Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

Optimum Conditions in Preparative Liquid Chromatography. II. Selection of Column Dimensions

Klaus-Peter Hupe[a]; Bernd Hoffmann[a]
[a] Waldbronn Analytical Division, Hewlett-Packard, Waldbronn, West Germany

To cite this Article Hupe, Klaus-Peter and Hoffmann, Bernd(1987) 'Optimum Conditions in Preparative Liquid Chromatography. II. Selection of Column Dimensions', Separation Science and Technology, 22: 8, 1869 — 1880
To link to this Article: DOI: 10.1080/01496398708057617
URL: http://dx.doi.org/10.1080/01496398708057617

SEPARATION SCIENCE AND TECHNOLOGY, 22(8-10), pp. 1869-1880, 1987

Optimum Conditions in Preparative Liquid Chromatography. II. Selection of Column Dimensions

KLAUS-PETER HUPE and BERND HOFFMANN

WALDBRONN ANALYTICAL DIVISION
HEWLETT-PACKARD GmbH
D 7517 WALDBRONN, WEST GERMANY

INTRODUCTION

The amount of substance that can be separated per unit of time (the production rate) and the purity of the isolated compounds are the determining quantities in preparative chromatography. The production rate in combination with a certain resolution (i.e., a certain selectivity and a certain number of theoretical plates) are therefore the overruling optimization criteria for the design and operation of a preparative chromatographic system.

During recent years the preparative separation of biopolymers like proteins, peptides, and polynucleotides has gained ever-increasing importance. These molecules, on contact with the stationary phase, tend to become irreversibly absorbed and/or denatured, which affects their recovery from the column. In such cases it is therefore of great importance to design the system so that, while maintaining the above-mentioned criteria, the separated substances come in contact with the least possible amount of stationary phase and "see" the stationary phase for the least possible time. Since solvents are always a source of impurities, it is also desirable that the isolated compounds are contained in the least possible amount of mobile phase; therefore, the conditions have to be chosen so that the sample on its way through the column is diluted to the least possible degree.

Table 1 summarizes the various optimization criteria. The theoretical treatment will show that with respect to the column dimensions, all criteria have their optima for the same values.

TABLE 1
Optimization Criteria for Preparative Separations[a]

Time (production rate)	G_{inj}/t_R	mg/h
Solvent consumption	G_{inj}/V_R	mg/mL
Dilution of sample	G_{inj}/σ_{Vtot}	mg/mL
Amount of stationary phase	G_{inj}/V_0	mg/mL

[a] G_{inj} = amount of sample.
t_R = retention time.
V_R = retention volume.
σ_{Vtot} = sample dispersion.
V_0 = column volume.

THEORY

For the determination of the column dimensions (length and diameter) it is assumed that all other chromatographic conditions have been defined on an analytical level and that for the given problem a column can be specified so that an infinitesimally small injection volume just generates sufficient resolution to separate the most critical pair of components. Such a column then has just the number of theoretical plates required for that separation. In the further course of these considerations, all parameters other than column length and diameter will be kept constant. This does not mean that preparative separations should always be carried out under identical conditions as analytical separations. These, for various reasons, may differ from each other substantially, as discussed elsewhere (*1*).

Figure 1 shows the two pathways that exist for scaling up the amount of substance to be separated: The first is achieved by overloading the column with sample and cutting fractions in order to compensate for the decreasing resolution. This so-called "three fraction technique" was first investigated by Haarhoff et al. (*2*). Figure 2, which has been taken from that work, shows the recovered amount of solute as a function of the available resolution R and the permitted level of impurity. The second pathway is accomplished by increasing the column volume and the amount of solute so that the resolution is kept constant. In practice, both means are applied simultaneously; for the theoretical treatment it is of advantage to consider them separately.

Following the path on the right side of Fig. 1, one is immediately confronted with the basic question of whether to increase the column volume by increasing column length or column diameter. In Fig. 3 an analytical column is scaled up by increasing the length on one side and

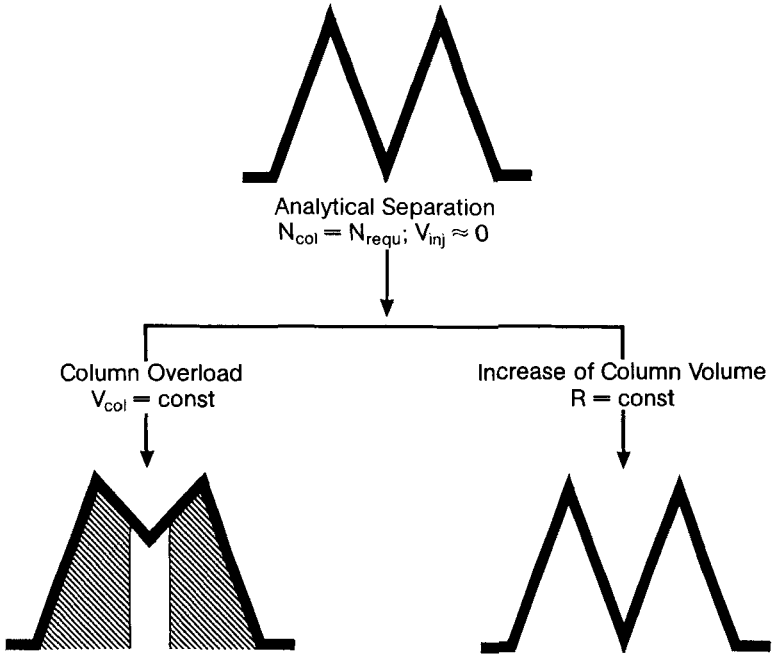

FIG. 1. Basic possibilities for scaling-up preparative separations.

by increasing the diameter on the other side, leading to two columns which have the same volume but which differ in dimensions. The question to be answered is which of the two columns will yield the higher production rate while maintaining the same resolution.

In order to answer this question a relation is employed which was derived in an earlier investigation (*1*):

$$P_{iN} = A\varepsilon_t \bar{u}_0 c_i D\left[\frac{1}{N} - \frac{H_0}{L}\right]^{1/2} \tag{1}$$

In this equation P_{iN} is the production rate for solute i, A is the column cross section, ε_t is the total porosity, $\bar{u}_0$ is the linear flow velocity, c_i is the initial sample concentration, D is the ratio of the injected volume to its volume standard deviation, N is the required plate number, H_0 is the theoretical plate height, and L is the column length.

This equation has been derived under the assumption that the sample load is increased by increasing the sample volume at constant concen-

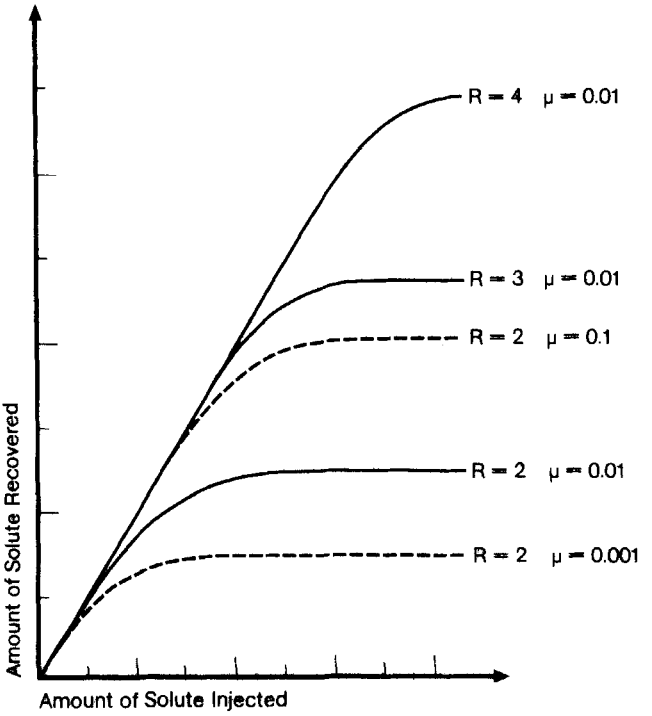

FIG. 2. Three fraction technique, according to Ref. *2*. R = resolution, μ = degree of impurity.

tration. If mass overload were assumed, the term in brackets would look different and show a dependence on the distribution isotherm. This term determines the relative magnitude of the dispersion caused by both the column and the sample. Under the assumption of volume overload, the optimum ratio of the total dispersion and the kinetic dispersion of the column is found to be 1.5. Knox and Pyper (*3*), assuming mass overload and a Langmuir type of distribution isotherm, found this value to be 3. Therefore, depending on the particular curvature of the distribution isotherm (rarely known in practical situations) and the region one is working in, one must expect different quantitative results. The conclusions, however, which can be drawn from the following considerations are of general validity.

According to Eq. (1), the production rate becomes zero if the term in brackets becomes zero. This is the case if

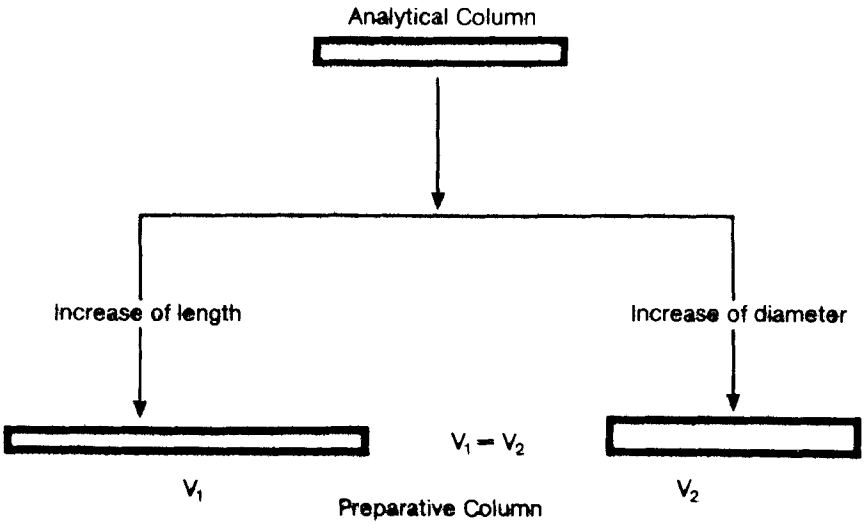

FIG. 3. Column volume scale-up.

$$1/N = H_0/L \qquad \text{or} \qquad L = L_0 = H_0 N$$

where L_0 is the initial column length.

This is the "analytical" case where the column has just enough length (L_0) to perform the desired separation with an infinitesimally small sample. L_0 is called the initial column length. Equation (1) shows that the production rate increases both with increasing cross section and increasing column length. While the increase with column cross section is linear with column length, it asymtotically approaches a maximum value for $L = \infty$.

For the further treatment the column cross-section A and the column length L are substituted by the column volume V_{col} respectively according to the relation

$$V_{col} = AL \tag{2}$$

Equation (1) then takes the forms:

$$P_{iN} = \frac{V_{col}}{L} \varepsilon_t \bar{u}_0 c_i D \left[\frac{1}{N} - \frac{H_0}{L}\right]^{1/2} \tag{3}$$

and

$$P_{iN} = A\varepsilon_t \bar{u}_0 c_i D\left[\frac{1}{N} - \frac{H_0 A}{V_{col}}\right]^{1/2} \tag{4}$$

The change in P_{iN} as a function of the column volume may be easily derived: The derivative of Eq. (3) is taken at constant column length and of Eq. (4) at constant cross section.

Setting the derived expressions equal:

$$\left(\frac{dP_{iN}}{dV_{col}}\right)_L = \left(\frac{dP_{iN}}{dV_{col}}\right)_A$$

one asks the question whether there is a point in the scaling-up procedure where an increase in column cross section yields the same increase in production rate as an increase in column length. As some simple calculations show, the answer is yes. This point is reached at a column length L which lies 50% above the initial column length L_0:

$$L = \frac{3}{2} L_0$$

As Figs. 4 through 6 show, below this point an increase in length yields a faster increase of productivity than an increase in cross section. Above this point this situation reverses.

This result may be better understood by considering Eq. (1) in Fig. 4. For $L = L_0$, the production rate is zero (analytical situation, infinitesimally small sample). From there on it increases rapidly with increasing length, finally reaching a maximum value for $L = \infty$. At $L = \frac{3}{2}L_0$, the curve is touched by the straight line going through the origin. As can be seen from Fig. 5 (a plot of production rate versus column volume), straight lines through the origin represent columns with constant length and increasing cross section. Starting at Point A, one sees that at this point an increase of the column cross section does not increase the production rate. One first has to increase the column length (going from A to B). At this point a further increase of the column volume by increase of the cross section would lead to point B′ while a further increase of the length leads to Point C. Columns B′ and C have the same volume. However, Column C has a much higher production rate, which means that at Point B a further increase of length is of more advantage. At Point C the situation starts to reverse. A further increase of the column volume by increase of the cross section yields a higher increase of productivity (Point C′) than an increase of the length (Point D). Figure 6 shows an

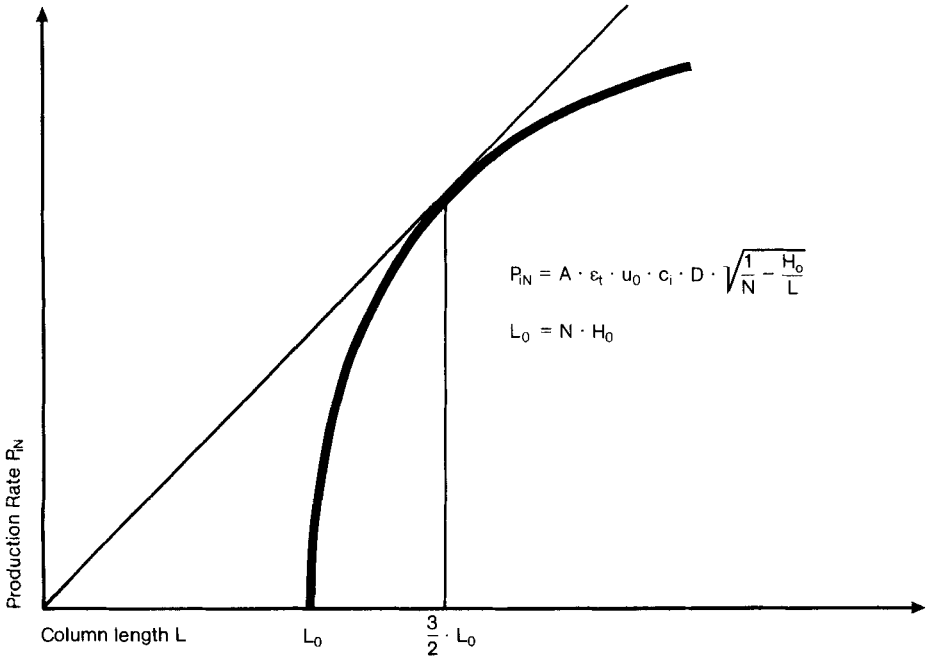

FIG. 4. Production rate as a function of column length.

example where it has been assumed that the analytical separation has been carried out with a column of 100 × 2.1 mm. In order to scale up this separation, one should use a column with a length of 150 mm and then further increase the diameter.

EXPERIMENTAL

Experiments where carried out using a Hewlett-Packard 1090 L Liquid Chromatograph. Columns used had the dimensions 100 × 2.1 mm, 200 × 2.1 mm, 100 × 4.6 mm, and 200 × 4.6 mm. Although columns of this size are normally used only for analytical purposes, they can be employed to advantage in the separation of μg-quantities of peptides and proteins used for kinetic studies, amino acid, or sequence analysis. The chromatographic conditions are given in the legends of the figures. The experiments were made to check whether the basic assumptions made in the theoretical treatment were sound.

Figure 7 shows the well-known dependence of the apparent plate

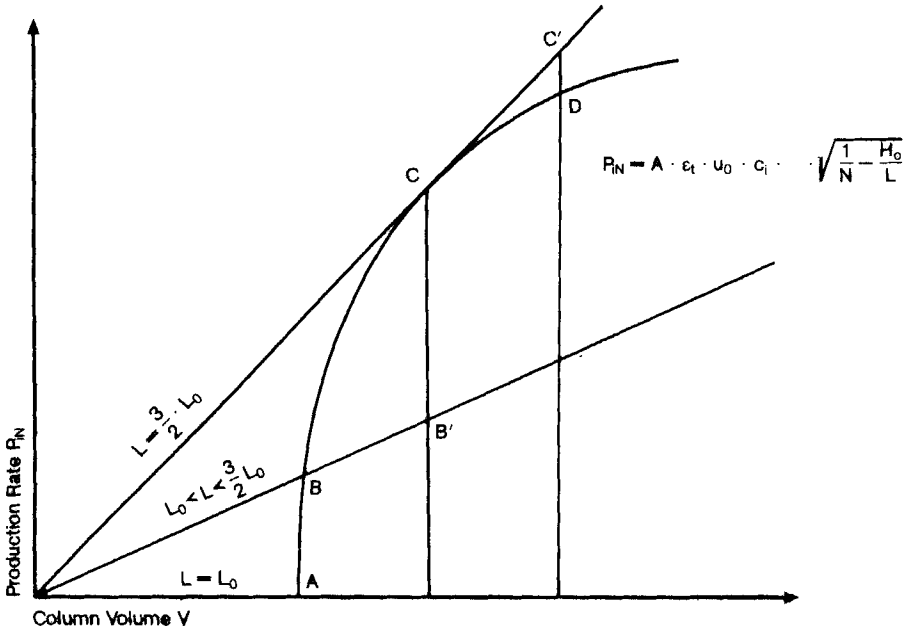

FIG. 5. Production rate as a function of column volume.

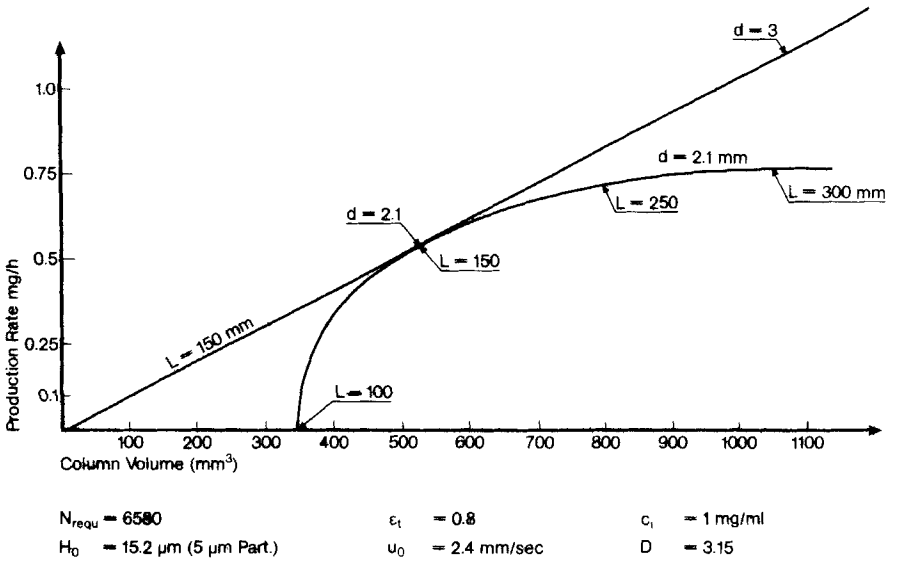

FIG. 6. Production rate as a function of column volume.

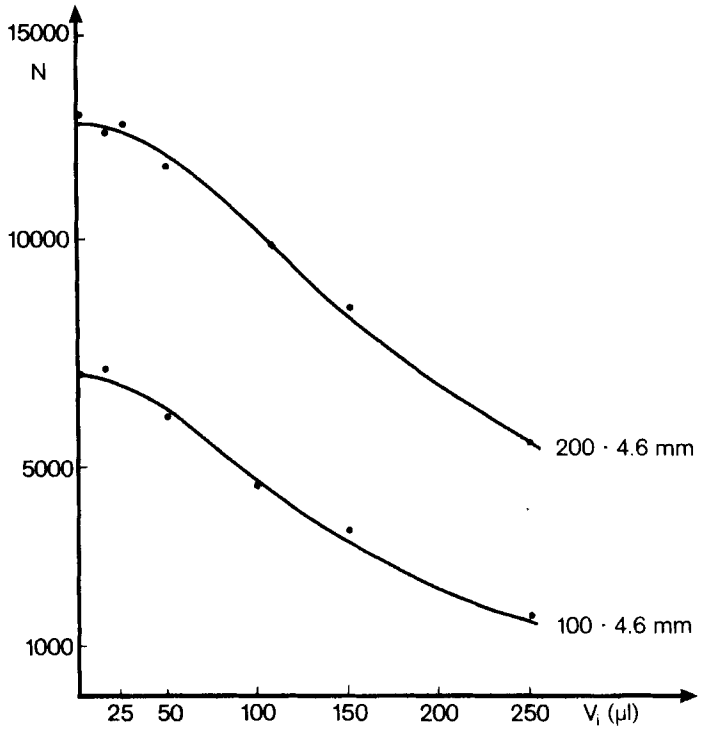

FIG. 7. Apparent plate number versus injection volume. Static phase: Shandon ODS Hypersil, 5 µm. Mobile phase: Acetonitrile/H_2O, 40/60 v/v; 2.4 mm/s. Sample: Nitrobenzene, 8 mg/100 mL. $k' = 1.81$.

number from the size of the injected sample (*4–6*). The same data were used to plot the reverse of the apparent plate number versus the square of the injection volume (Fig. 8). According to the equation given in Fig. 8, this must be a straight line if the variances of the dispersion of the column and the injected sample are additive. This obviously is the case. From the slope of these curves the value for D (the ratio of the injection volume and its volume standard deviation) can be calculated. No explanation was found for the fact that the D values differ for the two columns used in this experiment.

In Fig. 9 the total dispersion is plotted as its time variance versus the square of the retention time. According to the equation given in Fig. 9, the time variance $\sigma_{t\ ext}$ for the external system can be calculated from the intersect with the ordinate for $V_{inj} \approx 0$. The column plate number can be

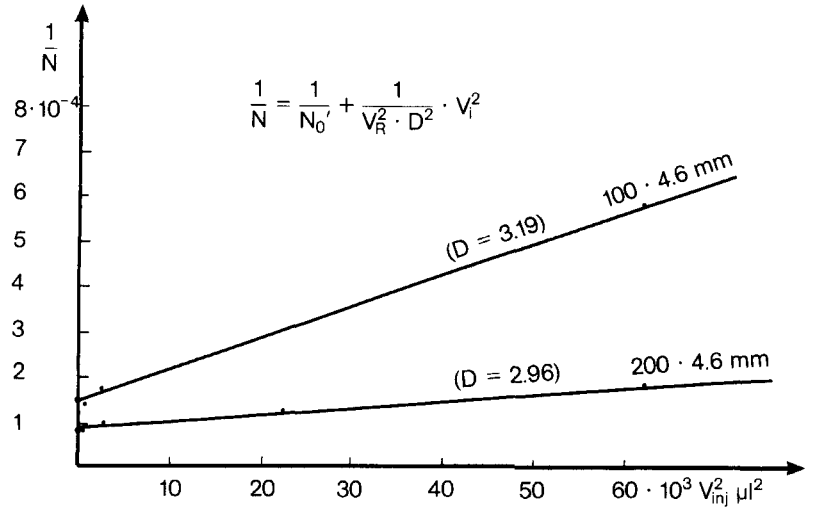

FIG. 8. Reverse of the apparent plate number as a function of the square of the injection volume (conditions as in Fig. 7).

derived from the slope and the D value from the difference of two values for different injection volumes.

Figure 10 shows the profiles of peaks with different injection volumes. The fact that the leading edges of all peaks have the same retention time suggests that the experiments were carried out in the linear range of the distribution isotherm. This was another assumption in the theoretical treatment.

CONCLUSIONS

In the scale-up procedure of liquid chromatographic columns, both length and diameter are of equal importance. Starting from the analytical situation, one should first increase the length and then the diameter. The theory, based on the assumption that the column is volume overloaded, suggests the use of a column which is 50% longer than the analytical column. The diameter is determined by the amount of substance to be separated and may have any value. The column dimensions are selected following these rules so that a given amount of sample

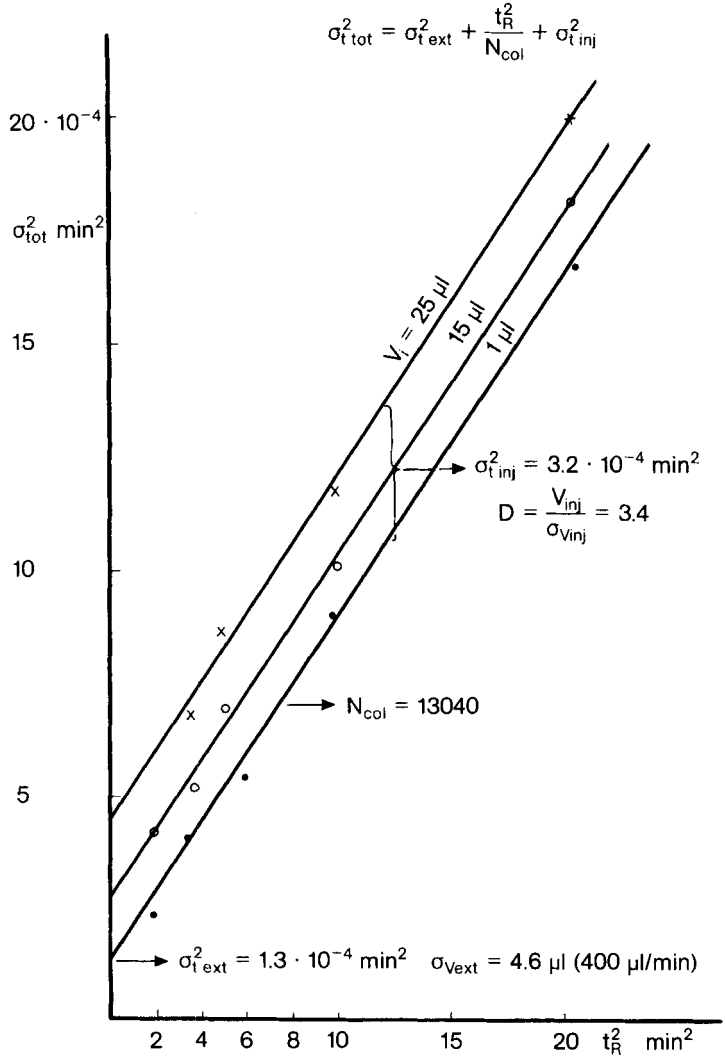

FIG. 9. Dispersion as a function of retention time. Column: 200 × 2.1 mm. Static phase: Shandon ODS Hypersil, 5 µm. Mobile phase: Acetonitrile/H_2O, 40/60 v/v; 400 µL/min. Sample: Nitrobenzene, 8 mg/100 mL.

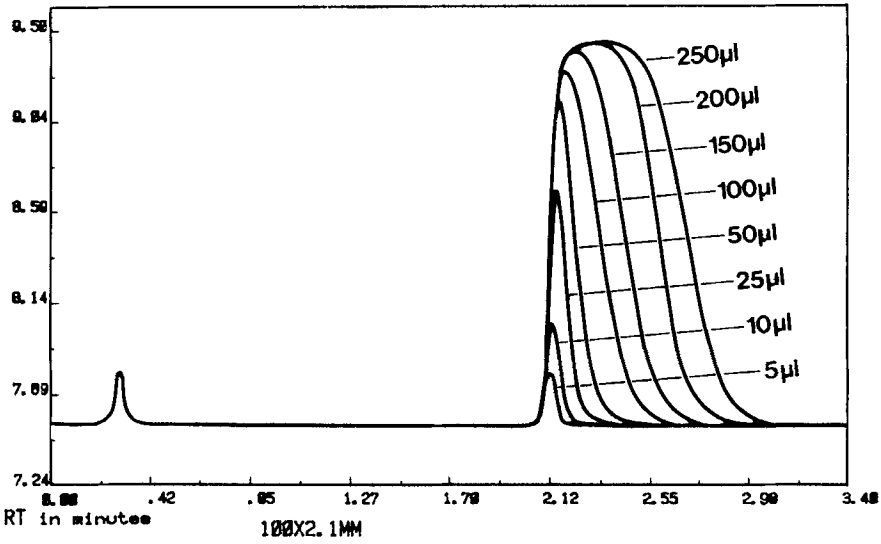

FIG. 10. Concentration profiles for different injection volumes. Column: 100 × 2.1 mm (conditions as in Fig. 9).

Is separated in the shortest possible time
With the smallest possible amount of solvent
On a column with the smallest possible volume
Is collected in the smallest possible volume of mobile phase

REFERENCES

1. K.-P. Hupe and H. H. Lauer, *J. Chromatogr., 203,* 41 (1981).
2. P. C. Haarhoff, P. C. van Berge, and V. Pretorius, *Trans. Faraday Soc., 57,* 1838 (1961).
3. J. H. Knox and H. M. Pyper, Paper Presented at the 1st International Symposium on Preparative and Up Scale Liquid Chromatography, Paris, France, January 1986.
4. A. Wehrli, *Anal. Chem., 47,* 289 (1975).
5. A. Wehrli, U. Hermann, and J. F. K. Huber, *J. Chromatogr., 125,* 59 (1976).
6. A. W. J. De Jong, H. Poppe, and J. C. Kraak, *Ibid., 209,* 432 (1981).