Journal of Chromatography, 200 (1980) 221–223
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 13,108

Note

Separation of urinary steroids by gas–liquid chromatography on packed and mixed-bed columns

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(Received April 22nd, 1980)

Gas–liquid chromatography (GLC) is one of the most widely used and effective techniques for group analysis of hormonal steroids. Packed columns with liquid phases OV-1, OV-17 and SE-30 are most often used and are most thermally stable[1–3]. OV-1 methylsiloxane polymer is a non-selective phase employed for separating steroids according to their molecular weights and shapes. The selective phase OV-225 is a methylphenylpolysiloxane containing 75% of phenyl groups: it is used for further differentiation of functional groups of steroid molecules. We mixed nine parts of OV-1 (1%) and one part of OV-225 (3%) both on Gas-Chrom Q (100–120 mesh) and thus obtained a mixed-bed column, which enabled a higher degree of separation of some clinically important steroid metabolites.

EXPERIMENTAL

All steroids were obtained from commercial sources. The gas chromatograph was a Packard-Becker Model 419 with a flame-ionization detector and a Hewlett-Packard Model 3380A integrator. The operating conditions were: OV-1 (Applied Science Labs., State College, PA, U.S.A.) on Gas-Chrom Q (100–120 mesh) (Serva, Heidelberg, G.F.R.); glass spiral column (3.76 m × 2 mm I.D.); column temperature held at 190°C for 5 min, then programmed at 1°C/min to 270°C; temperature of the evaporating area and of the detector, 270°C. For the mixed-bed columns (glass spiral, 2.7 m × 2 mm), nine parts of OV-1 (1%) and one part of OV-225 (3%) (Serva) on Gas-Chrom Q (100–120 mesh) were mixed. Conditions: column temperature held at 190°C for 10 min then increased at 1°C/min to 240°C followed by isothermal operation; temperature of the evaporating area and of the detector, 270°C; carrier gas (nitrogen) flow-rate, 30 ml/min (2.0 atm); chart speed, 0.5 cm/min.

The methoxime (MO) derivatives were prepared by dissolving the dried samples in 100 µl of 2% methyloxamine hydrochloride in pyridine, standing for 3 h at 90°C[4], followed by evaporation under nitrogen. The residue was treated with 50 µl of TMS-S Universal reagens (Serva) overnight at 75°C. A 2–3 µl volume of this mixture was injected into the gas chromatograph. The time of analysis on both columns was about 70 min.

0021-9673/80/0000–0000/$02.25 © 1980 Elsevier Scientific Publishing Company

RESULTS AND DISCUSSION

Recently a series of capillary columns was recommended for steroid analyses[5–7]. Their high numbers of theoretical plates and correspondingly high separation efficiencies were advantageous in separation of complex mixtures. The preparation of capillary columns is technically more difficult than that of packed ones, however. The packed columns with OV-1 and SE-30 separate the MO-trimethylsilyl (TMS) derivatives of fully silylated steroids in a similar way. The column with OV-17 does not separate 11-oxoandrosterone from 11-oxoetiocholanolone[1].

The separation results on our mixed-bed columns are shown in Table I and Fig. 1. The mixed-bed packed columns enabled separation of 11-oxoandrosterone from 11-oxoetiocholanolone, and of the substance tetrahydro-S from tetrahydrodeoxycorticosterone and allotetrahydro-S. The substance S and cortol have the same elution time, however. We also studied the separation in mixed-bed columns with 5, 10 and 15% OV-225. The best separation was achieved with 10% OV-225. Higher percentages of OV-225 shortened the retention times of corticosteroids, of the steroids

TABLE I

METHYLENE UNIT (MU) VALUES FOR SOME URINARY STEROID TMS AND MO-TMS DERIVATIVES

Abbreviations: A = androstane; P = pregnane; A′ = androstene; P′ = pregnene; C′ = cholestene.

Trivial name	*Systematic name*	*MU values*	
		OV-1	*Mixed bed*
Androsterone (A)	3α-Hydroxy-5α-A-17-one	25.02	25.35
Etiocholanolone (ET)	3α-Hydroxy-5β-A-17-one	25.22	25.60
Dehydroepiandrosterone (DHEA)	3β-Hydroxy-5-A′-17-one	25.68	26.02
11-Oxoandrosterone (11 KA)	3α-Hydroxy-5α-A-11,17-dione	26.05	26.70
11-Oxoetiocholanolone (11 KET)	3α-Hydroxy-5β-A-11,17-dione	26.05	26.88
11β-Hydroxyandrosterone (11 HA)	3α,11β-Dihydroxy-5α-A-17-one	26.90	27.05
11β-Hydroxyetiocholanolone (11 HET)	3α,11β-Dihydroxy-5β-A-17-one	27.02	27.30
Pregnanediol (PD)	5β-P-3α,20α-diol	27.52	27.62
Pregnanetriol (PT)	5β-P-3α,17α,20α-triol	27.88	27.88
Tetrahydro substance S (THS)	3α,17α,21-Trihydroxy-5β-P-20-one	28.58	28.65
Tetrahydrodeoxycorticosterone (THDOC)	3α,21-Dihydroxy-5β-P-20-one	28.58	28.88
allo-Tetrahydro substance S (a-THS)	3α,17α,21-Trihydroxy-5α-P-20-one	28.58	29.02
Pregnanetriolone (11 KPT)	3α,17α,20α-Trihydroxy-5β-P-11-one	29.05	29.02
5-Pregnenetriol	5-P′-3α,17α,20α-triol	29.45	29.40
Tetrahydrocortisone (THE)	3α,17α,21-Trihydroxy-5β-P-11,20-dione	29.58	29.82
Tetrahydrocorticosterone (THB)	3α,11β,21-Trihydroxy-5β-P-20-one	29.93	30.05
Tetrahydrocortisol (THF)	3α,11β,17α,21-Tetrahydroxy-5β-P-20-one	30.21	30.22
allo-Tetrahydrocortisol (a-THF)	3α,11β,17α,21-Tetrahydroxy-5α-P-20-one	30.32	30.35
Cortolone (HHE)	3α,17α,20α,21-Tetrahydroxy-5β-P-20-one	30.48	30.65
β-Cortolone (β-HHE)	3α,17α,20β,21-Tetrahydroxy-5β-P-20-one	30.78	30.92
Cortol (HHF)	5β-P-3α,11β,17α,20α,21-pentol	31.20	31.15
β-Cortol (β-HHF)	5β-P-3α,11β,17α,20β,21-pentol	30.78	30.70
Cholesterol (CH)	5-C′-3β-ol	30.78	30.92
Substance S (S)	17α,21-Dihydroxy-4-P′-3,20-dione	30.95	31.15
Cortisone (E)	17α,21-Dihydroxy-4-P′-3,11,20-trione	31.98	32.65
Cortisol (F)	11β,17α,21-Trihydroxy-4-P′-3,20-dione	32.65	32.92
5-Cholesten-3β-ol isobutyrate	5-C′-3β-ol isobutyrate	33.10	33.75

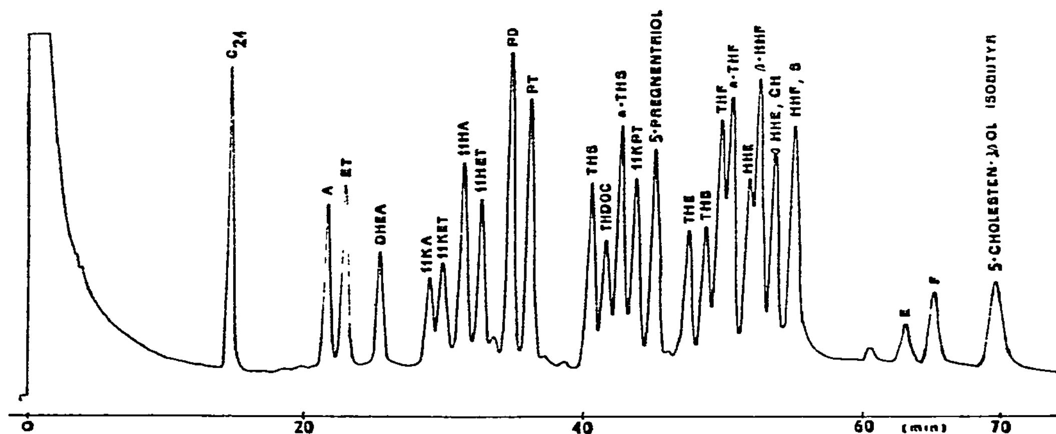

Fig. 1. A standard chromatogram. Peaks correspond to 240 ng of each steroid.

with a higher number of hydroxyl groups and an overlap with androgens occured. It is impossible to compute values of the methylene unit (MU) on a column with OV-225 only since the retention times are not linearly related to the number of carbon atoms in the alkanes[8]. The internal standard used for quantitative measurements was 5-cholesten-3β-ol isobutyrate; it has a shorter elution time than the often used cholesteryl butyrate.

We verified the clinical utilization of the described method in a study of the steroid spectrum in urine before and after adrenocorticotropin (ACTH) stimulation in three different types of arterial hypertension[9].

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