J. Pharm. Pharmacol. 1998, 50 (Supplement): 85

The use of a mixed derivative (TMS-TBDMS) for the identification of anabolic steroids using gc-ms

EDEMMA E. UDOH, ANTHONY C. MOFFAT AND ROGER D. JEE

Centre for Pharmaceutical Analysis, The School of Pharmacy, University of London, 29-39 Brunswick Square, London WC1N 1AX

There are about 7000 steroid preparations marketed world wide and the problems associated with steroid analysis and the issues surrounding anabolic steroid abuse are well documented. Regulatory bodies and governments have legislated for the control of anabolic steroids. Numerous derivatisation reagents and procedures have been developed for the analysis of these agents. TMS ethers and TMS enol ethers are generally the derivatives of choice for the screening of anabolic steroid abuse in sports using GC-MS (Donike & Zimmermann 1980). These methods are not without problems, some of which may be overcome by the use of TMS-TBDMS derivatives (Udoh et al 1997). This paper describes the use of a combined derivative (TMS-TBDMS) for the analysis of 10 commonly abused anabolic steroids using GC-MS (MID).

Samples were analysed using a HP5890 GC fitted with a capillary column (12 m x 0.22 mm) and automatic sampler. The mass selective detector (HP5970) was used in the electron impact mode. The column was programmed (45 °C to 290 °C, at 50 °C/min. analysis time 20 min). Each anabolic steroid standard (A, boldenone; B, bolasterone; C, epitestosterone; D, 11β-hydroxytestosterone; E, methandrostenolone; F, nandrolone; G, oxymesterone; H, stanolone; I, testosterone) was evaporated to dryness under nitrogen and 50 µl of TMS added and heated at 65 °C for 30 min. The solution was evaporated to dryness under nitrogen and 50 µl of TBDMS : TMIS: DTE (1000:3:1) mixture added. The solution was mixed and heated at 70 °C for 3hrs. This was then evaporated to dryness and reconstituted in 50 µl pyridine and 1 µl injected into the GC-MS.

Selective silylation of all the hydroxyl groups to form TMS ethers with a mass increment of 72 each, followed by enolization of the ketone groups to form TBDMS enol ethers with a mass increment of 114 each was observed. The total mass added therefore depended on the number of hydroxyl or ketone groups on the steroid and thus provided evidence of their type and number. TBDMS derivatisation stabilised the enol groups and, together with the TMS groups, gave characteristic ions in the mass spectrum for identification. In most cases the molecular ion was in high abundance - often above 60%. Other specific ions included M-15, M-57, and M-114. Table 1 gives the major ions in descending order and detection limits (signal / noise ratio of 3) of the 9 anabolic steroid as analysed by GC-MS (MID).

Table 1

S	M_r	x	y	RT	Significant ions	Lt
A	472	1	1	7.44	366, 367, 368, 282, 414, 415, 423, 208, 408, 350, 180, 264, 262, 149	5
B	502	1	1	9.25	502, 75, 503, 147, 445, 389, 504, 133, 390, 369, 180, 107, 196, 295	5
C	474	1	1	7.81	474, 475,476, 460, 75, 253, 461, 459, 251, 477, 91, 105,250, 179	1
D	562	2	1	10.5	500, 75, 560, 213,365, 81, 147, 133, 149, 501, 173, 99, 442, 249	4
E	486	1	1	8.35	309, 193, 147, 191, 489, 149, 486, 179, 192, 248, 341, 326, 487, 471	
F	460	1	1	7.85	460, 461, 462, 239, 445, 329, 236, 224, 403, 237, 404, 143, 446, 128	3
G	576	2	1	9.42	147, 576, 453, 577, 207, 221, 295, 578, 454, 124, 219, 429, 340, 281	1
H	476	1	1	7.91	419, 476, 75, 128, 420, 477, 185, 129, 421, 127, 105, 81, 93, 478, 461	1
I	474	1	1	8.05	474, 475, 415, 416, 459, 417, 430, 251, 418, 250, 431, 401, 456, 238,	0.5

S = Anabolic steroid; M_r = relative molecular mass; x = no. of TMS groups; y = no. of TBDMS groups; RT = retention time in minutes; Lt = detection limit in pg.

The proposed method gives good detection limits (≤5 pg) and can be conveniently used for the identification of synthetic and endogenous anabolic steroids.

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Udoh, E. E., Moffat, A. C., Jee, R. D .(1997) J. Pharm Pharmacol. 49, Suppl 4: 92