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INJECTORS FOR OPEN-TUBULAR COLUMN LIQUID CHROMATOGRAPHY WITH 10^6 THEORETICAL PLATES AT RETENTION TIMES IN THE MINUTE RANGE

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SUMMARY

A pressure pulse-driven stopped-flow injection system for use in open-tubular column liquid chromatography is described and is compared with a conventional split injector. For a non-retained sample component 10^6 theoretical plates are obtained in 220 s using a 1.3 m × 3.5 μm I.D. column. The loss of 25% in the number of theoretical plates corresponds to an injection volume of ⩽ 8 pl.

INTRODUCTION

In analytically relevant open-tubular column liquid chromatographic systems, extra-column contributions to signal broadening become very serious (for a reviews see ref. 1). As the volume of an open-tubular column 1 m long with I.D. 3.5 μm is only about 10 nl, the technical problems in achieving the maximum possible number of theoretical plates (980 000 in 4.2 min for non-retained components[2,3]) are substantial. Several theoretical and instrumental approaches to solving the problem of sample injection exist[4–16] but unfortunately, each of the methods tested to date has inherent limitations, making the injection process itself a frequent limiting factor to the resolving power of the chromatographic system. Here we describe a pressure pulse-driven stopped-flow injection device for injecting sample volumes in the picolitre range.

THEORETICAL

Extra-column effects

The total peak dispersion is generally expressed as the sum of the contributions from individual sources, *j*:

$$\sigma^2_{\text{total}} = \sum_j \sigma^2_j \qquad (1)$$

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where the variance σ_j provides a measure of peak dispersion. An acceptable limit for the extra column contributions i to the peak broadening σ_c of the column is

$$\frac{\sigma_i}{\sigma_c} \leqslant Z \tag{2}$$

where Z is an arbitrarily fixed limit. The additional contribution i to peak broadening $\Delta\sigma_{\text{total}}$ is

$$\frac{\Delta\sigma_{\text{total}}}{\sigma_c} \leqslant \sqrt{Z^2 + 1} - 1 \tag{3}$$

Knox and Gilbert[2] suggested a value of $Z = 0.5$, which corresponds to an extra-signal broadening of 12%. For an ideally delta function-shaped sample plug injection, the volume V_i injected is related to the variance as follows[4,17]:

$$V_i = \sqrt{12}\sigma_{v,i} \tag{4}$$

whereas for an exponential function the injection volume is equal to $\sigma_{v,i}$.

The variance can be expressed in units of length ($\sigma_{x,i}$), volume ($\sigma_{v,i}$) or time ($\sigma_{t,i}$). For systems without branches $\sigma_{v,i}$ and $\sigma_{t,i}$ are interchangeable; $\sigma_{x,i}$ should only be used in systems of constant flow cross-section. To describe split injectors, $\sigma_{t,i}$ is therefore the relevant quantity.

Laminar split injector

For a discussion of the effect of this type of injector on the peak broadening, the space [length L_i and diameter d_i (m) in Figs. 1 and 2] between the rotor of the injection valve and the open-tubular column is essential. The Reynolds number[18]

$$Re = \frac{\rho}{\eta} d_i u_i \tag{5}$$

where ρ is the density (kg m^{-3}), η is the viscosity (N s m^{-2}) and u_i the mean linear velocity of the mobile phase (m s^{-1}), gives a critical flow-rate u_i for the change from laminar to turbulent flow in a tube of circular cross-section if its value is > 2000. For a water-operated injector at room temperature ($\rho \approx 10^3$ kg m^{-3} and $\eta \approx 10^{-3}$ N s m^{-2}) and $d_i \leqslant 0.5$ mm the critical flow rate is $\geqslant 4$ m s^{-1} (> 47 ml/min^{-1}). It is therefore easy to maintain a laminar flow in this injector space. Assuming the validity of the Golay–Knox equation[19,20] for the injector and open-tubular column (length L_c, I.D. d_c) we can write

$$\left(\frac{\sigma_{t,i}}{\sigma_{t,c}}\right)^2 = \frac{\lambda_i}{\lambda_c} \cdot \frac{h_i}{h_c} \left(\frac{v_c}{v_i}\right)^2 \left(\frac{d_i}{d_c}\right)^4 \leqslant Z^2 \tag{6}$$

where $\lambda = L/d$ is the reduced length, $h = H/d$ the reduced plate height and $v = ud/D$ the reduced velocity or Péclet number of the mobile phase with respect to the injector

i or the column c; D is the diffusion coefficient of a solute in the mobile phase (m^2 s^{-1}) and H is the height equivalent to a theoretical plate (HETP). The most severe instrumental limitation is that the outer diameter D_c of the open-tubular column has to be smaller than d_i (see Fig. 2 and eqn. 6). For realistic systems, the laminar split injector leads to unacceptable peak broadening if open-tubular columns of I.D. $\leqslant$ 5 μm are to be used (see Table I and ref. 21).

TABLE I

CALCULATED BAND BROADENING, $\Delta\sigma_{total}/\sigma_{t,c}$, CAUSED BY A LAMINAR SPLIT-INJECTOR (L_i = 1 mm, d_i = 0.5 mm, F_i = 5 ml min^{-1}, v_i = 212000, h_i = 2210, λ_i = 2) FOR OPEN-TUBULAR COLUMNS OF DIFFERENT INNER DIAMETERS WITH CONSTANT COLUMN GEOMETRY, $\lambda_c = 10^5$ (N_{max} = 345000), OPERATED UNDER OPTIMAL CONDITIONS (h_c = 0.29, v_c = 13.8, k' = 0)

d_c (μm)	$\frac{\Delta\sigma_{total}}{\sigma_{t,c}}$ (%)	Z
10	<1	0.08
7	1	0.14
5	3	0.25
3.5	13	0.53
2.5	43	1.02
1.75	133	2.10
1.25	320	3.58

Pressure pulse-driven stopped-flow injector (PSI)

In the PSI procedure proposed here (Figs. 3 and 4), the following steps are involved:

(1) The sample (> 10 μl) is introduced by a syringe into the injection loop to contact the column inlet but avoiding a pressure difference between the column inlet and outlet. At this moment the diffusion of the sample into the open-tubular column inlet end starts.

(2) During a short time period a pressure difference between the column inlet and outlet is applied in order to force the desired volume element of the sample into the column.

(3) The injection loop is rinsed with the mobile phase, avoiding a pressure difference between the column inlet and outlet.

(4) The pressure difference used to run the elution is applied to the column inlet.

The product of pressure and time applied to the inlet of an open-tubular column defines a volume injected V_i:

$$V_i = \frac{\pi}{4} \cdot d_c^3 \cdot \frac{\Delta p_i \, \Delta t_i}{\lambda_c \eta \varphi} \tag{7}$$

or, expressed in reduced quantities[22]:

$$\Pi_i \tau_i = \lambda_c \lambda_i^* \tag{8}$$

where $\Pi_i = \Delta p_i d_c^2/(\eta D \varphi)$ is the Bodenstein number, $\tau_i = \Delta t_i D/d_c^2$ the Fourier number, $\lambda_i^* = z/d_c$ the reduced entrance length of the sample into the open-tubular column caused by the pressure pulse (Δp_i, Δt_i) and φ the Poiseuille number (equal to 32 for an open tube of circular cross-section).

Assuming ideally delta function-shaped pressure pulses and injection volumes we obtain for the tolerated injection volume (eqn. 4)

$$w_i \leqslant \frac{\pi}{4} \cdot Z\sqrt{12 h_c \lambda_c} \tag{9}$$

where $w_i = V_i/d_c^3$ is the reduced injection volume and h_c is the reduced plate height in the column; for example, the minimum $h_c = 0.29$.

Comparing the volume injected by the PSI (eqn. 8) with the tolerated injection volume (eqn. 9):

$$\Pi_i \tau_i = \frac{\Delta p_i \Delta t_i}{\eta \varphi} \leqslant Z\sqrt{12 h_i \lambda_c^3} \tag{10}$$

it becomes obvious that the maximal acceptable pressure pulse for operating the open-tubular column at the minimum plate height ($h = 0.29$) is exclusively given by the column geometry λ_c, *i.e.*, by the maximal possible plate number asked for. The results given in Table II corroborate the applicability of the PSI to open-tubular columns of high resolving power.

TABLE II

CALCULATED MAXIMALLY TOLERATED ($Z = \frac{1}{2}$) PRESSURE PULSE AND INJECTION VOLUME FOR OPEN-TUBULAR COLUMNS OPERATED UNDER OPTIMAL CONDITIONS (h_c = 0.29, v_c = 13.8) FOR DIFFERENT COLUMN GEOMETRIES, λ_c

λ_c	N_{max}	$\Delta p \Delta t$ (*bar s*)	*Column I.D., d_c*			
			1 μm		*3.5 μm*	
			V_i (*pl*)	L_c (*cm*)	V_i (*pl*)	L_c (*cm*)
10^5	350000	10	0.23	10	10	35
$2 \cdot 10^5$	690000	27	0.33	20	14	70
$5 \cdot 10^5$	1700000	107	0.52	50	22	175
10^6	3500000	305	0.73	100	31	350

Diffusion processes in the open-tubular column limit the time, Δt_{stop} (s), that is spent between sample injection and the start of the elution process:

$$\sigma_{x,stop} = \sqrt{2 D \Delta t_{stop}} \tag{11}$$

The tolerated stop-time interval is therefore

$$\tau_{stop} \leqslant \frac{Z^2 \lambda_c h_c}{2} \tag{12}$$

Table III indicates that this contribution to peak broadening becomes critical in practice only for $d_c < 2.5$ μm and relatively short columns.

TABLE III

CALCULATED MAXIMALLY TOLERATED ($Z = \frac{1}{2}$) STOP TIME FOR OPEN-TUBULAR COLUMNS OF DIFFERENT INNER DIAMETERS WITH DIFFERENT COLUMN GEOMETRIES OPERATED UNDER OPTIMAL CONDITIONS ($h_c = 0.29$, $v_c = 13.8$)

λ_c	*Column I.D., d_c (μm)*					
	1.25	*1.75*	*2.5*	*3.5*	*5*	*7*
10^5	5 s	11 s	22 s	44 s	1.5 min	3 min
$2 \cdot 10^5$	11 s	22 s	44 s	1.5 min	3 min	6 min
$5 \cdot 10^5$	30 s	1 min	2 min	3.5 min	7 min	15 min
10^6	1 min	2 min	4 min	7 min	15 min	30 min

EXPERIMENTAL

The chromatographic system[22] shown in Fig. 1 was used with the following components: a P-500 constant-flow high-precision pump ($p \leqslant 40$ bar) (Pharmacia, Uppsala, Sweden) with incorporated pressure gauge; a Haskel constant-pressure pump ($p \leqslant 400$ bar) (Ammann Technik, Kölliken, Switzerland) with incorporated pressure gauge; an EDR 212 piezoresistive pressure gauge (Haenni Messgeräte, Jegenstorf, Switzerland); and vitreous silica capillary columns of 14 and 3.5 μm I.D. (Scientific Glass Engineering, Ringwood, Australia). The split injection system (Fig. 2) was used with the following components: VICI injection valve, 0.2-μl internal loop,

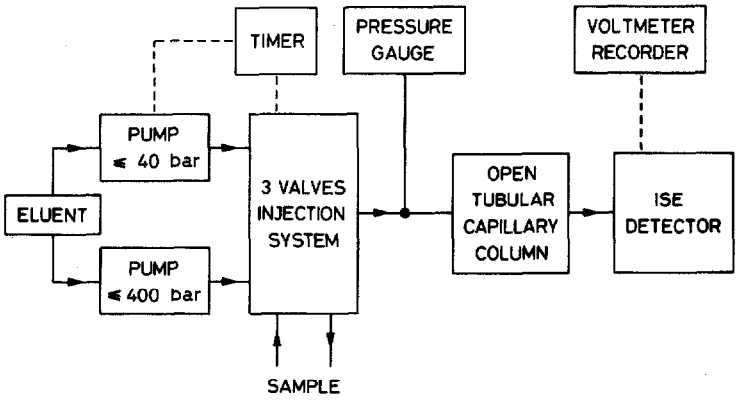

Fig. 1. Schematic diagram of the open-tubular column liquid chromatograph.

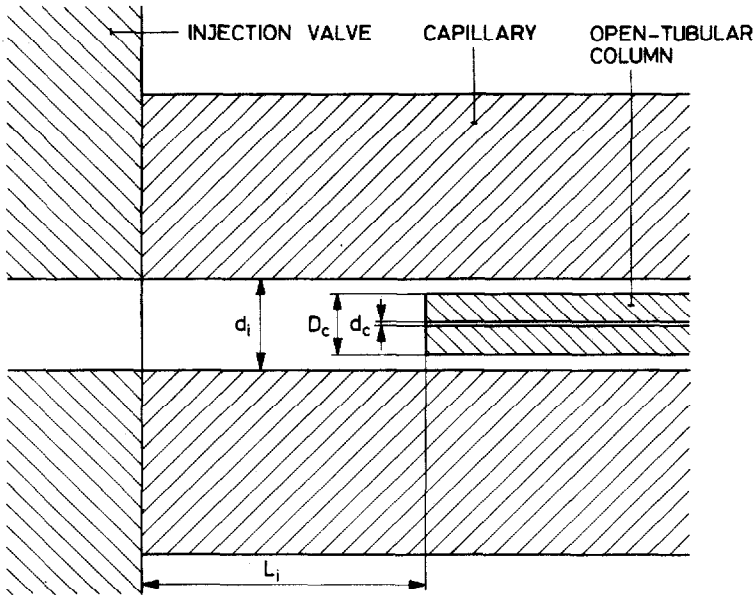

Fig. 2. Schematic diagram of the laminar split injector.

with electric actuator and VICI SD16 multi-position valve (Valco Instruments, Houston, TX, U.S.A.) equipped with a set of vitreous silica reference capillaries to control the open-tubular column inlet pressure from 0.1 to 300 bar at different split flow-rates; and Miconns T-piece and Teflon fittings (Antech, Bad Dürkheim, F.R.G.). The stopped-flow injection system shown in Fig. 3 consists of three VICI C6W switching valves with fast (< 200 ms) electric actuators (Valco Instruments); to control the switching according to Fig. 4a laboratory-made electronic timer was used. The ion-selective microelectrode detector, as previously shown in Fig. 3 in ref. 3, had a tip diameter of $\leqslant 1$ μm and was either inserted directly into the end of the open-tubular column or brought close to it using micromanipulators partially equipped with piezo-translators (Physik Instrumente, Waldbronn/Karlsruhe, F.R.G.), a WILD M8 microscope (Wild-Leitz, Heerbrugg, Switzerland) and fibre-optic cold light il-

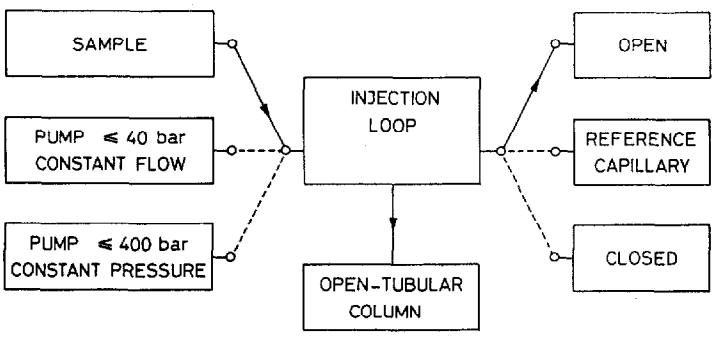

Fig. 3. Schematic diagram of the pressure pulse-driven stopped-flow injector.

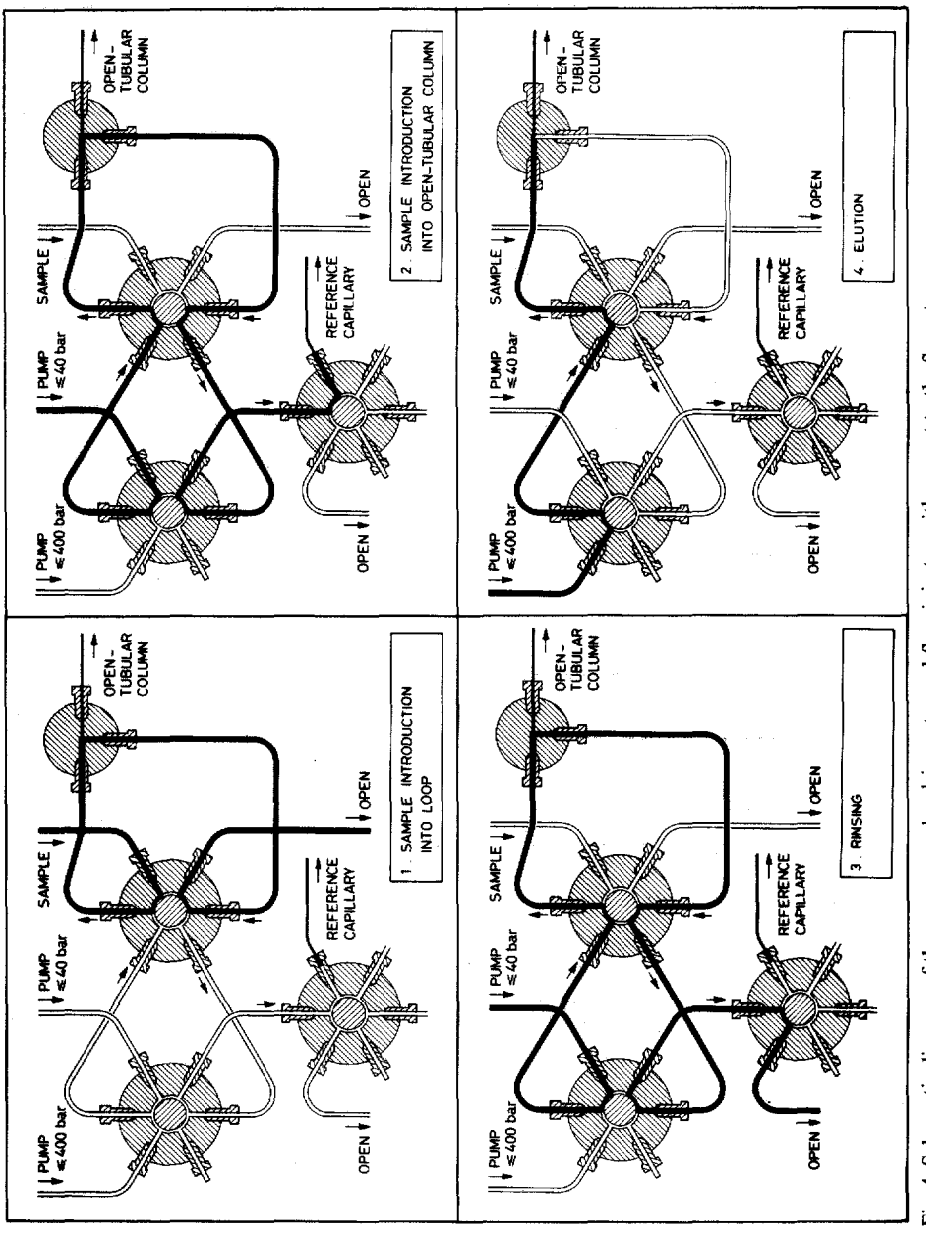

Fig. 4. Schematic diagram of the pressure pulse-driven stopped-flow injector with respect to the flow steps.

lumination (VOLPI, Urdorf, Switzerland). The whole apparatus was mounted on a pneumatic vibration isolation system (Physik Instrumente). The composition of the ion-selective membrane, the reference electrode and the response behaviour of the potentiometric detector have been described previously[3].

RESULTS AND DISCUSSION

Laminar split injector

The splitting ratio may be approximated by the ratio of the volume flow-rates through the open-tubular column and the tube of diameter d_i connected to the injection valve (Fig. 2). The amount of sample actually introduced into the column was measured by the injection of 0.2 μl of potassium chloride solutions into the injector tube and integration of the elution profile obtained with an ion-selective microelectrode (see also ref. 3 and Fig. 5). For columns of both 14 and 3.5 μm I.D. a good correlation between selected and measured splitting ratios is obtained (Fig. 5). At injection volumes of 0.2 μl and splitting ratios of $1{:}4 \cdot 10^5$–$1{:}4 \cdot 10^6$ the samples actually introduced into the open-tubular column correspond to volumes of 0.05 and 0.5 pl, respectively, if no dilution of the sample solution occurs. The plot of the reduced plate height as a function of the reduced linear velocity (Fig. 6) for a 3.5

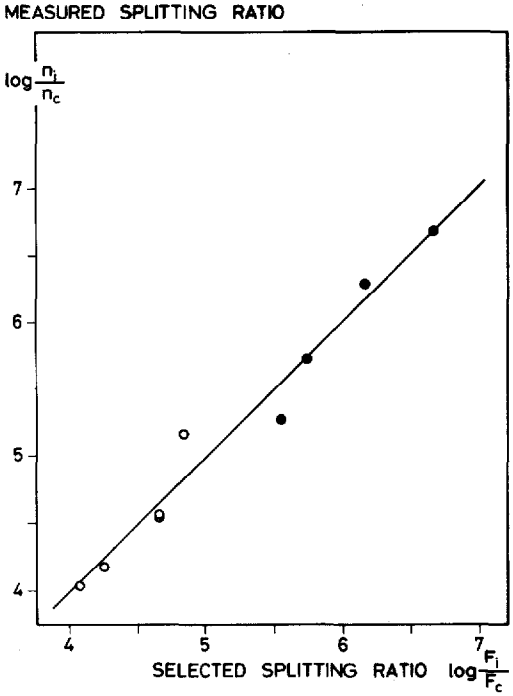

Fig. 5. Correlation between the splitting ratio calculated from the volume flow-rates and the splitting ratio calculated from the detector response. Mobile phase, 10^{-3} *M* potassium chloride solution. (●) Sample, 3 *M* potassium chloride solution; column, 133 cm × 3.5 μm I.D. (○) Sample, 0.1 *M* potassium chloride solution; column, 200 cm × 14 μm I.D.

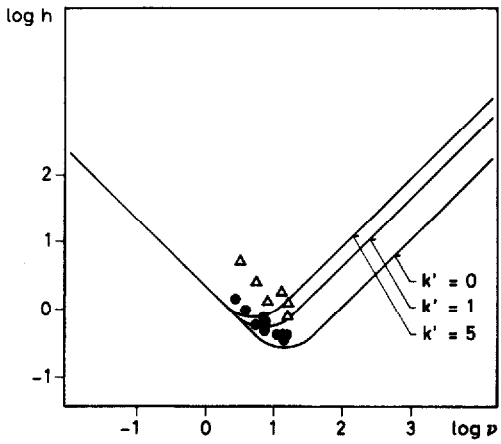

Fig. 6. Reduced plate height as a function of the reduced velocity for a 3.5-μm I.D. column used with either a stopped-flow (●) or a split injection system (△). Sample, 3 *M* potassium chloride solution; column, 130 cm × 3.5 μm I.D.; mobile phase, 10^{-3} *M* lithium acetate solution.

μm I.D. open-tubular column of length 1.3 m, however, indicates a minimum plate height with $h \approx 1$ only, corresponding to a $\Delta\sigma_{total}/\sigma_{t,c}$ of 86% (Table I; $Z = 1.6$). Using eqn. 1 a volume of 17 pl introduced into the open-tubular column can be calculated. Eqn. 6 therefore seems to give an optimistic estimate of the acceptable limits of a laminar split injector (see Table I).

Pressure pulse-driven stopped flow injection (PSI)

Utilizing the PSI the theoretical relationship between log h and log v is matched much better than when using the laminar split injector (Fig. 6). The deviations from the expected plate number (for zero retention) are 25–50%. Assuming the injector to be the only contributor to the dispersion, a volume introduced into the column of 8 pl (eqn. 1) can be calculated for the minimum plate height. Repetitive injection of 3*M* potassium chloride solution gave an overall precision of the measured signal maximum (50 mV) of ±1.1 mV, corresponding to ±4.5% in component concentration or activity.

The injection system described makes it possible to obtain 10^6 theoretical plates in 220 s for non-retained components and an open-tubular column of 3.5 μm I.D.[22].

LIST OF SYMBOLS

η	viscosity of the mobile phase (N s m^{-2});
λ, λ^*	reduced length;
v	reduced velocity of the mobile phase, Péclet number;
Π	reduced pressure, Bodenstein number;
ρ	density of the mobile phase (kg m^{-3});
$\Delta\sigma$	increase in standard deviation of the signal (m, s or m^3);
τ	reduced time, Fourier number;
φ	Poiseuille number;
D	diffusion coefficient of a solute in the mobile phase (m^2 s^{-1});

d inner diameter (m);
F volume flow-rate ($m^3\ s^{-1}$);
H height equivalent to a theoretical plate (m);
h reduced plate height;
k' capacity factor;
L length (m);
Δp pressure drop (Pa);
Re Reynolds number;
Δt time (s);
u linear velocity of mobile phase ($m\ s^{-1}$);
V volume (m^3);
w reduced volume;
Z arbitrarily fixed tolerance limit;
z distance to the end of the open-tubular column on the symmetry axis (m).

Subscripts

c open-tubular column;
i injection system;
stop stop per flow;
t time;
v volume;
x length.

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