimum detectable signal,

E, becomes 4×10^{-4} a.u. For nitrobenzene, ε is 10^4 (at 254 nm) and d is 1 cm for many UV detectors. Therefore c_p becomes 4×10^{-8} mol l^{-1} or 4.9×10^{-6} g l^{-1}. This is the lowest peak concentration of nitrobenzene which can be detected, independent of the retention volume.

What is the minimum concentration of nitrobenzene in the sample solution in order to obtain a detectable peak? If we inject the maximum allowed sample volume as calculated from eqn. 10, eqn. 19 is valid and we can rearrange it:

$$c_i = \frac{c_p \sqrt{2\pi}}{\theta K} \tag{19a}$$

With $\theta = 0.3$ (9% peak broadening) and $K = 2$, $c_i = 4.2\, c_p$. For nitrobenzene, the minimum detectable peak concentration was calculated above to be 4.9×10^{-6} g l^{-1}. Therefore the minimum detectable concentration of nitrobenzene in the sample is 21×10^{-6} g l^{-1} (0.021 ppm). This is only true if the maximum allowed sample volume is injected and is independent of the capacity factor of the peak.

It may be recalled that a detection limit for nitrobenzene of 0.1 ppm was calculated (page 203). This is five times higher than possible because the injection volume of 28 μl was adjusted to the first peak with $k' = 1$ and not to the nitrobenzene peak with $k' = 10$. As already mentioned, the maximum injection volume of the second peak is 157 μl, about five times higher than that of the first peak.

To summarize, the minimum detectable concentration of a solute in the sample solution depends only on the detector properties and on the optical properties of the solute, *i.e.*, its absorbance, if the maximum tolerable sample volume with respect to the retention volume of this solute is injected The minimum detectable concentration is independent of the column dimensions, plate number or capacity factor.

In contrast to the concentration, the minimum detectable mass depends on the capacity factor of the solute. The earlier a peak is eluted, the smaller is the maximum injection volume and, with the concentration of the sample solution being constant, the smaller is the absolute mass of solute injected. The same is true if the column inner diameter is reduced.

For trace analysis, this means that small bore columns should be used and low capacity factors are advantageous if the sample volume is limited, as is often the case in clinical or forensic chemistry. If enough sample is available, *e.g.*, in food analysis, it is not necessary to use small bore columns and low capacity factors. However, the analysis time, solvent consumption and column overload by accompanying substances (not discussed here) need to be kept in mind.

3.14. Eqn. 17

The calculation of the pressure drop expected is possible by use of the reduced flow resistance parameter, φ, as defined by Bristow and Knox[5], φ is about 500 for spherical and less than 1000 for irregular, slurry-packed HPLC materials. The pressure drop is markedly lower if organic solvents of low viscosity, *e.g.*, hexane, are used and it is higher for mixtures of methanol and water, the viscosities of which can reach 1.8 mPa s[4,5].

It may be of interest that the fourth reduced parameter according to Bristow

and Knox[5], besides the reduced plate height, h, the reduced velocity, v, and the flow resistance, φ, is the so-called separation impedance, E, which is defined as $E = h^2\varphi$. For the column discussed in this paper, E has the excellent value of 3125 ($2.5^2 \times 500$). The higher is E the poorer is the column.

4 CONCLUSIONS

It is obvious that the calculations described are interesting and moreover are useful for routine laboratory work They allow one to recognize some of the instrumental and chromatographic limitations and benefits of HPLC. However, the basic problem of chromatography is not solved with these considerations: What physicochemical conditions are needed to make possible the separation of the different types of molecules present in the sample mixture?

The different parameters which allow such a separation are to be found in the resolution equation, which is the basic equation of liquid chromatography·

$$R_S = \frac{1}{4}\left(\frac{\alpha - 1}{\alpha}\right)\sqrt{N}\left(\frac{k'}{1 + k'}\right) \tag{20}$$

In this equation there are only three parameters. Two of them, the plate number, N, and the capacity factor, k', have been met in the calculations described. In principle, they can be altered at will. The plate number can always be adjusted by using a shorter column or by coupling two or more columns, the capacity factor can be adjusted by using a mobile phase of greater or lower elution strength. In contrast, the relative retention, α, which represents the selectivity parameter, depends only on the physico-chemical properties of the separation system used, such as adsorption, hydrophobic or ion-exchange equilibria. Often these phenomena are not easy to understand and the chemist has to search for the best separation system by trial and error. Finding systems of high selectivity is the fine art of chromatography and the use of calculations such as those presented here is no more than an aid to this end

5 SUMMARY

Starting from the basic geometrical properties of a column used in high-performance liquid chromatography, it is shown how many data of practical interest can be calculated using the well known theory of liquid chromatography. The basic geometrical properties employed are the column length, column diameter and particle diameter of the stationary phase. Among the calculable data are the plate number of the column, the volume of a chromatographic peak, the maximum allowed injection volume and the dilution of the sample by the chromatographic process All calculations are illustrated with numerical examples Special attention is given to the problem of the injection volume and detection limit in trace analysis.

REFERENCES

1 L. R Snyder and J J Kirkland, *Introduction to Modern Liquid Chromatography*, Wiley, New York, 2nd ed , 1979

2 P. A Bristow, *Liquid Chromatography in Practice*, hetp, Wilmslow, 1977
3 J H Knox, J. N Done, A F Fell, M T. Gilbert, A Pryde and R A Wall, *High-Performance Liquid Chromatography*, Edinburgh University Press, Edinburgh, 1978
4 V Meyer, *Praxis der Hochleistungs-Flussigchromatographie*, 3 Auflage, Sauerländer, Aarau, 1984.
5 P A Bristow and J H Knox, *Chromatographia*, 10 (1977) 279
6 C R. Wilke and P Chang, *Am Inst Chem Eng J*, 1 (1955) 264
7 E. Grushka, *Anal Chem*, 42 (1970) 1142.
8 M Martin, C Eon and G Guiochon, *J. Chromatogr*, 108 (1975) 229
9 B. L Karger, M Martin and G Guiochon, *Anal Chem.*, 46 (1974) 1640
10 F Berthou, *Analusis*, 12 (1984) 3
11 J F. K Huber and R R Becker, *J Chromatogr*, 142 (1977) 765
12 C E Werkhoven-Goewie, U A. Th Brinkman and R. W Frei, *Anal Chem*, 53 (1981) 2072
13 J F K Huber, *Fresenius' Z Anal Chem*, 277 (1975) 341
14 R P W Scott and P Kucera, *J Chromatogr Sci*, 9 (1971) 641