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Gas chromatographic assay of methyltestosterone in tablets

N.A. ZAKHARI,*† M.I. WALASH,† M.S. RIZK,† S.S. TOUBAR,† C.J.W. BROOKS‡ AND W.J. COLE‡

† *Department of Analytical Chemistry, Faculty of Pharmacy, University of Mansoura, 33516 Mansoura, Egypt*
‡ *Department of Chemistry, University of Glasgow, Glasgow G12 8QQ, UK*

Abstract: A simple gas chromatographic procedure has been developed for the determination of methyltestosterone in bulk powders and in tablets. Two new silyl ether derivatives of methyltestosterone have been prepared using dimethylethylsilylimidazole (DMESI) and dimethylisopropylsilylimidazole (DMiPSI). The method is accurate and selective for methyltestosterone within the concentration range 0.1–1.5 μg μl^{-1}.

Keywords: *Gas chromatography; methyltestosterone; dimethylethylsilyl ether; dimethylisopropylsilyl ether.*

Introduction

Methyltestosterone is a widely used anabolic steroid and has also been encountered as a doping agent in sports. Several gas chromatographic methods are available for methyltestosterone doping control [1–4] and for analysis of its residues in meat [5, 6]. These methods depend on trimethylsilyl (TMS) ether derivatization of methyltestosterone. This paper describes a simple gas chromatographic procedure for the analysis of methyltestosterone in bulk powder and in tablets. The method depends on the formation of methoxime dimethylethylsilyl and dimethylisopropylsilyl ether derivatives of methyltestosterone.

These derivatives, which were developed by Miyazaki *et al.* [7, 8] for GC and GC–MS, have the advantage over the conventional TMS derivatives in that the compounds are shifted to a cleaner area in the GC trace due to a longer retention time and hence give good separation from volatile impurities and solvents. Moreover, the TMS ether derivatives of methyltestosterone show non-reproducible behaviour during derivatization, because of partial formation of 3-enol TMS ethers [9]. In the GC–MS-SIM technique, ions to be monitored are usually at higher m/z values (14 and 28 amu increase for each DMES and DMiPS group, respectively). Therefore, the possibility of contamination is reduced, especially when dealing with samples of biological origin.

Experimental

Solvents and reagents

Chloroform (HPLC-grade) was obtained from Rathburn Chemicals (Scotland), cyclohexane (spectroscopic grade) and toluene, (analytical grade) from Koch-Light Labs (UK). Ethyl acetate (Nanograde) was purchased from Mallinckrodt (St Louis, MO, USA). Pyridine (analytical grade, BDH Chemicals, Poole, UK) was dried by keeping overnight over potassium hydroxide pellets, then distilled. Methoxamine hydrochloride was obtained from Eastman Kodak (Kirkby, UK) and used as 0.3% (m/v) solution in dry pyridine. Dimethylethylsilylimidazole (DMESI) and dimethylisopropylsilylimidazole (DMiPSI) were obtained from Tokyo Kasei Kogyo Co. (Japan).

Steroids investigated

Norethandrolone (Nilevar) was purchased from Organon Labs (UK) and methyltestosterone from BDH Chemicals. Glossosterandryl tablets containing 10 and 25 mg methyltestosterone were marketed by Memphis Chem. Co. (Egypt). The purity of steroids was checked by TLC procedures using

* Author to whom correspondence should be addressed.

pre-coated Merck Silica Gel 60F-254 plates (BDH Chemicals) and chloroform–ethyl acetate (3:1, v/v) as solvent mixture [10, 11]. The amount applied on TLC was 10 μl containing 10 μg of steroid in ethyl acetate. Spots were visualized by spraying the plates with 10% aqueous sulphuric acid containing 1% ceric sulphate and heating at 110°C for 5–10 min. Only one spot for each steroid was detected. The purity was further confirmed by GC where each compound produced one peak indicating absence of impurities. The methoxime-TMS ether derivatives of the studied steriods were prepared according to Thenot and Horning [12] and used for GC (1% OV-1 and 1% OV-17, 6 ft column set at 230°C).

Gas–liquid chromatography

Packed gas–liquid chromatography was carried out with a Perkin–Elmer F-11 gas chromatograph equipped with a silanized glass column (6 ft × 4 mm i.d.) packed with 1% OV-1 coated on Gas Chrome Q 100–120 mesh (Phase Separations, Queensferry, UK). The column was heated isothermally at 230°C and the nitrogen carrier gas flow-rate was 45 ml min^{-1}. The instrument employed a flame ionization detector with the hydrogen flow at 40 ml min^{-1} and the air flow at 350 ml min^{-1}.

Gas chromatographic determination of pure steroid

Accurately measured volumes of the steroid solution in ethyl acetate equivalent to 10–150 μg were transferred into a series of 1 ml Reactivials (Pierce & Warriner, Chester, UK) containing 100 μl of Nilevar solution as the internal standard (i.s.). The solvent was evaporated to dryness under a slow stream of nitrogen; the residue was dissolved in 100 μl of methoxamine hydrochloride solution and the mixture was kept at 70°C for 1 h. The excess pyridine was evaporated under nitrogen and 10–20 μl of DMESI or DMiPSI reagent were added before the solutions were heated at 70°C for 2 h. The excess reagent was removed under nitrogen and the residue was extracted with 100 μl of cyclohexane. One microlitre of each solution was injected into the gas chromatograph. The peak height ratios of steroid to the i.s. were calculated for duplicate experiments as the mean value obtained from at least six injections for each concentration level. The mean per cent recovery was determined from the respective regression equation.

Analysis of tablets

One tablet containing 10 or 25 mg methyltestosterone was placed in a 10 ml screw-capped test tube containing 20 or 50 mg Nilevar, respectively (the i.s. solution was added in portions and the solvent was evaporated continuously under nitrogen to complete dryness). The tablet was disintegrated in 1 ml of distilled water, and the steroids were extracted with chloroform (4 × 2 ml). Each time the tube contents were shaken for 5–10 min, centrifuged and the chloroform extracts were combined and dried over anhydrous sodium sulphate. The dry chloroform extracts were collected into 20 and 50 ml volumetric flasks for 10 and 25 mg steroid/tablet, respectively, and the solution was made up to volume with dry chloroform. A 0.1 ml aliquot equivalent to 50 μg of steroid was taken for derivatization following the above mentioned procedures, after evaporating the chloroform at 40°C under a slow stream of nitrogen. A 1 μl aliquot was then injected for the GC analysis.

The peak height ratios were calculated for each preparation and the drug content was computed for each derivative from its respective regression equation.

Results and Discussion

Earlier studies by one of us [13] have discussed the optimum conditions for the preparation of DMES and MO–DMES ether derivatives of several hydroxy-steroids. These studies have shown that in many instances complete derivatization and formation of only one GC peak for each steroid which is completely separated from the i.s. peak can be achieved.

In the present investigation, Nilevar has been chosen as the i.s. because it is a simple structural isomer of methyltestosterone (retaining a tertiary alcohol group) and both hydroxy ketosteroids are closely similar in their chemical and physical properties. Nilevar was incorporated at the beginning of the assay so that there is compensation for possible losses at any stage in the procedure.

Figure 1 shows typical gas chromatograms of methyltestosterone and norethandrolone methoxime TMS, DMES and DMiPS ether derivatives prepared from pure samples. The retention times of the DMES and DMiPS ether derivatives are longer [7, 8] than those of the TMS ether derivatives, as indicated in

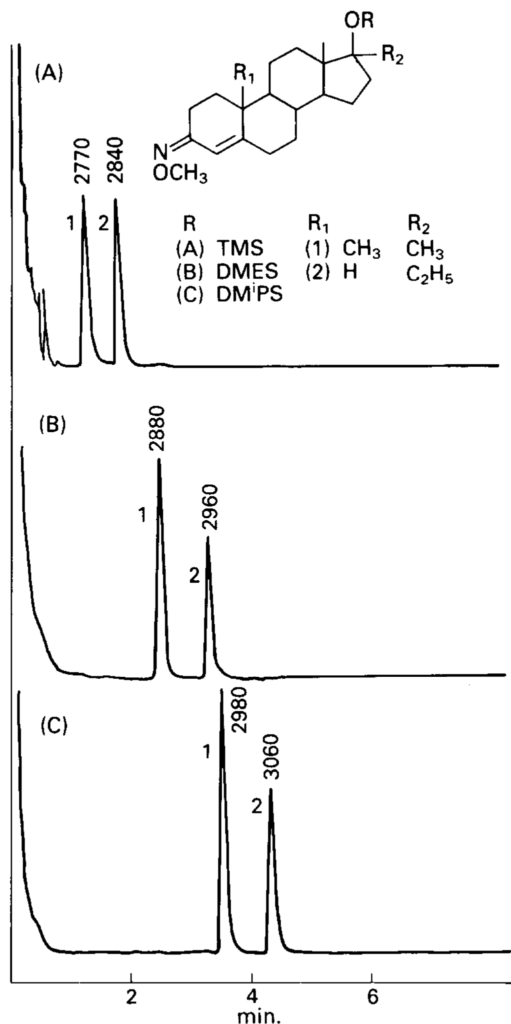

Figure 1
Typical gas chromatograms of (1) methyltestosterone, 1 μg μl^{-1} in A and B and 0.7 μg μl^{-1} in C; (2) norethandrolone 1 μg μl^{-1}, TMS, DMES and DMiPS ether derivatives (A, B and C, respectively); 6 ft column, 1% OV-1 and 230°C.

chromatograms B and C compared to chromatogram A. This advantage may be useful in the analysis of biological samples containing methyltestosterone because the compound is shifted to a cleaner area in the GC trace. Another advantage of the derivatives is that the presence of bulky or sterically crowded alkyl group improves the stability of steroid-silyl ether derivatives 10^2–10^4 times more than their corresponding TMS ether derivatives [8, 14, 15].

The i.s. peak was adequately separated from the methyltesterone peak with $I^{230°C}_{1\% OV\text{-}1}$ values of 2960 and 3060 for Nilevar DMES and DMiPS ethers, and 2880 and 2980 for the DMES and DMiPS ethers of methyltestosterone. The retention index values for the DMES and DMiPS ether derivatives are useful for the identification of these steroids even if there are other steroids present.

Seven different concentrations of the methyltestosterone DMES ether derivatives were prepared within the range 0.1–1.5 μg μl^{-1}. The lowest concentration limit afforded a reasonably measurable GC-peak without excessive tailing. The highest concentration limit was chosen to produce a peak height of about 80% of the recorder scale. No change in attenuation was made during the runs in order to keep the baseline stable all the time. In the case of the DMiPS ether derivative, six concentrations covering the range 0.1–1.0 μg μl^{-1} were prepared. The concentration of the i.s. was kept constant (1 μg μl^{-1}) with all steroid concentrations. Accordingly, the absolute concentration of methyltestosterone in μg μl^{-1} was used to replace the weight ratios in the graph (Fig. 2) and in the respective calculations.

The observed increased sensitivity of the detection of DMiPS ether which was about 17% more than the DMES ether (Fig. 2) may be attributed to the increased carbon content and to the decrease in polarity of the bulky isopropyl group [16]. The decreased polarity minimizes the interaction of the methoxime silyl ether with the GC stationary phase and reduces losses due to possible irreversible adsorption [17]. Moreover, bulky groups in-

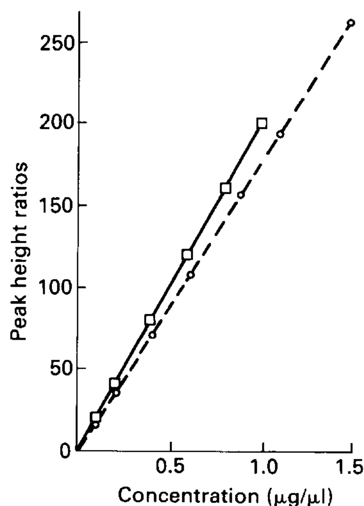

Figure 2
Calibration graph, regression equations and correlation coefficients of methyltestosterone methoxime by GC (6 ft column, 1% OV-1 and 230°C) using norethandrolone i.s. (1 μg μl^{-1}). ——— DMiPS ether. $Y = -0.007055 + 2.038493C$, $r = 0.999931$. – – – – DMES ether. $Y = 0.009889 + 1.746908C$, $r = 0.999919$.

Table 1
Assay results of methyltestosterone in tablets by GC (6 ft column, 1% OV-1 and 230°C)

Labelled methyltestosterone content/tablet	Amount injected μg μl^{-1}	Peak height ratios* and per cent recoveries						Average amount found per tablet	Mean per cent recovery ± RSD
Glosso-sterandryl tablets	0.5	0.88	0.871	0.889	0.89	0.876	0.893	10.000	99.98 ± 1.011
Memphis, Egypt 10 mg†		99.62	98.59	100.65	100.76	99.16	101.11		
Glosso-sterandryl tablets	0.5	0.882	0.855	0.886	0.878	0.892	0.90	24.960	99.86 ± 1.77
Memphis, Egypt 25 mg†		99.85	96.75	100.30	99.39	100.99	101.91		
Glosso-sterandryl tablets	0.5	1.0	1.05	1.02	1.027	1.011	1.001	10.060	100.58 ± 1.84
Memphis, Egypt 10 mg‡		98.80	103.71	100.77	101.45	99.88	98.90		
Glosso-sterandryl tablets	0.5	1.022	1.02	1.031	1.016	1.0	1.021	25.150	100.43 ± 0.821
Memphis, Egypt 25 mg‡		100.96	100.77	100.84	100.37	98.80	100.86		

* Using norethandrolone i.s. (1 μg μl^{-1}).
† Assayed as MO–DMES ether.
‡ Assayed as MO–DMiPS ether.

crease the thermal stability of hydroxy steroids for the GC analysis.

As shown from the correlation coefficient values quoted in Fig. 2, both derivatives have proved to be useful for the quantitative GC determination of methyltestosterone. The regression equations and the correlation coefficients quoted in the legend to Fig. 2 are calculated from the corresponding peak height ratios shown on the same figure. These equations were used to calculate the per cent recoveries of steroid in tablets (Table 1).

The method is adequately sensitive and only 1/200 of one tablet extract is required for analysis. Commonly used tablet excipients, fillers and diluents were found not to interfere with the proposed procedure.

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References

[1] M. Axelson, G. Schumacher and J. Sjövall, *J. Chromatogr. Sci.* **12**, 535–543 (1974).
[2] M. Donike and J. Zimmerman, *J. Chromatogr.* **202**, 483–492 (1980).
[3] M. Bertrand, R. Masse and R. Dugal, *Farm-Tijdschr. Belg.* **55**, 95–101 (1978).
[4] I. Bjorkhem, H. Ek and O. Lantto, *J. Chromatogr.* **232**, 154–160 (1982).
[5] H.J. Stan and B. Abraham, *J. Chromatogr.* **195**, 231–236 (1980).
[6] R. Verbeke, *J. Chromatogr.* **177**, 69–74 (1979).
[7] H. Miyazaki, M. Ishibashi, M. Itoh and T. Nombara, *Biomed. Mass Spectrom.* **4**, 23–35 (1977).
[8] H. Miyazaki, M. Ishibashi and K. Yamashita, *Biomed. Mass Spectrom.* **6**, 57–62 (1979).
[9] L.G.M.Th. Tuinstra, W.A. Traad, H.J. Keukens and R.J. Van Mazik, *J. Chromatogr.* **279**, 533–542 (1983).
[10] E. Houghton and P. Teale, *Biomed. Mass Spectrom.* **8**, 358–361 (1981).
[11] E. Stahl, in *Thin Layer Chromatography*, 2nd Edn, p. 339. Springer-Verlag, Berlin (1969).
[12] J.P. Thenot and E.C. Horning, *Anal. Lett.* **5**, 21–33 (1972).
[13] S.S. Toubar, Ph.D. Thesis, Mansoura University, Egypt (1988).
[14] H. Miyazaki, M. Ishibashi, K. Yamashita and Y. Nishikawa, *Biomed. Mass. Spectrom.* **8**, 521–526 (1981).
[15] L.H. Sommer, in *Stereochemistry Mechanism and Silicon*, p. 138. McGraw Hill, New York (1965).
[16] D.J. Harvey, *J. Chromatogr.* **147**, 291–298 (1978).
[17] M.A. Quilliam and J.B. Westmore, *Anal. Chem.* **50**, 59–68 (1978).

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