Estimation of the instrumental band broadening contribution to the column elution profiles in open tubular capillary liquid chromatography

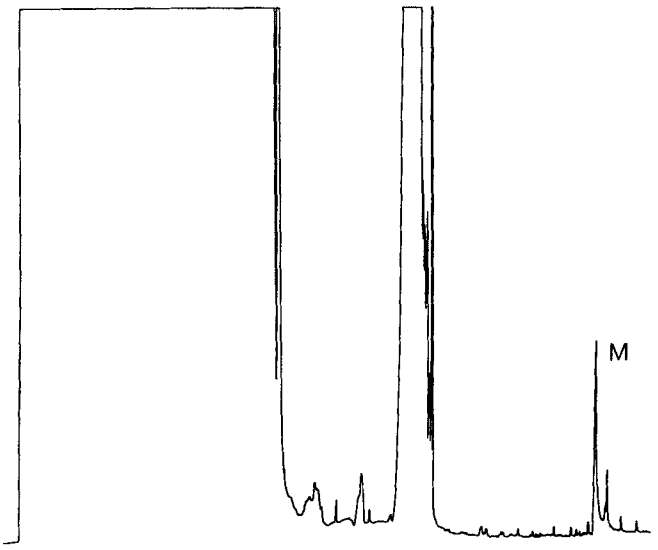

Figure 5

GC chromatogram of the morphine fraction of a urine containing 0.8 ppm morphine.

HPLC eluent. **Figure 4** shows a GC chromatogram from a blank urine, whereas in the urine shown in **Figure 5** a 0.8 ppm concentration of morphine was detected.

Acknowledgment

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Estimation of the Instrumental Band Broadening Contribution to the Column Elution Profiles in Open Tubular Capillary Liquid Chromatography

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Summary

In comparison with conventionally packed HPLC columns, from a theoretical point of view, open capillary liquid chromatography (OTLC) systems offer a number of advantages like high plate numbers and short analysis times. On the other hand, drastic changes have to be made to the instrumentation. In particular, the contribution to band broadening by the chromatographic equipment must be considerably reduced. In the present study an OTLC system was developed and evaluated, which yields satisfactory results for 26 μm i. d. columns. The determination of the contribution of the chromatographic equipment to the total band broadening is discussed.

1 Introduction

High performance liquid chromatography is a powerful analytical tool whose application areas are still growing. Typical packed columns of 3–25 cm length, internal diameters of 3–5 mm, and particle sizes of the stationary phase between 3–20 μm, yield 5–10.000 plates in 10–15 minutes.

In recent years several trends to miniaturization in HPLC could be observed. New types of columns like microbore, packed capillary, and open capillary columns were developed. Moreover, suitable instrumentation, which met the

specific demands of these colums, was designed [1,2]. In general, miniaturization in HPLC offers a number of advantages:

- a decreased consumption of stationary phase and eluent;
- less chromatographic dilution, which increases the sensitivity of concentration sensitive detectors;
- due to the low eluent flows, coupling with mass and infrared spectrometers, flame ionization detectors, *etc.* is facilitated;
- theoretical evaluations show that high separation power in short times for packed and open capillary columns can be expected [3-6].

Optimal results in OTLC, in terms of high plate numbers in short analysis times, can be reached by using columns with internal diameters between 1-10 μm [7].

This study deals with the development and evaluation of a chromatographic apparatus for OTLC. In this paper the influence and estimation of the extra-column band broadening contributions to the eluting profiles is discussed.

2 Theory

The performance of chromatographic columns can be reduced drastically by the contribution of the equipment to band broadening. In chromatographic practice, this is often underestimated. Because of the very small peak volumes in OTLC, the demands on the instrumentation are extremely high in this respect. Therefore, accurate methods must be available to determine the contribution of the equipment to the eluting profile.

The variance (σ_c^2) in flow through smooth-walled, non-coated circular tubes is given by the Taylor's equation [8]:

$$\sigma_c^2 = \frac{\pi^2 \cdot r^6 \cdot L \cdot u}{24\, D_m} \quad \text{(volume units)}^2 \qquad (1)$$

where L is the column length (m), r the column inner radius (m), D_m the diffusion coefficient of the component (m^2s^{-1}) in the mobile phase, and u the linear eluent velocity ($m.s^{-1}$). Due to flow patterns at both ends of the columns, possible adsorption effects of components, imperfections in the inner diameter of the column and roughness of inner wall, the term σ_c^2 must be extended with another term σ_p^2 to describe more realistic situations, where σ_p^2 represent the above influences. So the column contribution (σ_{cl}^2) equals:

$$\sigma_{cl}^2 = \sigma_c^2 + \sigma_p^2 \qquad (2)$$

Assuming that the different contributions are independent [9,10], the total variance (σ_t^2) of a chromatographic profile for open capillary liquid chromatographic systems equals:

$$\sigma_t^2 = \sigma_c^2 + \sigma_p^2 + \sigma_n^2 + \sigma_d^2 + \sigma_e^2 \qquad (3)$$

where:
$\sigma_n^2 = v_i^2/k_i^2$ = the contribution of the amount of sample and the geometry of the injector,
$\sigma_d^2 = v_d^2/k_2^2$ = the contribution of the cell volume and geometry of the detector,
$\sigma_e^2 = \tau_e.F^2$ = the contribution of the electronic time constants,
F = the flow rate of the eluent;
k_i^2 and k_2^2 are profile factors depending on the sample plug profile [11].

A generally accepted criterion for the contribution of the overall variance of the instrument (σ_i^2) is that this should not exceed 10 % of the column variance. This equals a loss of efficiency of 10 %, so:

$$\sigma_i^2 = \sigma_n^2 + \sigma_d^2 + \sigma_e^2 < 0.1\ \sigma_{cl}^2 \qquad (4)$$

From eq. (4) it can be derived that

$$\sigma_i \leq 0.32\ \sigma_{cl} \qquad (5)$$

The percentage contribution of band broadening of the equipment can be expressed as [10,12]:

$$\left(\frac{\sigma_t}{\sigma_{cl}} - 1\right).\ 100\,\% \qquad (6)$$

From eq. (4) it follows that:

$$\frac{\sigma_t}{\sigma_{cl}} \leq 1.05 \qquad (7)$$

This equals a maximum loss of chromatographic resolution of 5 %. Under identical chromatographic conditions flow patterns can be expected to be independent of the column length. The other mentioned contributions like adsorption of components, wall roughness, *etc.*, will depend on the column length. Therefore, the increase with the column length of the total variance σ_t^2 might differ from that of the calculated σ_c^2 as schematically presented in **Figure 1.**

Some of the contributions of the parts of the equipment can be derived theoretically, but others like influences of flow patterns at the entrance and exit of the column, geometry of

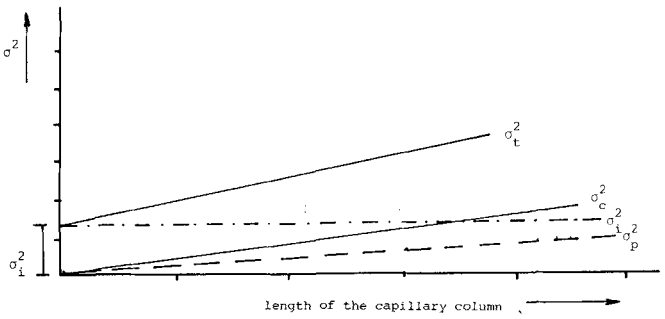

Figure 1

Schematic presentation of the different contributions to the total band broadening σ_t^2 of the chromatographic profile; the drawn lines of σ_c^2 and σ_t^2 can be calculated and determined respectively. The dotted lines of σ_i^2 and σ_p^2 cannot be estimated directly. The instrumental band broadening σ_i^2 is to be determined by linear extrapolation of σ_t^2 to column length = 0.

detector and injector, *etc.*, can only be determined experimentally.

Unfortunately most methods to determine the contribution of the equipment to the band broadening, provide only information about the total band broadening. These methods give less insight in the separate contributions of the different parts of the equipment. Particularly for packed HPLC systems a number of methods have been developed for the determination of σ_i^2 [9,10,13,14], including the replacement of the column by a capillary tube. In this work an attempt is made to determine σ_i^2 experimentally by varying the capillary column lengths. Under constant chromatographic conditions described in the experimental section, with several lengths of the capillary columns, a numer of chromatograms of phenol and 3,5-xylenol were made. From these chromatograms σ_t^2 was calculated according to the method of *Foley* and *Dorsey* [15,16] in order to determine correct values. By plotting of σ_t^2 versus the different lengths of the column, a straight line is obtained.

At the same time, from eq. (1) the contribution of the capillary tube can be calculated as a function of these column lengths. The slope of this line equals $(\pi^2 . r^6 . u)/D_m$. The differences between the experimentally determined line of σ_t^2 and the calculated one of σ_c^2 provide information about $(\sigma_i^2+\sigma_p^2)$.

Ultimately, extrapolation to zero column length yields the contribution of the equipment (σ_i^2) to the total band broadening.

For this method a number of conditions must be met:

- the injected amount of sample and the profile factor k_1^2 of the injector must be constant;
- the profile factor k_2^2 of the detector must be constant;
- the eluent flow must be constant, *inter alia* to keep the influences of wall roughness and flow patterns constant;
- the positioning of the column to the injector and detector must be performed reproducibly.

Under the same chromatographic conditions the above mentioned criteria are met, with the exception of the last point, where it is a matter of discussion as to how far the positioning of the column at the injector and detector can be done reproducibly.

3 Experimental

The OTLC equipment was constructed of several commercially available and custom-made parts. Fused silica columns with 26 μm i.d. were used (Chrompack International B.V., Middelburg, The Netherlands). A pneumatic amplifier liquid pump model DSTV-150 (Haskel Eng. and Supply Corp., Burbank, CA USA) provided with liquid pressure reducers was used for controlling a constant eluent flow. The pump system can operate between 2–1000 bar. The injection system consisted of a pneumatic actuated injection loop, internal loop volume 60 nl, model ACI 4W.06-AH 90 (Valco, Houston, TX, USA). The injector was provided with a speed-up kit model SQE (Humphrey Products, Kalamazoo, MI USA), which allowed small switching times (80–2500 ms) of the valve. By controlling the switching time of the valve sample volumes as low as 100 pl could be injected. With a vespel ferrule and liner the column was positioned directly to the sample loop, enabling 'on-top-of-the-column' sample injection.

Two home-made detectors were applied (1) A modified flame ionization (FID) detector with a cell volume of 50 pl. The eluent was deionized water and the test component 3,5-xylenol (k' = 0). The noise level R_n of the detector was $0.5.10^{-13}$ A. The injection volumes were 60, 16, 8.7 nl, respectively, and the sample concentration was 520 ppm. (2) A home-made 'free wall jet' ampèrometric detector, with a cell volume < 1 nl. The eluent consisted of 1.2 mmol sodium-phosphate buffer, ph 6.9 in water. The test component was phenol (k' = 0). The oxidation potential was +0.80 V (*vs.* Ag/AgCl) and the noise level R_n of the detector was 2.10^{-12}A. Injection volumes of 60 and 10 nl, and a sample concentration of 770 ppm were used. Details of the latter detector are described elsewhere [17].

The detector signals were recorded on a recorder model BD 40 (Kipp & Zn., Delft, The Netherlands). The chemicals were of analytical grade (Merck, Darmstadt, FRG).

Several lengths of the 26 μm i.d. capillary tube were cut off and positioned between the injector and detector. Repeated injections of the test solutions of 3,5-xylenol and phenol were carried out. The capacity factors (k') of the test components equaled zero and the diffusion coefficient, D_m, in the eluent $10^{-5}cm^2.s^{-1}$ [4]. To test the influence of the injection volume and injection profile, different volumes (60, 16, 10, 8.7 nl, respectively) of the test solution were injected.

To obtain a constant eluent velocity, a pressure gradient $\Delta P/\Delta L$ of 12.25 bar/m over the column was maintained, resulting in a flow of about 800 nl/min and a linear eluent velocity of 26.0 $mm.s^{-1}$. Moreover, these conditions prevented clogging of the outlet of the column, when applying the FID detector.

From the chromatograms the values of σ_t^2 were calculated.

These values, together with the σ_c^2 values calculated from eq. (1), were plotted versus the various lengths of the capillary columns. After extrapolation to column length zero σ_i^2 can be calculated.

4 Results and Discussion

In **Table 1** the minimum variances, due to the convective dispersion in the empty capillary columns used in this study, are summarized. In these values, the influences of

Table 1

Minimum theoretical values of σ_c^2 and σ_c according to eq. (1) for several lengths of the columns and at a number of eluent velocities; $D_m = 10^{-5}$ cm^2.s^{-1}.

Column	u (mm.s^{-1})	σ_c^2 (μl^2)	σ_c (nl)
24.5 m × 26 μm	0.533 (opt)	$2.59.10^{-5}$	5.09
	5.0	$2.43.10^{-4}$	15.6
	26.0	$1.26.10^{-3}$	35.6
15.8 m × 26 μm	0.533 (opt)	$1.67.10^{-5}$	4.09
	8.0	$2.51.10^{-4}$	15.8
	26.0	$8.15.10^{-4}$	28.6
8.6 m × 26 μm	0.533 (opt)	$9.10.10^{-6}$	3.01
	15.0	$2.55.10^{-4}$	16.0
	26.0	$4.44.10^{-4}$	21.1
2.72 m × 5 μm	2.66 (opt)	$7.26.10^{-10}$	26.0 pl
	3.0	$8.19.10^{-10}$	28.6 pl
	10.0	$2.73.10^{-9}$	52.3 pl

Table 2

Contribution of the extra-column σ_i^2-values to the total variances σ_t^2 at several lengths of columns and injection volumes; column inner diameter = 26 μm; linear eluent velocity = 26.0 mm.s^{-1}; detector, optimized FID; σ_c^2, calculated according to eq. (1); % instrumental contribution calculated according to eq. (6), assuming $\sigma_p^2 = 0$.

Column length (m)	v_i (nl)	σ_c^2 (μl^2)	$(\sigma_i^2 + \sigma_p^2)$ (μl^2)	Instrumental contribution (%)
24.5	8.7	$1.26.10^{-3}$	$3.35.10^{-4}$	12.5
	16.0		$6.17.10^{-4}$	22.1
	60		$7.6.10^{-3}$	164.0
15.8	8.7	$8.15.10^{-4}$	$2.87.10^{-4}$	16.3
	16.0		$4.96.10^{-4}$	26.8
	60.0		$8.0\ .10^{-3}$	229.6
8.6	8.7	$4.44.10^{-4}$	$2.47.10^{-4}$	24.8
	16.0		$3.98.10^{-4}$	37.7
	60		$8.4\ .10^{-3}$	347.2

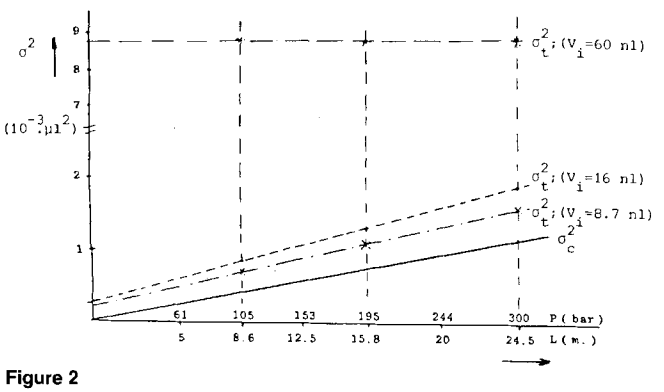

Figure 2

Linear extrapolation method for the determination of the equipment contribution to the band broadening. Detector: optimized FID; eluent velocity = 26 mm.s^{-1}; $D_m = 10^{-5}$ cm^2.s^{-1}; L = length of capillary tube; P = pressure at column inlet.

Table 3

Summary from Fig. 2 of the values of slopes, extra-column variances σ_i^2 and σ_i, calculated by extrapolation to column length zero, for three injection volumes; calculated slope of σ_c^2-curve = $5{,}16.10^{-8}$ (mm^5).

v_i (nl)	slope (mm^5)	σ_i^2 (μl^2)	σ_i (nl)
8.7	$5.71.10^{-8}$	$2.0.10^{-4}$	14.1
16.0	$6.53.10^{-8}$	$2.8.10^{-4}$	16.7
60	—	$8.8.10^{-3}$	93.8

Table 4

Contribution of the extra-column σ_i^2-values to the total variances σ_t^2 for several column lengths and injection volumes; column inner diameter = 26 μm; linear eluent velocity = 26.0 mm.s^{-1}; ampèrometric detector; σ_c^2, calculated according to eq. (1); % instrumental contribution calculated according to eq. (6), assuming $\sigma_p^2 = 0$.

Column length (m)	v_i (nl)	σ_c^2 (μl^2)	$(\sigma_i^2 + \sigma_p^2)$ (μl^2)	Instrumental contribution (%)
24.5	10	$1.26.10^{-3}$	$4.10.10^{-4}$	15.1
	60		$8.44.10^{-3}$	177.5
15.8	10	$8.15.10^{-4}$	$3.95.10^{-4}$	21.8
	60		$8.79.10^{-3}$	243.3
8.6	10	$4.44.10^{-4}$	$3.77.10^{-4}$	36.0
	60		$9.10.10^{-3}$	363.6

Table 5

Summary from Fig. 3 of the values of slopes, extra-column variances σ_i^2 and σ_i, calculated by extrapolation to column length zero, for two injection volumes; calculated slope of σ_i^2-curve = $5.16\ 10^{-8}$ (mm^5).

v_i (nl)	slope (mm^5)	σ_i^2 (μl^2)	σ_i (nl)
10	$5.43.10^{-8}$	$3.5.10^{-4}$	18.7
60	$1.02.10^{-8}$	$9.5.10^{-3}$	97.3

flow patterns at both ends of the columns, adsorbtion effects, *etc.*, are not taken into account.

In **Tables 2** and **3** and **Figure 2** the results are summarized of the calculations of σ_c^2 and measurements of σ_i^2 for different lengths of columns, applying the FID detector. Moreover, the percentage contributions according to eq. (6) are calculated.

In **Tables 4** and **5** and **Figure 3** the results are summarized applying the ampèrometric detector.

The effect of large injection volumes is illustrated by the apparent independency of σ_t^2 of the length of the column at 60 nl injections.

From **Tables 3** and **5**, and Figures 2 and 3, it can be derived that the slopes of the experimental σ_t^2-lines are steeper

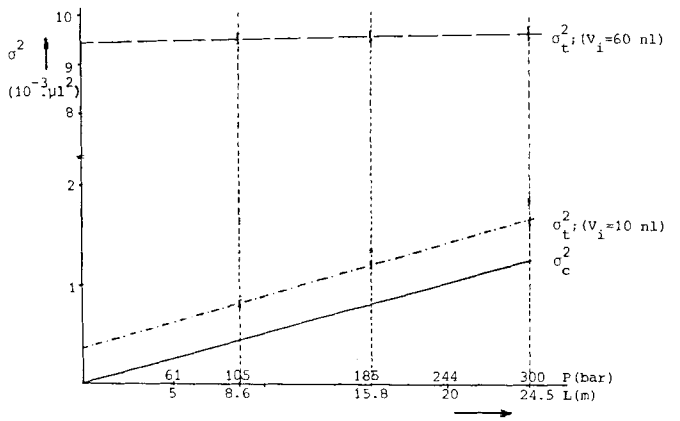

Figure 3

Linear extrapolation method for the determination of the equipment contribution to the band broadening. Detector: ampèrometric detector; eluent velocity = 26 mm.s^{-1}; $D_m = 10^{-5}$ cm^2.s^{-1}; L = length of the capillary tube; P = pressure at column inlet.

compared to the σ_c^2-lines. The influences of the earlier mentioned effects of adsorption, wall roughness and imperfection of the inner diameter of the column on σ_t^2 are dependent on the length of the capillary column, contrary to the flow pattern at the column ends. For injection volumes which are compatible with the applied capillaries, therefore, the slopes of the σ_t^2-lines might be somewhat larger than the calculated values of $(\pi^2.r^6.u)/D_m$ for the σ_c^2-lines.

Because σ_p^2 cannot be estimated, the real extra-column variance must be calculated by extrapolating the length of the column to zero.

Assuming that for the 8.6 m column, $\sigma_p^2 = 0$, u = 26 mm.s^{-1}, injection profile factor $k_i^2 = 1$, the total band broadening of the equipment is caused by the injector; then it can be calculated from eq. (4), that the maximum injection volume is 6.8 nl. It could be argued that the larger steepness of the σ_t^2-lines in comparison with the σ_c^2-lines could also be due to injection overload. However, it can be calculated that about 30 and 40 nl can be injected, under the same conditions in 15.8 m and 24.5 m columns. In the case that profile factors are larger than one, even larger injection volumes are allowed. The data in Figures 2 and 3 fit the idea of linear regression well. So it is unlikely that only injection problems cause the deviant steepness of the σ_t^2-lines.

From Tables 2 and 4 it can be seen that the lowest contribution of σ_i to the total standard deviation is 12.5 %, assuming $\sigma_p^2 = 0$. This is larger than the criterion of 5 % in the eqs. (4) and (7). The value of 12.5 % can be somewhat favourable because the real column standard deviation $\sigma_{cl}^2 > \sigma_c$.

5 Conclusions

Under practical conditions, applying columns of 26 μm i. d., at lengths > 20 m, and at injection volumes of about 10 nl, the criterion of 5 % loss in standard deviation may be satisfied. The approach to determine the variance of the equipment for OTLC in relation to the total eluting profile, from chromatographic experiments provides satisfying results.

Another method based on the measurement of the relative influence of the equipment band broadening on the eluting profiles of chromatographic peaks may be successful for OTLC equipment. This will be part of further study. The application of this method for equipment, designed for packed HPLC columns, was recently reviewed [18]. Current work includes the design and evaluation of instruments for capillary columns of smaller inner diameters than the 26 μm used in this study.

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