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Simple test for determination of the degree of distortion of the liquid-phase flow profile in columns for preparative liquid chromatography☆

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ABSTRACT

A simple method for the determination of the degree of distortion of the flow profile in a well designed preparative liquid chromatographic column is presented. The method consists in carrying out two test chromatographic analyses, one standard and one bidirectional elution. It was experimentally verified that similarity of the results of the two tests confirms a virtually plug flow profile of the mobile phase in a column. On the other hand, differences in the results of the two tests demonstrate that the mobile phase flow profile in a column bed is of non-plug character. In such instances mass dispersion in a packed bed is then much better characterized by the HETP value calculated on the basis of bidirectional column test.

INTRODUCTION

Liquid chromatography (LC) is being used increasingly often for obtaining pure, particularly biologically active, substances [1–9]. There is also growing interest in preparing preparative columns for one's own purposes. The number of published preparative column packing procedures is small [7,10–17]. In addition, it often happens that repetition of a procedure under different conditions, *e.g.*, different column geometry, sorbent type (particularly irregular particle shape and greater Δd_p) and instrumentation used for packing the column, yields much worse results. Preparative columns prepared under such conditions often reveal unsatisfactory efficiency and poor peak shapes, far from Gaussian even without overloading during efficiency testing.

This does not only happen with columns prepared and packed by the user, as commercial preparative columns also usually reveal tailing near the baseline [12,17,18]. This tailing can be avoided only when the columns are operated under "infinite diameter" conditions [12,18–22]. Hence is difficult or even impossible to obtain very pure substances (99.99% or more) when using the entire cross-section of the column during separation [18]. It seems, therefore, that it is necessary to continue investigations on the problems of the reproducible packing of efficient and stable preparative columns for LC.

Poor efficiency of preparative columns can be due in general to the following reasons: (1) poor structure of the column packing resulting from the deficiencies of the described packing methods: (a) non-plug flow profile of the mobile phase in the column bed, resulting from an irregular radial distribution of particles of various size, or an irregular radial distribution of the degree of packing of the particles [12,13,18,19,21,22]; these problems are often encountered in practice; (b) insufficiently stable packing of the particles or irregularities of the packing bed —large intergranular spaces, splitting of the packing bed in the column or a large dead space between the upper distribution head and the packing layer, resulting from settlement of the

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packing bed [12,13,18–21]; these problems rarely occur when the columns are provided with axial packing compression systems [10,12,13]. (2) Incorrect design of the distribution heads, resulting in: (a) distortion of the zone of the injected substances directly below the head outlet owing to a too great resistance of radial flow of the liquid in the head [23]; (b) a significant broadening of the zone of the sampled substances resulting from diffusion in the excessively large space in the distribution or the outlet head [21], or from the presence of a void volume and blockage of frits in the column heads.

There are a few methods that permit the diagnosis of the reasons for unsatisfactory peak shapes and poor efficiency of preparative LC columns with particle sizes ranging from 5 to 100 μm [12,13,21–27]. These methods, however, cause the destruction of the column packing [12,13,23–25] or are very complicated and require the application of special expensive equipment [22,26,27].

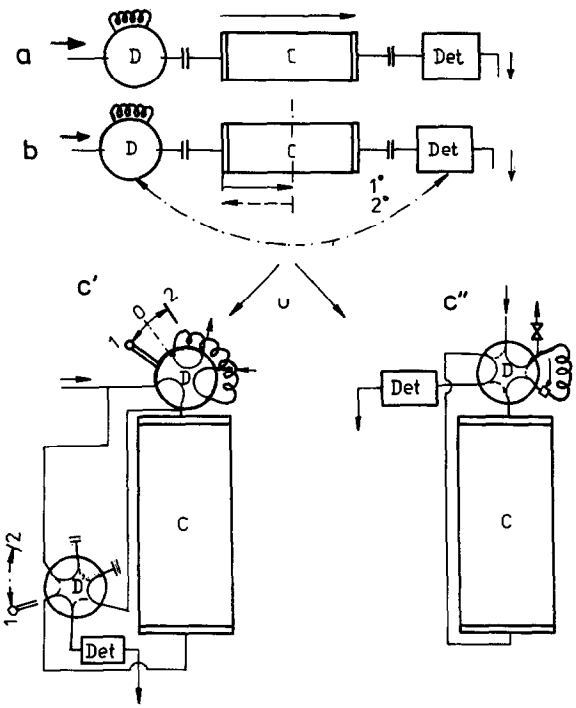
The need for the development of a simple method allowing the diagnosis of the major reasons for unsatisfactory column efficiency has stimulated rational investigations on the technology of column packing. The determination of the potentially attainable column efficiency for a particular sorbent, mobile phase flow-rate and separated substances is also important, particularly when looking for the optimum conditions of column packing with new sorbents. Under the conditions of a standard column efficiency test, such information can be obtained only when the profile of the mobile phase flow in the column bed is truly plug.

Calculations of the attainable column efficiency according to Knox *et al.* [22] are not reliable when the packing particles are irregular and the fraction of the particle size is relatively broad.

The paper presents a simple, two-stage test, which enables some conclusions to be drawn regarding the reasons for unsatisfactory column performance and/or the determination of the potentially attainable efficiency of a preparative LC column, provided that the column is correctly designed and packed in an optimum manner (the mobile phase flow profile in the column bed should be of the plug type). The proposed test can be also useful for the determination of the extra-column broadening of the bands of separated substances in analytical LC or with the use of microcolumns.

PRINCIPLE OF THE TEST

The test consists of two stages (see Fig. 1). The first stage is a standard test of the column efficiency, *i.e.*, a test chromatogram of a single substance or an easily separable mixture under the conditions when no overloading occurs (Fig. 1a and item 1 in the table of the positions of the six-port valve). The second stage involves bidirectional elution of a single test substance, initially as in the first stage, but only until a certain fraction of the column length



	c'	c"
1°	D: 1 υ 2 D': 1	D: ———
2°	D: 0 D': 2	D: – – – – –

Fig. 1. Illustration of the principle of the two-stage test [(a) conventional test; (b) bidirectional test] and instrument set-up facilitating the performance of the bidirectional test (c', c"): the asterisk denotes sampling of tracer to the sampling loop without the eluent flow. C = chromatographic column; D = six-port sampling valve; Det = chromatograpahic detector; D' = auxiliary six-port valve; 1, 0, 2 = positions of the valve lever, → and – · – · → denote directions of liquid flow.

(one to three quarters) has been reached. Next the direction of the eluent flow is reversed and the tracer is eluted back to the column inlet. The reversal of the eluent flow and the recording of the chromatogram during the reversed elution of the tracer are possible owing to an exchange of the positions of the sampling valve and the detector (Fig. 1b and item 2 in the table of the positions of the six-port valve). The exchange can be accomplished by a real reversal of the column (Fig. 1c) with respect to the sampling valve (D) and the detector (Det); application of a set of two six-port valves connected to the column and the detector according to scheme c′ in Fig. 1; or application of the system c″ in Fig. 1, introducing the tracer solution to the "sampling loop" through a membrane.

Both stages of the test are carried out properly only when a small amount of the tracer is introduced into the column in the form of a narrow rectangular pulse. The maximum volume of the tracer solution can be calculated from the equation

$$V_i^{max} \approx 0.7\, d_c^2\, L_c^{1/2}\, d_p^{1/2} \qquad (1)$$

In the case of application of the bidirectional test for the quantitative determination of the extra-column dispersion of the bands of the separated substances ($\Sigma\sigma_{vex}^2$), it is necessary to perform a series of experiments with bidirectional elution of the smallest possible amount of a non-retained substance. The elution should be carried out for various distances of elution of the tracer band in the column (*e.g.*, for a distance of one, two or three quarters of the column length). Next it is necessary to prepare a diagram of the $\sigma_v^2 = f(l')$ dependence (see Fig. 2 and the list of symbols) and to extrapolate the value of σ_v^2 for $l' = 0$ (best of all using the linear regression method).

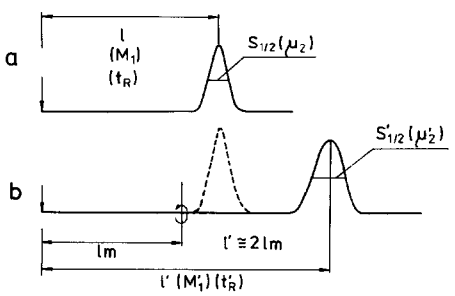


Fig. 2. Chromatograms obtained from (a) the conventional and (b) the bidirectional column test: illustration of eqns. 2 and 3.

PRINCIPLES OF CALCULATION OF THE DISPERSIVE PARAMETERS OF A COLUMN

According to the outline in Fig. 2, on the basis of the chromatograms obtained it is possible to calculate the dispersive parameters of the column separately for stages I and II of the test. Both the HETP (H) and the dispersion coefficient (D) can be used for this purpose. The calculations can be performed on the basis of peak width at half-height ($S_{1/2}$), or on the basis of statistical moments, which, according to Carbonell and McCoy [28], is more objective.

The following dependence for the bidirectional test can be written on the basis of definitions of H or D values [21,22,29], and taking into account the scheme in Fig. 2:

$$H = L_c \cdot \frac{\mu_2'}{M_1 M_1'} \qquad (2)$$

In cases when the peak width at half-height and the l and l' segments are measured on the chromatograms, this equation is simplified to

$$H = \frac{L_c}{5.54} \cdot \frac{(S_{1/2})^2}{l'} \qquad (3)$$

After calculating H, also the longitudinal dispersion coefficient D and the dispersive Peclet number can be calculated. The results can therefore be presented as $H = f(u_0)$ or $Pe_D^{-1} = f(ReSc)$, and hence as $h/2 = f(v)$.

MODELS OF DISPERSION OF TRACER ZONES IN MICROGRANULAR POROUS BEDS

Fig. 3 shows the models of possible mass dispersion conditions in a microgranular bed of a chromatographic column. Extreme conditions have been taken into account: from a bed containing a large plate number and characterized by a plug flow profile (Fig. 3, 1), through dense beds of irregular radial permeability distribution (Fig. 3, 2), to poorly packed beds (mainly as a result of excessive electrostatic interaction between the particles) of plug (Fig. 3, 3) and non-plug (Fig. 3, 4) permeability profiles. Apart from the column cross-sections, part a in Fig. 3 presents the local tracer concentration distribu-

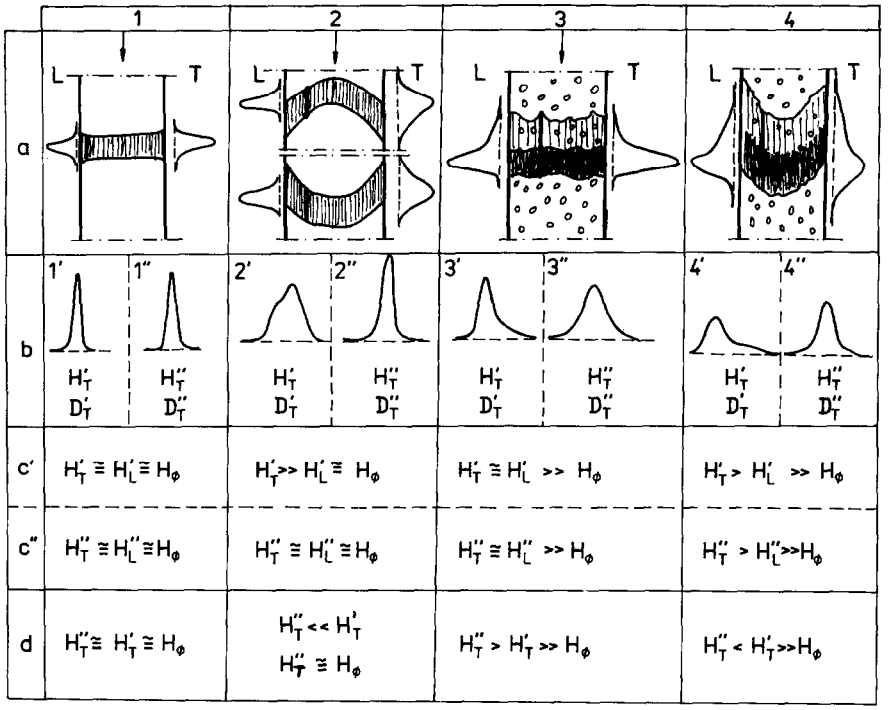


Fig. 3. Comparison of mass dispersion conditions in a microgranular layer (a) with the results of the conventional (b, 1', 2', 3', 4', c') and the bidirectional (b, 1", 2", 3", 4", c") column efficiency test. L = locally observed tracer concentration distributions; T = tracer concentration distributions averaged for the entire column cross-section (measured at the column outlet). Part d presents the dependences allowing the evaluation of the packing bed structure, based on a comparison of the results from both the stages of the test. Single prives denote results of the conventional test and double primes the results of the bidirectional test.

tions (L) and distributions averaged for the column cross-section (T). Part b illustrates the approximate peak shapes for the conventional test (denoted by single primes) and for the bidirectional test (denoted by double primes). Part c of Fig. 3 presents the expected dependences between the HETP values determined locally and at the column outlet for the conventional (c') or the bidirectional test (c"). Part d of Fig. 3 presents the theoretically predicted dependence between the HETP values from the conventional and the bidirectional tests, determined for the mentioned above four different dispersion conditions. The HETP values in Fig. 3c and d have also been related to the minimum attainable dispersion in the examined column (H_ϕ).

It can be expected from the comparison of the dependences between H''_T, H'_T and H_ϕ shown in part d of Fig. 3 that the comparison of the HETP values from the conventional and bidirectional tests, taking additionally into account the approximate HETP values calculated from theoretical dependences (*e.g.*, using the Knox equation [22]), should allow the prediction of the type of flow profile in the column and the degree of packing of the column bed.

At the same time the bidirectional test should allow the determination of the maximum attainable column efficiency (H_ϕ) for columns of a dense packing bed (Fig. 3, 1 and 2) in spite of a non-plug flow profile, as in Fig. 3, 2. It also follows from Fig. 3, 2 that the conventional test allows the determination of the HETP value describing the radial dispersion in the column packing only when the flow profile in the column is of a truly plug-type.

The above conclusions arising from the analysis of the model presented in Fig. 3 have been drawn assuming that the effect of radial dispersion on the results of the bidirectional test is negligible. Based on the results of Knox *et al.* [22], this assumption is

more valid the smaller is d_p and the higher is u_0. However, in cases of a strongly distorted flow profile in the column, the radial dispersion can cause a certain broadening of peaks obtained in the bidirectional test, particularly in the peak base region. In such cases the values of HETP determined on the basis of peak width at half-height in chromatograms obtained by the bidirectional test should be more useful for the calculation of H and D than values calculated on the basis of statistical moments.

EXPERIMENTAL

Materials

The column packings were silica gel 60 Å of mean particle diameter d_p = 17, 22, 33, 50 and 124 μm and with the particle size distribution given previously [13], LiChrosorb RP-18 (d_p = 10 μm), LiChroprep Si 60 (d_p = 25–40 μm) (E. Merck, Darmstadt, Germany) and Nucleosil C_{18} (d_p = 7 μm) (Macherey, Nagel & Co., Düren, Germany).

Methanol, methanol–water (8:2, v/v), hexane–dioxane (85:15, v/v) were used as mobile phases with reagents from POCh (Gliwice, Poland) and demineralized water.

Instrumentation

Columns of I.D. 17, 25, 33, 44 and 52 mm and length varying from 100 to 450 mm (stainless steel), equipped with moveable distribution heads with mechanical axial compression of the packing, were used. The design of the heads has been described [30]. Proper operation of the distribution heads of these columns has been checked previously [13].

A Model CHP 02 preparative chromatograph for column testing (Elkor, Gdańsk, Poland) was used, equipped with a two-head piston pump (5–300 ml/min, P_{max} = 150 bar), pulse damper, six-port sampling valve (20 μl–20 ml), a UV detector (254 nm), a refractive index (RI) detector (5 μl) connected to a split at the column outlet and a y–t recorder.

The columns were dry [13,15] or slurry packed [12,17] under both optimum and unfavourable conditions.

Methods

Preparative column tests were carried out according to the procedure described under Principle of the Test. The Model CHP 02 preparative chromatograph was modified for this purpose according to scheme c″ in Fig. 1.

Solutions of benzene and *o*-nitroaniline in hexane–dioxane (85:15) were sampled in the case of columns packed with silica gel in the first stage of the test (conventional test). The RP-18 columns were examined using benzene and naphthalene in methanol–water. Sample sizes were 20, 200, 400 and 500 μl for the columns of 17, 33, 44 and 50 mm I.D., respectively.

During the second stage (bidirectional test), samples of the same size, but containing one of the test substances dissolved in the mobile phase, were injected. For the determination of the dead volume of the column pure methanol was used as the mobile phase for benzene as the solute.

On the basis of the chromatograms obtained, the value of H was calculated as the measure of HETP taking into account the peak width at half-height.

In addition, two or three "bands" of Sudan I dye dissolved in dioxane (10%, w/v) were injected into some of the columns (without eluting them from the column). Owing to this, after pushing the packing out of the column it was possible to photograph the shape of the zones of the eluted substances in the axial cross-section of the column packing and to observe the changes in the zone shape during the elution of the dye bands along the column [12,13,23]. The illustrations in Fig. 6 (column C) were drawn on the basis of these photographs.

For the purposes of this paper, specially prepared distribution heads were also additionally used, *viz.*, heads with an enlarged volume of the space between the liquid inlet and the frit and heads with zero volume of this space (radial distribution plate removed), in which the radial flow of the liquid was virtually impossible.

RESULTS AND DISCUSSION

Figs. 4 and 5 present examples of chromatograms obtained during testing the columns by the conventional and the bidirectional tests (for benzene as the test substance). Fig. 4 presents the chromatograms obtained for two columns of 50 mm I.D., dry packed with LiChroprep Si 60 (d_p = 25–40 μm), and Fig. 5 the chromatograms obtained for two columns of 32 mm I.D., slurry packed with Nucleosil C_{18} (d_p = 7 μm). The columns were packed under the previously

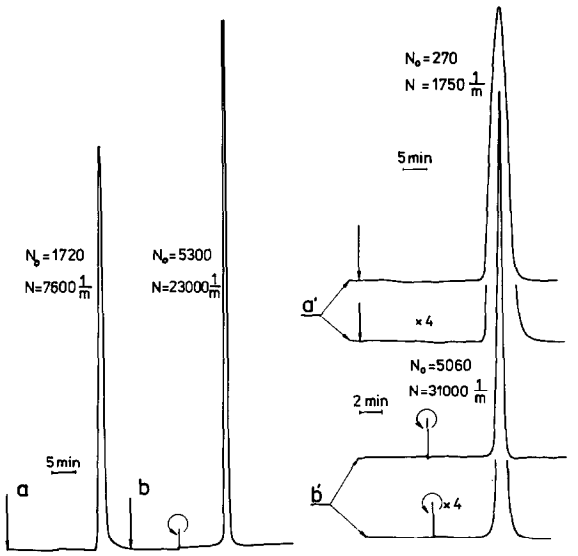


Fig. 4. Examples of test chromatograms of two preparative columns, d_c = 50 mm, L_c = (I) 22 cm and (II) 16 cm, dry packed with silica gel LiChroprep SI 60, d_p = 20–40 μm. a, a', Conventional test; b, b', bidirectional test; a, b, column I; a', b', column II. Parts a' and b' also present the bottom fragments of the respective peaks obtained at four times higher detector sensitivity. Flow-rate, 19 ml/min; mobile phase, methanol; test substance, *o*-nitroaniline.

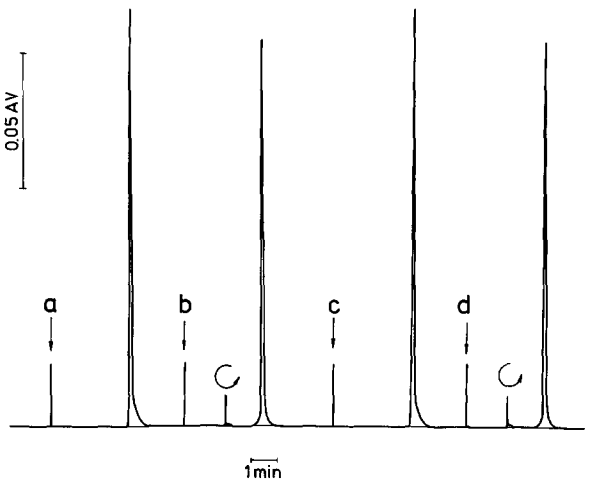


Fig. 5. Chromatograms obtained during (a, c) conventional and (b, d) bidirectional testing of efficiency of a 250 × 16 mm I.D. column packed by slurry method with Nucleosil C_{18} (7 μm). Mobile phase, methanol–water (8:2, v/v); sample, benzene; mobile phase velocity, u = 1.3 mm/s.

described conditions [12,13]. The results obtained are summarized in Fig. 6.

Column A in Fig. 6 presents typical examples of the results obtained in the first stage of testing the preparative columns packed with various methods under various conditions, and column B the results obtained in the second stage. Column C shows the shapes of two Sudan I zones retained during elution in the cross-section of the packing. Column D lists the dimensions of the columns (d_c, L_c) and packing particle sizes (d_p). The values of $h = H/d_p$ and $\upsilon = u_0 d_p/D_m$, obtained in the first and second stages, and the values of h_t calculated using the Knox equation [22] are listed by the respective chromatograms (values of A, B and C for these calculations are given at the bottom of Fig. 6). Arrows on the chromatograms indicate unsatisfactory peak shapes.

The results illustrated in Figs. 4–6, and other results obtained previously, lead to the following considerations. It is very difficult in practice to obtain preparative LC columns that produce ideal Gaussian peaks and at the same time have similar HETP values in the conventional and the bidirectional tests. Such a similarity often occurs for columns packed with the most reproducible slurry method when the peak width is measured at half-height. However, such preparative columns usually reveal tailing at the baseline when tested conventionally. Under the conditions of the bidirectional test this tailing appears on both sides of a peak at about half the width of the tailing part of a peak obtained during conventional testing. Tailing does not occur only in cases when the columns are tested under "infinite diameter" conditions (Fig. 6, 1, b; split = 50%, V_i = 50 μl). The results obtained are consistent with previous investigations [12,18]. It seems that slower migration of bands in the vicinity of the column walls results from excessive packing of the near-wall packing layer compared with the remaining part of less dense packing, not "leaning" against the rigid column walls.

Unsatisfactory peak shapes and poor column efficiencies are often observed in the conventional test also with columns dry-packed using the pre-

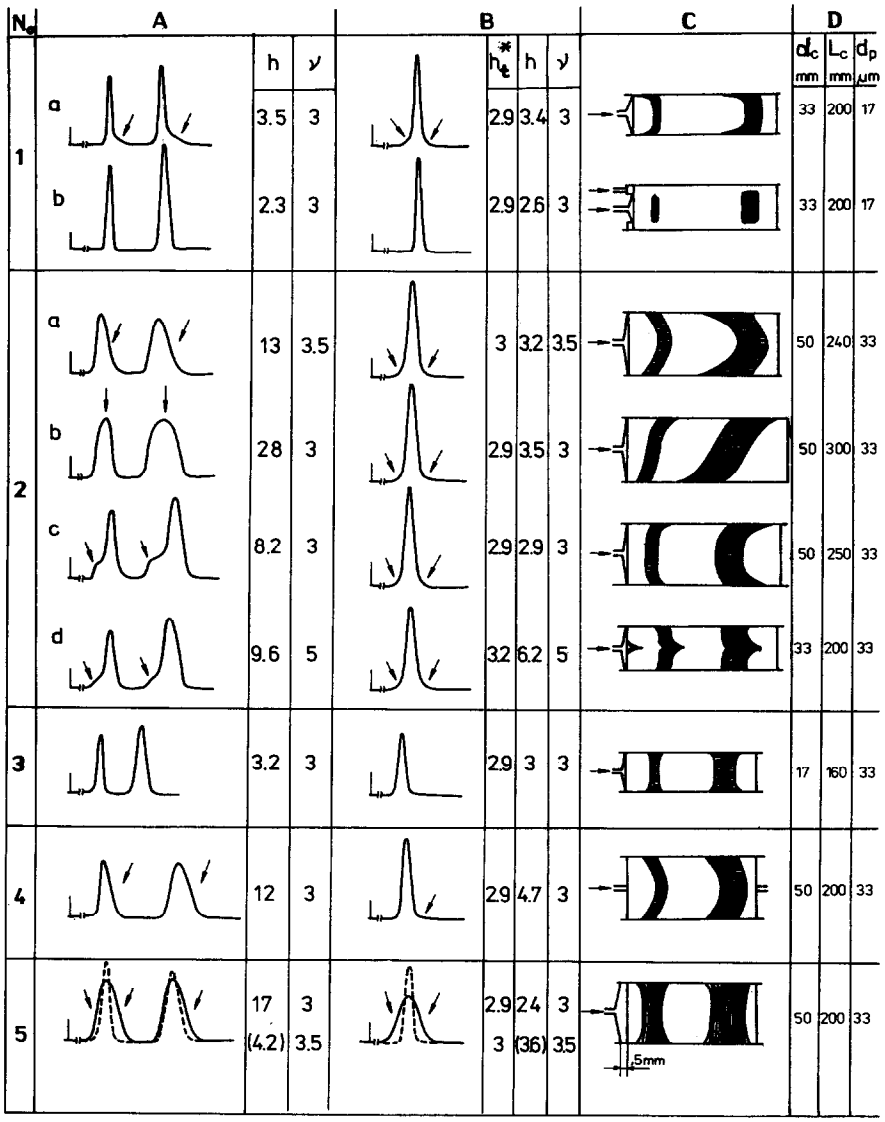


Fig. 6. Comparison of examples of typical results of the (A) conventional and (B) bidirectional tests of various preparative columns packed by the slurry method [12,17] (No. 1) and the impact method [13,15] (Nos. 2–5), under various conditions of application of the impact method, both optimum (Nos. 3, 4 and 5) and unfavourable (Nos. 2a–d). Column efficiency characterized by h for a given v. Arrows indicate unfavourable fragments of the chromatographic peaks. Distribution heads of an incorrect design were used for testing columns. Nos. 4 and 5. Experiments with column 1B were carried out under "infinite diameter" conditions (S = 50%, 50-μl sample). The dashed line in the case of column No. 5 illustrates testing with a proper distribution head (the respective values of h are given in parentheses). Column C illustrates the shapes of the zones of Sudan I dye retained during elution in a column (axial cross-section). * B = 1.6; A = 1.5; C = 0.06; $h_t = B/v + A \cdot v^{0.33} + C \cdot v$.

viously described impact method [13]. This method was used with mean packing particle diameter $d_p >$ 25–30 μm (Figs. 5 and 6, 2–5). The above statement particularly concerns long columns ($L_c >$ 25 cm) packed with an irregular packing material of a relatively broad particle size range (1.5 <

$d_{p\ max}/d_{p\ min} < 2$). Columns of poor efficiency are obtained particularly often when the packing material is introduced too slow (Fig. 6, 2, c) or too fast (Fig. 6, 2, a), or when a slant surface of the packing material is formed during packing (Fig. 6, 2, b). These difficulties are usually due to a non-plug liquid flow profile in the prevailing part or in the entire column cross-section (Fig. 6, 2, a–c). In spite of a wrong flow profile, under such conditions the bidirectional test yields HETP values that are very close to the theoretical values (column 2, B in Fig. 6).

Previous investigations [13] demonstrated that these phenomena are due either to spontaneous segregation of the packing particles during packing, intensified by the vibration of the column walls and of the packing (*i.e.*, too long packing time), or to a non-uniform radial interparticle porosity distribution of the packing bed [13] (*i.e.*, too short packing time). In the second case, the degree of packing density of the bed decreases with distance from the column wall owing to the vibrations of the walls and the resistance offered by them. Beds that are insufficiently packed and at the same time are not stabilized by the pressure of the heads, or dried beds, undergo splitting (Fig. 6, 2, d).

The bidirectional test almost completely eliminates these effects (Fig. 6, 2, a–d), as the molecules cover a similar route in the column in both directions. The certain broadening of peaks observed in this method mainly at the peak base is due to radial dispersion of mass in the column packing. Peak shapes (particularly at the base) and HETP values obtained from the conventional test were similar to those from the bidirectional test only in the very rare cases of a truly plug flow profile. This indicates that the unsatisfactory preparative LC column efficiency observed in practice is mainly due to a non-plug profile of liquid flow in the column (case 2 in Fig. 3).

Unfortunately, the results can be non-explicit and can lead to a conclusion that the liquid flow profile is incorrect also when the distribution head is incorrectly designed (too large radial flow resistance) (see Fig. 6, 4). However, an unnaturally rapid increase in the column operating pressure accompanying the increase in the mobile phase flow-rate is simultaneously observed in such instances, which helps in giving a proper diagnosis [an increase in the reduced permeability (Φ) ranging from 1300 for $v = 3$ to 3600 for $v = 78$ was observed for column 4 in Fig. 6; the decrease in permeability accompanying the flow-rate increase was eliminated by moving the upper head 2 mm away from the column packing].

On the other hand, when the distribution heads operate incorrectly owing to the so-called diffusional dead spaces inside the heads, the results of the bidirectional test with a well packed column are often worse than the results of the conventional test. In such cases the results of the bidirectional test can be better than the results of the conventional test when band broadening in the head is accompanied by a poor liquid flow profile in the column. Such a phenomenon occurs during conventional testing when the poor liquid flow profile influences the column efficiency to a larger extent than the diffusive broadening of the band in the head. Such effects have been observed, for example, when the head of column 5 (Fig. 6) was used for column 2b (Fig. 6). However, the value of h obtained from the bidirectional test, equal to *ca.* 25 ($v = 3$), although close to the value obtained from conventional testing ($h = 28$), is far from theoretical expectations ($h_t = 2.9$).

Examination of the proper operation of the distribution heads is nowadays less important, as commercial preparative LC columns usually have properly designed systems of radial distribution of the mobile phase to/from the packing surface and they do not have excessive dead spaces.

It is very important to check whether the column is completely packed and both the heads contact the packing bed before carrying out the bidirectional test, otherwise it is possible that the structure of the column packing bed will be destroyed owing to particle movement during the backward flow of the liquid phase. This statement means that the proposed bidirectional column test can be applied only for preparative columns with axial compression of the packing, achieved by mechanical, pneumatic or hydraulic pressing of the heads to the packing during column operation. It has been established experimentally that in such columns the packing bed was not destroyed by the reverse flow of the mobile phase. For instance, the HETP values determined for both directions of the mobile phase flow are similar provided that the heads are presented sufficiently strongly to the packing bed.

CONCLUSIONS

The described simple two-stage column test, consisting of a conventional test and a bidirectional test, produces repeatable results. It can yield a few practical benefits when it is performed on well designed columns (when proper operation of the distribution heads is certain and when the heads are strongly pressed to the packing bed), as follows.

(1a) The differences between the HETP values, and between the shapes of peaks obtained during the conventional testing (broad peaks) and bidirectional testing (narrow peaks), prove that the flow profile in the column is non-plug. The greater the difference between the peak widths from both tests, the further is the flow profile from plug flow. The HETP value obtained from the bidirectional test characterizes in such instances the potentially attainable column efficiency in the applied chromatographic system, while the HETP value calculated from the conventional test chromatogram has no simple physical sense, as it is influenced by the flow profile in the column, which is assumed to be plug flow in all definitions of HETP.

(1b) A similarity of peak shapes and HETP values obtained from both tests proves a satisfactory profile of liquid flow in the column. Should the HETP value be additionally close to the value calculated from the Knox equation, the structure of the column packing is satisfactory and the extra-column effects are negligible.

(2) When the liquid flow profile in the column is non-plug, the results of the bidirectional test characterize well the attainable column efficiency. This is very important when testing the applicability of new sorbents for preparative purposes.

(3) The bidirectional test permits the examination of the dependence of HETP on k', d_p, u_0 and other parameters, even when one cannot be sure whether the flow profile in the column is truly plug.

(4) The bidirectional test performed on analytical or microcolumns with a few elution distances can be one of the methods for the experimental determination of the extra-column broadening of bands of the separated substances.

In cases when proper operation of the distribution heads is doubtful:

(1) When it is certain that the structure of the packing and the flow profile are satisfactory, the differences between the HETP values in favour of the conventional test confirm the existence of large diffusion spaces in column heads, or large extra-column (out of the bed) band-broadening effects (*e.g.*, the existence of void volumes and/or blockage of the frit). On the other hand, a difference between the HETP values in favour of the bidirectional test can indicate excessive resistance of radial flow in the distribution head.

(2) When the occurrence of an incorrect liquid flow profile cannot be excluded, the comparison of the results of the conventional and the bidirectional test yields limited diagnostic data, and hence the application of additional methods of quantitative evaluation of the structure of the column packing is necessary in such instances in order to determine unequivocally the reasons for the observed poor column efficiency.

SYMBOLS

a'	value determined in the conventional test
a''	value determined in the bidirectional test
a	one of the following symbols: H_T, H_L, D_T (see Fig. 3)
A, B, C	constants in the Knox equation
d_c	column diameter
D	coefficient of axial dispersion in the column; $D \equiv \frac{1}{2} \cdot \frac{d\sigma_L^2}{dt} = \frac{1}{2} \cdot \frac{HL_c}{t_R}$
d_p	particle diameter (mm)
D_m	diffusion coefficient of sample substance in the mobile phase
H	height equivalent to a theoretical plate (HETP)
H_Φ	HETP attainable in the given column
H_T	HETP at the outlet of the column
H_L	HETP calculated on a basis of a local width of a band "stopped" in the column
h	reduced HETP ($h = H/d_p$)
L	elution distance along the column
L_c	column length
L_m	length of migration path of a sample in the column in one direction
l_0	retention distance for a non-retained substance on a conventional test chromatogram

l, l'	distance between the start and the peak maximum on a chromatogram (see Fig. 2)
l_m	distance between the start and the moment of flow direction reversal, measured on a chromatogram from the two-stage test
M_1, M'_1	first ordinary statistical moment of a chromatographic peak
Pe_D	dispersive Peclet number, $Pe_D = (ud_p)/D$
Re	Reynold's number, $Re = (u_o d_p \rho)/\eta$
Sc	Schmidt's number (diffusive), $Sc = \eta/(D_m\rho)$
$S_{1/2}$, $S'_{1/2}$	peak width at half-height (see Fig. 2)
t_0	retention time of a non-retained substance in the conventional test
t_R, t'_R	retention time of the test substance (see Fig. 2)
u_0	mobile phase linear flow velocity, $u_0 = L_c/t_0$
u	apparent rate of tracer migration along the column axis
V_R	retention volume
η	mobile phase viscosity
ρ	mobile phase density
ν	reduced mobile phase flow velocity, $\nu = u\, d_p/D_M$
μ_2, μ'_2	second central moment of a chromatographic peak
σ_v^2	variance of the concentration distribution in a peak, calculated in terms of volume
$\Sigma\sigma_{vex}^2$	tracer extra-column dispersion, calculated in terms of volume
σ_L^2	variance of tracer band broadening, calculated along the column

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