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Evaluation of packed capillary liquid chromatography columns and comparison with conventional-size columns

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Abstract

Apart from extracolumn effects peak dispersion in liquid chromatographic columns is caused by the column inlet, the packed bed, and the column outlet. A strategy applicable for independent evaluation of the individual sources of column band broadening was developed on the basis of the linear extrapolation method (LEM). This method was applied to compare the performance of packed capillary LC columns from various commercial suppliers with conventional-size columns. The columns differed widely in their performance with respect to peak shapes and widths for standard substances. The capillary columns were found well packed, but in some cases overall performance would benefit from improving the design of the area between the packed bed and the connecting capillaries, containing frits as well as dead volumes.

Keywords: Stationary phases, LC; Packed capillary columns; Linear extrapolation method; Column performance; Alkylbenzenes; Toluene; Uracil

1. Introduction

Particularly in the field of pharmaceutical quality control where usually a large number of structurally similar substances are to be separated high chromatographic resolution is mandatory. Satisfactory results are only achievable on the basis of high performance chromatographic instrumentation and separation columns. In contrast to conventional-size LC where suitable and well-matured equipment is commercially available for most applications this is actually not the case in miniaturized LC techniques, e.g. capillary LC.

Chromatographic resolution is always compromised by band broadening. The individual sources of peak dispersion should be independently assessed and optimized in order to obtain the best possible performance of the LC system.

If the column properties (stationary phase, length, particle diameter), the composition and linear velocity of the mobile phase and the separation temperature are equal, chromatographic behavior should be independent from column i.d., provided that the quality of the columns and the equipment are comparable. Although the transfer of analytical HPLC methods from conventional-size LC with typically 3.0–4.6 mm i.d. columns to capillary size dimensions of 0.1–0.5 mm i.d. columns promises several advantages such as significant reduction of the consumption and disposal of solvents, the ability to work with limited sample amounts and easier and more efficient interfacing with other instruments, e.g. electrospray ionization (ESI) MS [1–3], mainly the lack of reliable instrumentation and the limited availability of high quality capillary columns have prevented this technique from gaining more widespread acceptance. Only a limited number of successful transfers to smaller bore columns are described in literature in chiral [4–8], reversed-phase [9,10] and size-exclusion chromatography [11].

Recent work performed in our laboratory illustrated that band broadening generated by a commercial capillary LC system with some modifications is in the same range as by a conventional-size LC system [12]. The present work focuses on the efficiency of commercially available columns packed with C_{18} modified stationary phases. A strategy is described to separately assess band broadening due to the packed bed and dispersion caused by the inlet and the outlet configuration of a column, respectively. This method provides a quick and simple evaluation of the performance of the individual column parts. It was applied to investigate the performance of 0.3 mm i.d. capillary columns in comparison with conventional-size columns.

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Several packing techniques for capillary columns have been developed [13–19], but column packing still remains a challenge. Reasons are probably technical difficulties due to the small amounts of material handled and the often fragile tubing used. Furthermore, the so called 'wall effect' causes changes in packing density near the column wall and, hence, influences column efficiency [20–24]. This might be more important in small bore columns because the ratio particles near the column wall and particles in the core region are larger compared with conventional-size columns. Another challenge is the construction of devices to retain the packing material, e.g. immobilization owing to polymeric matrices, sintered frits or metal screens [25–28] and the design of the transition areas to the inlet and outlet capillaries. Frits have been shown to contribute significantly to band broadening in LC [29] and the volumetric sizes in capillary dimensions are even more unfavorable.

1.1. Theory and approach

For overall performance comparison of conventional-size and capillary columns packed with equal type of stationary phases the chromatographic resolution, R_S, was applied. The values needed for its calculation can be obtained directly from the chromatogram and no assumptions as in case of theoretical plate number, N, (explained later) are necessary. The following equation has been used for calculation:

$$R_S = \frac{2(t_{r2} - t_{r1})}{w_1 + w_2} \tag{1}$$

where t_{r1} and t_{r2} are the retention times of the peaks of interest and w_1 and w_2 the corresponding basis peak widths.

Band dispersion in a chromatographic run is the sum of extracolumn or instrumental band broadening and dispersion inside the column. The instrumental band broadening can easily be determined by measuring the variance of the signal obtained by replacing the column with a zero-dead-volume union. Band broadening inside the column can be further differentiated in dispersion due to the packed bed and due to the part between the packed bed and the inlet or outlet capillary. Detailed investigations of the column band broadening are more complex since the individual parts cannot be tested separately. It can be assumed that the packed bed behaves according to the van Deemter equation and the remains, e.g. dead volumes and frits, like extracolumn parts. Thus their influence on overall band broadening effects each peak to the same extent, in contrast to the retention time-dependent dispersion due to the packed bed. The linear extrapolation method (LEM) offers a possibility for separate evaluation of the quality of the packed bed and of the other column parts, respectively [30–35]. The basic assumption is that the true N-value for a column, N_{col}, is independent of retention time. This is only correct if the conditions pointed out by Claessens et al. are met [32]:

(i) the total variance is the sum of the column and instrument variances,
(ii) the instrument variance is independent of the capacity factors of the components,
(iii) all components have the same true plate height (equal diffusion coefficients and negligible plate height dependency on capacity factor),

where the column variance means the variance due to the packed bed and the instrumental variance includes the variance of the remaining column parts, e.g. dead volumes and frits.

Starting from the basic equation:

$$N = \frac{t_r^2}{\sigma^2} \tag{2}$$

where t_r is the retention time directly taken from the chromatogram and σ^2 is the measured overall variance, the following equation can be derived:

$$N_{col} = \frac{t_{r,col}^2}{\sigma_{col}^2} \tag{3}$$

where $t_{r,col}$ is the time which the analytes spend inside the packed bed region (column retention time), and σ_{col}^2 is the peak variance due to the packed bed (column variance). With the relationships:

$$t_{r,col} = t_r - t_{ex} \tag{4}$$

and

$$\sigma_{col}^2 = \sigma^2 - \sigma_{ex}^2 \tag{5}$$

where t_{ex} is the time, the analytes spend outside the packed bed, and σ_{ex}^2 the corresponding variance, Eq. (3) can be rewritten as:

$$N_{col} = \frac{(t_r - t_{ex})^2}{\sigma^2 - \sigma_{ex}^2} \tag{6}$$

and transformed to:

$$\sigma^2 = \frac{(t_r - t_{ex})^2}{N_{col}} + \sigma_{ex}^2 \tag{7}$$

By plotting the measured variance, σ^2, versus the column retention time squared, $t_{r,col}^2$, of a number of analytes a straight line graph can be obtained with $1/N_{col}$ as the slope. By linear extrapolation of the graph σ_{ex}^2 appears as the intercept (Fig. 1). Homologue compounds are suggested for that approach due to their similar chemical and physical properties.

The values N_{col} and σ_{ex}^2 express apparent separation characteristics and depend on several chromatographic conditions, e.g. the nature of the analytes, the type and velocity of the mobile phase, and column temperature. This can be used for an estimation of the band broadening in front and after the packed bed by performing two types of analysis, one under non-stacking and the second under stacking conditions. By using non-stacking conditions all extracolumn bed dispersion should be manifested in the value for the intercept.

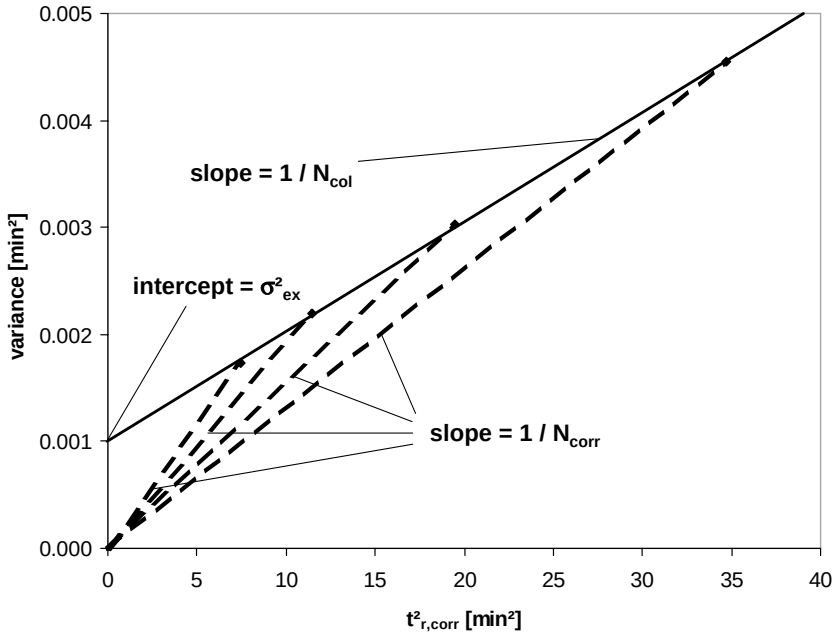

Fig. 1. Illustration of LEM. Toluene, ethyl-, *n*-propyl-, and *n*-butylbenzene data points are depicted. Conditions: test mixture 2 on XTerra RP 18, 100 mm × 0.32 mm, 3.5 μm particles (Waters); mobile phase, acetonitrile–water (60:40, v/v); flow rate, 4.8 μl/min; UV detection wavelength, 210 nm. $t_{r,corr}$ is the retention time corrected for the time the analytes spend outside the packed bed and N_{corr} the plate number based on $t_{r,corr}$.

On the other hand, the result of the precolumn dispersion is reduced or even eliminated owing to the enrichment of the analytes on the column head under stacking conditions. In that case the remaining intercept is related only to the post-column bed dispersion.

The focus of this work is mainly on the packing and the column hardware quality. Detailed examinations of packing material characteristics are out of the scope. Nevertheless, if only columns packed with different batches of stationary phase were available, the material characteristics had to be considered.

An unretained compound remains uninfluenced by the retention and exchange characteristics of the stationary phase. Its dispersion should only be due to the column parts beside the packed bed and the eddy diffusion determined by the particle diameter and the packing quality. With the dispersion of an unretained compound near the value of the LEM intercept determined under non-stacking conditions representing the dispersion outside the packed bed, a good packing quality can be assumed, even in case of a high value for the reduced height of a theoretical plate, *h*.

2. Experimental

2.1. Chemicals and chromatographic conditions

Gradient-grade methanol, gradient-grade acetonitrile (LiChrosolv, Merck, Darmstadt, Germany), and water obtained through a Milli-Q system (Millipore, Bedford, MA, USA) were used for preparing mobile phases by premixing of appropriate volumes and degassing 5 min in an ultrasonic bath. Test compounds, all of analytical-reagent grade quality, were obtained from Fluka (Buchs, Switzerland). Test mixture 1 contained uracil (36 ng/ml), methylparabene (35 ng/ml), ethylparabene (31 ng/ml), propylparabene (43 ng/ml) and butylparabene (39 ng/ml) dissolved in methanol–water (65:35, v/v). Test mixture 2 contained uracil (28 ng/ml), toluene (0.05 μl/ml), ethylbenzene (0.1 μl/ml), *n*-propylbenzene (0.1 μl/ml), *n*-butylbenzene (0.1 μl/ml), *n*-pentylbenzene (0.2 μl/ml) and *n*-hexylbenzene (0.2 μl/ml) dissolved in acetonitrile–water (50:50, v/v).

Chromatography under non-stacking conditions was performed with test mixture 1, methanol–water (65:35, v/v) as mobile phase and a detection wavelength of 254 nm. For stacking conditions test mixture 2, acetonitrile–water (60:40, v/v) as mobile phase and a detection wavelength of 210 nm were used. A column temperature of 30 °C and a data acquisition rate of 1 point/s were applied in general. The mobile phase flow rates used (Table 1) supply an equivalent linear velocity for all columns assuming a comparable packing density and can be converted using the scaling factor, $f = d_1^2/d_2^2$, where d_1 and d_2 are the column diameters. The same factor had to be applied to the injection volumes (Table 1) to provide an equivalent analyte load.

The characteristics of the investigated columns are given in Table 1.

2.2. Equipment

The ‘low dispersion’ capillary LC set-up described previously was used for capillary LC experiments [12]. It consists

Table 1
Columns and chromatographic conditions applied in performance study

Sign	Column	i.d. (mm)	Length (mm)	Particle size (μm)	Inlet configuration[a]	Outlet configuration	Flow rate (μl/min)	Injection volume (μl)
A1	XTerra RP18 (Waters, Milford, MA)	4.6	100	3.5	1/16 in. female	1/16 in. female	1000	24
A2	XTerra RP18 (Waters, Milford, MA)	0.32	100	3.5	1/16 in. female	1/16 in. female	4.8	0.1
B1	Prontosil 120 ace-EPS C_{18} (VDS optilab, Berlin, Germany)	4.0	150	3	1/16 in. female	1/16 in. female	760	18
B2	Prontosil 120 ace-EPS C_{18} (VDS optilab, Berlin, Germany)	0.3	150	3	1/16 in. female	1/16 in. female	4.3	0.1
C1	Sapphire 110 C_{18} (Grom, Rottenberg-Hailfingen, Germany)	4.0	150	5	1/16 in. female	1/16 in. female	760	18
C2	Sapphire 110 C_{18} (Grom, Rottenberg-Hailfingen, Germany)	0.3	150	5	150 μm i.d. steel capillary of 6 cm length	75 μm i.d. fused silica capillary of 30 cm length	4.3	0.1
D1	YMC Pro C_{18} (YMC, Tokyo, Japan)	4.6	150	3	1/16 in. female	1/16 in. female	1000	24
D2	YMC Pro C_{18} (YMC Europe, Schermbeck, Germany)	0.3	150	3	50 μm i.d. fused silica capillary of 50 cm length	50 μm i.d. fused silica capillary of 50 cm length	4.3	0.1
E1	Inertsil ODS 3 (GL Science, Tokyo, Japan)	4.6	150	3	1/16 in. female	1/16 in. female	1000	24
E2	Inertsil ODS 3 (LC Packings, Amsterdam, The Netherlands)	0.3	150	3	PEEK shielded fused silica capillary of 5 cm length	50 μm i.d. fused silica capillary of 50 cm length	4.3	0.1
F	Protecol C_{18} (SGE, Melbourne, Australia)	0.3	150	3	PEEK shielded fused silica capillary of 20 cm length	PEEK shielded fused silica capillary of 10 cm length	4.3	0.1
G	Polaris C_{18} A (Unimicro Technologies, Pleasanton, CA)	0.32	150	3	Only bare column without connections	Only bare column without connections	4.8	0.1
H	HotSep Kromasil C_{18} 100 (G&T Septech, Oslo, Norway)	0.32	150	3.5	50 μm i.d. fused silica capillary of 25 cm length	50 μm i.d. fused silica capillary of 25 cm length	4.8	0.1

For columns B and C the same packing material batch was used in capillary and conventional-size LC.
[a] 1 in. = 2.54 cm; PEEK: polyether ether ketone.

of a CapLC-System from Waters (Waters, Milford, MA, USA) equipped with a model C2-1006D six-port injection valve from VICI (Schenkon, Switzerland) and a 25 μl sample syringe. The injections were performed using the timed injection mode, in which the sample is first transferred by the sample syringe into the sample loop. The volume injected is now determined by the time the injection valve is left in the INJECT position and by the flow rate. Back switching of the valve to the LOAD position ensures a sharp, tailing free rear end of the injected sample zone. Connections were mostly constructed with fused silica capillaries of 50 μm i.d. (Polymicro, Phoenix, AZ, USA) in different lengths. For detection a ^{3D}CE diode array detection (DAD) system from Agilent (Waldbronn, Germany) equipped with a perpendicular flow cell was used. The perpendicular flow cell was prepared by burning off the polyimide coating over a small distance from a 50 μm i.d. fused silica capillary to provide an optical window. The window was cleaned with methanol and introduced into the green (aperture 620 μm × 40 μm) Agilent CE interface. For column temperature control the CapLC-System included oven was used.

The conventional-size chromatograph consists of an Alliance 2690 and a 2487 UV-detector with 10 μl flow cell (Waters). Connections were performed with 125 μm i.d. stainless steel tubing (VICI). The column was hold at constant temperature with a column-thermostat Jetstream 2 plus from VDS Optilab (VDS Optilab, Berlin, Germany).

2.3. Data acquisition and calculation

Data acquisition was performed with ChemStation Software 6.1 (Agilent) in the case of capillary LC and with Millennium 4.0 Software (Waters) in the case of conventional-size LC. The variances were examined from basis peak widths, w, determined by the tangent method, by $\sigma^2 = (w/4)^2$. The tangent method was preferred to second central moments, although the resulting variances are only accurate for Gaussian peaks, because this method represents the apparent resolution in a better way [36,37]. Furthermore, it is more robust with regard to start and end points of integration and more widely used. It underestimates, however, the variance of peaks showing foot tailing and consequently underestimates the intercept σ^2_{ex} in certain cases. Methods taking into account peak asymmetry, e.g. the Foley–Dorsey equation, were not applied as they do not correctly describe Gaussian peaks with foot tailing, because the tailing appears mainly below five percent of total peak height.

3. Results and discussion

3.1. Correction of retention time

For accurate calculations the retention time read from the chromatogram, t_r, must be reduced by the time the analytes spend outside the column bed, t_{ex}. Otherwise, the t_r-dependent values, e.g. plate number, N, are misleading. Table 2 illustrates contradictory results produced by evaluation of resolution, R_S, and plate number, N, of corresponding columns, i.e. capillary and conventional-size columns packed with the same type of stationary phase. The conventional-size column gives higher values for R_S in correlation with lower ones for N compared with the capillary column, although the net retention times are similar. The reason for this contradictory result is the higher extent of t_{ex} in capillary LC owing to the limited possibilities of its reduction. The internal diameter of connecting tubing must remain in functional appraisal to clogging tendency and back pressure. This becomes evident in calculation of N and increases the value considerably, even if the peak variance is slightly higher.

To overcome the problem, the measured retention time must be corrected. In conventional-size LC, the column was replaced by a zero-dead-volume union and the retention time of the obtained signal was taken as t_{ex}. In capillary LC, this solution is not applicable because the inlet and/or outlet capillaries are frequently solidly connected to the separation column (Table 1) and cannot be removed without damaging the column. Additionally, opposite to conventional-size columns the volume between the packed bed and the capillaries (frits, dead volume) contributes noticeably to t_{ex}. Assuming similar packing density and linear flow velocity in

Table 2
Illustration of the advantage when retention times are corrected for extracolumn time in the calculation

	Resolution		t'_r		N		N_{corr}	
	0.32 mm i.d.	4.6 mm i.d.	0.32 mm i.d.	4.6 mm i.d.	0.32 mm i.d.	4.6 mm i.d.	0.32 mm i.d.	4.6 mm i.d.
Uracil					2875	1188	628	865
Toluene	11.5	12.7	1.81	1.82	8336	5268	4333	4705
Ethylbenzene	3.6	3.8	2.45	2.46	8955	6258	5191	5707
n-Propylbenzene	5.1	5.3	3.49	3.49	9883	7541	6429	7024
n-Butylbenzene	6.0	6.2	4.96	4.96	10621	8607	7630	8158
n-Pentylbenzene	6.8	7.0	7.05	7.04	11031	9428	8598	9061
n-Hexylbenzene	7.6	7.7	10.11	10.07	11096	9794	9237	9516

Conditions: test mixture 2 on XTerra RP 18, 100 mm, 3.5 μm particles (Waters); mobile phase, acetonitrile–water (60:40, v/v); flow rates, 4.8 and 100 μl/min, respectively; UV detection wavelength, 210 nm.

corresponding columns the column retention time, $t_{r,col}$, of uracil is the same in both cases. To determine t_{ex} in capillary LC, the $t_{r,col}$ value of the uracil peak obtained with the corresponding conventional-size column was subtracted from the capillary LC uracil retention time, t_r, to calculate t_{ex} (Eq. (4)). For the remaining capillary columns, the mean of the t_{ex} values (1.3 min) observed with the 150 mm columns (B2–E2) was taken assuming similar behavior.

Further calculations containing retention times, e.g. LEM, were done using the corrected $t_{r,col}$ values.

3.2. LEM

All chromatographic runs were performed in duplicate and the mean values were used for calculation of N_{col} and σ^2_{ex} by LEM. Individual values varied by not more than 2% for t_r and not more than 5% for w. Most of the resulting graphs showed non-linear behavior. Examples are shown in Fig. 2. In the case of parabenes, a convex derivation appeared due to the butylparabene signal and is probably related to a stacking effect for that compound, which reduces dispersion. In contrast, alkylbenzenes pentyl- and hexylbenzene often effected a concave derivation which is probably related to a higher mass transfer inhibition between mobile and stationary phase resulting in increased band broadening. Therefore, only the homologues methylparabene to propylparaben and toluene to butylbenzene, respectively, could be used for performance evaluation by LEM as the others obviously fail to meet the conditions mentioned earlier. The correlation coefficients of the graphs considering the remaining compounds were in the range of 0.9983–0.9999 and thus sufficient for this project.

A 150 μm i.d. fused silica capillary of 100 mm length (Polymicro) was placed between column and detector to effect extensive extracolumn dispersion in order to challenge the applicability of the LEM. The chromatograms recorded with and without additionally connected capillary were evaluated for N_{corr} and N_{col} (Table 3). Opposite to N_{corr}, N_{col} was only slightly affected, despite the large extent of additional extracolumn dispersion.

Table 3
N_{col} stability in presence of additional extra column dispersion

	N_{corr}	
	Without	With capillary
Uracil	628	304
Toluene	4333	2429
Ethylbenzene	5191	3260
n-Propylbenzene	6429	4367
n-Butylbenzene	7630	5695
n-Pentylbenzene	8598	6935
n-Hexylbenzene	9237	8054
N_{col}	9733	8770

Conditions: test mixture 2 on XTerra RP 18, 0.32 mm × 100 mm, 3.5 μm particles (Waters); mobile phase, acetonitrile–water (60:40, v/v); flow rate, 4.8 μl/min; UV detection wavelength, 210 nm; capillary 150 μm i.d., 100 mm length.

3.3. Column performance

The results of the performance investigation are depicted in Figs. 3 and 4. Serving as overall performance indicator for non-stacking conditions, the resolution of the early eluting peak pair methyl/ethylparabene was chosen, which takes into account the extracolumn bed dispersion to a substantial extent. In the case of stacking conditions, the later eluting peak pair propyl/butylbenzene was selected for better characterization of the individual column bed performance.

The reduced height of a theoretical plate, h, was preferred to N to describe column bed performance because it enables better comparison between the individual columns. The variable h takes different column lengths, l, and particle sizes, d_p, into account with the following relationship:

$$h = \frac{N}{l d_p} \tag{7}$$

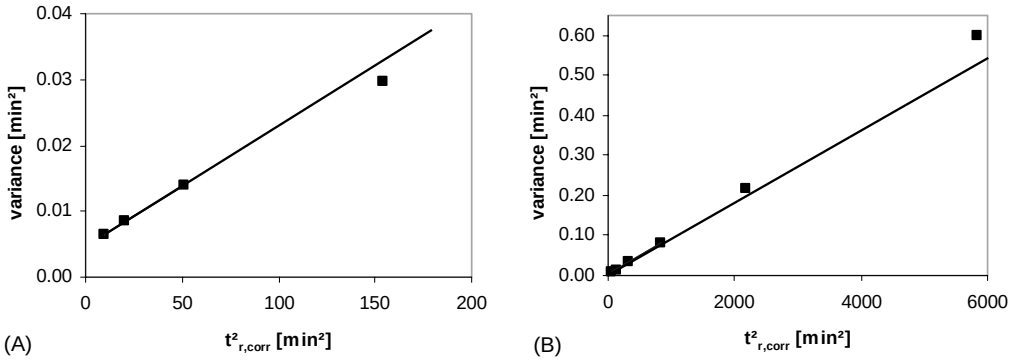

Fig. 2. Non-linear behavior of longer retained compounds using LEM. Conditions: Sapphire 110 C_{18}, 150 mm × 0.3 mm, 5 μm particles (Grom); flow rate, 4.3 μl/min; injection volume 0.1 μl, column temperature, 30 °C. (A) Convex derivation; methyl-, ethyl-, *n*-propyl-, and *n*-butylparabene data points are depicted. Conditions: test mixture 1; mobile phase, methanol–water (65:35, v/v); UV detection wavelength, 254 nm. (B) Concave derivation; toluene, ethyl-, *n*-propyl, *n*-butyl-, *n*-pentyl-, and hexylbenzene data points are depicted. Conditions: test mixture 2; mobile phase, acetonitrile–water (60:40, v/v); UV detection wavelength, 210 nm.

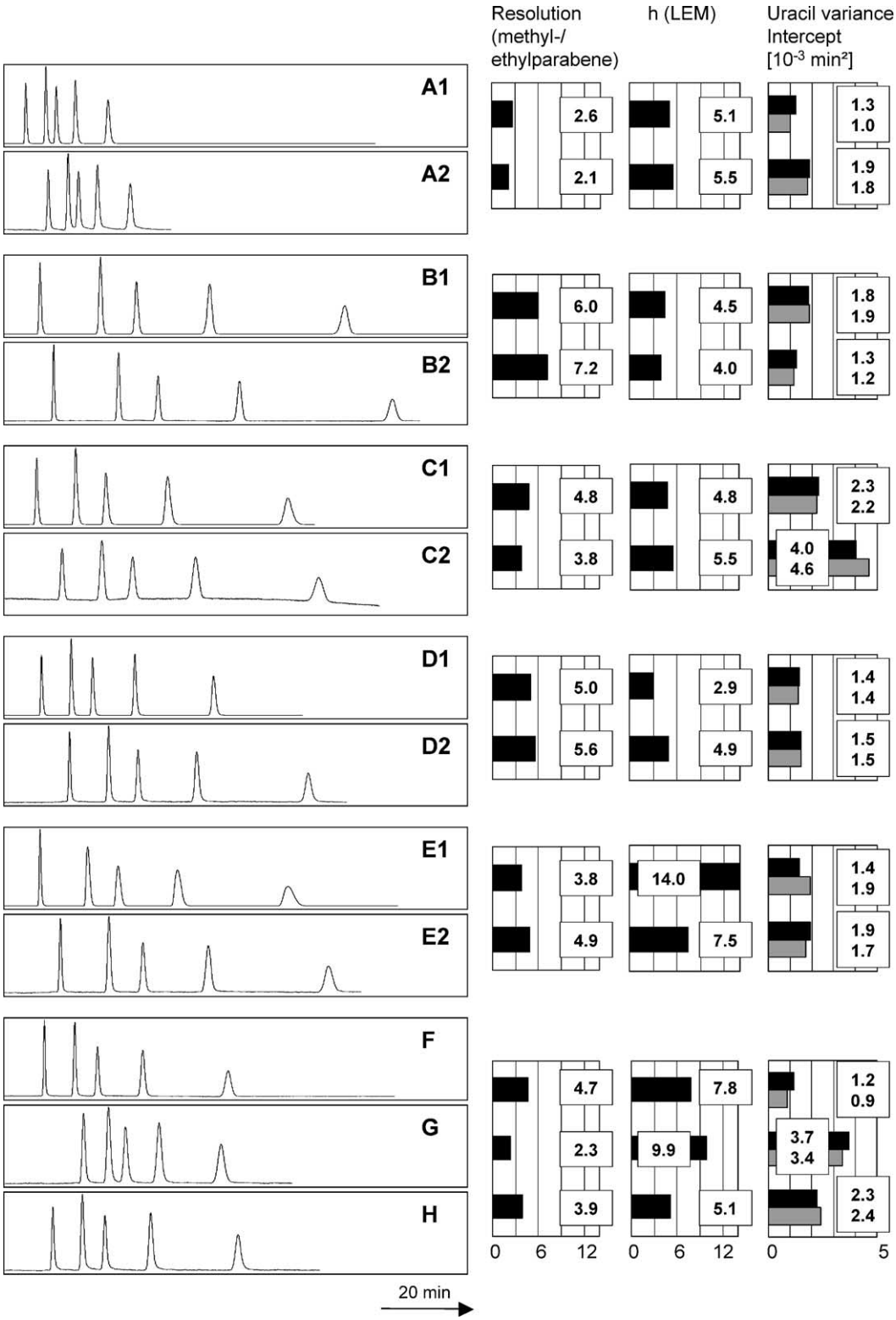

Fig. 3. Performance investigation under non-stacking conditions. Conditions: test mixture 1; mobile phase, methanol–water (65:35, v/v); UV detection wavelength, 254 nm. For flow rates and injection volumes see Table 1, except column G. In case of column G only a flow rate of 3.8 μl/min could be applied due to limited back pressure stability with used connections. *y*-axes represent arbitrary units and are scaled according the largest peak.

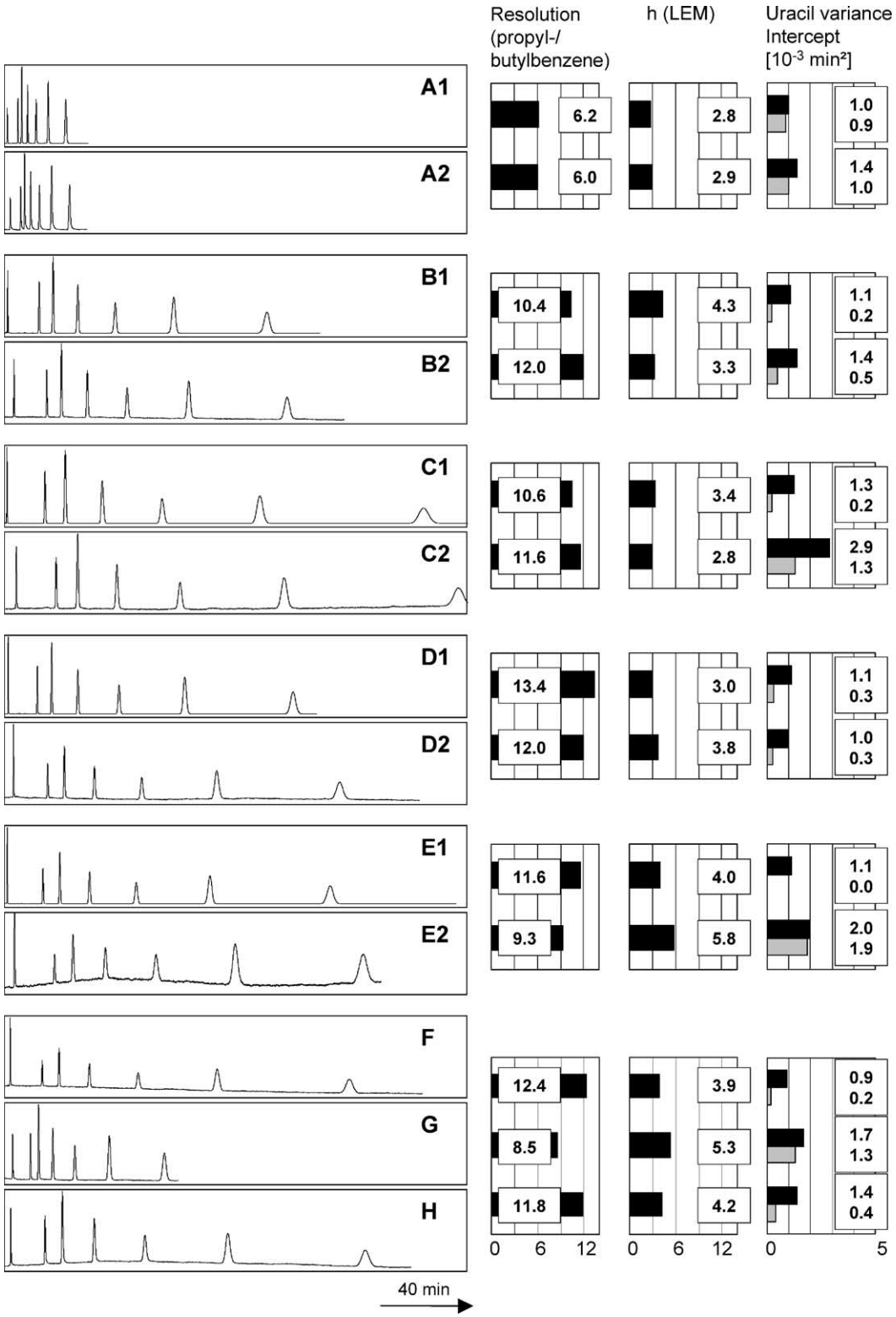

Fig. 4. Performance investigation under stacking conditions. Conditions: test mixture 2; mobile phase, acetonitrile–water (60:40, v/v); UV detection wavelength, 210 nm. For flow rates and injection volumes see Table 1. The *y*-axes represent arbitrary units and are scaled according the largest peak.

The variances of the uracil peaks were analyzed to enable a direct comparison with the corresponding intercepts calculated by the LEM. This analysis shows that the variances of each pair are in the same range under non-stacking conditions, indicating good packing quality for all examined columns. In addition, a reduction of mobile phase viscosity, as in the case of changing from methanol–water to acetonitrile–water mixtures, leads to a decrease of the uracil variances. In most cases a significant part of extracolumn dispersion occurs in front of the column because a considerable difference between the uracil variance and the intercept becomes apparent when stacking conditions are applied.

The columns of type A show similar resolution of the benzene peak pair combined with comparable values for h and σ^2_{ex}. The foot tailing of the capillary column, underestimated by the tangent method, should also be taken into account. The lower resolution of the parabene peak pair of the capillary column in connection with an almost doubled intercept variance indicates a higher extent of precolumn band broadening. This, as well as the observed foot tailing can be explained by enlarged dispersion occurring within the area between packed bed and connecting capillary in case of the capillary column.

With columns of type B performance of the capillary column was superior over the corresponding conventional-size column. The reason for the slightly longer retention times in conjunction with a lower h value of the capillary column is probably a higher packing density, because the linear flow velocity and the packing material batch are the same in both columns.

In the case of columns of type C, a large extent of dispersion occurs in front of the capillary column, which is probably due to the steel tubing serving as connector (Table 1). Using stacking conditions this drawback could be overcome as the resolution comparison of the benzenes shows. Alternatively columns with a fused silica capillary inlet are commercially available.

With columns of type D large differences in net retention times were attained which are probably related to batch variations of the packing material since other parameters influencing net retention times were controlled. This results in a higher resolution value for the capillary column in case of the parabene peak pair although its h is higher and the calculated σ^2_{ex} in the same range compared with the conventional-size column.

The performance differences of the columns of type E can mainly be explained by a higher dispersion due to the outlet of the capillary column and probably by dissimilar packing material characteristics, which become apparent by the distorted parabene peaks in the conventional-size column.

In general, the capillary columns F, G, and H show similar characteristics to the former columns. In the case of columns G and H foot tailing and, especially by column G, a relatively large intercept was detected, indicating additional band broadening caused by frits or dead volumes. Column F generates highly symmetrical peaks.

4. Conclusion

The developed approach was successfully applied to evaluate the performance of several capillary LC columns.

Capillary columns of high quality packed with C_{18} type stationary phases covering a wide range of selectivity are commercially available from various suppliers. The differences of the packing quality of the column bed between capillary and conventional-size columns were found negligible, which is in contradiction to our predictions pointing on the possibly enhanced ‘wall effect’ and technical challenges by the reduction of scale.

The design of the area between the packed bed and the connecting capillaries, including the construction of devices to retain the packing material, has been identified as the most challenging part in manufacturing high performance capillary columns.

The described method should be further applicable to evaluate other types of columns like nano and monolithic ones and offers potential for the development of new packing procedures or to improve the design of column hardware.

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