CHROM. 6256

ANALYSIS OF STEROIDS BY HIGH RESOLUTION GAS–LIQUID CHROMATOGRAPHY

I. PREPARATION OF APOLAR COLUMNS

G. A. F. M. RUTTEN AND J. A. LUYTEN
Laboratory of Instrumental Analysis, Eindhoven University of Technology, Eindhoven (The Netherlands)

(Received June 30th, 1972)

SUMMARY

Several aspects of the use of glass capillary columns in the gas–liquid chromatography of steroids are discussed. The preparation of these columns is described in detail. It consists of two steps: pretreatment of the glass and coating with an apolar phase.

Etching, silanisation and the use of surface-active agents were studied as pretreatment steps. An intermediate test permits an evaluation of the pretreatment and indicates the actual behaviour of the glass wall. Treatment with a surface-active agent, in combination with a "static" coating procedure, is most satisfactory.

The performances of some of the columns obtained (both bad and good) are presented and several chromatograms illustrate the possibilities for steroid analysis.

INTRODUCTION

Despite their excellent resolving power, open-hole capillary columns are not commonly used in the gas–liquid chromatography (GLC) of steroids. VÖLLMIN[1,2] demonstrated the successful use of glass capillary columns for the analysis of steroids in several cases of disorders in steroid metabolism and showed that is it worthwhile to use the capillary column technique. In our opinion the reluctance to use this type of column in clinical chemistry may be attributed to the following difficulties: catalytic activity, coating, injection and instrumentation.

The stainless-steel capillary columns that are nowadays quite common in hydrocarbon analysis cannot be applied to steroid analysis at elevated temperatures, on account of the catalytic activity of steel. For that reason one is forced to work with less active materials, like glass. Nevertheless glass still needs a pretreatment before coating, at least for apolar stationary phases.

Until now the preparation of capillary columns has often proved to be governed by unknown parameters that are difficult to control. More reliable and reproducible

procedures have gradually been developed. At present, glass is coming more and more into use as a column material. It demands a subtle way of preparation. This comprises amongst other things at least one pretreatment of the glass before the real coating.

By its strongly hydrophilic nature, glass attacks all steroid derivatives to some degree. The substances are adsorbed and consequently somewhat decomposed. This results in tailing elution peaks, low yields and a "hilly" baseline. According to our experience, silanisation of the glass surface or treatment with surface-active agents can eliminate these effects.

Coating exercises a more direct influence on the resulting resolution. The coating procedure often presents difficulties. A good coating should produce a thin, uniform layer of stationary phase on the inner wall. We found the method of BOUCHE AND VERZELE[3] most satisfactory.

Special attention should be paid to the injection technique on capillary columns. The injection of small samples cannot, due to the low sample capacity of capillary columns, be carried out in combination with splitting systems in the case of high boiling compounds present in dilute solutions. GROB AND GROB[4] have reported a method of direct injection on to capillary columns and later[5] they described the principles, applications and technique in detail. In our laboratory an all-glass solid sampling device for capillary columns has been constructed[6]. This system is used for the injection of urinary steroids on to glass capillary columns.

In most commercially available gas chromatographs the connections to injector and detector require special adaptations for glass capillary columns. Shrinkable PTFE tubes may be used for the direct attachment of the glass column to injector and detector tubings.

These points are extensively discussed in the papers of NOVOTNÝ AND ZLATKIS[7] and MERLE D'AUBIGNE *et al.*[8].

In the present paper we describe our method of preparation for glass capillary columns. These columns are mainly used for the analysis of steroids. However, it must be emphasized that the overall performance of capillary columns is not only a matter of column preparation; instrument adaptation and injection technique are equally important. If these points are neglected, an excellent capillary column will produce disappointing and discouraging results.

MATERIALS AND METHODS

Preparation of glass capillary columns

The preparation of glass capillary columns may be considered to consist of two steps: (1) pretreatments; (2) coating with the stationary phase. Both steps have characteristic effects on the final result, and both make their own demands as to evaluation. After the pretreatment or, better, after each step of the pretreatment, an intermediate test may be applied to determine whether the pretreatment has had the desired result. The preparation is continued or, if need be, the pretreatment repeated.

The pretreatment has two aims: it should deactivate the glass surface and promote the spreading of the stationary phase.

Etching

It can be assumed that the inner wall of a glass capillary is essentially smooth.

Thin films can be applied, provided the stationary phase spreads spontaneously across the glass surface. For SE-30 (and other methylsilicone oils) this seems to be the case. For non-spreading stationary phases, the contact angle between the wall and the phase can be reduced by etching the surface. The stationary phase will occupy the cracks and holes. Capillaries coated in this way with non-spreading liquids will exhibit a reasonably good capacity ratio, but the plate number will be low.

Glass may be etched with alkali (NaOH, aq. NH_3) or with acids (HCl, HF) in aqueous solution. This results in a glass wall with strong adsorptive properties and a pronounced hydrophilic nature. NOVOTNÝ AND TESAŘÍK[9] emphasized the use of non-aqueous etching methods (dry HCl, HF or a milder method with methyl trifluorochloroethyl ether, decomposition of the ether at elevated temperatures produces hydrofluoric acid).

Studies on the behaviour of methylsilicone oils indicate that such liquids spread spontaneously across glass[10]. So etching, intended to promote spreading, might not be necessary for SE-30 or OV-101. However, etching was included in our series of experiments for comparative purposes.

The procedure is that at 40° a gentle nitrogen stream, saturated with methyl trifluorochloroethyl ether, is passed through the column. Both ends of the column are then sealed and the column is heated at 350° for 24 h. Then the column is purged with a nitrogen stream at the same temperature.

Deactivation

Plain, smooth glass requires deactivation before steroid analysis, as it contains reactive silanol groups and metal oxides on its surface. From the numerous methods available, the most notable are silanisation, interlayers of polymers, surface-active agents, corrosion inhibitors and carbonisation of the wall (*cf.* GROB[11]). For the deactivation of packed column supports, silanisation is commonly used. When applied to the glass surface, the active silanol groups are modified and the wall is shielded, thus preventing adsorption and decomposition of the steroids. Many reagents and working procedures are known. The method of NOVOTNÝ *et al.*[12] yields a surface apparently quite wettable by apolar phases.

About 25% of the column volume is filled with a mixture of hexamethyldisilazane (HMDS)–trimethylchlorosilane (TMCS) (5:1, v/v). After pressing this liquid through the column both ends are sealed and the column is maintained for a few days at 150° or higher. Next, drying and elimination of the excess reagent are carried out by purging with nitrogen at the same temperature.

The molecules of surface-active agents adhere to the inner wall of a capillary column, thus forming a monomolecular layer. Provided that shielding of the surface is complete and that the adhesion is sufficiently strong at higher temperatures, this might represent a simple and versatile method of deactivation. The surface-active agents are added to the stationary phase or used separately to pretreat the wall. The first method is a one-step preparation, but one cannot study the effect of the agent on the wall. We therefore favour the latter method, as suggested by GROB[11], but using Gas-Quat L (trioctadecylmethylammonium bromide) in accordance with METCALFE AND MARTIN[13] or benzyltriphenylphosphonium chloride (BTPPC) as introduced more recently by MALEC[14]. The gravimetric reagent for potassium, Kalignost (sodium tetraphenylborate) (KGn), having a molecular structure somewhat "antagonistic"

to that of BTPPC, was included in our experiments as proposed by FRANKEN[20]. Kalignost is an anionic agent; the other two are cationic.

About 15% of the column volume is filled with a 1% (w/w) solution of the agent. After passing this quickly through the column, the column is dried with a nitrogen stream. The column is then rinsed with more solvent and dried again. The whole procedure may be repeated. Gas-Quat L and BTPPC are dissolved in dichloromethane, Kalignost in acetone.

Intermediate test

In order to check the pretreatment, the column is next subjected to an intermediate test. We found that the coating of insufficiently deactivated glass capillaries causes bad column performances in steroid analysis. The intermediate test indicates whether the nature of the glass allows successful coating or whether the pretreatment must be repeated. Instead of testing the coated columns as GROB[11,15] did, we test the uncovered columns using the same compounds. The elution peak shapes indicate if the column will be suitable for steroid analysis after coating.

The column is mounted in the oven of a chromatograph equipped with a splitting device. At 190° and a split ratio of 1:750, *ca.* 0.5 μl of each of the following compounds is injected, the determinations being carried out in triplicate: (1) *n*-undecane, (2) dibutyl ketone (DBK), (3) *cis*/*trans*-2-propylcyclohexanol (PCH), (4) 2,6-dimethylaniline (DMA). They comprise a series of compounds of different adsorptive properties and different acidities. The alcohol, as expected, adsorbs most strongly. Columns exhibiting an asymmetric elution peak for this or any other compound are insufficiently deactivated.

Coating procedures

The coating efficiency is determined by the thickness and uniformity of the layer of stationary phase. In the case of thin layers, adsorption on the glass wall predominates; thick films, on the other hand, are not stable. Usually 0.1–1.0 μm layers are applied. Sometimes it is better to use wider-bore capillary tubes, up to 1 mm, so as to apply thicker layers without increasing the capacity ratio too much. However, this method only obscures the undesirable effects of the wall and will theoretically yield columns with performances lower than those obtained with narrow-bore capillary tubes.

For glass columns two methods of coating are popular: the "dynamic method" and the "static method" described by BOUCHE AND VERZELE[3].

The dynamic method, introduced by DIJKSTRA AND DE GOEY[16] is quite simple: a solution containing the stationary phase is passed through the column in such a way that a thin film is deposited on the wall. Next the solvent is evaporated and the stationary phase remains. The thickness of the film depends on the solution velocity, surface tension and concentration, as indicated by several authors. For a review see TESAŘÍK AND NEČASOVÁ[17].

The dynamic procedure is that about 15% of the column length is filled with a 5% (w/w) solution of SE-30 or OV-101 in isooctane. The liquid is then passed through the column, keeping the flow-rate as constant as possible. By means of a "pig-tail" dummy column, a rise in the flow-rate of the liquid when leaving the column is prevented. As soon as the liquid has left the column, the pressure is increased to

prevent the formation of secondary plugs of liquid. When dry, the column is ready for conditioning.

The static method is somewhat cumbersome. An important advantage is that the phase ratio is known accurately. According to older methods (GOLAY[18]), the column was slowly introduced into an oven. Recently an apparatus has been constructed by ILKOVA AND MISTRYUKOV[19] which permits the same method for glass columns.

For glass capillary columns the method of BOUCHE AND VERZELE[3] is used. The capillary is closed off at one end, and a vacuum is applied at the other end. The results are erratic for various solvents and stationary phases. Once the evaporation proceeds, no further difficulties are usually encountered. The deposition is carried out at room temperature. In the case of viscous, non-spreading stationary phases the film formed in this way may break up on subsequent heating and contract to tiny droplets scarcely visible with the naked eye. This phenomenon was observed for poly(*meta*-phenyl ether). Fortunately, when SE-30 is used difficulties of this kind are not encountered. With *n*-hexane as solvent and a vacuum of about 2 cm of Hg, the coating of a 20-m column can be completed within 2 to 3 days. Only some modified points of BOUCHE AND VERZELE's method will be discussed.

The static procedure is that the waterglass (needed for sealing) and a 0.3–0.7% (w/w) solution of SE-30 or OV-101 in *n*-hexane are separately degassed in a vacuum desiccator. The column is then filled very carefully either by suction or by pressure. A stream of hot air is passed over the filled column which is dipped into the waterglass. Some liquid is driven out of the column by expansion and, on cooling, some waterglass is sucked up. Care must be taken that no air bubbles enter the column. After drying overnight, the seal is hardened and evacuation can be started.

Testing of the coated column

After the usual conditioning period the column is tested at various inlet pressures. A mixture of *n*-alkanes (C_{20}, C_{22}, C_{24}, C_{28}), dissolved in hexane and a synthetic mixture of steroid TMS derivatives (see Table I) are injected by means of an all-glass

TABLE I

SYNTHETIC STEROID MIXTURE FOR TESTING THE COLUMNS

The names are given in order of elution (as TMS derivatives) from SE-30 at 230°.

Trivial name	*Systematic name*	*Abbreviation*
	Docosane	*n*-C_{22}
Androsterone	5α-Androstane-3α-ol-17-one	A
Etiocholanolone	5β-Androstane-3α-ol-17-one	E
Dehydroepiandrosterone	5-Androstene-3β-ol-17-one	DHEA
Testosterone[a]	2,4-Androstadiene-3,17β-diol	T*
	4-Androstene-17β-ol-3-one	T
Estradiol	1,3,5(10)-Estratriene-3,17β-diol	E II
11-Hydroxyetiocholanolone	5β-Androstane-3α,11β-diol-17-one	11 HE
Allopregnanediol	5α-Pregnane-3α,20α-diol	aPD
Pregnanediol	5β-Pregnane-3α,20α-diol	PD
Estriol	1,3,5(10)-Estratriene-3,16α,17β-triol	E III

[a] Silanisation of testosterone with BSA–TMCS gives rise to two derivatives.

solid injection system and are analysed at 230°. From the chromatograms several column parameters can be calculated.

The results in Tables II–VI are for *n*-alkanes only, and the possibilities for steroid analysis are indicated in the last columns of Tables II–IV. The separation number (*SN*), according to KAISER[21], has proved to be a useful parameter, immediately indicating the separating power of the column. In the tables, the separation number between n-C_{24} and n-C_{28} is given:

$$SN_{24-28} = \frac{t_{28} - t_{24}}{w_{28} + w_{24}} - 1 \qquad (1)$$

where w is the width at half height, and t_{28} and t_{24} are the retention times of the two alkanes.

The speed of separation is taken into account by calculating the square root of the number of effective plates per second ($\sqrt{N_{eff}}/t_R$), $\sqrt{N_{eff}}$ being directly related to the resolution (t_R = total retention time). The coating efficiency was calculated according to ETTRE[22], using the formula:

$$\text{coating efficiency} = 100 \times \frac{\text{HETP}_{min,obs.}}{\text{HETP}_{min,calc.}} \% \qquad (2)$$

where $\text{HETP}_{min,calc.} = r\sqrt{\frac{1 + 6k + 11k^2}{3(1 + k)^2}}$, r = radius of the column, and k = capacity ratio.

EXPERIMENTAL

All columns, ¼ mm I.D. and 1 mm O.D., were drawn from Pyrex glass tubes. Lengths ranging from 20 to 30 m were used. A Model 5750 Hewlett-Packard gas chromatograph with a flame ionization detector (FID) was used, with special adaptations for the mounting of capillary columns[23]. Narrow-bore glass tubes, the outer diameter of which tapers off to 1 mm, were used to adapt the detector junction to the capillary columns. A slighty modified, horizontally positioned, all-glass injection system[6] penetrates through the injection tubings into the oven. The modification consists of an extension from the point where the injection piece narrows for the attachment of the column, by means of a glass tube (5 × 0.4 mm) of sufficient length.

Connections to the injector and the detector adapter were made by shrinkable PTFE tubes. To prevent unwanted peak broadening due to insufficient flushing by the small effluent stream from the capillary column, additional purge gas, nitrogen, was added at the detector junction at up to 40 ml/min. The detector temperature was 270°.

When splitting was necessary, as for the intermediate test, a specially built gas chromatograph equipped with a Hamilton splitter was used.

RESULTS AND DISCUSSION

Silanisation

Firstly, columns will be discussed that were subjected to silanisation only. The intermediate test adequately indicates the influence of silanisation. (The results of this test will be discussed extensively under *Etching plus silanisation.*) For example,

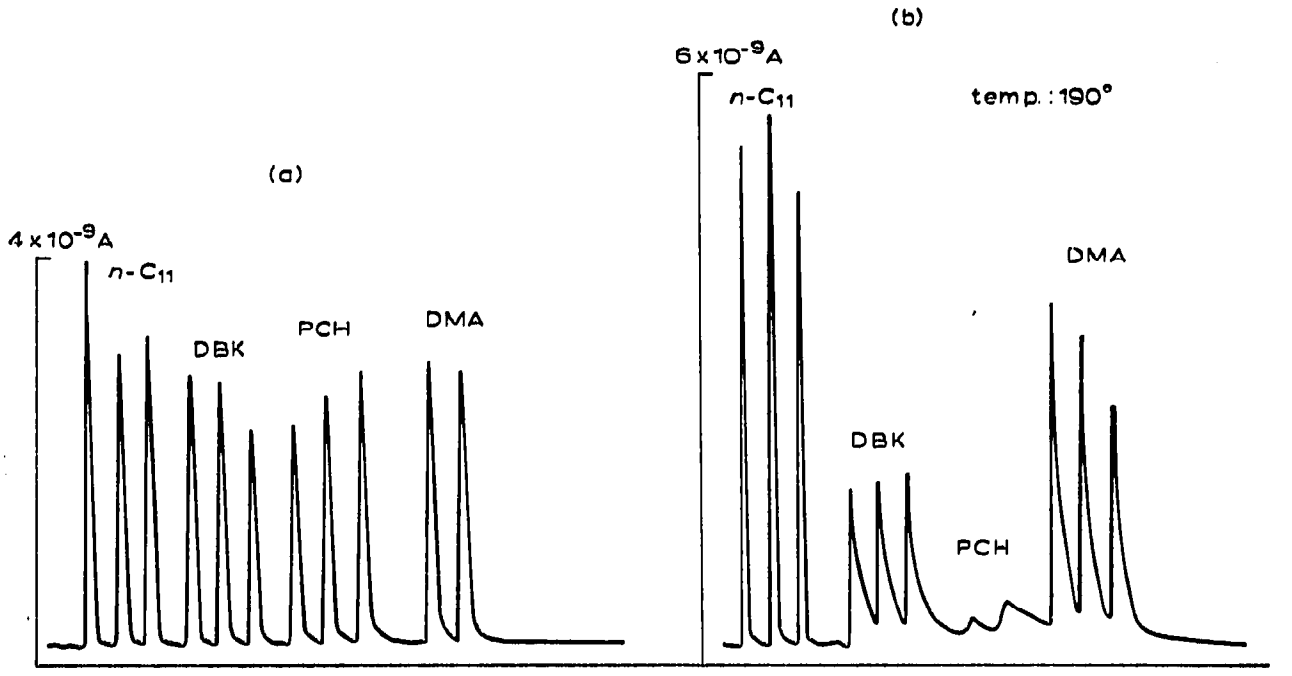

Fig. 1. Peak shapes of the test compounds after (a) sufficient and (b) after insufficient silanisation. (For abbreviations see text.)

Figs. 1a and 1b show the peak shapes after sufficient and insufficient silanisation, respectively.

Table II illustrates the effect of silanisation temperature. Initially columns were silanised at 150°. Columns silanised at 150° (*e.g.* column No. 1) showed a fast deterioration: *SN* decreased within three days from 35.1 to 27.7 and the plate number for *n*-C_{24} from 2250 to 1200. Columns silanised at 200° (*e.g.* column No. 2) did not exhibit fast degradation. It is striking that the *SN* and the plate number are of the same order of magnitude as those for columns silanised at 150° after deterioration. During this deterioration the capacity ratio remained constant. Therefore we concluded that the film is not stable enough. These results can be explained by the assumption that, during silanisation and drying of the column, certain reaction products are deposited on to the glass wall. These products probably form a layer favourable to the spreading of stationary phase. On heating to 250° these products degrade, as can be observed from the FID zero current, thus rendering the surface repellant. It seems that such products are not formed on silanisation at 200°. Thenceforth all columns were silanised at temperatures of about 200°.

Table III gives the results of some silanised and subsequently coated columns. These columns are selected to illustrate the silanisation procedure. They should not be regarded as representative as regards the number of rejects. Table III clearly reveals the advantages of the static coating procedure. Columns 4, 5 and 6 show higher capacity ratios, higher plate numbers, higher separation numbers and higher coating efficiencies than columns 3 and 7. The square root of the number of effective plates per second ($\sqrt{N_{eff}}/t_R$) is not significantly higher, because the higher capacity ratio entails longer retention times. All figures are based on *n*-alkanes. They do not inform us about the behaviour of the columns towards steroids. Their quantitative yields are to a

TABLE II

EFFECT OF SILANISATION TEMPERATURE ON COLUMN AGEING

All figures for n-C_{24} at 250° on SE-30 (carrier gas: nitrogen).

Column number	*Length (m)*	*Silanisation temp. (°C)*	*Coating method*	*Age (days)*	*Linear velocity of carrier gas (cm/sec)*	*Capacity ratio*	*Theoretical plates*	*Theoretical plates/m*	*Square root of effective plates/sec*	*SN C_{24}–C_{28}*	*Behaviour towards steroids*
1	23.5	150	dynamic	0	19.7	1.7	52,970	2250	0.452	35.1	good
				3	19.6	1.6	28,120	1200	0.329	27.7	tailing
2	26.0	200	dynamic	0	14.7	1.6	33,450	1290	0.245	23.3	fair
				3	14.7	1.5	34,230	1320	0.245	24.0	fair

TABLE III

PERFORMANCES OF SOME SILANISED COLUMNS

All figures for n-C_{24} at 230° on SE-30 (carrier gas: nitrogen).

Column number	*Length (m)*	*Silanisation*		*Coating method*	*Linear velocity of carrier gas (cm/sec)*	*Capacity ratio*	*Theoretical plates*	*Theoretical plates/m*	*Square-root of effective plates/sec*	*SN C_{24}–C_{28}*	*Coating efficiency (%)*	*Behaviour towards steroids*
		Time (h)	*Temp. (°C)*									
3	22.0	72	200	dynamic	17.8	4.8	20,400	930	0.163	26.7	27	good
4	22.3	72	190	static	19.3	7.5	57,220	2560	0.214	47.5	62	very good
5	20.3	72[a]	190[a]	static	16.1	7.9	42,710	2100	0.164	42.3	51	low yields
6	20.0	64	190	static	18.8	7.6	38,660	1930	0.190	37.6	58	low yields
7	19.7	91	230	dynamic	26.0	7.0	14,620	740	0.176	24.1	16[b]	no analysis possible

[a] Performed twice.

[b] Not at optimum conditions.

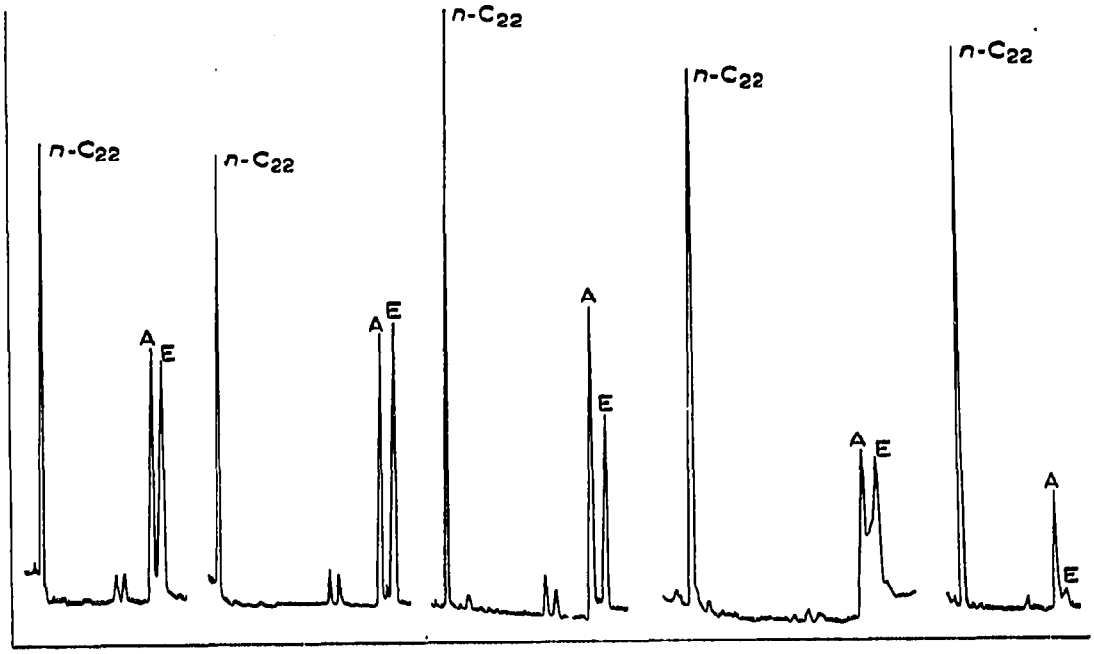

Fig. 2. Peak shapes of docosane, androsterone and etiocholanolone from the columns Nos. 3–7. Conditions: columns coated with SE-30, oven temperature 230°.

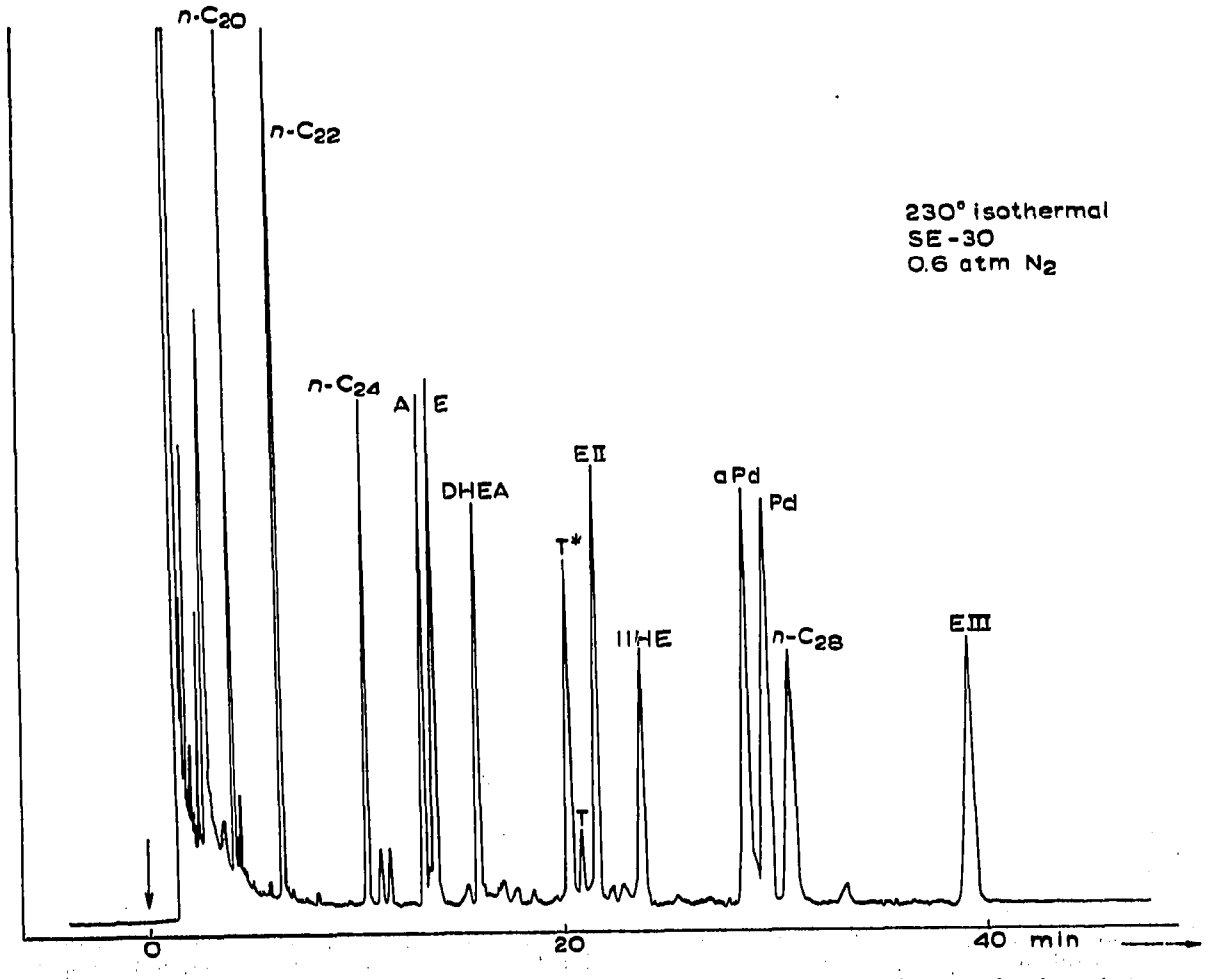

Fig. 3. Successfully silanised and coated column (column No. 4); synthetic mixture of steroids.

much greater extent dependent on the success of the pretreatment: Fig. 2 shows the isomeric pair androsterone–etiocholanolone accompanied by n-C_{22} as eluted from columns 3 to 7. The mutual peak heights vary considerably. We observed the same phenomenon for the pair allopregnanediol–pregnanediol. Comparison with the yield of n-C_{22} reveals considerable differences in the average yield of the steroids as a whole.

Insufficient silanisation of the inner wall causes low yields and a "hilly" appearance of the chromatogram, rather than asymmetric or strongly tailing peaks. The same can be noted for other methods or surface deactivation. This effect is independent of the coating efficiency. Fig. 3 illustrates the results for a successfully silanised and coated column. The steroids do not show any tailing or asymmetry. The isomeric pairs androsterone–etiocholanolone and allopregnanediol–pregnanediol have the same peak heights, in accordance with the original amounts weighed. Such a chromatogram permits a reliable quantitative interpretation.

Silanised columns deteriorate only after several months of use, when smaller yields of etiocholanolone and pregnanediol, and then of the other steroids, are obtained; finally the plate number for the n-alkanes diminishes, indicating that the film is degrading.

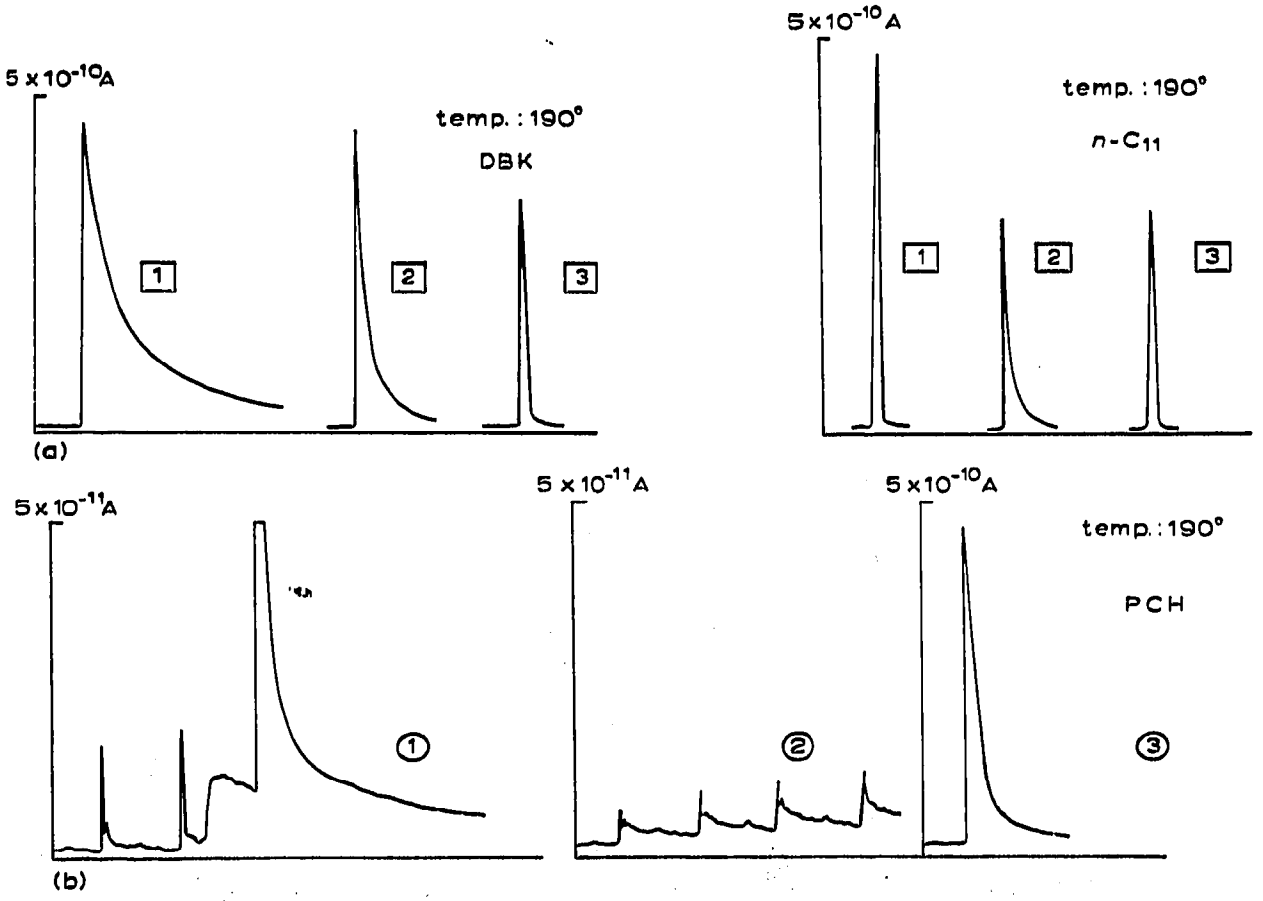

Fig. 4. Peak shapes of the test compounds after etching and subsequent silanisation: (1) before etching; (2) after etching; (3) after silanisation. (a) n-Undecane and dibutyl ketone. (b) Propylcyclohexanol.

Etching plus silanisation

Etched and silanised columns were prepared to examine the influence of etching on the separation. The effects of etching on the properties of the glass wall can be seen quite well from the intermediate test. Figs. 4a and 4b show the behaviour of the test compounds. After etching, n-C_{11} is apparently adsorbed on to the enlarged surface area; the peak shape of DBK, a compound of weakly acidic character, improves as the pH of the wall decreases. PCH, having a weakly basic character, is always adsorbed, although more strongly after etching (note the sensitivity of the FID). Even after silanisation PCH still tails. Repeated silanisation results in further improvement of the peak size and shape (see Fig. 5).

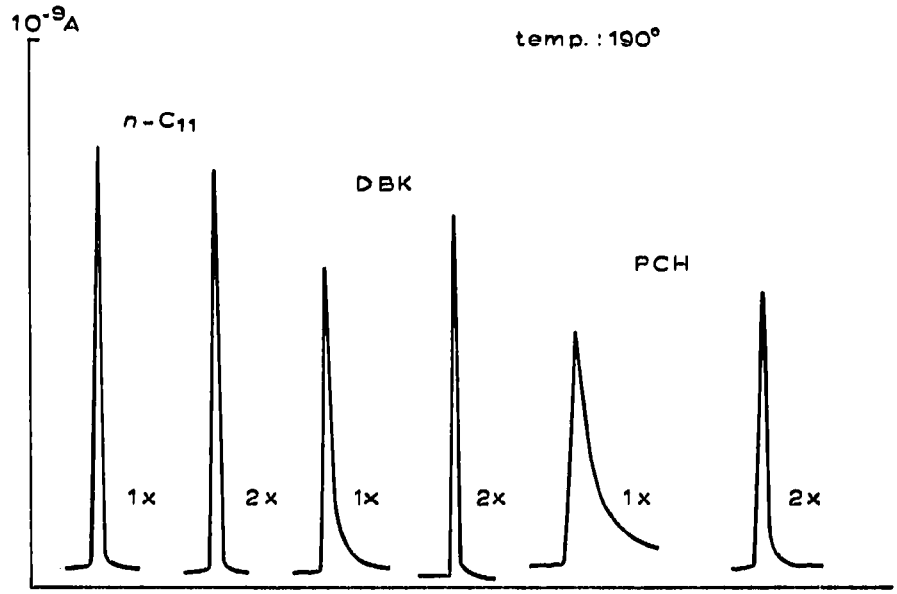

Fig. 5. Peak shapes of the test compounds after repeated silanisation. 1 × : After the first silanisation. 2 × : After a second silanisation.

Etched columns silanised at 200°, which passed the intermediate test (*i.e.*, had a symmetrical PCH peak), after coating performed a reasonable steroid separation. Thermostability was good (see Table IV) even for periods longer than six weeks.

Once again, etched columns silanised at 150° showed a fast deterioration within 24 h. Table IV gives typical results.

We required a sufficient yield and a symmetrical peak shape for PCH before proceeding to coating. This requirement may be thought rigorous and so we coated some columns which were insufficiently deactivated. Insufficiently silanised columns (see Fig. 6 for the intermediate test) showed a fast deterioration with regard to steroids, but not for n-alkanes. The typical changes in the yield of steroids for such columns are given in Fig. 7.

On comparing etched plus silanised columns with columns that were only silanised, it is found that the percentage of rejects in the first group is higher and the performances in the first group are not significantly better than those of the second group, and durability is even less. The preparation of the first group is more time-consuming and the silanisation of etched columns is more difficult. These conclusions

J. Chromatogr., 74 (1972) 177–193

TABLE IV

DETERIORATION OF ETCHED PLUS SILANISED COLUMNS

All figures for n-C_{24} at 255° on SE-30 (carrier gas: nitrogen).

Column number	*Length (m)*	*Silanisation temp. (°C)*	*Coating method*	*Age (days)*	*Linear velocity of carrier gas (cm/sec)*	*Capacity ratio*	*Theoretical plates*	*Theoretical plates/m*	*Square root of effective plates/sec*	*SN C_{24}–C_{28}*	*Behaviour towards steroids*
8	33.0	150	dynamic	0	24.1	2.0	52,650	1600	0.375	31.4	good
				1½	24.3	1.9	23,040	700	0.251	24.2	selective adsorption
10	21.0	200	dynamic	0	20.8	1.6	26,460	1260	0.379	24.0	good
				1½	20.4	1.6	22,640	1080	0.344	22.8	good

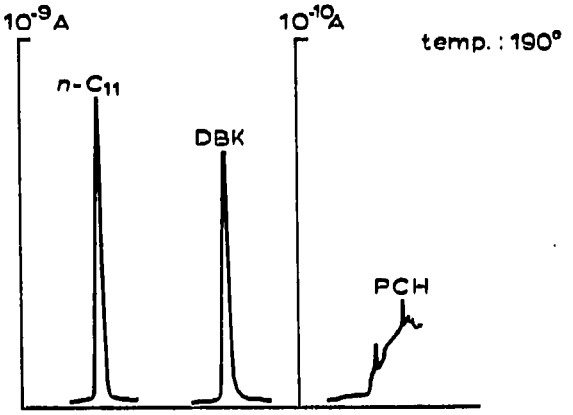

Fig. 6. Intermediate test applied to an insufficiently silanised column (No. 9).

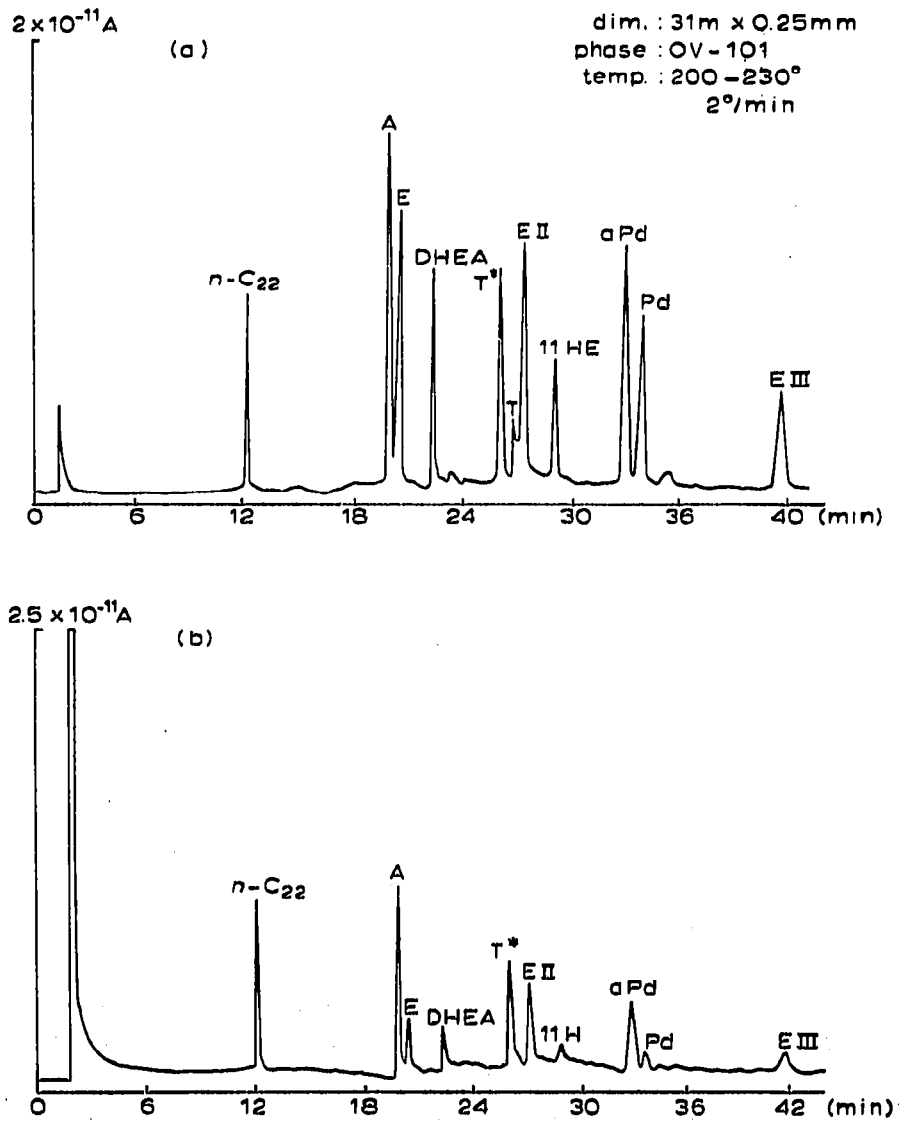

Fig. 7. Typical changes in the yield of steroids during deterioration of an insufficiently silanised column (No. 9). (a) Immediately after conditioning. (b) After 1¼ days operation at 250°.

TABLE V

COMPARISON OF SEVERAL PREPARATION PROCEDURES

All figures for n-C_{24} at 230° on SE-30 (carrier gas: nitrogen).

Column number	*Length (m)*	*Pretreatment*	*Coating method*	*Linear velocity of carrier gas (cm/sec)*	*Capacity ratio*	*Theoretical plates*	*Theoretical plates/m*	*Square root of effective plates/sec*	*Separation number C_{24}–C_{28}*	*Coating efficiency (%)*
3	22.0	sil.	dynamic	17.8	4.8	20,400	930	0.163	26.7	27
4	22.3	sil.	static	19.3	7.5	57,220	2560	0.214	47.5	62
11	21.7	BTPPC	dynamic	11.9	4.2	33,290	1540	0.155	34.0	30
12	21.3	BTPPC	static	14.3	7.0	53,380	2500	0.168	43.3	53
13	23.0	KGn	dynamic	14.5	4.0	25,520	1110	0.161	29.7	22
14	21.0	KGn	static	11.8	7.8	45,480	2170	0.121	49.1	50

TABLE VI

SOME BTPPC-PRETREATED AND STATIC COATED COLUMNS

All figures apply to n-C_{24} at 230° on SE-30 (carrier-gas: nitrogen).

Column number	*Length (m)*	*Pretreatment*	*Coating method*	*Linear velocity of carrier gas (cm/sec)*	*Capacity ratio*	*Theoretical plates*	*Theoretical plates/m*	*Square root of effective plates/sec*	*Separation number C_{24}-C_{28}*	*Coating efficiency (%)*
15	30.0	BTPPC	static	18.6	4.8	77,200	2570	0.246	53.6	58
16	21.0	BTPPC	static	14.3	6.5	68,540	3260	0.207	51.8	70
17	67.0	BTPPC	static	16.4	3.7	166,150	2480	0.170	72.7	60

are based on experiences with a large number of columns in our laboratory. Therefore, though it is difficult to assess its value, we do not favour etching as a pretreatment for apolar columns.

Surface-active agents

The silanisation procedure is in general cumbersome and takes several days. Surface-active agents are easily applicable to the wall. Complete deactivation can be achieved by repeated rinsings with a solution of the agent, as is depicted in Fig. 8.

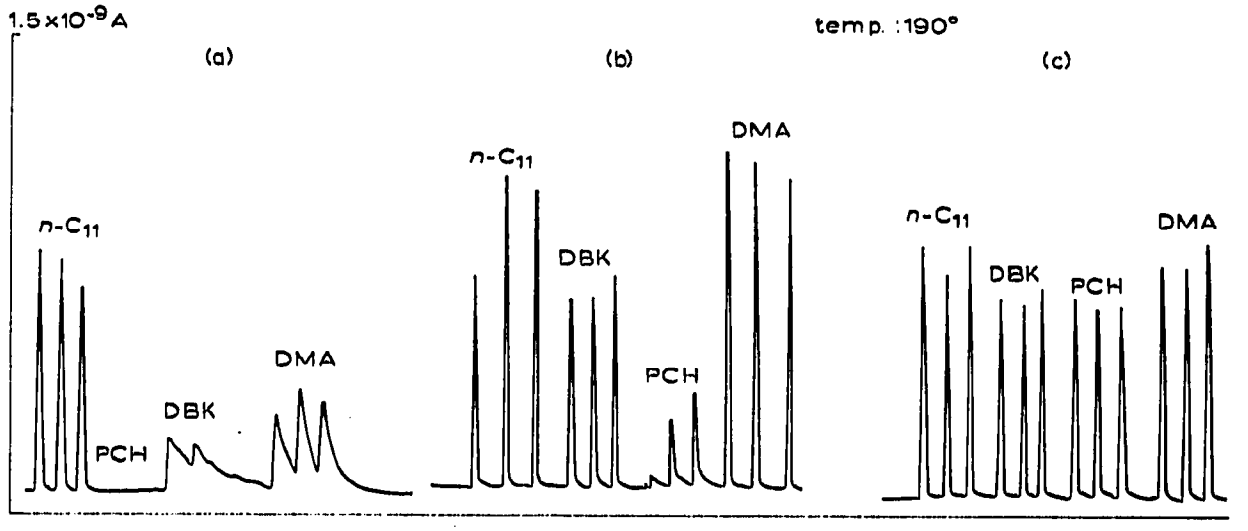

Fig. 8. Deactivation of the glass wall by repeated rinsings with BTPPC. (a) Before rinsing. (b) After one rinsing (PCH still tailing; typical "saturation"). (c) After a second rinsing.

Surprisingly, both BTPPC and Kalignost treatments resulted in the same peak shapes for the testing compounds. It seems that adsorption strength, rather than chemical character, influences the peak shapes. At first we used Gas-Quat L. Repeated rinsings with this agent resulted in good deactivation. However, MALEC[14] deduced from thermogravimetric studies that Gas-Quat L decomposes at temperatures above 220°. Our columns treated with Gas-Quat L did not show deterioration, even after prolonged use between 230° and 250°. MALEC indicates that BTPPC is stable up to 350°. Indeed, columns rinsed with this surface-active agent did not show any sign of degradation even after operation with severe temperature programming over a period of six months. Furthermore, columns treated with Kalignost are also stable. Our experiences only cover a ten month period, but the Kalignost treatment seems to be very promising, as no sign of degradation has yet been observed.

For coating columns treated with surface-active agents, we used the static method of BOUCHE AND VERZELE[3] and the dynamic method. A clear relationship exists between the velocity of the liquid plug in dynamic coating and capacity ratio. No correlation can be observed between these two parameters in the case of etched and silanised columns (Fig. 9). This permits prediction of the film thickness. Generally a plug velocity of 1 cm/sec was chosen.

Some typical figures are presented in Table V, which also includes some data for

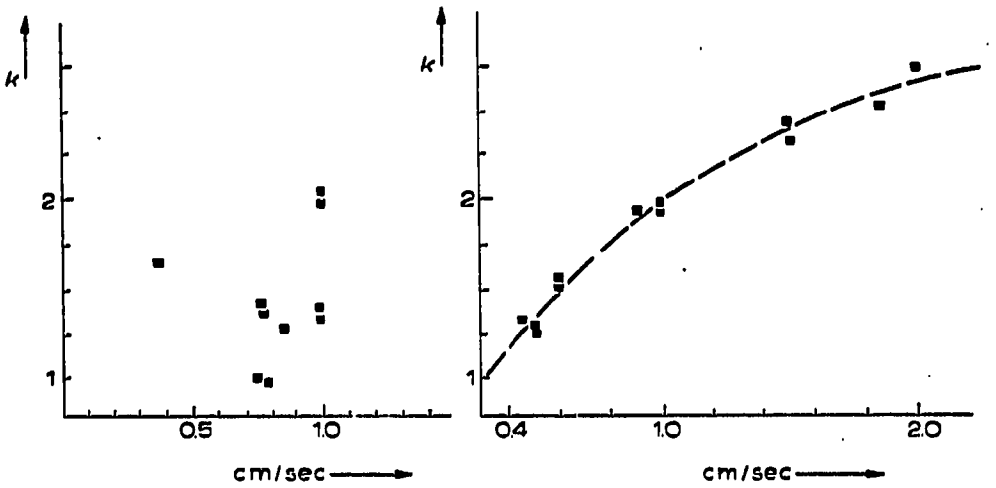

Fig. 9. Correlation between liquid plug velocity and resultant capacity ratio for n-C_{24} at 250°. Left: Gas-Quat L treated columns; right: silanised columns.

silanised columns. Again, the superiority of BOUCHE AND VERZELE's method is obvious. Very good coating efficiencies are obtained. The steroids are eluted in good yields, permitting quantitative determinations. Finally, Table VI summarises results for columns of different lengths and capacity ratios, prepared by rinsing with BTPPC and subsequent static coating, which is the method of preparation we prefer. Fig. 10 shows the test-mixture on column No. 17.

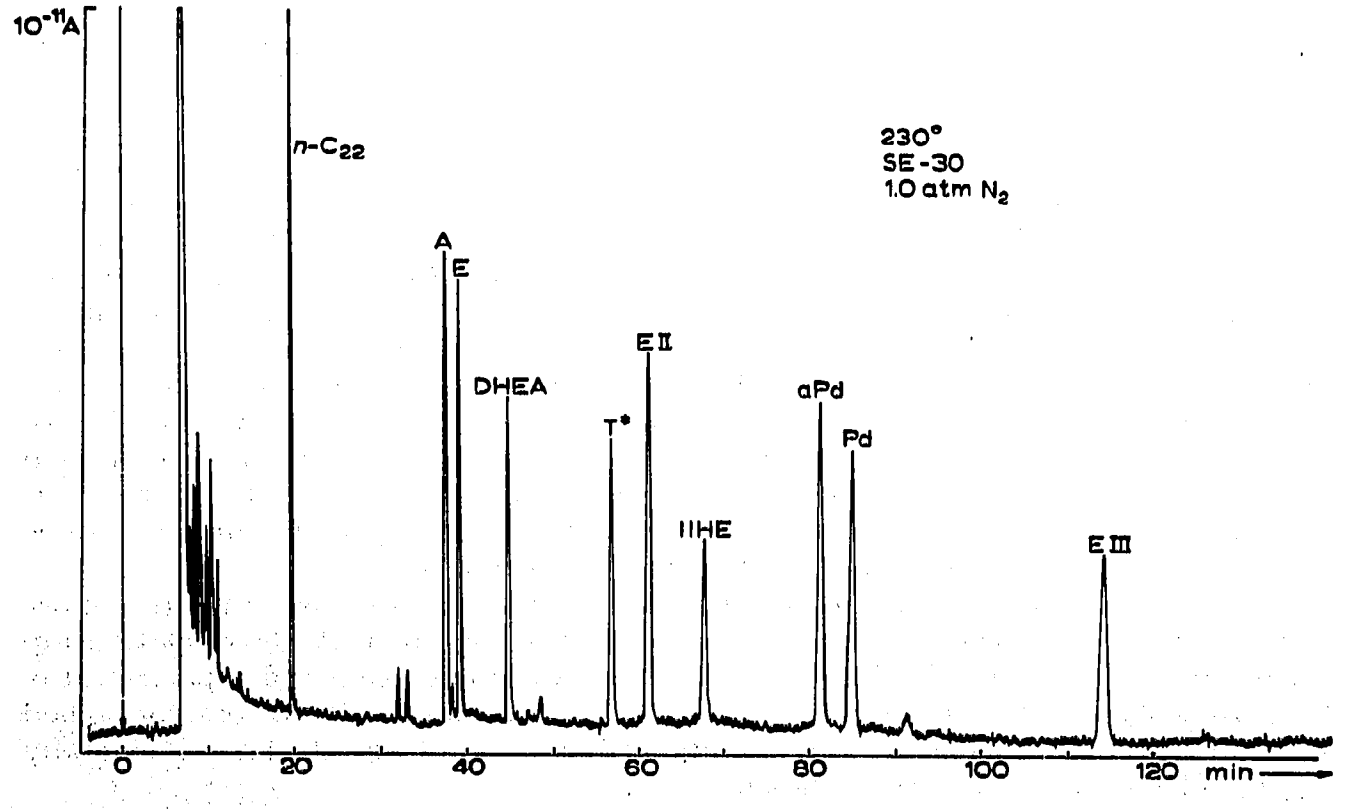

Fig. 10. Chromatogram of the test mixture on column No. 17.

CONCLUSIONS

It has been shown that glass capillary columns which are to be coated with apolar phases, such as SE-30 and OV-101, must receive a deactivating pretreatment. Etching in the course of pretreatment gives no obvious improvement in column performance after coating and encumbers the deactivation considerably. Insufficiently deactivated columns excercise a selective adsorption for some steroids, independent of coating efficiency. Testing the column with *n*-alkanes reveals the success of the coating procedure. This gives a useful indication of film uniformity.

We favour the versatility of pretreatment with surface-active agents. In combination with the static coating procedure this yields good columns for steroid analysis. The longer preparation procedure when using BOUCHE AND VERZELE's method is compensated by better performances.

Columns showing somewhat less separation power can be prepared within one day by the dynamic coating method.

REFERENCES

1 J. A. VÖLLMIN, *Chromatographia*, 3 (1970) 233.
2 J. A. VÖLLMIN, *Clin. Chim. Acta*, 34 (1971) 207.
3 J. BOUCHE AND M. VERZELE, *J. Gas Chromatogr.*, 6 (1968) 501.
4 K. GROB AND G. GROB, *J. Chromatogr. Sci.*, 7 (1969) 584.
5 K. GROB AND G. GROB, *Chromatographia*, 5 (1972) 3.
6 P. M. J. VAN DEN BERG AND TH. P. H. COX, *Chromatographia*, 5 (1972) 301.
7 M. NOVOTNÝ AND A. ZLATKIS, *Chromatogr. Rev.*, 14 (1971) 1.
8 J. MERLE D'AUBIGNE, C. LANDAULT AND G. GUIOCHON, *Chromatographia*, 4 (1971) 309.
9 M. NOVOTNÝ AND K. TESAŘÍK, in H. G. STRUPPE (Editor), *Gas Chromatographie 1968*, Akademie-Verlag GmbH, Berlin, 1968, p. 575.
10 W. D. BASCOM, R. L. COTTINGTON AND C. R. SINGLETERRY, *Advan. Chem. Ser.*, No. 43 (1969) 335.
11 K. GROB, *Helv. Chim. Acta*, 51 (1968) 718.
12 M. NOVOTNÝ, K. D. BARTLE AND L. BLOMBERG, *J. Chromatogr. Sci.*, 8 (1970) 390.
13 L. D. METCALFE AND R. J. MARTIN, *Anal. Chem.*, 39 (1967) 1204.
14 E. J. MALEC, *J. Chromatogr. Sci.*, 9 (1971) 1318.
15 K. GROB AND G. GROB, *Chromatographia*, 4 (1971) 422.
16 G. DIJKSTRA AND J. DE GOEY, in D. H. DESTY (Editor), *Gas Chromatography 1958*, Butterworths, London, 1958, p. 56.
17 K. TESAŘÍK AND M. NEČASOVÁ, *J. Chromatogr.*, 65 (1972) 39.
18 M. J. E. GOLAY, in D. H. DESTY (Editor), *Gas Chromatography 1958*, Butterworths, London, 1958, p. 67.
19 E. L. ILKOVA AND E. A. MISTRYUKOV, *J. Chromatogr. Sci.*, 9 (1971) 569.
20 J. J. FRANKEN, private communication, 1972.
21 R. KAISER, *Z. Anal. Chem.*, 189 (1962) 11.
22 L. S. ETTRE, *Open Tubular Columns in Gas Chromatography*, Plenum Press, New York, 1959, p. 15.
23 J. J. FRANKEN AND H. L. VADER, *Chromatographia*, in press.