MICROANALYTICAL SEPARATIONS BY GAS CHROMATOGRAPHY IN THE SEX HORMONE AND BILE ACID SERIES

W. J. A. VandenHeuvel, C. C. Sweeley and E. C. Horning

Laboratory of Chemistry of Natural Products
National Heart Institute
Bethesda, Maryland

Received July 11, 1960

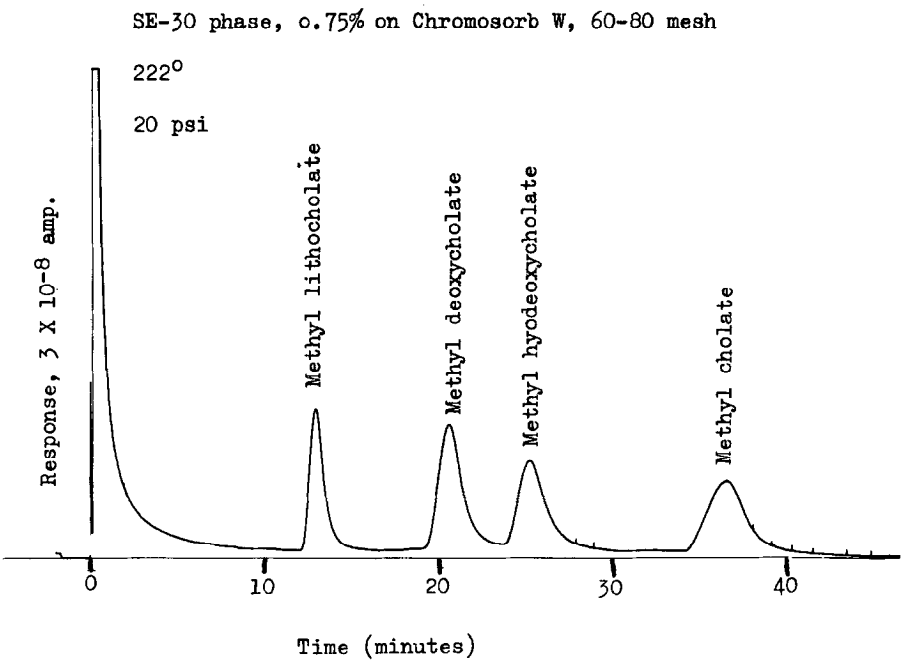
The microanalytical separation of steroids by gas chromatography may be carried out by conventional methods with thermostable liquid phases at 260-280°. This is the basis of the work of Sweeley and Horning (1960) with an ethylene glycol-isophthalic acid polyester as a polar stationary phase, and Beerthuis and Recourt (1960) with a silicone oil as a non-polar phase. Satisfactory separations for a number of steroids were achieved in both instances, but the conditions precluded extension of these methods to polyfunctional compounds and substances of limited thermal stability.

Experiments with silicone polymer SE-30 (Silicone Products Department, General Electric Co.) coated in low concentration on a Chromosorb W support provided a new approach to the problem. Retention times of 5-50 minutes were found for a variety of steroids at a column temperature of 222° (flow rate, 30 ml./min.). Cholesterol, cholestanol, β-sitosterol and stigmasterol, as well as a number of androstane and pregnane derivatives, were chromatographed under these conditions (VandenHeuvel, Sweeley and Horning, 1960). The effectiveness of this method may depend on the thin-film effects described by Hishta, Messerly and Reschke (1960).

Two classes of steroids of particular interest in biological and biochemical work are sex hormones and bile acids. In order to determine if this powerful and highly sensitive analytical separatory procedure was applicable to these compounds, a series of columns was prepared and tested under a variety of conditions. The liquid phase was SE-30 silicone polymer.

The coating procedure was carried out with a toluene solution (0.75, 1.5 and 3.0%) according to the method of Horning, Moscatelli and Sweeley (1959). Chromosorb W, 60-80 and 80-100 mesh, was used as the support. The columns were 6 ft. x 4 mm.; theoretical plate values were 1800-2200. An argon ionization detection system was used. The steroid hormones were used without chemical modification but the bile acids were converted to methyl esters with diazomethane before chromatography.

Well-defined peaks with no evidence of decomposition were obtained for all compounds. The retention times were consistent with previous observations of the effects of the functional groups present in these substances. The C-18, C-19 and C-21 hormones were eluted relatively rapidly (all were eluted before cholestane). Bile acid methyl esters showed longer retention times. Methyl cholate was collected after chromatography, and the infrared spectrum of the collected material was identical with that of the starting material, indicating that no change occurred during the operation.



RELATIVE RETENTION TIMES[a]

SEX HORMONES	TIME, 222° 1.5%	TIME, 222° 0.75%
Estrone	0.54	
Estradiol	0.62	
Estriol	0.95	
Progesterone	0.90	
Androsterone	0.41	
Testosterone	0.56	
BILE ACID DERIVATIVES		
Methyl lithocholate		2.15
Methyl deoxycholate		3.43
Methyl hyodeoxycholate		4.20
Methyl cholate		6.10
REFERENCE COMPOUND		
Cholestane	1.00[b]	1.00[c]

[a] Argon ionization detector, 6 ft. x 4 mm. I.D. columns.

[b] Time, 12.6 min. for 60-80 mesh Chromosorb W, 1.5% SE-30, 20 psi. (120 ml./min.).

[c] Time, 6.0 min. for 60-80 mesh Chromosorb W, 0.75% SE-30, 20 psi. (109 ml./min.).

Retention times relative to cholestane are given in the Table. All retention times were measured at 222°. A 0.75 or 1.5% coating on 60-80 mesh support, used with inlet pressures of 10-20 lbs., was found most useful. Under these conditions the hormone separations required only a few minutes. The bile acid separations required a somewhat longer time.

Adrenal cortical steroid hormones are currently under investigation to determine if additional polyfunctional steroids can be separated in the same way.

REFERENCES

Beerthuis, R. K. and Recourt, J. H., Nature, 186, 372 (1960).

Horning, E. C., Moscatelli, E. A. and Sweeley, C. C., Chem. and Ind., 751 (1959).

Hishta, C., Messerly, J. P., Reschke, R. F., Fredericks, D. H. and Cooke, W. D., Anal. Chem., 32, 880 (1960); Hishta, C., Messerly, J. P. and Reschke, R. F., Abstracts 137th A. C. S. Meeting, April, 1960, p. 29B.

Sweeley, C. C. and Horning, E. C., Nature, in press.

VandenHeuvel, W. J. A., Sweeley, C. C. and Horning, E. C., J. Am. Chem. Soc., in press.