Journal of Chromatography, 564 (1991) 385–391
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 5715

Comparison of two mass-selective detectors with special reference to the analysis of residues of anabolics

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ABSTRACT

In the analysis of residues of anabolic agents the use of combined techniques, especially gas chromatography–mass spectrometry, is of increasing interest. When this method is used for the analysis of residues of anabolics it is very important to have a knowledge of the characteristics of the different types of apparatus on the market and to be sure that the results are not "apparatus-dependent". In this study two mass-selective detectors, the Hewlett-Packard 5970 and the ion trap detector 800, were compared by injecting the same samples of derivatized anabolics (standard solutions) into the two systems.

INTRODUCTION

The use of steroids as growth promotores in the fattening of animals is prohibited in all EEC member states. In Belgium the control on the illicit application of anabolics is carried out by the Institute of Veterinary Inspection (IVK-IEV) according to the EEC directives [1,2]. For the analysis of residues of anabolics, routine high-performance thin-layer chromatographic (HPTLC) methods have been described [3–11]. Nowadays, the use of combined techniques, especially gas chromatography–mass spectrometry (GC–MS), for the analysis of residues of anabolic agents is of increasing interest, because of the need to increase the qualitative accuracy of the analysis: to obtain a more unequivocal determination of the identity of the residue [12–14].

The use of GC–MS in routine analysis of anabolics is hampered for two main reasons: (1) the cost of GC–MS apparatus (purchase and maintenance) is high; (2) there is no routine "multi-residue" GC–MS method available (a GC–MS method by which most anabolics are detected in one run and which has been generally accepted and successfully tested by several laboratories).

However, so-called "affordable" GC–MS apparatus has recently appeared on the market (price of purchase *ca.* $2–3 \cdot 10^6$ BEF or $6–9 \cdot 10^4$ US $. This equivalent

0378-4347/91/$03.50

is mostly sold in the form of detectors (*e.g.* mass-selective detector or ion trap detector). In the near future it can be foreseen that such detectors will be affordable for routine analysis on the condition that enough samples have to be screened for a large enough number of analytes [15].

According to Belgian law, a so-called "positive" result on one sample, obtained in one laboratory, can be challenged by the owner of the animal. A second analysis in a second independent laboratory should be carried out. In different laboratories different GC–MS systems will be in use. When GC–MS is used for the analysis of residues of anabolics it is very important to have a knowledge of the characteristics of the different systems on the market. It is very important to be sure that GC–MS results are not "apparatus-dependent".

In this study two mass-selective detectors, the Hewlett-Packard 5970 (HP 5970) and the ion trap detector 800 (ITD 800) were compared by injecting the same samples of derivatized anabolics (standard solutions) into the two systems.

EXPERIMENTAL

Apparatus

Hewlett-Packard system. An HP 5970 (Hewlett-Packard, Palo Alto, CA, U.S.A.) mass-selective detector was coupled to a Hewlett-Packard Model 5890 gas chromatograph with a 7673 split–splitless autoinjector. The transfer line temperature was set at 260°C. The column used was an Ultra 1 (Hewlett-Packard) (25 m × 0.2 mm I.D., film thickness 0.11 μm). The GC parameters used were: injection temperature, 130°C; initial time, 2 min; temperature increase, 8°C/min to 250°C; platform time, 7 min; temperature increase, 30°C/min to 300°C; platform time, 4 min. Helium was used as carrier gas at a flow-rate of 1 ml/min. The detector was used in the selected-ion monitoring (SIM) mode (dwell time 100 ms).

Finnigan system. A Finnigan ITD 800 (Finnigan Matt, San Jose, CA, U.S.A.) ion trap mass spectrometer was coupled to a Varian 3700 gas chromatograph, through a flexible heated transferline (280°C), equipped with a J & W "cold on-column" injector (J & W, Folsom, CA, U.S.A.). The column used was a J & W DB-5 (30 m × 0.25 mm I.D., film thickness 0.25 μm). The column was coupled directly to the ion trap (without using the open split interface). The GC parameters used were: injection temperature, 180°C; temperature increase, 10°C/min to 300°C; 10 min hold at 300°C. Helium was used as carrier gas at a flow-rate of 1 ml/min. The acquisition parameters of the detector were: full scan range, 130–500 a.m.u.; AGC (automatic gain control) on background mass, 130 a.m.u.; B-sensitivity setting, 7000; emission current, 10 μA (with beam restrictor removed).

Reagents and reference compounds

The reference steroids nortestosterone (NORT), methyltestosterone (MET) and ethinylestradiol (EE2) were obtained from Steraloids (Wilton, NH, U.S.A.). N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) and trimethylchlorosilane

(TMCS) were obtained from Pierce (Rockford, IL, U.S.A.). All other reagents were reagent-grade products from E. Merck (Darmstadt, Germany).

Derivatized steroid solutions

From a stock solution of 1 mg/ml, derivatized standard solutions of three important anabolic agents (NORT, MET and EE2) were obtained by conversion of 20 μl into trimethylsilyl (TMS) derivatives using BSTFA and TMCS in pyridine according to the RIKILT standard operating procedure [16]. The standard solutions (20 μl = 2000 ng) were evaporated to dryness under a mild nitrogen stream at 30°C. The derivatization reagent was prepared by mixing 0.5 ml of BSTFA, 50 μl of TMCS and 4.5 ml of pyridine (dried over potassium hydroxide), and 100 μl of the reagent were added to the standards. The mixture was allowed to stand for 1 h at 60°C. After cooling, the vials were evaporated to dryness at 30°C. A mixture of iso-octane–decane (4:1) (200 μl) was added to the derivatized standards) resulting in a concentration of 10 ng/μl. This standard solution was divided into two portions of 100 μl. Appropriate dilutions of the stock solution were used for preparing standard solutions varying from 1 to 500 pg/μl).

RESULTS AND DISCUSSION

Comparison of spectra

In the full-scan mode mass spectra of relative large amounts of NORT, MET and EE2 (10 ng) were obtained with both systems. The results are shown in Fig. 1.

With both systems the same diagnostic ions [16] were found. However, the ratios of these ions to the base peak differed between the two: an example is given in Table I.

For NORT it should be mentioned that an *m/z* value of 418 was found with the ITD 800 but not with the HP 5970. This *m/z* value corresponds to the di-TMS derivative of NORT. The reason for this phenomenon was not found. Possibly partial disilylation occurs in the time between the injections into the two systems, or within the cold on-column injector.

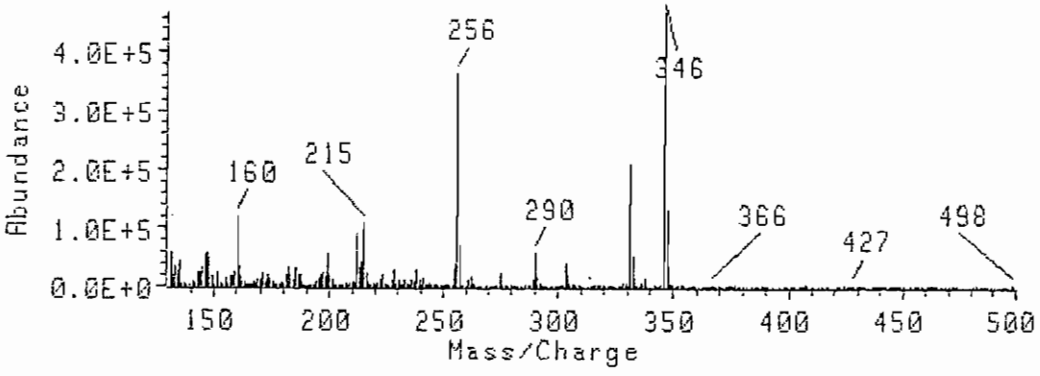
Limits of detection

For EE2, the performance of both systems at lower concentrations was tested. Both were used under their standard operating procedures: the HP 5970 was operated in the SIM mode for the four diagnostic ions 440, 425, 300 and 285. The ITD 800 was tested in the full-scan mode in the range 130–500 a.m.u. A concentration range of 1–500 pg was tested by the injection of 1 μl of the same samples of EE2 into both systems.

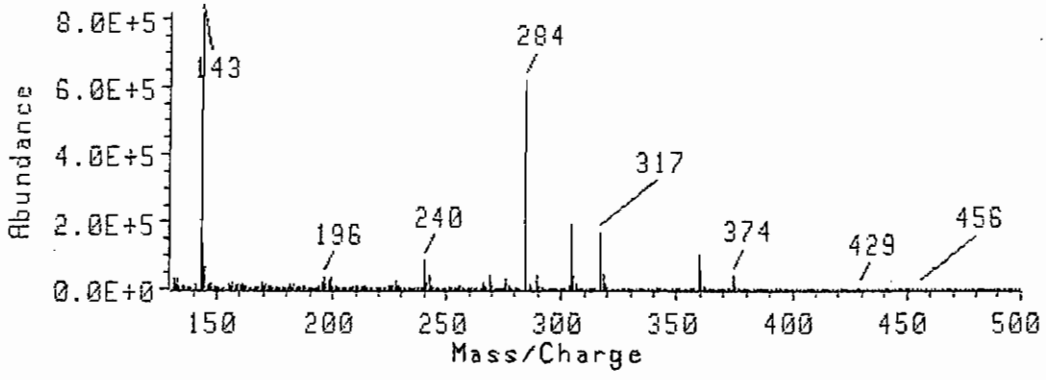
With the HP 5970, the criteria for low-resolution MS are fulfilled at least down to 30 pg (relative intensities of the four diagnostic ions within ±10%).

With the ITD 800, full-scan spectra of EE2 could be obtained down to at least 30 pg: the variations of the relative intensities of the three major ions (285, 300

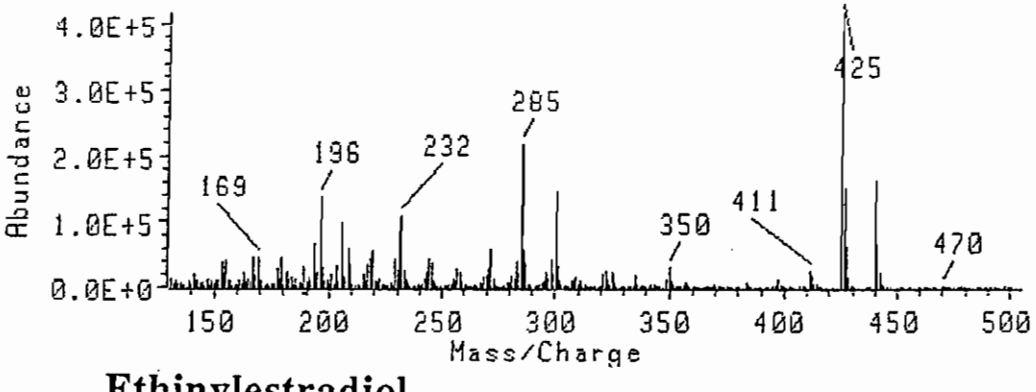
A **SPECTRA on the HP 5970**



Nortestosterone



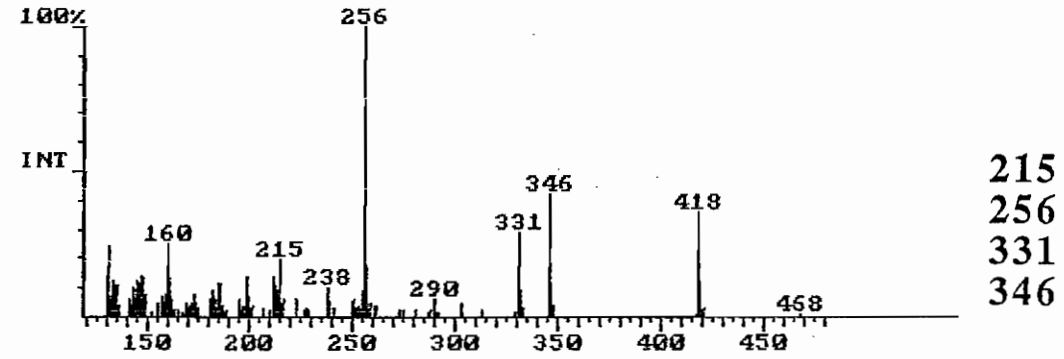
Methyltestosterone



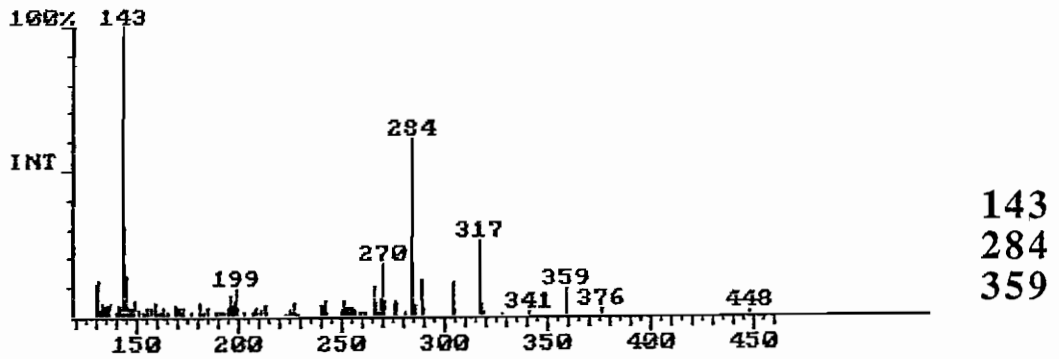
Ethinylestradiol

Fig. 1.

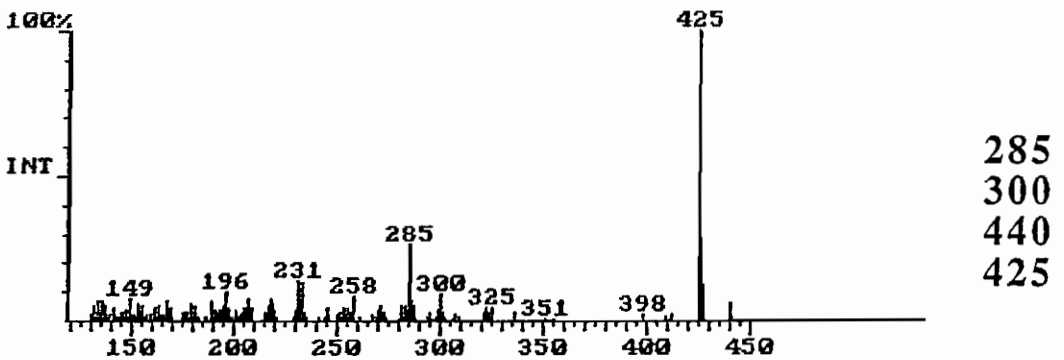
B SPECTRA on the ITD 800



Nortestosterone



Methyltestosterone



Ethinylestradiol

Fig. 1. Comparison of the spectra of large (10 ng) amounts of three anabolics (nortestosterone, methyltestosterone, ethinylestradiol). (A) Obtained with the HP 5970; (B) obtained with the ITD 800.

and 425) are within ± 10% (EEC criteria). The real detection limit of the ITD 800 will strongly depend on the instrument settings (emission current, multiplier setting, etc.). Also, the chemical background in the trap formed by bleeding of the column is of very great importance. In the present experiment the beam restrictor

TABLE I

RATIOS OF THE IONS TO THE BASE-PEAK

Ion (m/z)	Ratio to base peak	
	ITD 800	HP 5970
NORT (to 346)		
215	73	28
256	300	79
331	80	49
MET (to 143)		
284	61	5
359	11	12
EE2 (to 425)		
285	27	54
300	9	35
440	7	39

was removed and all settings were tuned for optimum sensitivity (and allowable loss of resolution).

CONCLUSION

The sensitivity of both systems if sufficient to meet EEC criteria: detection of residues at the level of 2 ppb. This level is reached by injection of the equivalent of 25 mg of matrix. However, it is possible that different quality criteria should be used for the two systems. The criteria based on the relative intensities of four (three) diagnostic ions [being within $\pm 10\%$ (or $x\%$)] are set for SIM apparatus and are thus suitable for this type of apparatus. For sull-scan apparatus, such as the ITD 800, other criteria could be more valuable because the whole spectrum is available. One of the possible criteria is the degree of fit between a "sample" spectrum and a "standard" spectrum calculated by the computer during a library search. For example, a fit of 80% indicates that two spectra are very alike (100% being a perfect fit). The difference between those two criteria (and eventually other types) should be tested in the future.

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