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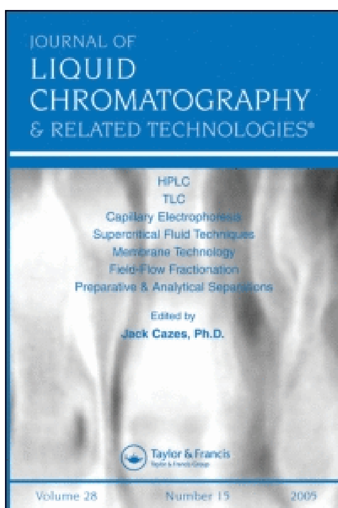

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THE EFFECT OF COLUMN DIAMETER ON HPLC SEPARATIONS USING CONSTANT LENGTH COLUMNS

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ABSTRACT

The effect of column dimensions on resolution, sample capacity, mobile-phase use and efficiency using both constant flow-rate and constant linear velocity was studied. The four columns selected have the same length (238 mm), but different diameters (column A: 4.6 mm; column B: 4.0 mm; column C: 3.2 mm and column D: 2.1 mm). It was observed that at constant flow-rate the reduced plate height was the lowest for columns B and C, higher for column A and highest for column D. On the other hand increasing the injected quantity while keeping injected volume constant revealed columns B and C to have a better efficiency only at the low concentrations. Column A with the largest diameter was superior at high concentrations and thus offers the best load-ability. Column D offered, as expected, poor loadability. Examination of the columns at the same calculated linear velocity, showed no appreciable changes in efficiency for the four columns. Preferable detection limits and big solvent savings were obtained when small column diameters were used.

INTRODUCTION

The column dimensions and the packing materials play an important role in chromatographic separations. Although there have been many studies dealing with support materials and their modifications, few studies have been devoted

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to the effect of column dimensions (length, internal diameter and volume) on resolution and loadability. Berg and McNair (1) showed how efficiency varied with columns' length and diameter. They found that constant column length efficiency increased as the internal diameter increased, and that constant column internal diameter (i.d.) N/m decreased with an increase in column length. This last statement contradicts what was previously reported by Mellor (2) that columns of the same diameter, 4.5 mm but various lengths (5 cm to 25 cm) packed with the same particle size material (3 μm or 5 μm ODS) gave almost the same efficiency/metre. Wolf (3) examined preparative scale columns of the same length (5 cm) but different internal diameters 0.77 to 2.36 cm. He found a linear relationship between the square root of the column i.d. and the number of theoretical plates when they were used at constant linear carrier velocity. The peak variance as a function of column length and diameter was evaluated (4). It was found that the observed decrease in variance resulting from decreased column length agreed with theory for 22 cm and 10 cm but not 3 cm x 4.6 mm columns. Also, when the column's i.d. was decreased from 4.6 to 2.1 mm, the variance as a function of column length was consistent with theory. Lauer and Rozing (5) used 30 x 4.6 mm and 144 x 2.1 mm columns (both have the same volume) to study the influence of column dimensions and sample size on detection by evaluating the relationship between external band spreading, injection volume, column band broadening and solute dilution on small volume columns. They concluded (5) that detection limits can be improved by using small volume high efficiency columns which generate more plates than required for a particular separation problem. Issaq, et al (6) studied the effect of column dimension on resolution, sample capacity, retention time, efficiency and mobile phase composition, using both constant flow rate and constant linear velocity. The four columns selected (A = 238 x 3.2 mm, B = 153 x 4.0 mm, C = 116 x 4.6 mm and D = 50 x 7 mm) had the same volume. K' values were found to be constant, within experimental error, for all columns. At constant linear velocity, the retention time was found to be a linear function of column length, while at constant flow rate retention time

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was constant for all columns. The longest column (A) generated the largest N values while columns B and C gave the lowest H values, for dilute solutions. On the other hand, it was observed that as the sample size increased, N generated by column (A) decreased rapidly and eventually fell below the values generated by columns B and C. These two columns (B and C) can tolerate a larger sample size with less reduction in N value than the longest column. It is important to note that although there were minor differences in performance between columns B and C, there were significant differences between them (B and C) and the other two columns (A and D). Column A offered the highest sensitivity (narrower peaks) for dilute solutions, while columns B and C offered higher loadability. The volume of organic modifier in the mobile phase affected the retention equally in the four columns. It was also found that equal separation (α) was obtained for each column at constant flow rate and constant linear velocity, however, with the latter the retention times were longer.

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The above studies (1-4) deal with the effect of column diameter and/or length on chromatographic performance, using columns of widely different diameters from analytical to preparative or lengths which are not those mostly used for analytical applications. A survey of the literature indicated that the most widely used columns would have a diameter of ~4 mm and are 15-30 cm long. For this study, four commercially available analytical columns having the same length but different diameters were selected to evaluate the effect of column diameter on column performance. The effect of column diameter on Rs, H and N will be evaluated, as well as, the effect of solute quantity on column efficiency and loadability.

EXPERIMENTAL

Materials:

The four columns selected for this study have the same length, 238 mm, and the following internal diameters: column A: 4.6 mm; column B: 4.0 mm;

column C: 3.2 mm; and column D: 2.1 mm. Each column was prepacked with 5 µm spherical, reversed-phase C_{18} bonded silica of the same batch by the same slurry method (Advanced Separations Technologies, Inc., Whippany, NJ). The test solutes were biphenyl (BP) and 2-phenylphenol (2PP) from Aldrich Chemical Co., Milwaukee, WI. The mobile phase was 25% distilled water in acetonitrile (glass distilled Burdick & Jackson, Muskegon, MI). The mobile phases were filtered and degassed before use and maintained under helium throughout the experiments. Effluents were monitored at 254 nm. Flow rates, solution concentrations, and injection volumes are as specified in the figure legends.

Apparatus:

A Hewlett-Packard liquid chromatograph (Model 1090M) equipped with a photodiode array detector, an autoinjector and a computing control system (Chem. Station) was used in this study.

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RESULTS AND DISCUSSION

Column Selection:

In the last five years, many chromatographers used smaller internal diameter and/or column length for several reasons; (a) to decrease analysis time while maintaining a high number of theoretical plates; (b) to improve the detection at the trace level; and (c) to scale up the separation process in order to achieve a cleaner product. This is especially true in the production and purification of biomolecules where the emphasis is on throughput rather than on resolution.

These realities, lead us to examine the effect of solute quantity (loadability) of columns having different dimensions. The column length selected, 238 mm, is close to the most widely used column length, 250 mm, and will be the same for the four columns selected. The column diameters of 4.6, 4.0, 3.2 and 2.1 mm are in the diameter range of the most popular columns

used. The decrease in the diameters from 4.6 to 2.1 mm gives a five-fold decrease in the volume of the empty columns which will lead to a savings in the amount of mobile phase used.

Column Testing:

For each column, a chromatogram of a 5 µl injection of the two test solutes Biphenyl (BP and 2-phenylphenol (2PP), was obtained at flow-rate of 0.7 ml/min. The sample concentration which was in the linear range of the isotherm was sufficiently low to establish the low surface coverage of the stationary phase by the sample. The dead time was determined using a dilute solution of $NaNO_2$ (1.85×10^{-4} Molar) as a non-retained solute and detected at 210 nm. In this case, the residence time decreased approximately 4.5-4.8 times when the 2.1 mm i.d. column was used in place of the 4.6 mm i.d. column. This is close to the empty tube volume ratios, which leads to an increase in the linear velocities as i.d. decreased. The linear velocities measured were as follows: column A: 1.98 mm/s; column B: 2.27 mm/s; column C: 3.4 mm/s; and column D: 6.86 mm/s.

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These results were used to evaluate the separation potential of the various columns. The so-called column permeability k_o, and the separation impedance, E, introduced by Bristow and Knox (7) appeared to be satisfactory criterias for the column performance.

$$E = t_r \Delta p / N^2 \eta (1+k') = H^2/k^o = h^2 \phi \quad (1)$$

$$k_o = \eta \mu L / dp^2 \Delta p \quad (2)$$

The value of E in equation 1 takes into consideration the column pressure drop, Δp; the column efficiency, N; the viscosity, η; as well as the retention time, t_r; and capacity ratio, k'. It is further shown that the separation impedance is equal to the square of the plate height H divided by the column permeability k_o, given by equation 2. Alternatively, E is equal to the square of the reduced plate height, h, multiplied by the column resistence factor ϕ.

The values of the column permeability can be evaluated using the equation of Kozney-Carman, which relates the column permeability to porosity, eq. 3, or by directly using Darcy's law, eq. 1, which requires more information.

$$k^\circ = \varepsilon^3/180\ (1-\varepsilon)^2 \qquad (3)$$

In this equation, the external porosity ε is the fraction of the column volume not occupied by the particles and is therefore the fraction available to the solvent flowing around the particles.

ε can be estimated using the following equation:

$$\varepsilon = Ft_o/\pi r^2 L \qquad (4)$$

where F is the flow-rate, r is the radius and L is the length of the column.

The column parameters were calculated for the test solute 2PP, for all the columns and are presented in Table 1.

Several important observations are revealed from the results (Table 1) about the performance of the different columns. It is noted that h value is the lowest for columns C and B while it is a little higher for column A, but very high for column D. This is due to the high linear velocity measured at a flow-rate of 0.7 ml/min. This means that the three columns A, B and C can be

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Table 1. Columns Performance Parameters at the same Flow Rate (0.7 ml/min)

Column Dimensions mm	h	$k^\circ\ 10^{-3}$	ϕ	E	ε
A (238 x 4.6)	3.54	0.59	1695	21241	.354
B (238 x 4.0)	3.19	1.07	929	9475	.408
C (238 x 3.2)	2.67	1.32	759	5410	.427
D (238 x 2.1)	5.89	2.54	394	13660	.491

used at different linear velocities without an appreciable loss in theoretical plates.

The values of k^0 and the related parameter ϕ for columns B and C fall between the typical range of well packed columns, 1×10^{-3} for k^0 and 1000 for ϕ. The low value of k^0 for column A and the high value for column D are caused mainly by changes in the porosity, because k^0 values increase and decrease rapidly with increasing or decreasing porosity as apparent from equation 3. This leads to the conclusion that the packing of columns having different column internal diameters at different pressure drops result in different porosities.

The larger E value for column A is a result of the high value of ϕ, rather than h. As expected, the lowest value of E belongs to column C where the ε-value and the h-value are close to the typical values for HPLC conventional columns. The H values for the different columns at the same flow-rate and different linear velocities are shown in Figure 1.

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It was of interest to evaluate the column performance parameters for all the columns at the same linear velocity of column A (1.98 mm/sec) i.e. the following flow rates: A = 0.7 ml/mm; B = .53 ml/min; C = .34 ml/min. The calculated linear velocities for D = .146 ml/min. Columns B, C and D are based on data from column A, which assumes the same retention times for the unretained and the test solutes BP and 2PP. However, deviations were observed as column diameters decreased, Table 2. This can also be seen from Figures 2 and 3.

The first important point is the deviation in the observed linear velocity from the calculated which is within experimental error for columns B and C but high, -20% for column D. This leads to the comparison of the first three columns. The value of h for the solute pp increased with the increase in diameter, i.e. the number of theoretical plates decreased as column diameter increased. These results are the opposite of those observed by Berg and McNair (1) and by Bartow and Knox (7). This can be explained either by

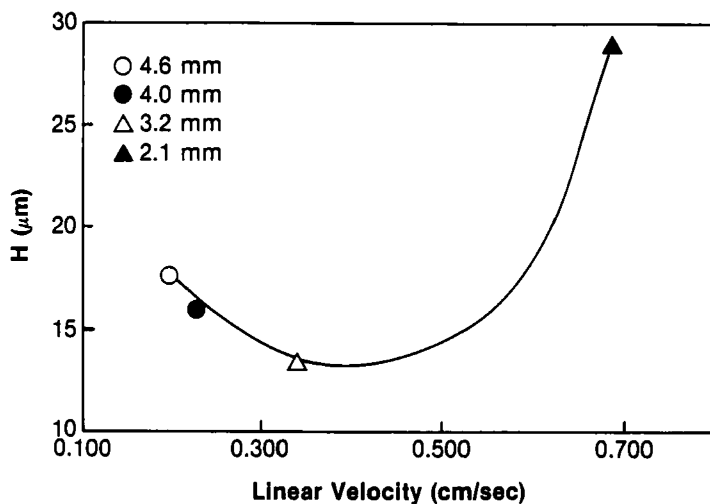

Figure 1 Height equivalent to theoretical plate versus linear velocity of the different columns at the same flow-rate (0.7 ml/min) for the solute 2-phenylphenol. Mobile phase 75% acetonitrile/water. Each point represents different column diameter and velocity.

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Table 2. Columns Performance Parameters at the same Linear Velocity

Linear Velocity mm/sec	Column Dimensions (mm)	h(pp)	k° 10^{-3}	ϕ	E	ε
1.98	A (238 x 4.6)	3.54	.59	1695	21241	.354
1.95	B (238 x 4.0)	3.12	.645	1550	15141	.362
1.85	C (238 x 3.2)	2.43	.784	1275	7559	.379
1.61	D (238 x 2.1)	3.23	1.447	691	7233	.436

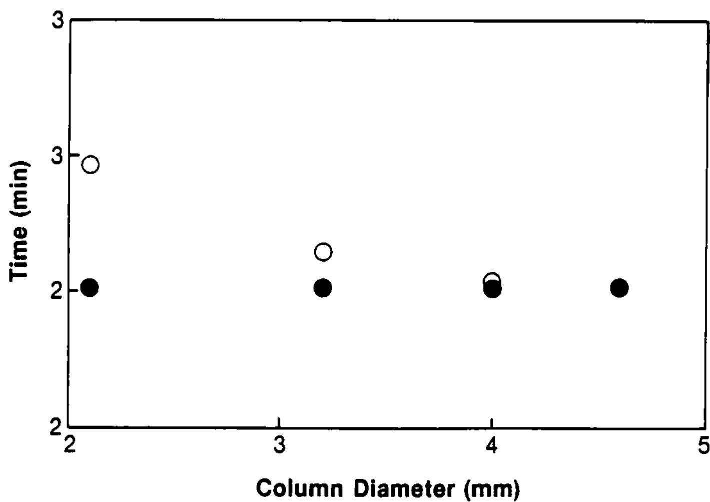

Figure 2 Calculated and experimental dead time versus column internal diameter.

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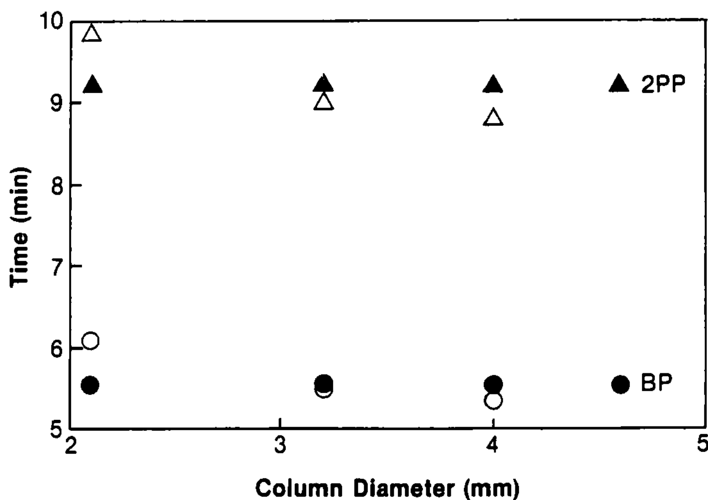

Figure 3 Experimental and expected retention times for the solutes biphenyl (BP) and 2-phenylphenol (2PP) versus column internal diameter.

the porosity values which increased with a decrease in column diameter affecting the values of k° (lowest for column A) or by the small deviations of the linear velocity because these points fall near the optimum where small changes of μ yield high changes in the values of N.

The important point to remember is that changing from wider diameter columns to narrower diameter columns while keeping linear velocities the same led to non appreciable changes in theoretical plates, but to a savings in the solvents used, assuming all other experimental conditions were kept the same.

Effect of Sample Quantity on N, H and Rs:

The decrease of efficiency with increasing injected quantity either by increasing injected volume or by increasing solute concentration while keeping injected volume constant, is of great concern in chromatography. It is even more critical in preparative chromatography where the optimum sample size is determined by the corresponding minimum adsorbent/sample ratio which would yield adequate separation with a given degree of pority. It was established that the adverse overload effect appears more rapidly on a more efficient column compared to a less efficient column. Furthermore, it was shown that large samples can result in a nonlinear Isotherm which leads to wider bands and to shifts of the retention time. Both of these effects will lead to a decrease in resolution.

In this work, we attempted to compare the four columns with respect to efficiency and resolution as function of the sample quantity injected. The injected volume was kept constant while increasing the concentration to very high quantities relative to the column loadability. The columns were examined at the same flow-rate , 0.7 ml/min, which gave different linear velocities. The second point which this study covered was the degree of increase in detection limits while keeping all the extra column broadening effects constant. Figure 4 shows a plot of detector response against column internal diameter at constant flow-rate for the two solutes BP and 2PP. The results clearly show

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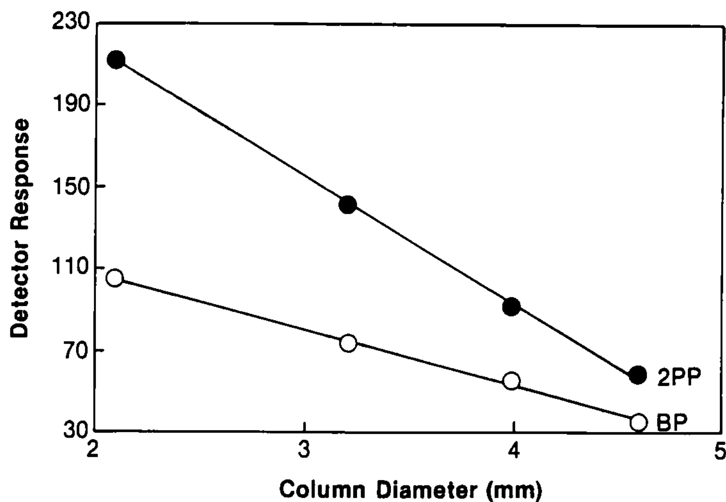

Figure 4 Effect of column diameter on detection limits for the solute 2PP and BP.

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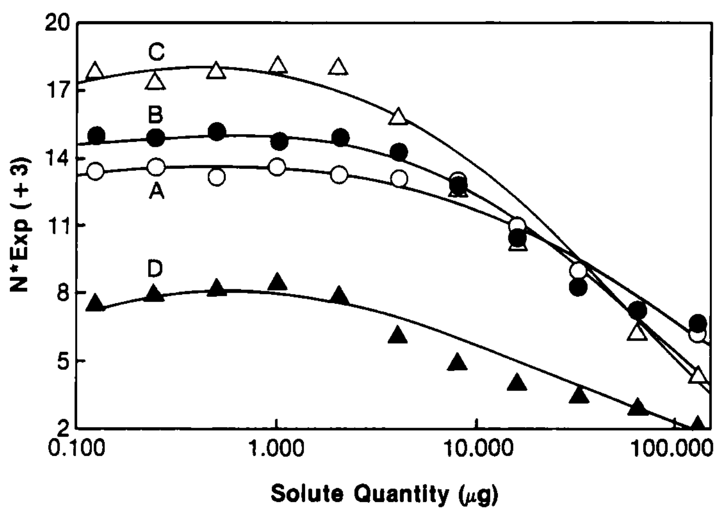

Figure 5 Effect of sample quantity on number of theoretical plates, at the following conditions: flow-rate = 0.7 ml/min, mobile phase ACN/H_2O (75:25), solute 2PP, T = 35°C.

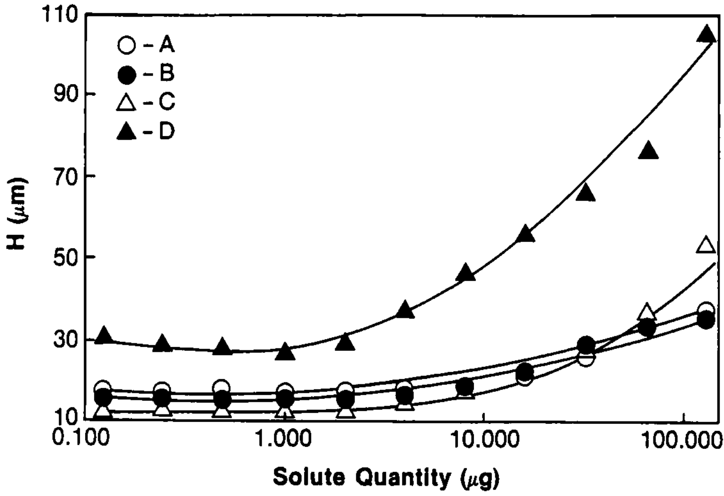

Figure 6 Effect of sample quantity on the height equivalent to theoretical plate. All conditions are the same as for Figure 5.

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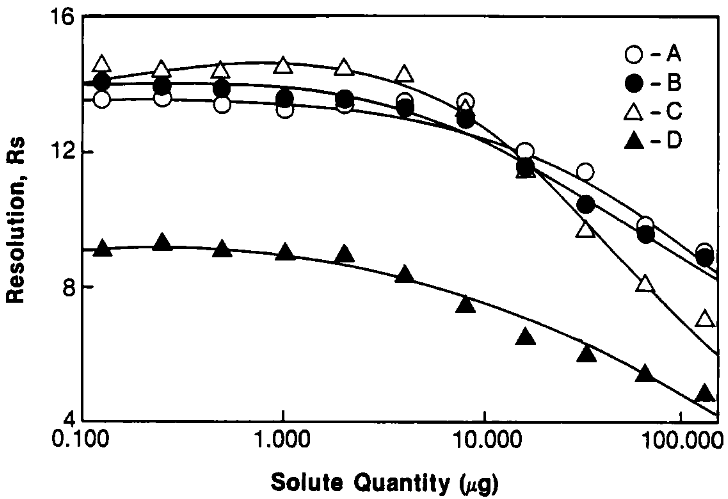

Figure 7 Effect of sample quantity on resolution. All conditions are the same as for Figure 5.

that the detection limit is 4-5 times greater for the 2.1 mm i.d. column than for the 4.6 mm i.d. column. This is very important in cases where traces must be detected and quantitatively determined or in cases where the trace component tails the major eluting component.

Figure 5 shows a plot of N (theoretical plate height) versus sample quantity (calculated by the 5 µl injected). Examination of this figure revealed that columns C and B gave better efficiency than columns A and D when low solute quantities were injected. This effect was mainly caused by linear velocity dependence of efficiency and porosity of the columns as mentioned earlier. At higher injected sample quantity, column A with the widest internal diameter is superior to columns B and C. Columns A, B and C offered good loadability in the whole range of injected quantities, while column D gave a poor efficiency, Figure 6, but better detection limits. These results lead to the conclusion that any one of the three columns A, B or C gives equivalent results, but column C is superior when solvent consumption is considered. Figure 7 shows the effect of the amount of solute injected on resolution. It is clear that columns A, B and C gave equal resolution when small quantities up to 10 µg of either BP or 2PP were injected. However, the resolution obtained on column A dropped faster than on columns B and C at solute concentrations above 10 µg. These results are caused mainly by the behavior of N against injected quantity. Column D gave lower resolution than the other three columns, which can be attributed to the high linear velocity causing a drop in efficiency and the small loading capability.

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