Journal of Chromatography, 122 (1976) 317–329

CHROM. 9090

USE OF AN 18-mm I.D. COLUMN FOR ANALYTICAL- AND SEMI-PREPARATIVE-SCALE HIGH-PRESSURE LIQUID CHROMATOGRAPHY

E. GODBILLE and P. DEVAUX
Centre de Recherches, Roussel Uclaf, 93230 Romainville (France)

SUMMARY

This paper describes the operation and performance of an 18-mm I.D. column of variable length in high-performance liquid chromatography. Special emphasis is directed towards ease of packing and both analytical- and preparative-scale capabilities. The packing is effected by using a piston that moves up and down in the column.

A few examples are presented to illustrate the speed of packing and reproducibility of the method in liquid–solid chromatography. The influence of the packing pressure upon efficiency in terms of permeability of the column is also considered. The performance of the column is discussed from both analytical and preparative points of view.

The analytical properties of the chromatographic unit in liquid–solid chromatography are shown to be comparable to those obtained with narrower columns. For example, using nordienolone as solute ($k' = 0.65$), the minimum reduced plate height is about 2.5 at a reduced fluid velocity of 3. A few examples of separations in reversed-phase liquid chromatography are also given.

The maximum amount of support that can be loaded into the column is about 50 g. Therefore, preparative separations can be carried out over the range 1–50 mg or more. The preparative properties of the column are discussed in terms of variation of the HETP and variation of retention as a function of the amount of sample injected.

INTRODUCTION

Recent developments in preparative high-performance liquid chromatography (HPLC) have shown that columns of large internal diameter are also suitable for analytical separations. For example, a regular increase in plate number with increasing internal diameter at constant linear flow velocity has been reported by Wolf[1], and a reduced plate height ($h = H/d_p$) of about 4 has been obtained[2] with an 8-cm I.D. column.

In addition, the concept of an "infinite-diameter column", introduced by Knox and Parcher[3], led to several investigations on the influence of this parameter. Using this approach, superior performances of columns of large internal diameter (7.94 and 10.9 mm) over narrow-bore columns have been reported[4,5].

A third factor favouring the use of columns of large internal diameter is the

improvement of packing procedures and the use of a moderate pressure drop to obtain high efficiency[6,7].

In this paper, the performances of an 18-mm I.D. column in liquid–solid and reversed-phase liquid chromatography are described. This type of instrument has been developed with a view to both analytical and semi-preparative HPLC applications. Examples of separations are given in order to illustrate the possibilities of such a column.

EXPERIMENTAL

Chromatographic apparatus

All work was carried out on a laboratory-built chromatographic unit. The solvent is delivered by means of a single-stroke, displacement-type pump. The reservoir is constructed of stainless steel and has a capacity of 4 l. The displacement of a cylinder pushed by a constant nitrogen pressure inside the reservoir allows the liquid phase to flow through the column at a constant flow-rate. The rate of delivery of the mobile phase is easily modified by changing the downstream back-pressure. A three-way valve at the top of the reservoir permits either filling of the vessel or delivery of the liquid phase.

The sample is introduced into the column through a six-port valve (Chromatronix). Various external loops allow the introduction of sample volumes of 0.1–2 ml. Two types of injectors have been used, specially designed for either on-column introduction or for swept injection. In the first mode (Fig. 1), the sample is directly injected into the centre of the column packing; in the second mode (Fig. 2), the sample is introduced over the whole cross-section of the stationary phase. The injectors can

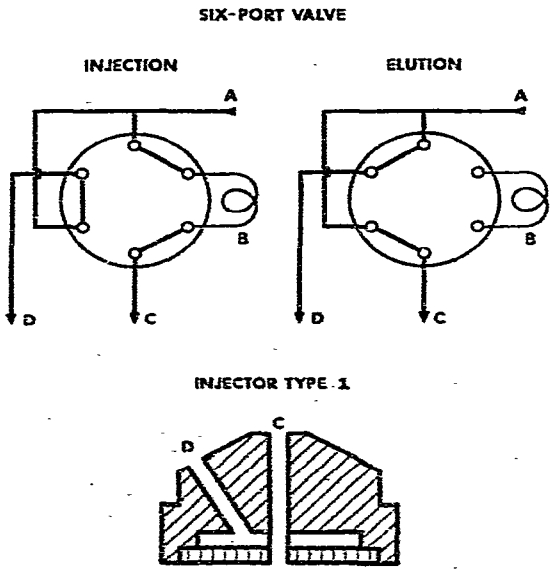

Fig. 1. Injection procedure with the "on-column" injector. A, From solvent reservoir; B, sample loop; C, to the injector; D, mobile phase.

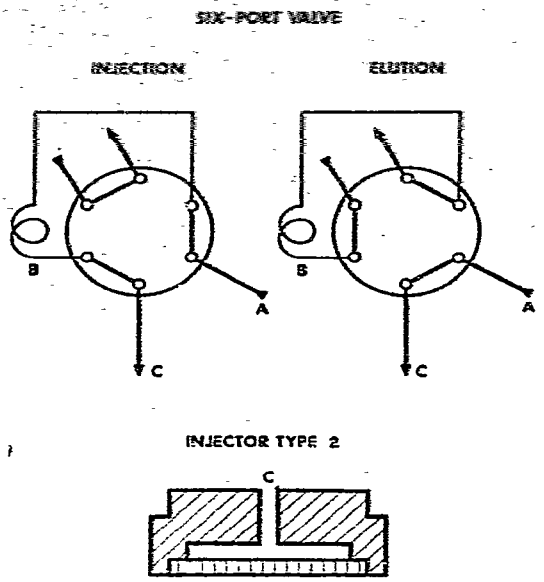

Fig. 2. Injection procedure with the swept injector. A, From solvent reservoir; B, sample loop; C, to the injector.

easily be removed from the top of the column by unscrewing them. As discussed later, the column efficiency and the amount of sample volume that can be injected are very dependent upon these two types of injections.

The column was prepared from a 70-cm length of 32 mm O.D. (18 mm I.D.) stainless-steel tubing. A piston moved by a pneumatic jack allows the support to be compressed during elution. The packing pressure may vary from 1 to 8 bar. The head of the piston is equipped with a porous sintered stainless-steel disc and is designed to assure a tight fit with the internal wall. Such a system, which has been described for an 8-cm I.D. column[2], provides a very homogenous packing density, eliminates dead volume near the injector and facilitates the packing procedure.

The detector was a variable-wavelength UV detector (Gilson Spectrochrom M) equipped with an 80-μl cell.

The chromatographic apparatus is shown schematically in Fig. 3 and a photograph of the whole unit is shown in Fig. 4.

Preparation of column

To pack the column, the piston is set to its lowest position and the injection port removed. The packing material in suspension in the mobile phase is then poured into the column. The injector is replaced and the piston is allowed to compress the support until a selected packing pressure is reached. During that period, the excess of solvent is discarded through the six-port valve.

This procedure, which takes about 10 min, provides a column efficiency comparable with that obtained with a narrower bore column using a balanced slurry packing procedure.

To remove the packing material, the injector is removed from the column and the piston is allowed to push out the compressed adsorbent.

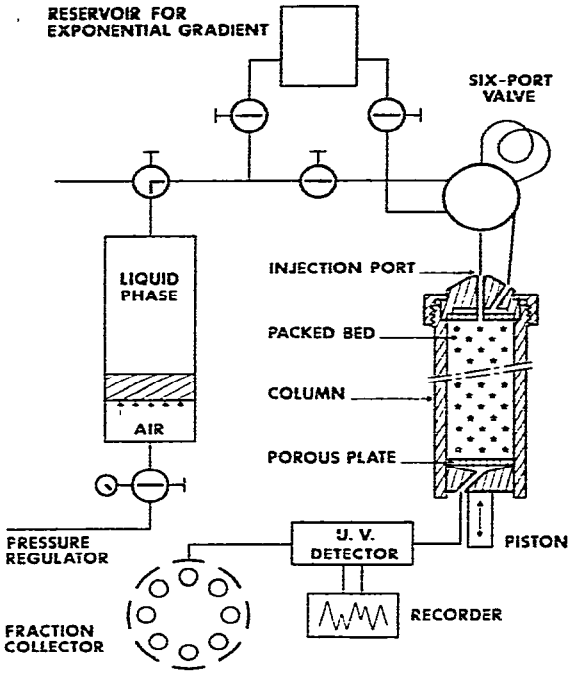

Fig. 3. Schematic diagram of the apparatus.

Reagents and chromatographic conditions

All work was performed with a constant amount of packing material (35 g) corresponding to a column length of 28.5 cm. The packing material was used without any pre-treatment. All measurements were made at room temperature.

Analytical performances were evaluated by injecting 200 μl of a standard solution (20%, v/v) of nordienolone (9,10-dehydronortestosterone) in the eluent. The detection wavelength was 258 nm. Preparative performances were investigated by injecting 1 ml of a solution of progesterone and testosterone in the mobile phase at various concentrations ranging from 0.1 to 100 mg/cm^3.

Liquid–solid chromatography. The silica gel was LiChrosorb 60 (Merck, Darmstadt, G.F.R.) of average particle diameter 10 μm. The mobile phase consisted of methylene chloride containing 5% (v/v) of methanol. The capacity factor of the solute nordienolone ($k' = 0.65$) was determined using undecane as a non-retained solute (refractive index detection).

Reversed-phase liquid chromatography. LiChrosorb RP8 was employed as stationary phase (average particle diameter ca. 10 μm). The liquid phase was a mixture of methanol and water (65:35, v/v).

RESULTS AND DISCUSSION

The performance of this column in both liquid–solid (adsorption) and reversed-phase (partition) liquid chromatography was investigated.

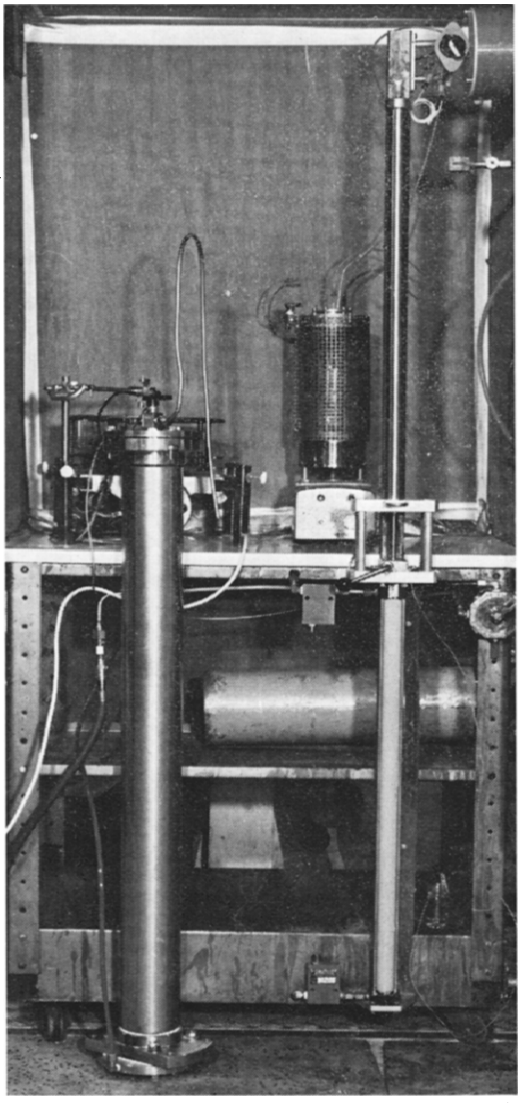

Fig. 4. Photograph of the chromatographic unit.

Liquid–solid chromatography

Analytical conditions. It has been shown[8,9] that the characteristics of a chromatographic column or a stationary phase could be evaluated by the variation of the reduced plate height (h) as a function of the reduced fluid velocity (v). These two chromatographic parameters are expressed by the equations[10]

$$h = \frac{H}{d_p} \tag{1}$$

and

$$v = u \cdot \frac{d_p}{D_M} \tag{2}$$

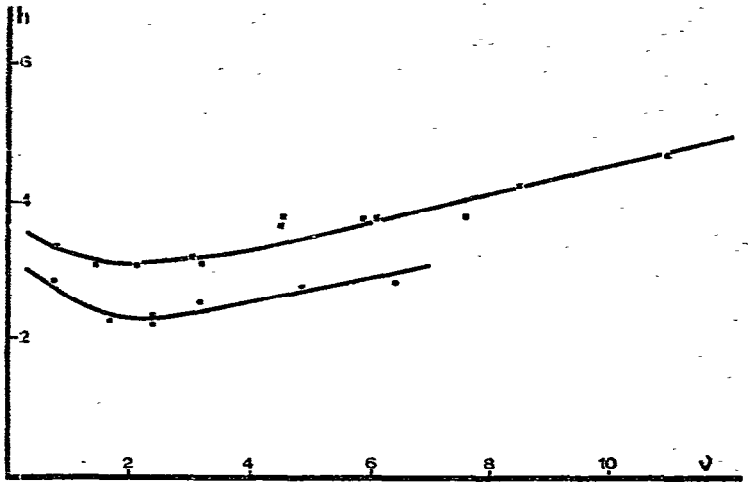

Fig. 5. Plot of h *versus* ν for nordienolone with the two injectors. ●, First type of injector; ■, second type of injector. Amount of solute injected: 40 μg.

where H, d_p, u and D_M are the HETP, particle diameter, linear flow velocity of the mobile phase and diffusion coefficient of the sample in the mobile phase, respectively. D_M was $1.45 \cdot 10^{-5}$ cm^2/sec, as estimated by the Wilke–Chang equation[11] for nordienolone in the eluent methylene chloride–methanol (95:5, v/v).

The variation of h with ν was studied with the two injectors and the results are represented in Fig. 5.

The efficiency of this column is excellent and the value of h_{min} (2.3) corresponds to the theoretically optimal value ($1 < h < 3$). Moreover, h_{min} is obtained for a ν value of 2.5, which is the value predicted by the Knox equation[12]. Taking into account the high column to particle diameter ratio ($1.8 \cdot 10^3$), this column can be considered to be of the "infinite diameter" type.

According to Knox and Parcher[3], only 5% of the solute injected into the centre of the packing will reach the internal wall of a column, where a disturbing effect on the flow velocity occurs, if

$$A = \frac{d_c^2}{d_p L} \geqslant 2.4 \qquad (3)$$

where d_c, d_p and L are the column internal diameter, the particle diameter and the column length, respectively, and A is the Knox and Parcher coefficient. Recent investigations have confirmed this theory. For example, a regular increase in column efficiency with increasing A values up to 45 has been observed[13].

In the present work, the existence of an "infinite diameter" is certain, as all of our experiments were carried out with an A value near 115. However, this is not the only factor that contributes to the high efficiency. If one uses an injector of the second type, where the solute is injected over the cross-section of the packing, thus cancelling out all of the properties of the "infinite diameter" column, the efficiency is reduced, but not to a great extent, and its minimum value is still about $h = 3.2$. The presence of a constant packing pressure contributes to the performance of this column by avoiding

TABLE I

CHROMATOGRAPHIC PARAMETERS AT THE OPTIMAL EFFICIENCY

Parameter	*Value*	*Parameter*	*Value*
Packing pressure	6 bar	Flow-rate	4.3 cm^3/min
Column length	28.5 cm	Pressure drop	3 bar
Number of theoretical plates*	12,500	Retention time	23.5 min
Fluid velocity	$3.2 \cdot 10^{-2}$ cm/sec	Column permeability, K**	$1.3 \cdot 10^{-9}$ cm^2

* First type of injector.

** K was determined from the equation $K = uL\eta/\Delta p$, assuming a mobile phase viscosity of 0.45 cP and by determining the slope of the Δp *versus* u curve.

irregular flow profiles and by minimizing the influence of dead volume near the injector and the end of the column.

Table I shows the principal chromatographic parameters for nordienolone at the optimal column efficiency.

Table I shows that because of its large internal diameter, the flow-rate in the column is relatively high, which means that from a preparative point of view a large volume of solvent must be handled, but the loss of efficiency due to the detector cell volume and the connection between the end of the column and the detector is reduced. A very small pressure drop is necessary in order to achieve about 44,000 theorical plates per metre. The use of a low pressure has several advantages, as discussed by Martin *et al.*[7]. The retention time is long, but (for a given column length) it can be reduced without affecting the efficiency too drastically by increasing the flow velocity, as can be seen in Fig. 5. For example, nordienolone, which has a retention time of 24 min at the optimal efficiency (12,500 theoretical plates), can be eluted in 8 min with a still acceptable plate number (10,500 theoretical plates).

For quantitative applications, the main disadvantage of such a column is the large retention volume of eluted bands, *e.g.*, V_R (nordienolone) = 100 cm^3 at h_{min}. Therefore, the sample will be more diluted with this type of column than with narrower bore columns[14].

The column permeability is in close agreement with reported data[15] ($K = 1.17 \cdot 10^{-9}$ cm^2 for the same particle diameter but using the balance density packing method). This parameter can be used as a measure of the average particle diameter of the support. The specific column permeability, K_F, is defined as follows:

$$K_F = \frac{F \eta L}{\pi R^2 \Delta p} \tag{4}$$

where F is the flow-rate (cm^3/sec), η the mobile phase viscosity (poise), L the column length (cm), R the column radius (cm) and Δp the pressure drop (dyne/cm^2).

Experimental data[15] indicate that

$$K_F = d_p^2 \cdot 10^3$$

Therefore,

$$d_p = \left(\frac{F \eta L}{10^3 \pi R^2 \Delta p}\right)^{\frac{1}{2}} \tag{5}$$

TABLE II

INFLUENCE OF THE PACKING PRESSURE ON FLOW-RATE AND REPRODUCIBILITY OF THE EFFICIENCY

This work was performed with the second type of injector B.

Packing pressure (bar)	*Pressure drop (bar)*	*Flow-rate (ml/min)*	*No. of theoretical plates per metre*
6.5	2	2.6	31,600
	3	4.2	32,280
	4	5.6–5.3	32,180–31,900
	8	10.3	27,050
8	2	2.6–2.6	31,130–33,000
	3	3.8–4.25	33,000–32,600
	4	5.6–5.7	32,650–30,300
	8	10.7–11.3	25,580

Under our experimental conditions, eqn. 5 gives $d_p = 10.6\ \mu m$. The very close agreement between this value and that stated by the manufacturer ($d_p = 10\ \mu m$) confirms that the packing procedure described in this work is very similar to the balanced density packing method, as far as the permeability of the column is concerned.

As shown in Table II, the packing pressure has no influence on the flow-rate (the column permeability is constant) or the efficiency. Duplicate measurements performed each time with a new batch of adsorbent illustrate the reproducibility of the packing procedure and column performance.

As an illustration of the analytical capabilities of the column, Fig. 6 illustrates the separation of several steroids of different polarities at the optimal efficiency. It can be seen that the shapes of the peaks are highly symmetrical. This parameter has been quantitatively estimated by measuring the symmetry index (S.I.)[9], defined by the following equation:

$$\text{S.I.} = \frac{\text{Trailing peak half-width}}{\text{Leading peak half-width}}$$

of nordienolone at various fluid velocities.

The symmetry index is obtained by drawing a perpendicular from the intersection of the tangents at the inflexion points of the peak to the base-line. The distance on this base-line between the intersection of this perpendicular and the two tangents gives the peak half-width. According to the nature of the injector, the calculated S.I. values are different: for the first type of injector, S.I. $= 1.08 \pm 0.07$, and for the second type S.I. $= 1.35 \pm 0.1$.

These data confirm our first observation (see Fig. 5) concerning the dependence of the HETP on the injection mode.

Preparative conditions. In preparative liquid chromatography, the volume of sample injected on to the column is very important because the poor solubility of the material to be analyzed may necessitate the use of a large sample volume. The influence of this parameter was evaluated by observing the variation of column effi-

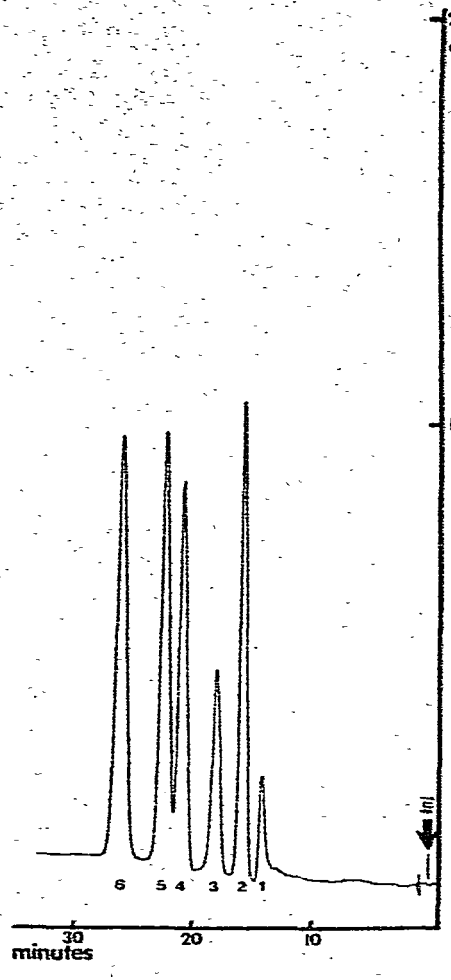

Fig. 6. Separation of several steroids by liquid–solid chromatography. Mobile phase, methylene chloride–methanol (95:5, v/v); flow-rate, 4.3 cm^3/min; amount of each steroid, 40 μg; column length, 28.5 cm; detection wavelength, 248 nm; pressure drop, 3 bar; volume injected, 200 μl. 1 = Progesterone; 2 = testosterone acetate; 3 = 17α-methyltestosterone; 4 = dexamethasone acetate; 5 = testosterone; 6 = androstadienolone. do = Optical density.

ciency (HETP) as a function of sample volume. A constant amount of nordienolone (40 μg) was injected at various concentrations in the mobile phase.

The results are given in Table III. The efficiency is markedly affected by the volume injected, as reported earlier[4,5], but not to the same extent for the two injectors.

Large volumes injected by the first type of injector (injection into the centre of the packing) produce a sharper decrease in efficiency than with the second type of injector. This phenomenon can be explained by considering that, at the inlet of the column, the flow velocity of the sample is much higher with the "on-column" injector than with the swept injector. Therefore, extensive band spreading is likely to occur with increasing injected volume.

TABLE III

INFLUENCE OF SAMPLE VOLUME ON COLUMN EFFICIENCY

Constant flow-rate: 4 cm³/min.

Injector type	*Sample volume injected (ml)*	*HETP (μm)*
Second	0.1	34.5
	0.2	33.5
	0.5	31
	1	40.5
	2	49.5
First	0.1	24.5
	0.2	25
	0.5	30
	1	58
	2	290

The preparative performance of a column is very difficult to define precisely. The maximum sample size can be estimated quantitatively by means of either of the following definitions:

(1) the linear capacity[16] corresponds to the value of the ratio of weight of sample to weight of adsorbent (W_S/W_A) that gives a 10% decrease in the retention time of any solute relative to its retention time at the minimum detectable amount;

(2) the W_S/W_A value that corresponds to a 100% increase in column efficiency[15].

Under our experimental conditions, the retention volume of the solutes studied (testosterone and progesterone) were independent of the weight of sample injected (m) up to 100 mg. Therefore, only the variation of HETP with sample size (m) was examined. For the reasons discussed above, an injector of the second type was used for this study. The variations are shown in Fig. 7, and are in agreement with published results[15,16].

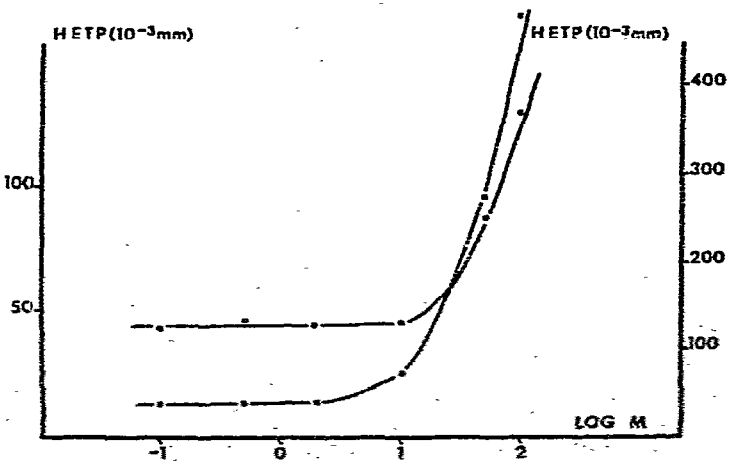

Fig. 7. HETP as a function of the logarithm of the weight of sample injected (mg). Volume injected, 1 ml; flow-rate, 8 cm³/min; injector, second type. ●, Curve relative to progesterone (left-hand scale); ■, curve relative to testosterone (right-hand scale).

The sample loadability of this column, using the second definition given above, was estimated as $W_S/W_A = 1.4 \cdot 10^{-3}$ for progesterone ($k' = 0.2$) and $0.3 \cdot 10^{-3}$ for testosterone ($k' = 0.8$). It is interesting to note that these values are very close to those reported by Endele *et al.*[15] of $1.7 \cdot 10^{-3}$ for benzene ($k' = 0.3$) and $0.15 \cdot 10^{-3}$ for nitrobenzene ($k' = 6.3$). The sample loadability of the column seems to be very dependent upon its retention value. This result should be confirmed by further studies, because it could be of great interest in preparative liquid chromatography.

An interesting observation occurred concerning the variations of the peak shape of the two solutes with increasing amounts injected: progesterone exhibited an increasingly tailing peak, whereas testosterone was characterized by an increasingly leading peak. The partition coefficient, α (ratio of the concentration of the sample in the stationary phase to its concentration in the mobile phase), behaves differently with increasing solute concentrations: for progesterone α tends to decrease, which means that the specific sites of the silica gel become saturated very rapidly, whereas for testosterone α increases, so that the stationary phase is overloaded and/or the solubility of this sample in the eluent is low.

Reversed-phase liquid chromatography

Only preliminary analytical investigations concerning the performances of this column in partition liquid chromatography are reported here. The column efficiency was evaluated as a function of the flow-rate of the mobile phase and the type of injection. For this purpose, the experimental conditions used were identical with those described in the first section, with the exception of the nature of the stationary phase and eluent. The variation of HETP of nordienolone with flow-rate is shown in Fig. 8 for the two injection procedures. This preliminary result suggests that the difference between the two procedures is not significant, contrary to the result observed in liquid–solid chromatography.

In both types of liquid chromatography, the column efficiencies are nearly identical using the second type of injector and they differ when the other type of in-

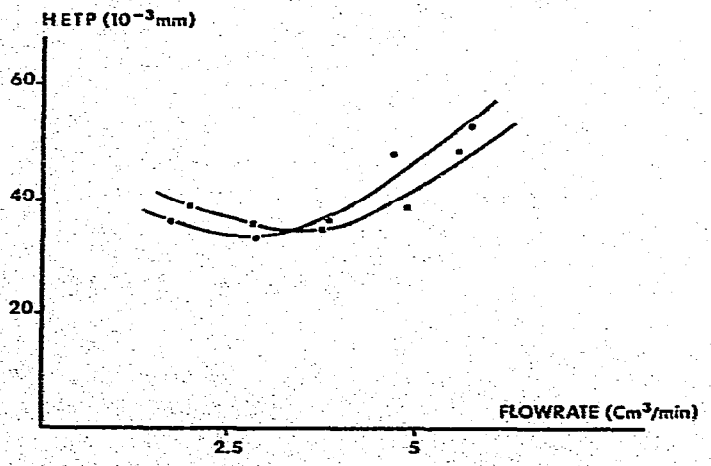

Fig. 8. HETP of nordienolone in reversed-phase liquid chromatography as a function of the mobile phase flow-rate for the two injectors. Amount of sample injected, 40 μg; mobile phase, methanol–water (65:35, v/v). ■, First type of injector; ●, second type of injector.

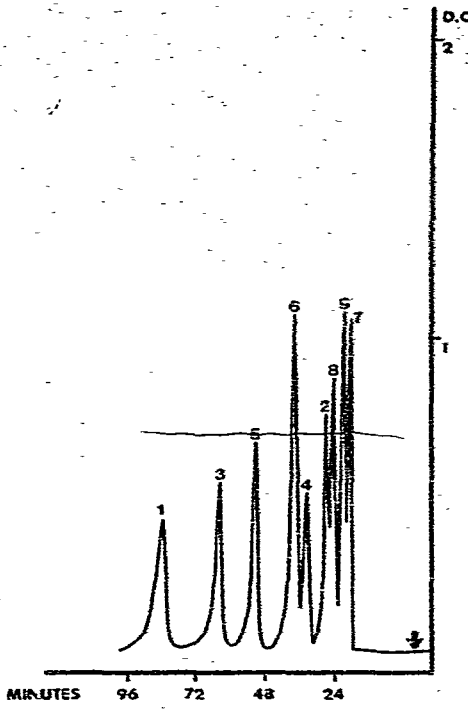

Fig. 9. Analysis of several steroids by reversed-phase liquid chromatography. Mobile phase, methanol–water (65:35, v/v); flow-rate, 2.5 cm³/min; amount of each steroid, 40 μg; column length, 12.3 cm; detection wavelength, 248 nm; pressure drop, 6 bar; volume injected, 200 μl. Steroids: 1–6, as in Fig. 6; 7, Δ-cortisone; 8, dexamethasone; 9, Δ-hydrocortisone. D.O. = Optical density.

jector is employed. The column efficiency is higher in adsorption than in partition chromatography. Work is in progress to confirm this first observation.

The peak shapes are also different, as they exhibit a slight tailing in partition chromatography, as expressed by their S.I. values: for the first type of injector S.I. = 1.42 ± 0.6 and for the second type S.I. = 1.68 ± 0.4.

Fig. 9 shows an example of analytical separation of a few steroids by reversed-phase liquid chromatography. As expected, the order of elution is reversed relative to the order in adsorption chromatograpyh, and the peaks are less symmetrical (*cf.*, Fig. 6).

CONCLUSION

The main conclusion is that excellent analytical results can be obtained with columns of large internal diameter (18 mm). Their good performance is due to the fact that such columns can be considered to be of "infinite diameter" and that the support is constantly compressed during the elution process.

Permeability data have shown that the packing procedure used is very similar to the balanced density packing method. From a practical point of view, these columns are very easy to pack in a very short time (10 min) and give reproducible results.

The use of two different types of injectors allows the column to be employed for either analytical or preparative separations. Samples of up to 100 mg can be analyzed (semi-preparative scale HPLC), which is a very suitable amount for spectroscopic identification.

The main disadvantages are that a large amount of support is needed and that the sensitivity is lower than with columns of small internal diameter. Also, the chemically bonded stationary phase appears to be less efficient than silica gel, but the results of this first investigation has to be confirmed in both analytical and preparative chromatography before definite conclusions can be drawn.

REFERENCES

1 J. P. Wolf, *Anal. Chem.*, 45 (1973) 1248.
2 E. Godbille and P. Devaux, *J. Chromatogr. Sci.*, 12 (1974) 564; *Fr. Pat. Appl.*, No. 2,219,797.
3 J. H. Knox and J. F. Parcher, *Anal. Chem.*, 41 (1969) 1599.
4 J. J. Destefano and H. C. Beachell, *J. Chromatogr. Sci.*, 8 (1970) 434.
5 J. J. Destefano and H. C. Beachell, *J. Chromatogr. Sci.*, 10 (1972) 654.
6 S. T. Sie and N. van den Hoed, *J. Chromatogr. Sci.*, 7 (1969) 257.
7 M. Martin, C. Eon and G. Guiochon, *J. Chromatogr.*, 99 (1974) 357.
8 J. M. Done and J. H. Knox, *J. Chromatogr. Sci.*, 10 (1972) 606.
9 G. J. Kennedy and J. H. Knox, *J. Chromatogr. Sci.*, 10 (1972) 549.
10 J. C. Giddings, *Anal. Chem.*, 35 (1963) 1338.
11 C. R. Wilke and P. Chang, *Amer. Inst. Chem. Eng. J.*, 1 (1955) 264.
12 J. H. Knox and M. Saleem, *J. Chromatogr. Sci.*, 7 (1969) 614.
13 J. Vermont, M. Deleuil, A. J. de Vries and L. L. Guillemin, *Anal. Chem.*, 48 (1975) 1329.
14 M. Martin, G. Blu, C. Eon and G. Guiochon, *J. Chromatogr. Sci.*, 12 (1974) 438.
15 R. Endele, I. Halász and K. Unger, *J. Chromatogr.*, 99 (1974) 377.
16 L. R. Snyder, *Anal. Chem.*, 39 (1967) 698.