# Gas Chromatography of Trimethylsilyl Ethers of Some 17-Ketosteroids

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Trimethylsilyl ethers of 17-ketosteroids were separated by gas chromatography through the use of NGS, and QF-1 phases. Relative retention times found in the mentioned phases with innert supports Chromosorb W, and Gas Chrom Q are compared. Advantages, and an availability of described phases for the separation of 17-ketosteroids occuring in human urine are discussed.

The gas chromatographic technique proved to be an available method also for the separation of different mixtures of steroids, and sterols. Mixtures of various naturally occuring C-19 steroids containing the keto group on carbon 17, known also as 17-ketosteroids (17-KS) have been separated with different results [1, 2]. The best results have been obtained with the separation of trimethylsilyl ethers (TMSi-ethers) of 17-KS [3—6].

In our paper we present the relative retention times of TMSi-ethers of 17-KS naturally occuring in human urine. The results obtained with NGS, and QF-1 phases indicate that it is possible to determine many most important 17-KS by means of gas chromatographic technique through the use of mentioned phases.

## Experimental

### *Nomenclature of used 17-KS*

| | | Abbreviation |
|---|---|---|
| Androsterone | 5α-Androstane-3α-ol-17-one | A |
| Testanolone (etiocholanolone) | 5β-Androstane-3α-ol-17-one | E |
| Isoandrosterone | 5α-Androstane-3β-ol-17-one | epi—A |
| Dehydroepiandrosterone | Androst-5-ene-3β-ol-17-one | DHA |
| 11β-Hydroxyandrosterone | 5α-Androstane-3α,11β-diol-17-one | 11—OH A |
| 11β-Hydroxyetiocholanolone | 5β-Androstane-3α,11β-diol-17-one | 11—OH E |
| 11-Ketoandrosterone | 5α-Androstane-3α-ol-11,17-dione | 11=O A |
| 11-Ketoetiocholanolone | 5β-Androstane-3α-ol-11,17-dione | 11=O E |

### *Material*

Reference samples were obtained from commercial sources: A, E, epi—A, 11—OH A, 11=O A, 11=O E from the Medical Supply, Prague; DHA, and 11—OH E from the Koch-Light Lab. Ltd. Hexamethyldisilazane, trimethylchlorosilane, neopentylglyco-

succinate (NGS), and the silicone polymer QF-1 were obtained from the C. Erba. Chromosorb W (AW-DMCSi), 100—120 mesh was purchased from J. Manville, and Gas Chrom Q, 100—120 mesh from Applied Science Labs. Chloroform was dried by calcium chloride. Aceton was analytical grade.

### *Apparatus*

Gas chromatographic separations were made with Chrom 3 instrument (Laboratory Instruments, Prague) equipped with a flame ionization detector with a glasss column adapted for a direct introduction of a sample.

### *Experimental conditions*

Mixtures of TMSi-ethers of 17-KS were chromatographed under the following experimental conditions: Two columns were used for the analysis by gas chromatography. One column with a length of 210 cm and internal diameter 4 mm contained Chromosorb W 100—120 mesh with QF-1 phase at a 3% level. The column was operated at 205°C and 215°C, 45 ml/min of nitrogen flow rate, 50 ml/min of hydrogen flow rate, and 600 ml/min of air flow rate.

The second column with a length of 180 cm, and internal diameter 4 mm contained Gas Chrom Q 100—120 mesh with NGS phase at a 1,5% level. The column was operated at 220°C, 55 ml/min of nitrogen flow rate, 60 ml/min of hydrogen flow rate, and 600 ml/min of air flow rate.

The samples 4 μl volume were injected directly into the column by means of a microsyringe.

### *Preparation of TMSi-ethers*

TMSi-ethers of 17-KS were prepared according to the method of *Luukkainen* [7] by reaction of the steroid with hexamethyldisilazane, and trimethylchlorsilane in chloroform.

## Results and Discussion

The gas chromatographic separation of TMSi-ethers etiocholanolone, and isoandrosterone on the silicone polymer QF-1 is shown in Fig. 1. The possibility of their separation by this method is especially important as these compounds owing to an identical mobility in most of routinely used mixtures are unseparable by paper chromatography. The highly effective support Chromosorb W with 3% of QF-1 polymer enables owing to its high inertion a satisfactory separation of TMSi-ethers of the studied 17-KS. The separation carried out under the same experimental conditions on the support Gas Chrom Q was unsatisfactory.

Up to the present time procurable supports fail to separate isoandrosterone, and dehydroepiandrosterone. Therefore we used for the separation of these compounds QF-1 phase at a 3% level, and Chromosorb W as the support. The separation of isoandrosterone, dehydroepiandrosterone as well as of other 17-KS (androsterone, etiocholanolone, 11-hydroxyandrosterone, 11-hydroxyetiocholanolone, 11-ketoandrosterone, and 11-ketoetiocholanolone) is shown in Fig. 2.

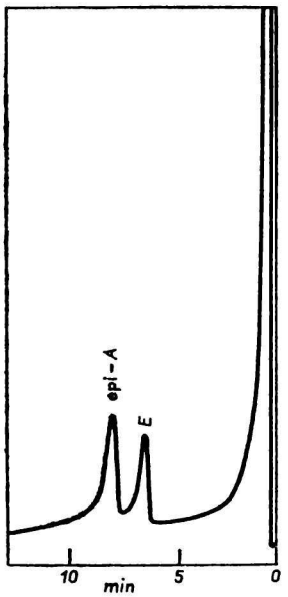


*Fig. 1.* Separation of isoandrosterone (epi—A), and etiocholanolone (E) on the support Chromosorb W 100—120 mesh, with QF-1 phase at a 3% level. Column temperature 205°C; $N_2$ flow rate 45 ml/min.

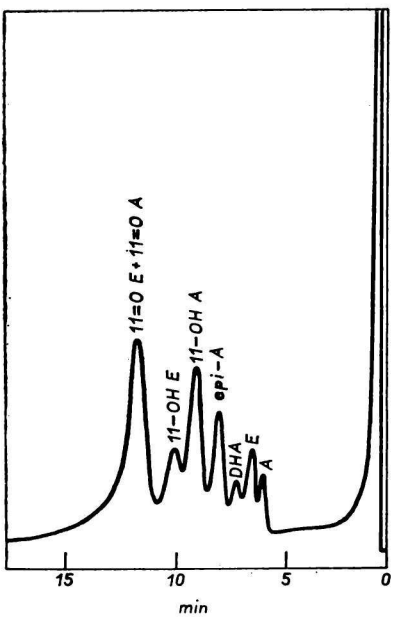


*Fig. 2.* Record of the chromatographic analysis of reference samples A; E; DHA; epi—A; 11—OH A; 11—OH E; 11=O E; 11=O A. The column with a length of 210 cm contained Chromosorb W 100—120 mesh, with QF-1 phase at a 3% level. Column temperature 215°C; $N_2$ flow rate 45 ml/min.

*Lehnert, et al.* [3] obtained a good separation of dehydroepiandrosterone, androsterone, and etiocholanolone with the NGS phase. We used this phase also for the separation of some other 17-KS. The pattern of the separation of A, E, DHA, 11=O A, 11=O E, 11—OH A, and 11—OH E is shown in Fig. 3.

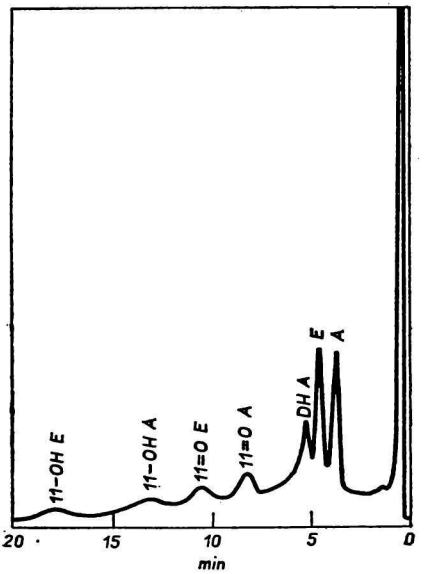


*Fig. 3.* Separation of 17-KS: A, E, DHA, 11=O A, 11=O E, 11—OH A, 11—OH E on a column containing Gas Chrom Q 100—120 mesh, with NGS phase at a 1,5% level. Column temperature 220°C; $N_2$ flow rate 55 ml/min.

*Table 1*

Relative retention times of TMSi-ethers of 17-KS to cholestane

| TMSi-ethers | NGS | QF-1 |
|---|---|---|
| androsterone | 1.00 | 1.13 |
| etiocholanolone | 1.29 | 1.23 |
| isoandrosterone | 1.55 | 1.40 |
| dehydroepiandrosterone | 1.55 | 1.53 |
| 11$\beta$-hydroxyandrosterone | 2.48 | 1.74 |
| 11$\beta$-hydroxyetiocholanolone | 3.16 | 1.98 |
| 11-ketoandrosterone | 3.93 | 2.23 |
| 11-ketoetiocholanolone | 5.38 | 2.23 |

5$\alpha$-Cholestane on NGS 3 min. 25 sec., on QF-1 5 min. 15 sec.

Relative retention times obtained with the NGS phase and with QF-1 phase are listed in Table 1. The values of relative retention times indicate that it is advantageous to separate on NGS phase a mixture of 17-KS which does not contain isoandrosterone, because its elution time is identical with that of dehydroepiandrosterone. In the case of the separation of isoandrosterone, and dehydroepiandrosterone QF-1 phase with Chromosorb W should be used. Under these conditions all 17-KS with exception of 11-ketoandrosterone, and 11-ketoetiocholanolone may be separated.

Our results indicate that by the method of gas chromatography with NGS, and QF-1 phases in a combination with various supports it is possible to determine 17-KS naturally occuring in human urine. However it is necessary to mention that various purification procedures, especially paper chromatography, or thin layer chromatography should precede the gas chromatographic analysis.

## References

1. Sweeley C. C., Horning E. C., *Nature* ***187***, 144 (1960).
2. Sparagana M., Keutman E. H., Mason W. B., *Anal. Chem.* ***35***, 1231 (1963).
3. Lehnert G., Mücke W., Valentin H., *Endokrinologie* ***46***, 241 (1964).
4. Vanden Heuvel W. H. A., Greech B. G., Horning E. C., *Anal. Biochem.* ***4***, 191 (1962).
5. Kirschner A. M., Lipsett M. B., Collins D. R., *J. Gas Chromatogr.* ***2***, 360 (1964).
6. Curtius H. Ch., *Z. Klin. Chem.* ***4***, 114 (1966).
7. Luukkainen T., Vanden Heuvel W. J. A., Haahti E. O. A., Horning E. C., *Biochim. Biophys. Acta* ***52***, 599 (1961).

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