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Extracolumn band broadening in capillary liquid chromatography

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Abstract

A commercially available capillary LC instrument was modified to investigate and control the contribution of different instrument components on extracolumn band broadening. Quantitative estimations of dispersion induced by several equipmental parts were carried out. Injection parameters could be optimized to achieve the theoretical value of 12 for a profile factor describing a rectangular sample profile. Additionally, an additive injector flow channel dependent dispersion effect was found. A practical approach for minimizing instrumental effects in capillary LC is suggested. The results were compared with those obtained with an HPLC instrument designed for conventional size columns.

Keywords: Extracolumn band broadening; Band broadening; Capillary columns

1. Introduction

Capillary LC with packed 0.1–0.5 mm inner diameter (i.d.) columns has been established as an alternative technique to conventional sized LC [1–9]. The increased mass sensitivity enables the analysis of expensive, limited and highly diluted samples. The reduced scale gives the opportunity to use expensive separation phases and limits consumption of hazardous organic solvents. The ability to work with minute sample sizes and small volumetric flow rates allowing direct coupling with special detectors (MS, NMR) are the most important benefits. Opposite to other miniaturized techniques like capillary electrophoresis, up- and downscaling of capillary LC methods is possible to adjust the separation to the actual analytical requirements, e.g. with respect to the available sample amount. Currently, HPLC methods using 3.0 and 4.6 mm i.d. columns are still dominating the field of sensitive and selective determination of drugs and drug products for quality control in pharmaceutical industry. Lack of suitable miniaturized equipment and high-quality columns prevented capillary LC from widespread operation in analytical laboratories. In recent years, miniaturized analytical systems and columns from a number of suppliers became available, so that it should be possible to use the mentioned advantages of miniaturization in pharmaceutical analysis. In order to introduce capillary LC in pharmaceutical quality control all requirements for validation by

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the authorities must be met. Therefore, development of sensitive, selective and robust capillary LC methods for routine work according to GMP is necessary.

Peak dispersion deteriorates selectivity and sensitivity of separation methods. Peak volumes are directly related to the square of the column diameter, therefore, the effect of various HPLC instrument band broadening sources becomes more evident with smaller columns and smaller injection volumes. Extracolumn band broadening can compromise separations and is one of the major challenges in miniaturized LC [10–15]. Furthermore, column packing and design are more critical.

This work presents a detailed investigation of dispersion sources of commercially available capillary LC equipment including different injection modes, injection valves, sample loops, connecting capillaries, detector flow cells and connectors. A general procedure to identify the most important dispersion effects of the used instrumentation is described and strategies for their minimization are suggested. The performance of the optimized instrumental set-up was compared with conventional size equipment with special emphasis to extracolumn band broadening.

1.1. Theory

The effect of extracolumn band broadening on separation efficiency has been studied by a number of workers and specific equations and methods have been developed for the determination of instrumental dispersion [16–26]. The overall performance of a chromatographic system is given by the performance of the column itself and by the instrumental contribution to the band broadening. Assuming that all contributions to peak variance are independent, the total variance of a peak is the sum of these contributions:

$$\sigma^2_{\text{tot}} = \sigma^2_{\text{col}} + \sigma^2_{\text{cap}} + \sigma^2_{\text{inj}} + \sigma^2_{\text{det}} + \sigma^2_{\text{other}} \quad (1)$$

where σ^2_{tot} is the observed peak variance, σ^2_{col} the column variance, σ^2_{cap} the variance originating from connecting capillaries, σ^2_{inj} the variance originating from the injection plug and the injection process, σ^2_{det} the variance originating from the detector cell volume and contributions of the detector time constant, and σ^2_{other} the contributions from the remaining components, e.g. unions. The variances can be given in time, e.g. min^2, or volume, e.g. μl^2 units and can be converted with the square of the flow rate.

Several methods have been used to calculate extracolumn band broadening, e.g. without a column in the system by direct coupling of the injection and detection device or with a column in the system by the linear extrapolation method (LEM) [20,22,27] or whole column detection chromatography [28]. The last mentioned procedure, where the sample plug dispersion is measured with photodiodes placed along a transparent column, is not applicable for most HPLC columns. The LEM is based on a correlation of peak variances and capacity factors of a number of homologues and takes the dispersion contributions associated with frits and the flow profile at the entrance and exit of a column into account. The first method allows the determination of the variance only due to the instrument and was used for this work by performing triplicate injections of an uracil solution into various capillary LC set-ups.

1.2. Connecting capillaries

A connecting capillary is usually necessary between the injector and the column and the column and the detector, respectively. The theory of sample plug dispersion in open tubes is known and the volume variance can theoretically be calculated with the Taylor–Aris equation [22,29]:

$$\sigma^2_{v,\text{cap}} = \frac{r^4 l \pi F}{384 D_{\text{m}}} \quad (2)$$

where $\sigma^2_{v,\text{cap}}$ is the variance originating from the capillary in volume units, r the radius, l the length of the capillary, D_{m} the diffusion constant of the analyte in the mobile phase, and F the flow rate. The dispersion is diffusion controlled and results in Gaussian peak shapes. The determination is practically more difficult as the capillary radius may vary along the length and a value for the diffusion constant of the analyte in the mobile phase is often not available. For practical purposes Eq. (2) can be simplified to:

$$\sigma^2_{v,\text{cap}} = r^4 l K \quad (3)$$

where

$$K = \frac{\pi F}{384 D_{\text{m}}} \quad (4)$$

1.3. Injection

The contribution of sampling to the total peak variance depends on the profile and size of the sample plug [17,18,22,26,30]. It is usually assumed that the contribution to the band variance of the sample introduction is given by the equation:

$$\sigma^2_{v,\mathrm{inj}} = \frac{V^2_{\mathrm{inj}}}{D^2} \quad (5)$$

where $\sigma^2_{v,\mathrm{inj}}$ is the sample plug variance in volume units, V_{inj} the sample volume and D^2 is a constant for each injection technique. The constant D^2 is related to the sample profile and equals 12 for a rectangular sample plug. This represents the lowest possible contribution from the injection process.

1.4. Detection

UV detection has remained one of the most popular LC detection principles, especially in pharmaceutical quality control. The flow properties of the detector cell, its volume, other construction features, and the detector response time can contribute to band broadening. Due to its importance, band broadening caused by detection has often been topic of interest [31–37]. The theory of dispersion in detection devices is more complex as the impact of the parabolic velocity profile of the liquid passing through the cell, the logarithmic dilution effect resulting from the finite cell volume, diffusion contributions as well as data acquisition and electronic characteristics must be considered [17,18].

Two kinds of flow cells are generally applied for capillary LC: perpendicular or cross flow cells and longitudinal or parallel flow cells (Fig. 1) [35,36]. The band broadening caused by proper perpendicular flow cells should be negligible as the effective cell volume is reduced to a minimum, but the short path lengths result in a substantial loss of concentration sensitivity. The geometry of the longitudinal flow cells provide a relatively long optical path length, leading to a higher sensitivity, but unfortunately increasing dispersion. Extended light path (bubble) cells were developed for sensitive, low dispersion detection in capillary electrophoresis (Agilent, Waldbronn, Germany) and should be applicable for capillary LC, too. In the case of extended light path cells, a small fraction of a narrow capillary is enlarged by etching to offer a two to three times longer light path (Fig. 1). Nevertheless, this enhancement is not sufficient to attain sensitivity as known from conventional LC.

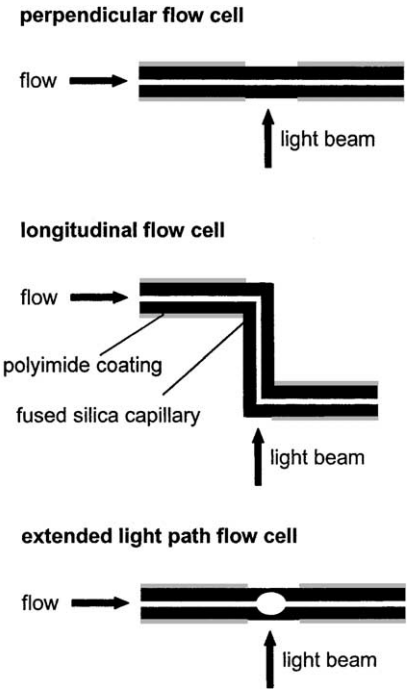

Fig. 1. Flow cell geometries.

2. Experimental

2.1. Chemicals and chromatographic conditions

Gradient-grade methanol and water (LiChrosolv, Merck, Darmstadt, Germany) 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) and dissolved in mobile phase. Unless otherwise mentioned, methanol with a flow rate of 5 μl/min was used as mobile phase and a methanolic uracil solution (33 ng/ml) to indicate the dispersion effects.

2.2. Equipment

Capillary LC experiments were performed on a CapLC-System from Waters (Waters, Milford, MA).

The conventional size chromatograph consists of an Alliance 2690 and a 2487 UV-detector with 10 μl flow cell (Waters).

The autosampler of the CapLC-System was equipped with a model C2-1006D 6-port-injection valve from VICI (Schenkon, Switzerland) including stators and rotors of 150 and 250 μm internal passage diameters, respectively, as well as a 25 μl sample syringe.

Sixty-two micrometers i.d. polyether ether ketone (PEEK) tubes (UpChurch, Oak Harbor, WA) and fused silica capillaries of 25, 50, 75, and 150 μm i.d. (Polymicro, Phoenix, AZ) in different lengths served as connecting capillaries. In case of fused silica capillaries, the capillary ends were covered by fitting 1/16 in. o.d. PEEK-sleeves (Upchurch) and fixed with stainless steel fittings (VICI). Stainless steel zero-dead-volume unions of 150 and 250 μm bores (VICI) were used for calculating the impact of connections.

UV detection was performed at 254 nm. As detection devices the CapLC-System included DAD with a 250 nl longitudinal flow cell of 5 mm optical path length (Waters), a API 785 A detector (Applied Biosystems, Foster City, CA) equipped with a 35 nl longitudinal flow cell of 8 mm path length (LC Packings, Amsterdam, The Netherlands) and a 3D CE DAD from Agilent (Agilent, Waldbronn, Germany) equipped with extended light path (Agilent) and perpendicular flow cells were used. The perpendicular flow cells were prepared by burning off the polyimide coating over a small distance from the 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. In connection with the extended light path cell a red (aperture 145 μm × 145 μm) Agilent CE interface was used.

2.3. Data acquisition

To minimize electronic effects on peak shape and band width, a high data acquisition rate of 40 points/s (3D CE) and 20 points/s (other detectors) was chosen for extracolumn band broadening studies. Data acquisition was performed with ChemStation Software 6.1 (Agilent) in case of CE detector, with Millennium 4.0 Software (Waters) in case of API detector and with MassLynx Software 3.5 (Micromass, Manchester, UK) in case of CapLC detector.

The volume variances were determined by multiplication of the time based second central moments, M2, calculated by the ChemStation Software with the square of the flow rate. Second central moments are accurate for all peak shapes and Gaussian peaks, necessary for other calculation methods, could not be assumed in most cases. The comparison of capillary and conventional equipment was carried out under typical operation conditions with a data acquisition rate of 2 points/s and by examining the time variance from basis peak widths, w, by $\sigma^2 = (w/4)^2$, because this method is more commonly used. It was also applied to data obtained with the CapLC-System DAD since a calculation of M2 for these data was impossible due to software incompatibilities.

3. Results and discussion

3.1. Connecting capillaries and unions

A number of fused silica capillaries varying in i.d. and length was tested. To determine the constant K and to prove the applicability of the relationship given in Eq. (2), linear regression of the measured variances and $r^4 l$ was performed. For constant K a value of 0.54 mm could be found as the slope. The intercept represents the residual variance due to the remaining system, e.g. injection and detection, and could be determined as 0.004 μl^2 (Fig. 2). These values show that connecting capillaries are an important source of extracolumn band broadening and should be carefully chosen. The reduction of i.d. and length is necessary to get low dispersion, but limited due to back pressure and clogging problems and the arrangement of certain system parts. Under the applied conditions an optimum capillary i.d. of 50 μm was found. It must be noted that the constant K depends on the analyte and on the type and velocity of the mobile phase. Fig. 3A shows peaks obtained with different connecting capillaries under otherwise equal conditions. It is seen that dispersion due to capillaries leads to a Gaussian contribution. The increasing absorbance observed with capillaries of larger i.d. is a result of the longer optical path length during perpendicular detection. The good linearity of the regression line and the shape

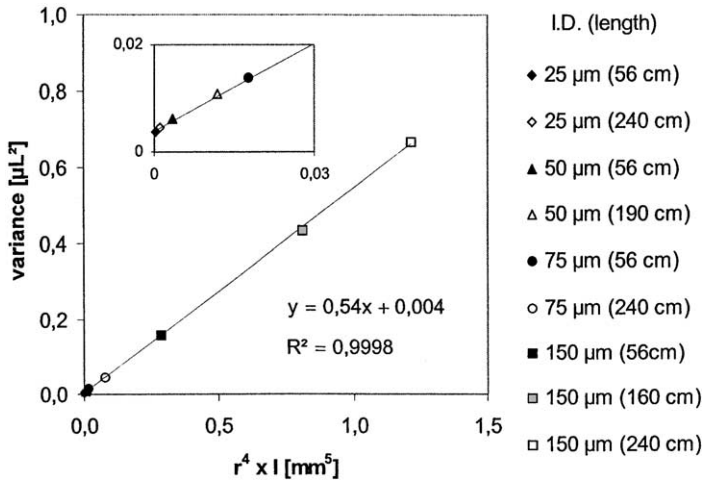

Fig. 2. Peak variances obtained with different connecting capillaries. Conditions: 250 μm bore injection valve with 10 μl sample loop; 0.1 μl injection volume placed by timed injection; perpendicular detection. Each data point is the mean of three measurements. Fault indicators are not given due to clarity reasons. The small diagram represents the enlarged left lower corner of diagram 1.

of the resulting peaks indicate that peak dispersion as predicted from theory could be achieved. To examine the band broadening effect of connections and unions, a capillary was cut in the middle and both ends were joined using a zero-dead-volume union. Two zero-dead-volume unions with different inside bores were tested. It was found that the contribution of the additional two connections and the union to the total bandwidth is insignificant in both cases. This means that high-quality connections are not a major issue to extracolumn band broadening in capillary LC.

3.2. Injection

In the injection process the injection hardware including injection valve and sample loop, the injection mode, and the injection volume can be influenced by the chromatographer. The dispersion of the sample zone depends on the injection mode and on the valve. Decreasing the stator passage i.d. can greatly enhance injection performance [11,39,41]. Bakalyar et al. described, that the dispersion caused by the injector is the sum of the sample plug variance, which depends only on the sample volume and on the variance caused by dispersion of the sample zone as it passes through and out of the injector [26]. In this work, micro-valves with 150 and 250 μm stator bores and the appropriate rotors were used. A 10 μl stainless steel sample loop of 250 μm i.d. and a 1 μl fused silica sample loop of 100 μm i.d. (laboratory made) served as sample chambers. A scheme of the injection valve is given in Fig. 4.

The following injection modes were applied: partial loop injection, in which the volume injected is determined by the amount dispensed from the sample syringe and timed injection, in which the volume injected is determined by the time the injection valve is left in the INJECT position and the flow rate [42,43]. Full loop injection of sample volumes in the nanoliters range cannot be performed with external loop valves as it is impossible to construct the required sample chamber without pressure problems effecting the sample syringe. Microliter pickup injection, in which an additional transport solvent is used to place the sample volume into the sample loop usually results in poor peak shape and low reproducibility of sample size and cannot be used without stacking procedures. Due to these findings the last two injection modes were not considered during further investigations.

To investigate the influence of injection configuration on extracolumn band broadening, four sample volumes from 0.1 to 1.0 μl were injected. An injection volume of 0.1 μl was the lower limit with the timed injection technique due to limitations by the switching time of the valve. Without on-column focussing procedures a large volume injection of 1.0 μl placed on a

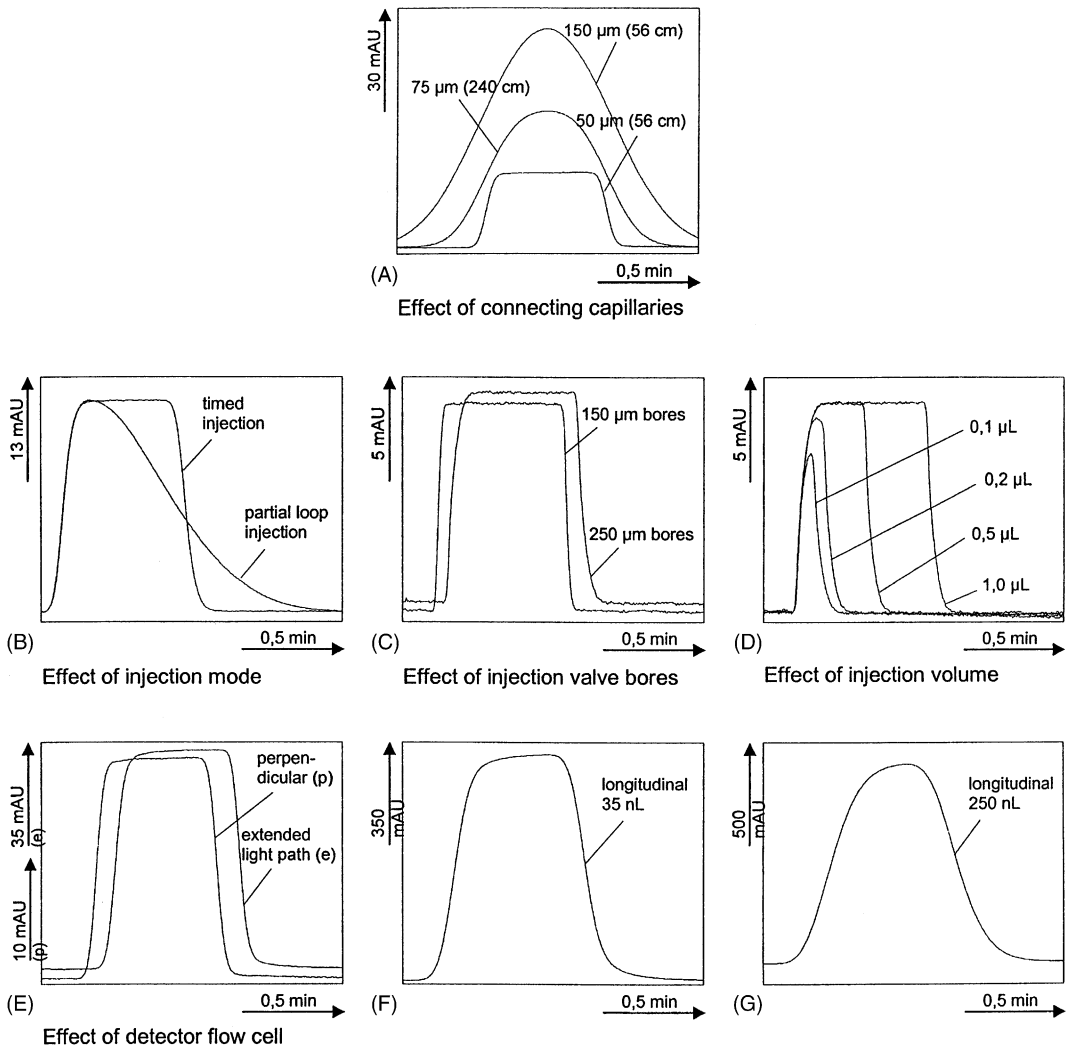

Fig. 3. Contribution of various extracolumn band broadening sources on peak shape and dispersion. To point out the different effects, peaks resulting from large volume injections of 1.0 μl are depicted. The peak profiles are arranged to allow the best comparison of the characteristic distortion. Test solution, uracil (33 ng/ml) in methanol; mobile phase, methanol; flow rate, 5 μl/min; UV detection wavelength, 254 nm. For further explanations see text. Conditions: (A) 250 μm bore injection valve, timed injection technique, perpendicular detection; (B) 150 μm bore injection valve, 50 μm i.d. connecting capillary of 56 cm length, perpendicular detection; (C) timed injection technique, 25 μm i.d. connecting capillary of 56 cm length, perpendicular detection; (D) 250 μm bore injection valve, timed injection technique, 25 μm i.d. connecting capillary of 56 cm length, perpendicular detection; (E)–(G) 150 μm bore injection valve; timed injection technique, 50 μm i.d. connecting capillary of 56 cm length.

0.3 mm capillary column would result in an enormous decrease of chromatographic resolution and is therefore practically not recommended. But it allows the analysis of the complete front and rear boundaries of a peak and, hence, conclusions of the main source(s) of extracolumn dispersion.

In Fig. 5 three injection configurations are compared. Linear regression calculations were performed to determine the constants D^2 as the reciprocals of the slopes and the variances of the remaining system as the intercepts (Eqs. (1) and (5)). In case of the timed injection mode the theoretical D^2 value of 12 for a

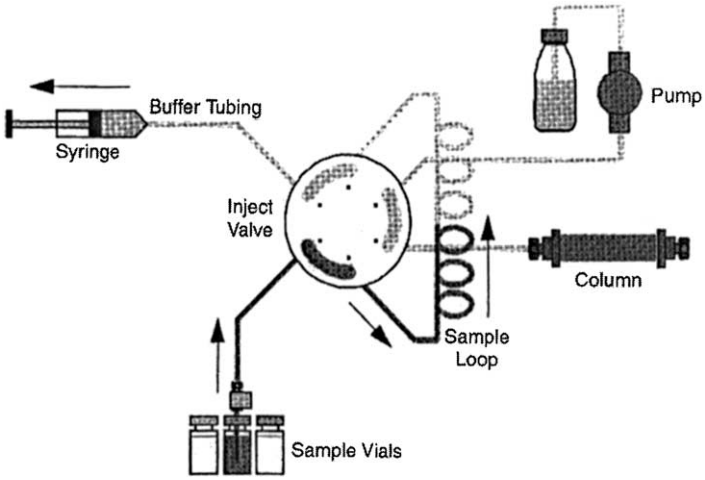

Fig. 4. Injection valve scheme according to Waters CapLC-System manual: “Installation and Maintenance Guide”.

rectangular sample plug could be observed with both stators. With the partial loop technique only a value of 6 and a considerably higher intercept value were found. Additionally, a trend to increasing D^2 values with increasing injection volumes was observed confirming the results of Lauer and Rozing [20] and Claessens [38].

In Fig. 3B the partial loop and the timed injection mode are compared. The front boundary of the sample plug is not effected by the injection mode, in contrast to the strongly influenced rear boundary. A closer look into the injection processes allows the location of reasons for that behavior. The first process, loading the injector, is the same in both injection modes except the drawn volume. The sample volume drawn by the syringe is the programmed volume in the partial loop mode and three times the programmed volume in the timed injection mode predicted by the CapLC-System.

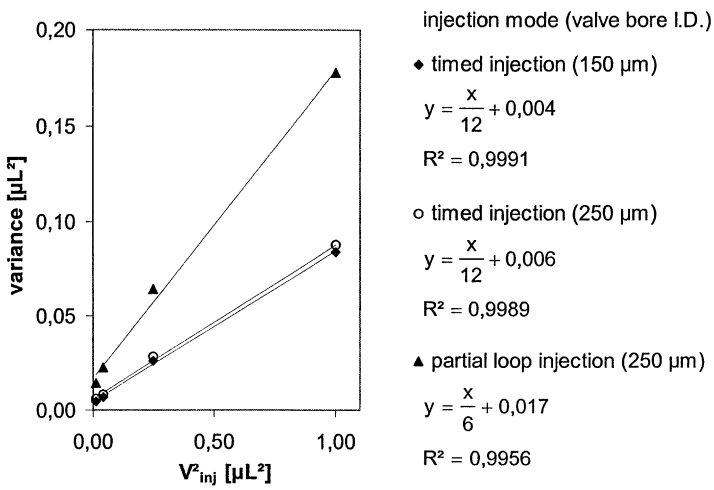

Fig. 5. Peak variances obtained with different injection procedures. Conditions: valves equipped with 10 μl sample loop; perpendicular detection; 50 μm i.d. connecting capillary of 56 cm length. Each data point is the mean of three measurements. Fault indicators are not given due to clarity reasons.

The sample fraction drawn first is mostly dispersed as it has to pass through the stator bore and the largest part of the sample loop. During that route mixing occurs with the previous solvent. The last drawn sample fraction, however, remains in the stator bore and is therefore almost undispersed after loading. The last drawn sample fraction enters the detector first and forms the front boundary, whose shape is only determined by the geometry of the stator passage and following band broadening sources, e.g. capillaries and detection device. That means, it should result the same shape with both injection modes as found experimentally.

The last injected sample fraction must be treated differently. Using the partial loop technique, the sample zone already dispersed during loading must pass through the loop and the stator bore for a second time and underlies dispersion due to the parabolic flow profile again. This part of the sample zone is cut in the timed injection mode by backswitching of the valve into the LOAD position and, therefore, the rear boundary, too, is only effected by the geometry of the stator passage and band broadening due to the remaining system. This results in enhanced injection performance.

Regarding Eqs. (1) and (5) the intercepts of the three regression lines should correspond to the dispersion effect of the remaining system and thus be equal under the applied experimental conditions. The observed discrepancy, however, can only be explained by an additional injection volume independent dispersion effect due to the injection process. Analysis of the peak shapes confirms this hypothesis. Although the theoretical value of 12 for D^2 indicating a rectangular sample plug is determined with both stators, Fig. 3C shows characteristic distortion of the two peaks. The front and rear boundaries of the peaks can be transferred into each other by reflection and rotation by 180°. This shape cannot be explained by dispersion due to connecting capillaries or detection, but only by dispersion due to the parabolic flow profile formed in the stator bores (Fig. 6) and is possibly effected by the movement of the valve. The distortion decreases considerably with decreasing i.d. of the bores due to the less developed parabolic flow profile in smaller i.d. tubings, noticeable as a reduction of the determined value for the intercept of the regression line.

In Fig. 3D four different sample volumes injected using the same conditions are overlaid. It is seen that

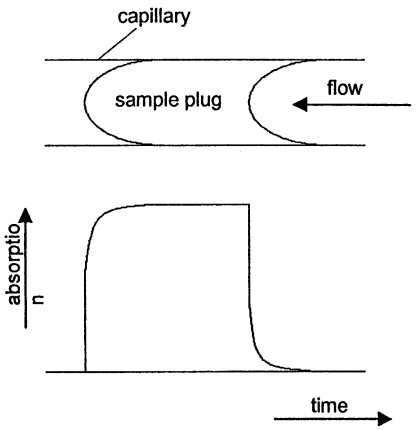

Fig. 6. Peak distortion due to parabolic flow profile.

the front and the rear boundaries are unaffected by the injection volume and the additional volume is added as a rectangular plug between these boundaries, if the injection volume is large enough. That explains the determined value 12 for D^2. With smaller volumes a plateau state cannot be reached as the dispersion influencing the rear of the sample zone acts to a sample which had been already diluted by the dispersion effect occurring to the front boundary. The overall dispersion effect is reduced, because mobile phase in front of the initial sample zone partly mixes with mobile phase thereafter, the effect becomes more evident with increasing dispersion and decreasing injection volumes and leads to a convex behavior of the variance versus squared injection volume curve and consequentially to a lower value for D^2 and for the intercept during linear regression analysis of relatively small injection volumes. In case of the injection processes this behavior can be observed for the partial loop injection mode.

Vissers et al. obtained an optimal value of 100 μm for sample loop i.d. in capillary LC [40]. In this work, the effect of sample loop i.d. was studied using the timed injection mode and no difference in dispersion was observed. The shapes of the peaks were only governed by the i.d. of the valve bores and following band broadening sources. It is conceivable that the behavior is different with the partial loop mode as the rear boundary is much effected by dispersion inside the loop. Further investigations of that topic, however, are beyond the scope of this paper.

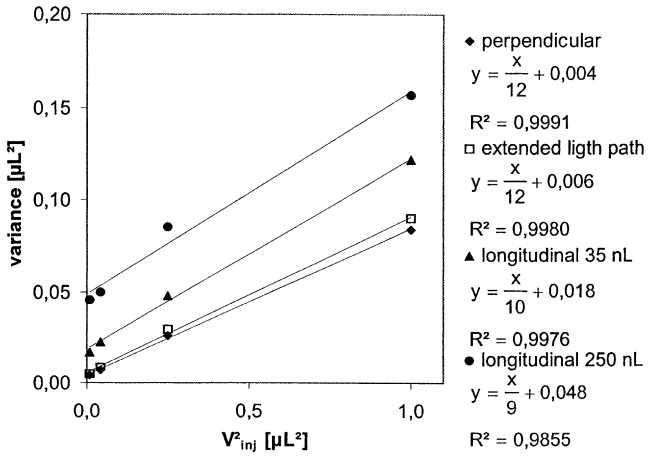

Fig. 7. Peak variances obtained with different detectors. Conditions: 150 μm bore injection valve with 10 μl sample loop; 0.1 μl injection volume placed by timed injection; 50 μm i.d. connecting capillary of 56 cm length. Each data point is the mean of three measurements. Fault indicators are not given due to clarity reasons.

3.3. Detection

Three types of detection cells were tested, perpendicular, extended light path, and longitudinal cells (Fig. 1). Fig. 7 shows the variance versus squared injection volume graphs for the four tested flow cells. In particular, the data points obtained with the longitudinal cells form convex curves reflecting a dispersion effect depending on the length of the sample zone. This behavior results in calculation of D^2 values deviating from the true value of 12 as already discussed, although the injection process is identical. Linear regression analysis of these graphs leads to a misinterpretation of the injection performance and indicates that Eq. (1) is not strictly applicable. Determination of D^2 to calculate the influence of injection on extracolumn dispersion should be carried out with a low dispersion detecting system to avoid such interference.

Example peak profiles are depicted in Fig. 3E–G. Due to the different detectors used, the absorbance units (AU) given in the figures E–G should not be compared. The contribution of the perpendicular cell to the observed peak dispersion can be assumed to be negligible. It is seen that with the extended light path cell the sensitivity is increased by a factor of approximately 3.5 due to the longer light path and the larger aperture area of the interface while leaving the peak shape almost unaffected. The high sensitivity offered by longitudinal flow cells is compromised by a higher extent of band broadening induced by this cell type. Comparing the behavior of the 35 and 250 nl cell, it becomes evident that the reduction of dispersion resulting from the 35 nl flow cell was quite drastic. Band broadening due to flow cells influences both boundaries, but in a different way and leads to unsymmetrical peaks.

3.4. Determination of main band broadening source(s) of an unknown capillary LC system

Fig. 3 illustrates the main band broadening sources in LC and the resulting peak shapes. The particular distortion characteristics were mentioned in previous sections. This part describes the analysis of an extracolumn peak produced by large volume injection. The possibility to study the complete front and rear boundaries can be employed to find out the most important dispersion source(s) of the capillary LC instrumentation used. Ten times the recommended injection volume was sufficient in this investigation. It must be noted that the injection volume should be increased till a plateau state is reached. Otherwise, the interpretation is more complicated as the boundaries cannot be evaluated separately, because the dispersion becomes combined. A high data acquisition rate is also recommended.

An almost rectangular sample plug as depicted in Fig. 3C (150 μm bores peak) indicates a practically dispersionless system. If both boundaries are dispersed to a high extent, the symmetry of the peak must be taken into account. If the peak is symmetrical, the connecting capillaries are probably the main cause of dispersion and should replaced (Fig. 3A). In case of an unsymmetrical peak, the detector flow cell is the most likely band broadening source and more problematical to optimize (Fig. 3F and G). To ensure that the connecting capillaries are appropriate, the experiment should be repeated with thinner ones, as it is easier to exchange a connecting capillary instead of a flow cell. If no distinct improvement is visible, the flow cell can be assumed to be the major issue for dispersion.

If the first part of the front boundary is steep, but the upper part distorted as can be seen in Fig. 3C for the 250 μm bore stator, the stator bores are probably the origin. A steep rear boundary with foot tailing as visible to a minimal extent in Fig. 3C (250 μm bores peak) confirms the stator bores as most imported band broadening source. The combination of a steep front and a flat rear boundary indicates an unfavorable injection mode (Fig. 3B). At last the injection volume should be adjusted as compromise between sensitivity and selectivity.

In practice, the interpretation may become more complicated since various band broadening effects appear simultaneously and peak shape characteristics may differ due to other injection and detection systems.

3.5. Practical considerations

For practical purposes, it is more convenient to express the extracolumn band broadening extent in loss of column resolution than in volume variances [19]. The characteristics of three exemplary capillary LC set-ups and the corresponding extracolumn variances are shown in Table 1. System A represents the instrumentation as it was delivered by the manufacturer. System B represents a high sensitivity set-up optimized with regard to extracolumn dispersion. System C corresponds to a set-up optimized for very low extracolumn dispersion but limited sensitivity. These systems were used to calculate the amount of column resolution that is lost by additional dispersion from the particular instrumentation. Chromatographic resolution, R_S, of two comparable peaks can be determined by:

$$R_S = \frac{\Delta t_r}{4\sigma} \tag{6}$$

where Δt_r is the retention time difference and σ the standard deviation of the last considered peak. The quantity of resolution loss, $R_{S,loss}$, depends on the amount of instrumental dispersion relative to the dispersion level caused by the column and can expressed by the following equation:

$$R_{S,loss}\,(\%) = \left[1 - \frac{R_{S,tot}}{R_{S,col}}\right] \times 100\% = \left[1 - \frac{\sigma_{col}}{\sigma_{tot}}\right] \times 100\% \tag{7}$$

Table 1
Characteristics of three capillary LC set-ups

System designation	Injection valve bores; injection mode; injection volume	Connecting capillary; material	Detection	Variance[a] (min^2)
System A (original status)	250 μm; partial loop; 0.2 μl[b]	62 μm i.d., 80 cm length; PEEK	Longitudinal 250 nl cell	0.00372[c]
System B (highly sensitive)	150 μm; timed; 0.1 μl	50 μm i.d., 56 cm length; fused silica	Longitudinal 35 nl cell including 75 μm i.d. capillary of 28 cm	0.00055
System C (low dispersion)	150 μm; timed; 0.1 μl	50 μm i.d., 56 cm length; fused silica	Perpendicular cell	0.00017

These systems were used to illustrate the performance of different capillary LC set-ups with regard to effects on chromatographic resolution due to extracolumn dispersion.

[a] Mean of three measurements.

[b] With partial loop mode 0.2 μl are the lower limit to receive reproducible peak areas.

[c] Determined by basis peak width.

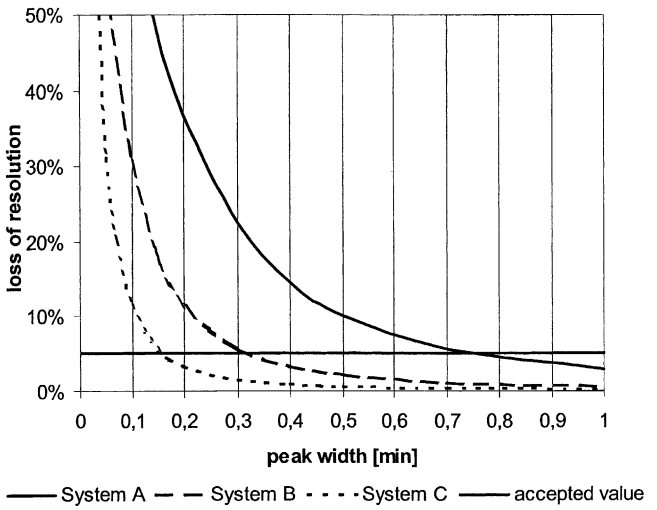

Fig. 8. Loss of resolution due to extracolumn dispersion with different capillary LC set-ups. Characteristics of the set-ups are listed in Table 1. For further explanations see text.

where $R_{S,col}$ is the theoretically obtainable resolution with a certain column and $R_{S,tot}$ the resolution visible in the chromatogram. σ_{col} and σ_{tot} are the corresponding standard deviations, which were determined using Eqs. (8) and (9).

$$\sigma_{col} = \frac{1}{4}w \tag{8}$$

$$\sigma_{tot} = \sqrt{\sigma_{sys}^2 + \sigma_{col}^2} \tag{9}$$

where w is the basis peak width and σ_{sys}^2 the measured system variance given in Table 1. A loss of 5% resolution corresponding to a loss of 10% in plate number due to extracolumn band broadening is generally accepted as tolerable. The dependence of resolution loss on peak width depicted in Fig. 8 shows large differences between the three test systems. Peaks wider than 0.15 min (peak basis) are almost unaffected by system A. This set-up can be used for analysis requiring

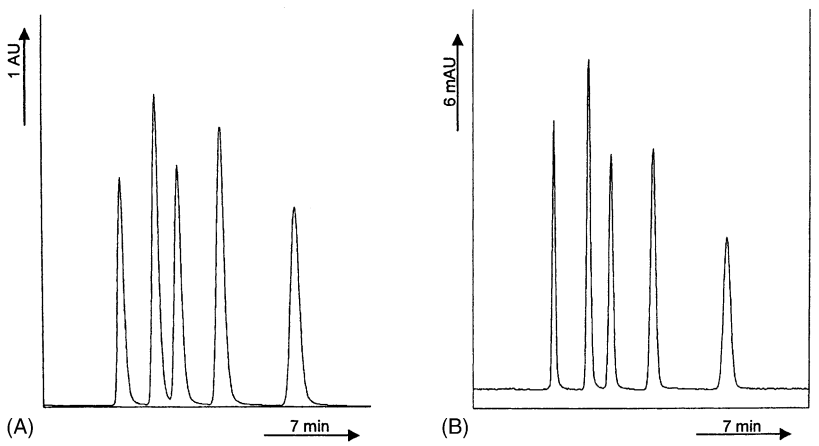

Fig. 9. Capillary LC chromatograms of a test mixture containing uracil (36 ng/ml), methylparabene (35 ng/ml), ethylparabene (31 ng/ml), propylparabene (43 ng/ml) and butylparabene (39 ng/ml) in mobile phase. The separations were performed on system A (A) and system C (B). The improved selectivity obtained with system C is obvious. Column, Protecol C18, 150 mm × 0.3 mm, 3 μm particle (SGE, Australia); mobile phase, methanol–water (70:30, v/v); flow rate, 5 μl/min; UV detection wavelength, 254 nm.

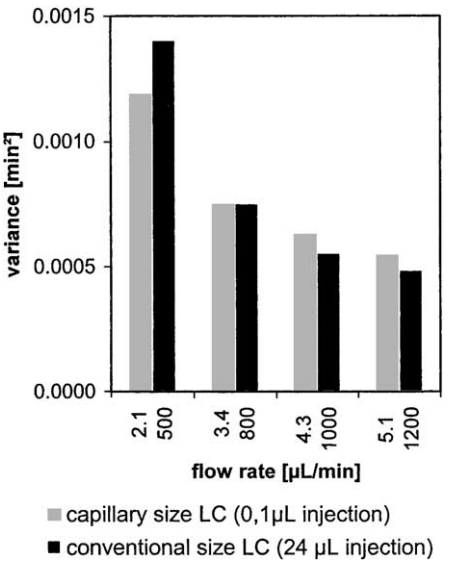

Fig. 10. Extracolumn dispersion of capillary and conventional size systems.

high resolution and moderate sensitivity. System B is suitable for most applications; in contrast, system C significantly decreases column performance in a range of peak widths usually observed with HPLC. Fig. 9 represents a comparison of two chromatograms acquired with identical chromatographic conditions, but different instrumentation. The improved selectivity obtained with system C is obvious.

This low dispersion system was compared with a conventional LC system using real chromatographic conditions. Fig. 10 shows that it is possible to attain comparable performance regarding to extracolumn dispersion of conventional and capillary LC equipment over a wide range of corresponding flow rates. The flow rates were referred to columns of 4.6 and 0.3 mm i.d., respectively, and converted using the scaling factor, f:

$$f = \frac{d_1^2}{d_2^2} \tag{10}$$

where d_1 and d_2 are the column i.d.

4. Conclusions

This paper describes a detailed investigation on extracolumn band broadening in capillary LC and suggests an approach to identify the most important dispersion sources by analysis of peak shapes. Connecting capillaries, injection, and detection were taken into account. The Taylor–Aris relationship was confirmed for fused silica capillaries of dimensions typically used in capillary LC. For flow rates of about 5 μl/min connecting capillaries of 50 μm i.d. were found to be the best regarding to low dispersion and moderate back pressure. High-quality connections by zero-dead-volume unions produce no additional band broadening. Detailed investigations of the injection process resulted in archiving the theoretical value of 12 for constant D^2 indicating an rectangular peak profile in case of timed injection mode. Nevertheless, distorted peak shapes were found. This is explained by an additional, injection volume independent term arising from dispersion due to the parabolic flow profile in the injection valve bores. Injection performance can be considerably enhanced by using an appropriate injection mode and an injection valve with small bores. The optimization of the detection side is challenging due to a compromise between high sensitivity and low dispersion. Careful selection of the flow cell is mandatory since all dispersion associated with detection is manifested in the chromatogram and cannot be overcome by focussing procedures as in case of dispersion occurring in front of the column. A sample zone depended dispersion effect of longitudinal detection flow cells was also observed, which influences the determination of D^2. Better chromatographic performance is obtained when the capillary LC system is adjusted according to the required conditions. If optimized capillary LC instrumentation is used, extracolumn band broadening in capillary LC and conventional LC is comparable. These results prove that careful optimization of instrumental parameters and hardware for capillary LC may lead to better results for the desired applications. Although the studies were focussed on capillary size LC and specific instrumentation, the basic principles should also be applicable to other LC dimensions and other equipment.

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