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Use of ion trap gas chromatography–tandem mass spectrometry for detection and confirmation of anabolic substances at trace levels in doping analysis

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Abstract

A procedure for detecting and confirming 23 anabolic substances and/or metabolites has been developed using a GC–MS–MS ion trap system in full-scan mode. The process used to select the precursor ion, and the optimization of the system parameters used to obtain the daughter ion spectra, are explained. Urine samples were prepared using solid-phase extraction and enzymatic hydrolysis, and after TMS derivatives had been formed, they were injected into the mass spectrometer. This method permits confirmation of the presence of anabolic substances at low ng ml^{-1} levels without the need of further purification procedures on the samples. This procedure has been used on more than 2000 urine samples collected from sporting competitions and has made it possible to confirm more than 45 true positive cases which could not have been confirmed using routine GC–MS methods. © 1997 Elsevier Science B.V.

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1. Introduction

Androgenic steroids and β_2-agonists with anabolic effect are the pharmacological agents most frequently used as doping substances in sport. The International Olympic Committee and other organisations responsible for anti-doping control have banned these substances because of their effects on the performance of the athletes and consequently on the results of competitions, and above all because of their adverse effects on the athletes' health [1].

Different analytical methodologies have been reported for detecting anabolic steroids and their metabolites [2,3]. Most of them are based on mass spectrometric detection. Currently, gas chromatography–mass spectrometry (GC–MS) is the most frequently used technique to confirm and quantify these compounds [4,5].

The biological fluid used in sports doping control is urine. This matrix contains a great variety of components, many of which are unknown, in different concentrations which are co-extracted to some extent with sample preparation procedures and introduced, together with the anabolic substances, into the GC–MS system. This results in interferences coeluting with analytes and in high background noise conditions, giving rise to false signals in mass

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spectra and therefore to possible erroneous interpretations. The use of high-resolution mass spectrometry (HRMS) has been proposed [6,7] to solve these problems and lower the detection limits of anabolic agents in urine samples. Furthermore, several procedures have been thoroughly described which use a combination of HRMS with ion affinity chromatography (IAC) [8] or with high-performance liquid chromatography (HPLC) [9] to eliminate interferences due to the urine matrix, and thus attain lower limits of detection and more reliable confirmation of the presence or absence of anabolic agents. Comparative studies of the efficiency of analysing anabolic agents using different alternative techniques [10] like HRMS and tandem mass spectrometry have also been carried out and have achieved similar results. Bowers and Borts [11] carried out research in the field of anabolic agents using ion trap tandem mass spectrometry and have reported on the use of their technique in the selected ion storage (SIS) mode for improving detection limits.

This paper presents a method for detecting and confirming 23 substances and/or metabolites with anabolic effects using an ion trap GC–MS–MS system in full scan mode. It also describes the optimization of those parameters which regulate secondary fragmentation. This technique has been used to detect and confirm anabolic steroids in urine samples of athletes in sporting competitions with excellent results.

2. Experimental

2.1. Reagents and chemicals

The following anabolic substances: 7α,17α-dimethyl-5β-androstane-3α,17β-diol (bolasterone metabolite), 5β-androst-1-en-17β-ol-3-one (boldenone metabolite), 6β-hydroxy-4-chloro-dehydromethyltestosterone (4-chlorodehydromethyltestosterone metabolite), 4-chloro-androst-4-en-3α-ol-17-one (clostebol metabolite), 2α-methyl-5α-androstan-3α-ol-17-one (drostanolone metabolite), 9α-fluoro-17α-methyl-androst-4-ene-3α,6β,11β,17β-tetrol (fluoxymesterone metabolite), 1α-methyl-5α-androstan-3α-ol-17-one (mesterolone metabolite), 17β-methyl-5β-androst-1-ene-3α,17α-diol (metandienone metabolite), 1-methylene-5α-androstan-3α-ol-17-one (metenolone metabolite), 17α-methyl-5α-androstane-3α,17β-diol and 17α-methyl-5β-androstane-3α,17β-diol (methyltestosterone metabolites), 5α-estran-3α-ol-17-one and 5β-estran-3αol-17-one (nandrolone metabolites), 17α-ethyl-5β-estrane-3α,17β-diol (norethandrolone metabolite), 17-epioxandrolone (oxandrolone metabolite), 3′-hydroxystanozolol and 16β-hydroxystanozolol (stanozolol metabolites) were donated by Dr Schänzer from the Institute of Biochemistry, German Sports University, Cologne, Germany. Clenbuterol, 17β-hydroxy-17α-methylandrost-4-en-3-one (methyltestosterone) as an internal standard (I.S.) and furazabol were obtained from Sigma (St. Louis, MO, USA). All reagents were of analytical-reagent grade. β-Glucuronidase from *Escherichia coli* was obtained from Boerhringer (Mannheim, Germany), Serdolit AD-2, dithyoerytritol and MSTFA(*N*-methyl-*N*-trimethylsilyl-trifluoroacetamide) from Serva (Heidelberg, Germany) and NH_4I from Merck (Darmstadt, Germany).

2.2. Stock solution and internal standard solution

Stock solutions were prepared in methanol at a concentration of 1000 ng ml^{-1}. These solutions were further diluted to yield appropriate working solutions for the preparation of the calibration standard. The solutions were sealed and refrigerated at 4°C until use. Methyltestosterone was used as an internal standard (I.S.), dissolved in methanol at 1000 ng ml^{-1} and diluted to 10 ng ml^{-1}.

2.3. Instrumentation

A Saturn 4D GC–MS–MS System (Varian Associates, Walnut Creet, CA, USA) coupled with a Varian 3400 gas chromatograph equipped with a 8200 CX autosampler and a Model 1078 programmable temperature split/splitless injector was used to carry out all the experiments. The whole system was controlled by standard Varian Software (Version 5.2), MS–MS conditions operating CID non-resonant and CID resonant excitation form were developed using Ion Trap MS–MS ToolKit software for Windows (Version 1.0). The instrumental conditions are shown in Table 1.

Table 1
Instrumental conditions

Gas chromatograph	
Column:	HP Ultra-1 (Crosslinked Methyl Silicone Gun) 25 m×0.2 mm, 0.11 μm film
Flow rate:	1 ml min^{-1}
Oven program:	120°C hold 1.6 s, 50°C min^{-1} until 200°C at 2°C min^{-1} until 245°C 245°C at 25°C min^{-1} until 300°C, hold 5 min
Injector temperature:	280°C
Transfer line temperature:	300°C
Injection mode:	Splitless, hold 1 min
Injection volume:	2 μl
Mass spectrometer (MS)	
Mass range:	130–600
Scan rate:	2 scan s^{-1}
Background mass:	45 m/z
Ion trap temperature:	220°C
Axial modulation:	4 V
RF level:	100 m/z
Filament:	60 μA
Multiplier:	Autotune + 100 V
AGC target:	Autotune
Mass spectrometer (MS–MS)	
Filament:	90 μA
Multiplier:	Autotune + 200 V
AGC target:	5000

2.4. Sample preparation

The sample was prepared according to the procedure described by Schänzer and Donike [5]. Briefly, 250 μl I.S. solution was added to 2 ml of urine. The urine was passed through a Pasteur pipette (230×7 mm) which contained 20 mm Serdolit AD-2 resin. The resin was washed with 2 ml of de-ionised water and eluted with 2 ml of methanol. After evaporation of the solvent, the residue was dissolved in 1 ml of a 0.2 *M* sodium phosphate buffer, pH 7 and 25 μl of β-glucoronidase from *Escherichia coli* were added. The mixture was incubated for 1 h at 55°C. The hydrolysate was cooled to room temperature and 250 μl of potassium carbonate solution (pH 9–10) were added. The mixture was extracted with 5 ml of diethyl ether on a mechanical shaker for 5 min. The organic phase was centrifuged for 5 min at approximately 300 *g* and dried under vacuum. The dried residue was derivatized with 50 μl of MSTFA–NH_4I–dithioerythritol (1000:2:4, v/w/w) for 30 min at 60°C.

3. Results and discussion

The mass spectra of the majority of the compounds revealed differences between the relative abundances of mass peaks obtained by ion trap MS and those obtained by quadrupole MS: fragments of greater m/z were generally more abundant in the ion trap spectrometer than in the quadrupole. Furthermore, in some cases the proportions of these fragments were changed due to the time the ions remained in the trap, with the most unstable ions becoming the most stable as a result of the geometry of the molecules. The experimental conditions used for the first fragmentation in the ion trap spectrometer are shown in Table 1.

The parent ion for each anabolic substance was

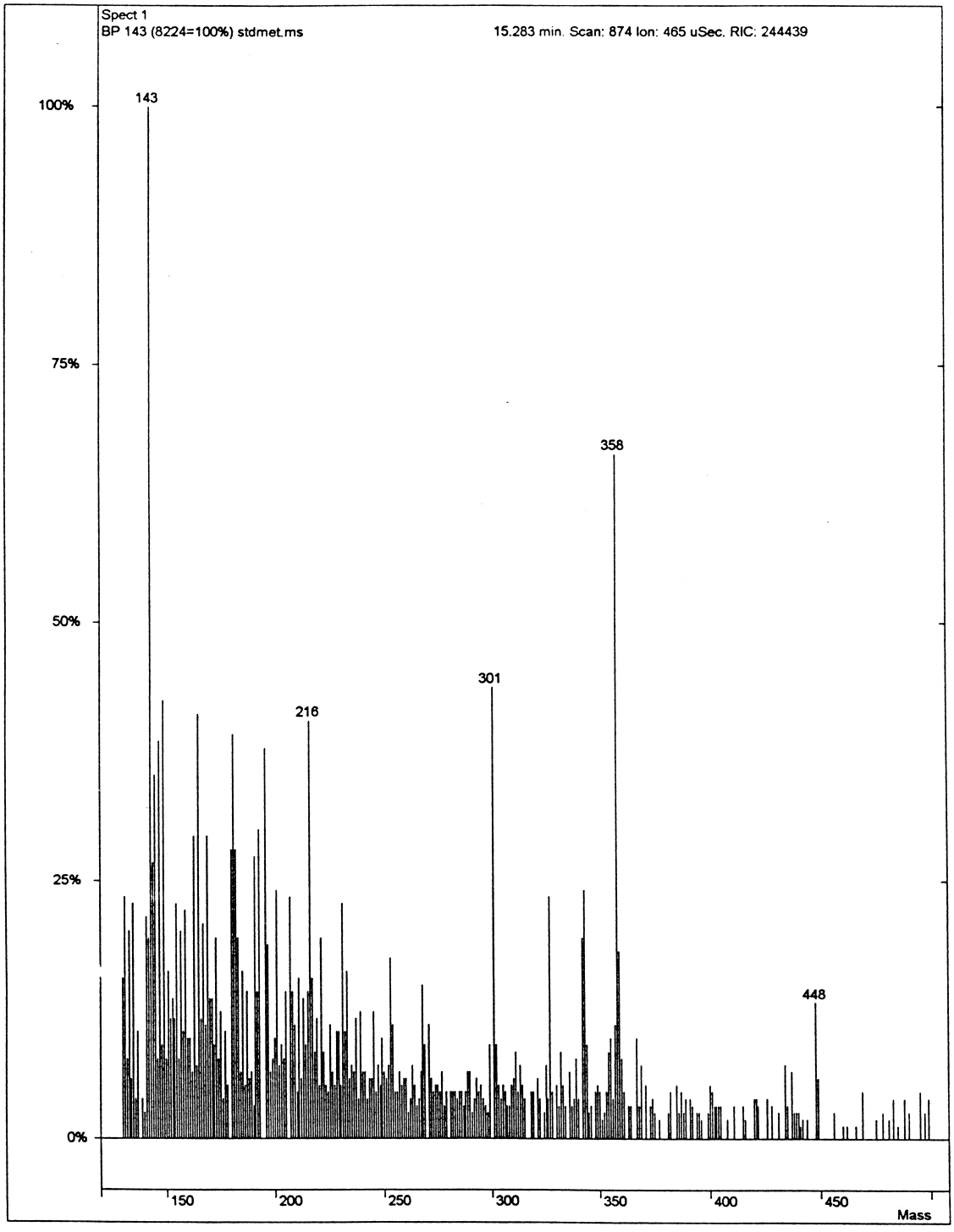

Fig. 1. Mass spectra of TMS derivative of epimethendiol.

chosen with the aim of obtaining the greatest possible selectivity. Thus parent ions were chosen that were not present in the background and did not commonly interfere. Moreover, in order to make confirmation possible with structurally informative spectra, ions with the highest possible m/z ratio were selected. Finally, another important criterion for selecting the parent ion was that the ion chosen should have a more intense ionic current to permit greater sensitivity. Fig. 1 shows the electronic impact mass spectrum of epimethendiol, a methandinone metabolite. In this case the m/z 358 ion was chosen as the precursor ion.

Fragmentation of the precursor ion was performed by collision-induced dissociation (CID) with helium molecules, the carrier gas which fills the ion trap. CID fragmentation can be performed using non-resonant or resonant excitation mode. Resonant excitation mode produced lesser fragmentation, greater concentration of ionic current, possibly greater sensitivity, but yielded less structural information and was rather more complex to optimize. On the other hand, non-resonant excitation produced greater fragmentation and was easier to optimize. Once the excitation mode has been selected, the main parameters determining the fragmentation behaviour of an ion are: excitation time, dissociation energy and the storage radio frequency, the last two parameters being the ones which have to be carefully optimized to ensure optimum performance of the technique.

Using software it was possible to change the dissociation conditions of the precursor ion, scan by scan, during a single analysis, thus permitting a rapid optimization of the fragmentation conditions. The fragmentation parameters were programmed into an ion preparation method (IPM). Each analysis was divided into segments, and within each segment a different IPM was used, so that in each analysis segment optimal dissociation conditions existed for one or more ions. It is also possible to keep the specific fragmentation conditions of up to ten ions in each IPM, although to achieve the highest possible degree of selectivity it is advisable not to include more than three or four ions in each segment. Fragmentation studies were performed to ascertain the CID behaviour of each precursor ion. Fig. 2 shows the dissociation behaviour of the m/z ion 358 of epimethendiol. The Y axis shows the variations of

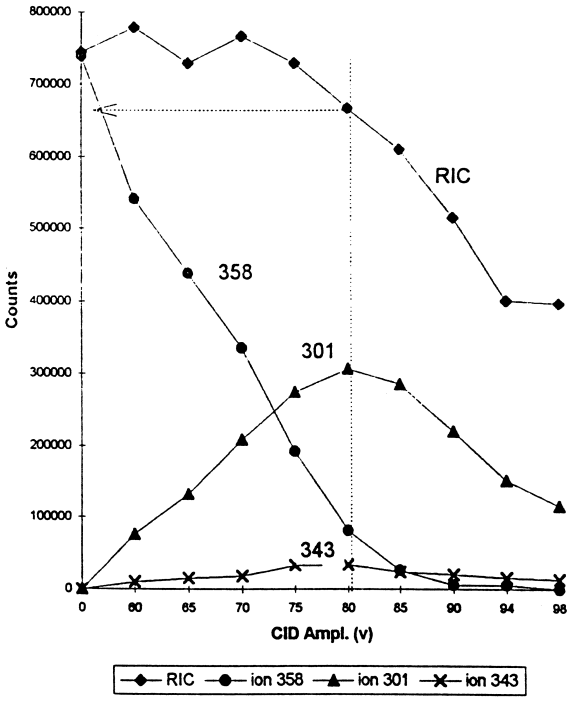

Fig. 2. Ion intensities as a function of CID voltage for precursor ion and product ions.

the reconstructed ionic current (RIC), and those of the m/z 358 precursor ion and the m/z 301 and 343 product ions, while the X axis shows the increase in voltage, while radio frequency was fixed at 130 m/z. Initially, when zero volts were applied, there was no fragmentation, thus permitting measurement of the initial ionic current of the precursor ion, which coincided with reconstructed ionic current. When voltage increased, m/z ion 358 began to dissociate into its product ions, which gradually gained ionic intensity. The voltage selected was that which made it possible to obtain a spectrum with a highly abundant base peak, that of m/z ion 301, a minimal initial ionic current loss, and the presence of representative fragments m/z ions 343 and 358.

Having carried out a similar study of dissociation behaviour for each of the precursor ions of each anabolic compound included in the screening, Table 2 shows substance, main excreted metabolites, retention time, parent ion, CID conditions and main daughter ions with their abundance. In all cases, a non-resonant excitation mode was used except for

Table 2

Substances with anabolic effect	Main excreted substance: parent or metabolite	t_R min	Parent Ion m/z	CID parameters			Main daughter ions m/z (abundance %)
				Amplitude (V)	RF m/z	Form	
Bolasterone	Bolasterone PC	22.3	445	55	100	NR	225(100%)/355(95%)/265(30%)/445(10%)
	7α,17α-Dimethyl-5β-androstane-3α,17β-diol	24.49	374	65	180	NR	284(100%)/374(92%)/269(60%)/214(32%)/247(30%)
Boldenone	Boldenone PC	16.05	430	85	140	NR	430(100%)/325(58%)/415(20%)
	5β-Androst-1-en-17β-ol-3-one	21.20	432	78	160	NR	417(100%)/432(65%)/19430%)/327(12%)/342(10%)
4-Chlorodehydro-methyltestosterone	6β-Hydroxy-4-chloro-dehydromethyl-testosterone	29.52	315	82	115	NR	227(100%)/241(80%)/315(65%)/279(15%)
Clenbuterol	Clenbuterol PC	9.24	300	85	110	NR	284(100%)/300(40%)/210(35%)/285(15%)/226(5%)
Clostebol	4-Chloro-androst-4-en-3α-ol-17-one	22.39	451	80	130	NR	325(100%)/451(90%)/361(70%)/235(50%)/271(40%)
Drostanolone	2α-Methyl-5α-androstan-3α-ol-17-one	18.69	433	65	100	NR	253(100%)/343(75%)/181(42%)/171(32%)/271(40%)
Fluoxymesterone	9α-Fluoro-17α-methyl-androst-4-ene-3α,6β,11β,17β-tetrol	26.86	552	80	150	NR	462(100%)/319(55%)/407(32%)/447(20%)/552(18%)
Furazabol	Furazabol PC	28.06	387	73	130	NR	297(100%)/387(57%)/161(8%)
Mesterolone	1α-Methyl-5α-androstan-3α-ol-17-one	20.11	433	62	100	NR	253(100%)/261(63%)/343(55%)/171(42%)/433(42%)
Metandienone	17β-Methyl-5β-androst-1-ene-3α,17α-diol	16.06	358	80	130	NR	301(100%)/358(22%)/343(12%)/357(8%)/268(8%)
Metenolone	Metenolone PC	22.61	446	62	105	NR	208(100%)/193(70%)/446(40&)/177(12%)/341(10%)
	1-Methylene-5α-androstan-3α-ol-17-one	19.44	431	62	103	NR	431(100%)/446(30%)/432(20%)/341(17%)/251(12%)
Methyltestosterone	17α-Methyl-5α-androstane-3α,17β-diol	20.39	435	75	150	NR	255(100%)/435(50%)/345(12%)/213(10%)/199(5%)
	17α-Methyl-5β-androstane-3α,17β-diol	20.71	435	75	150	NR	345(100%)/255(70%)/435(60%)/269(15%)/
Nandrolone	5α-Estran-3α-ol-17-one	15.72	405	70	110	NR	225(100%)/315(45%)/155(25%)/143(18%)/169(15%)
	5β-Estran-3α-ol-17-one	16.97	405	70	110	NR	315(100%)/225(85%)/169(22%)/143(22%)155(20%)
Norethandrolone	17α-Ethyl-5β-estrane-3α,17β-diol	22.48	421	75	150	NR	331(100%)/241(60%)/421(40%)/199(5%)
Oxandrolone	17-Epioxandrolone	21.81	363	75	130	NR	273(100%)/363(98%)/213(37%)/161(30%)/227(18%)
Salbutamol	Salbutamol PC	8.8	369	63	100	NR	207(100%)/191(75%)/163(60%)/369(30%)
Stanozolol	3ᶻHydroxystanozolol	31.3	545	0.35	135	R	455(100%)/277(12%)/387(10%)/439(10%)/347(8%)
	16β-Hydroxystanozolol	33.5	560	72	140	NR	560(100%)/231(8%)/328(6%)

The CID window and the CID time were fixed and their values were 3 m/z and 20 ms respectively.
NR: CID non-resonant excitation form; R: CID resonant excitation form.

3′-hydroxystanozolol, for which, given the high stability shown by its precursor ion, the much more aggressive resonant excitation mode was used. Fig. 3 shows the first page of MS–MS screening analysis after applying it to a sample spiked with a mixture of anabolic compounds at a concentration of 10 ng ml^{-1} in urine. To control each anabolic compound, the three most abundant fragments from the available full-scan mass spectrum were chosen.

The performance of the described analytical meth-

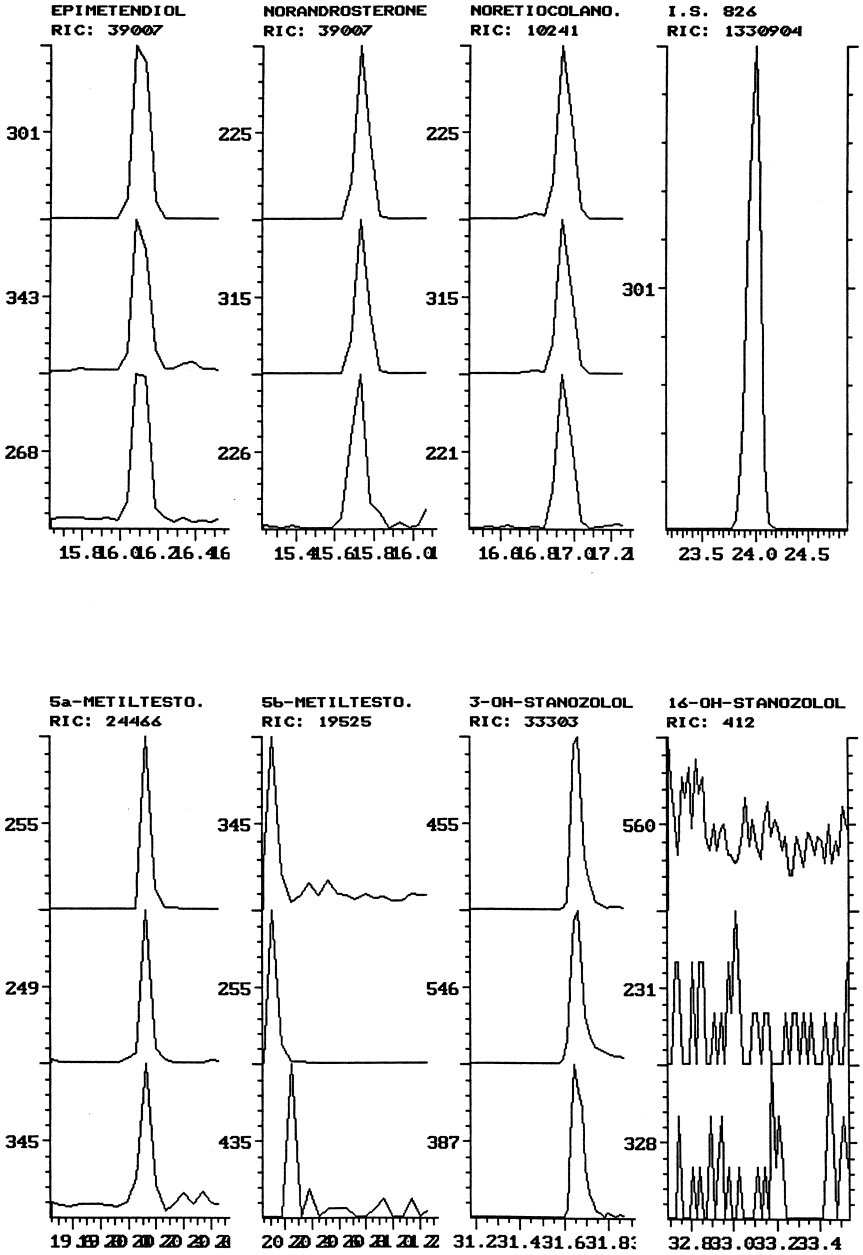

Fig. 3. First page of MS–MS screening analysis after applying it to a urine sample spiked with a mixture of anabolic agents at a concentration of 10 ng ml^{-1}.

od was evaluated on the compounds: epimethendiol, 5α-methyltestosterone, 5β-methyltestosterone and 3′-hydroxystanzolol, in a range of concentrations in urine of between 1 ng ml^{-1} and 10 ng ml^{-1}. Table 3 shows the linear regression equations for each anabolic substance and the corresponding r^2. In the case of 3′-hydroxystanozolol, the range of concentrations was from 2 ng ml^{-1} to 10 ng ml^{-1}, as it was not possible to detect 1 ng ml^{-1}. Intra-day and inter-day precision and accuracy were checked after constructing the calibration line for each anabolic substance, by preparing urine samples spiked with the above-mentioned anabolic substances in concentrations at 2 and 8 ng ml^{-1}, and calculating their experimental concentrations. In the intra-day assay the same sample was injected consecutively ten times, whilst in the inter-day assay the sample was injected once a day for two weeks. Likewise, in the inter-day and the intra-day assays the variation of the signal-to-noise ratio was studied as well as a series of spectral comparison parameters, namely: Fit, reverse fit and purity.

The purity is defined as 1000*TL*TL/(T2*L2) where:

Square of Targets Norm: T2 = sum (Tm*Tm)

(sum of all masses m)

Square of Librarys Norm: L2 = sum (Lm*Lm)

Dot product Target.Library: TL = sum (Tm*Lm)

The fit is defined in the same way, but only by taking into account masses that occur in the library spectrum (instead of all masses). The reverse fit is defined in the same manner, but taking into account masses that occur in the target spectrum. The use of these parameters to measure the spectral correlation between reference spectra (library or pure standards) and experimental spectra permits the establishment of cut-off criteria for confirmation. In our case it was shown that a value of 800/1000 ensured a spectral similarity between sample and reference which was sufficient to accurately verify the presence of the anabolic compound in the urine. Fig. 4 shows an MS–MS spectrum of a sample spiked with 1 ng ml^{-1} of 5α-methyltestosterone (upper), the MS–MS spectrum for pure standard 5α-methyltestosterone (middle), and the difference between them (lower). The upper part of the figure shows the spectral comparison parameters.

Table 3
Linear regression analysis

Substances	Equation[a]	r^2
5α-Methyltestosterone	$y = 2260.4x + 2128.4$	0.9996
5β-Methyltestosterone	$y = 2399.8x + 227.67$	0.9590
Epimethendiol	$y = 243.1x + 7.9705$	0.9913
3′-Hydroxystanozolol	$y = 237.23x + 716.5$	0.9898

[a] y = Peak area; x = concentration (ng ml^{-1}).

Table 4 shows the results of the intra-day and inter-day assays. The signal-to-noise ratio was shown to be a highly variable parameter. Thus it was decided to measure sensitivity in terms of spectral comparison parameters, given the high reproducibility shown by these parameters in comparison with the signal-to-noise ratio measure.

Table 5 shows the sensitivity study for all the anabolic compounds included in the screening. Sensitivity was measured in terms of confirmation limits and expressed as the concentration of the anabolic compound needed to permit a matching of more than 800/1000 when compared with the pure standard. With regard to the interferences, it should be remembered that the main characteristic of the MS–MS technique is its selectivity. However, a large number of endogenous compounds can be found in the urine matrix such as vitamins, proteins, salts and, above all, endogenous anabolic substances, all in much higher concentrations than those of the exogenous substances which are subject to control. For this reason if the parent ion of certain anabolic compounds is not carefully chosen, there may be a loss of selectivity and consequently sensitivity. In spite of this, if a suspect signal appears in the MS–MS screening analysis, it can be confirmed whether it is an interference or a positive result by seeking the suspect signal in the analysis and obtaining its mass spectrum, as a full scan screening is being used.

Fig. 5 shows the result of analysing a positive sample containing epimethendiol obtained from an athlete with the MS–MS screening analysis (left). Three aligned fragments made us suspect the pres-

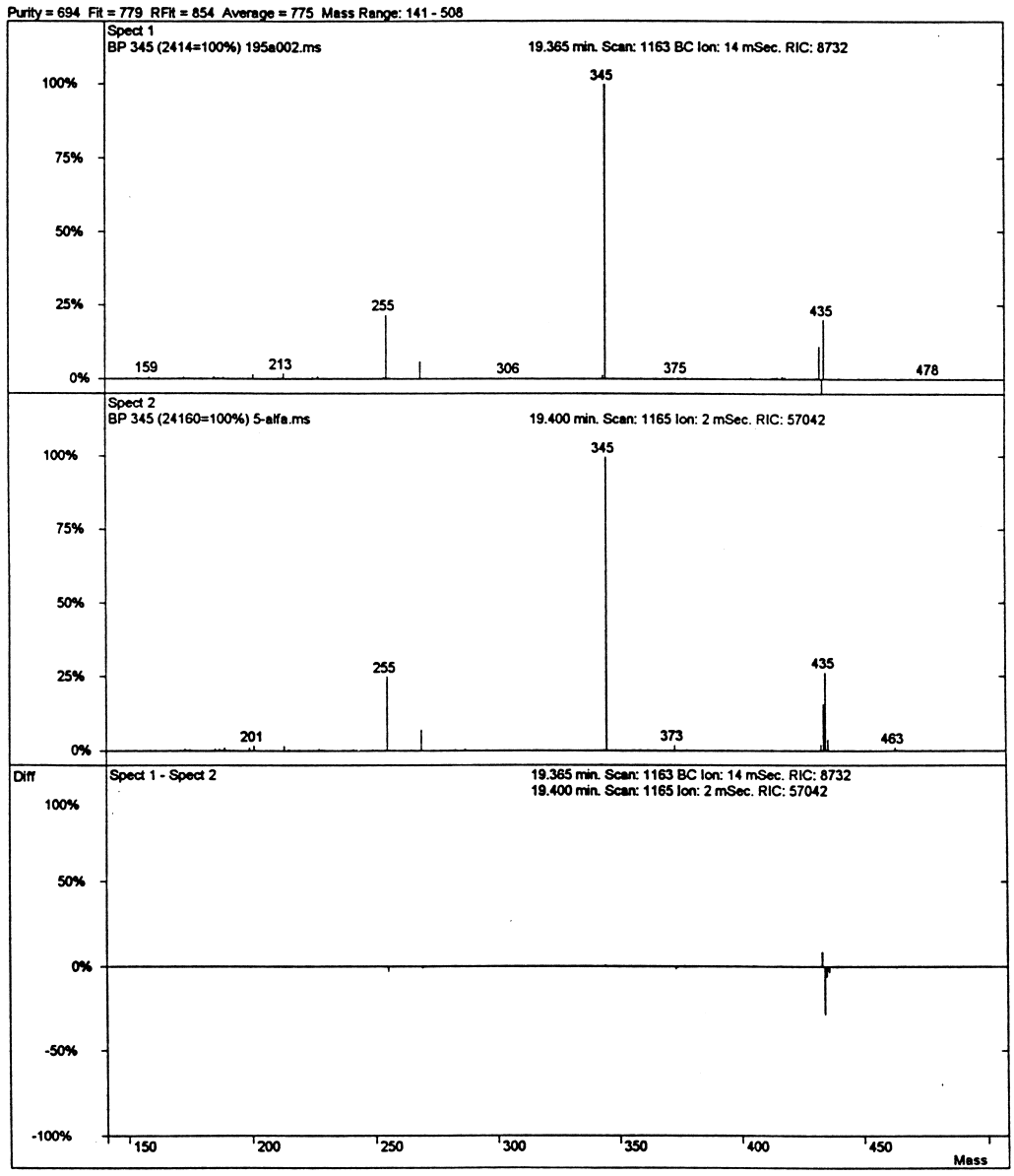

Fig. 4. Mass spectrum of TMS derivative of 5α-methyltestosterone in urine at a concentration of 2 ng ml^{-1} (upper), mass spectrum of a pure standard of TMS derivative of 5α-methyltestosterone (middle) and difference between the two mass spectra (lower).

ence of an anabolic substance, thus without needing to re-inject, the mass spectrum shown in Fig. 5 (right) was obtained, confirming the presence of the anabolic substance in the sample. Fig. 6 shows two injections of the same sample with epimethendiol, (upper) by MS–MS, m/z ion 301 and (lower) by MS, m/z ion 358. Comparison of these two signals shows how in this case the selective expulsion of the unwanted ions with the MS–MS technique enhances the signal-to-noise ratio more than twenty-fold.

Table 4
Results of the intra-day and inter-day assays

Concentration ($ng\ ml^{-1}$)	Substance	Amount found ($ng\ ml^{-1}$)		*S/N* ratio		Reverse fit		Fit		Purity	
		Mean	% R.S.D	Mean	%R.S.D.	Mean	% R.S.D.	Mean	% R.S.D.	Mean	% R.S.D.
Intra-day precision and accuracy assay (n=10)											
2	5α-Methyltestosterone	1.90	4.91	380	21.03	908	1.61	847	2.35	825	4.17
	5β-Methyltestosterone	1.99	4.07	454	20.34	841	1.68	839	2.29	814	3.35
	Epimethendiol	1.37	0.26	60	26.37	976	0.14	974	0.21	953	2.34
2.5	3Ḿydroxystanozolol	2.02	3.26	8	21.70	807	2.29	812	3.22	905	1.16
8	5α-Methyltestosterone	8.89	5.36	2011	19.12	968	0.25	929	0.21	914	0.68
	5β-Methyltestosterone	8.20	5.11	1590	25.36	962	0.40	962	0.40	925	0.67
	Epimethendiol	8.01	0.77	325	41.48	955	1.57	978	0.17	935	1.14
	3Ḿydroxystanozolol	8.34	3.25	31	15.16	950	1.17	961	0.88	906	1.53
Interday precision and accuracy assay, (n=10)											
2	5α-Methyltestosterone	1.90	9.89	18	12.13	876	5.06	891	4.36	864	4.16
	5β-Methyltestosterone	2.21	9.55	424	6.70	881	3.47	850	5.83	849	4.27
	Epimethendiol	1.20	6.73	72	15.80	971	0.97	973	0.27	967	0.62
2.5	3Ḿydroxystanozolol	2.89	5.90	6	23.00	806	3.14	817	3.90	810	2.56
8	5α-Methyltestosterone	8.19	19.32	1895	9.51	953	1.27	954	1.07	943	0.82
	5β-Methyltestosterone	8.32	6.68	1647	20.92	952	1.31	954	0.59	941	0.88
	Epimethendiol	7.53	8.74	299	12.40	974	1.13	979	0.40	970	0.73
	3′-Hydroxystanozolol	8.24	7.45	29	13.67	945	0.89	956	0.56	913	1.11

Table 5
Retention times and limits of detection for the anabolic compounds listed below

Substances with anabolic effect	Main excreted substance: parent or metabolite	t_R (min)	Approximate limit of confirmation (ng ml^{-1})
Bolasterone	Bolasterone PC	22.41	3
	7α,17α-Dimethyl-5β-androstane-3α,17β-diol	24.49	1.5
Boldenone	Boldenone PC	16.05	1
	5β-Androst-1-en-17b-ol-3-one	21.20	4
4-Chlorodehydro-methyltestosterone	6β-Hydroxy-4-chloro-dehydromethyl-testosterone	29.52	2
Clenbuterol	Clenbuterol PC	9.24	1
Clostebol	4-Chloro-androst-4-en-3α-ol-17-one	22.39	2
Drostanolone	2α-Methyl-5α-androstan-3α-ol-17-one	18.69	3
Fluoxymesterone	9α-Fluoro-17α-methyl-androst-4-ene-3α,6β,11β,17β-tetrol	26.86	2
Furazabol	Furazabol PC	28.06	1
Mesterolone	1α-Methyl-5α-androstan-3α-ol-17-one	20.11	3
Metandienone	17β-Methyl-5β-androst-1-ene-3α,17α-diol	16.06	1
Metenolone	Metenolone PC	22.61	*
	1-Methylene-5α-androstan-3α-ol-17-one	19.44	1
Methyltestosterone	17α-Methyl-5α-androstane-3α,17β-diol	20.39	1
	17α-Methyl-5β-androstane-3α,17β-diol	20.71	1
Nandrolone	5α-Estran-3α-ol-17-one	15.72	0.5
	5β-Estran-3α-ol-17-one	16.97	0.8
Norethandrolone	17α-Ethyl-5β-estrane-3α,17β-diol	22.48	1
Oxandrolone	17-Epioxandrolone	21.81	1
Salbutamol	Salbutamol PC	8.45	0.7
Stanozolol	3′-Hydroxystanozolol	31.74	3
	16β-Hydroxystanozolol	33.15	*

The assay was carried out with spiked urine samples ($n = 10$).
* It was not possible to calculate the LOC, since the reference compound was not available.

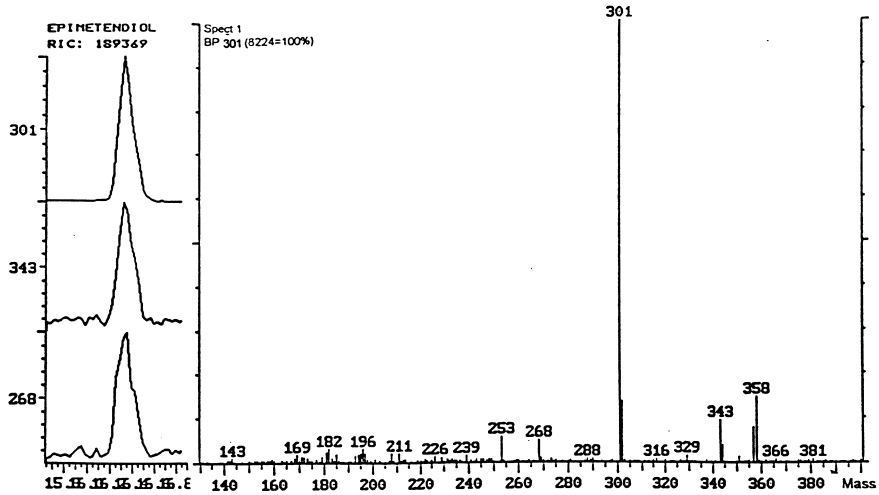

Fig. 5. MS–MS screening analysis of a positive urine sample with epimethendiol (left) and mass spectrum of epimethendiol obtained from the same sample in the same injection.

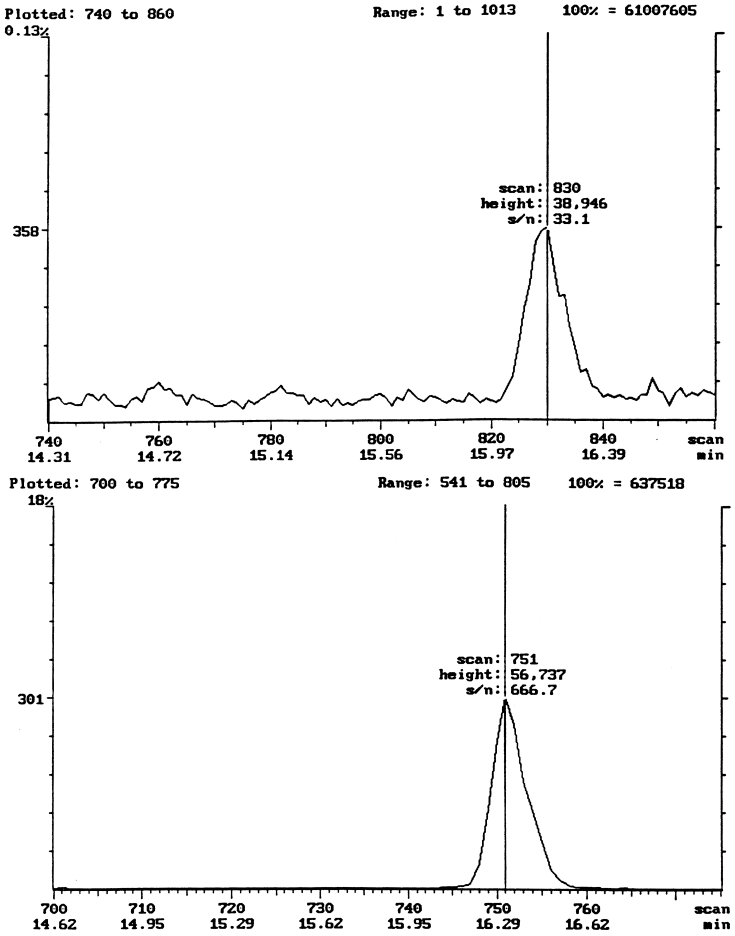

Fig. 6. MS–MS analysis of a positive urine sample with epimethendiol (upper) and MS analysis of the same sample (lower).

4. Conclusion

These results prove that the use of the GC–MS–MS ion trap system is effective in the analysis of complex matrices such as urine. This technique has been shown to be a powerful tool for the detection and confirmation of anabolic substances at low concentrations in urine samples arising from sporting competitions. The analytical method used is highly sensitive, accurate and reliable, easy to optimize, specific in confirming the anabolic substances and makes it possible to save time in routine sample analysis. Therefore GC–MS–MS is a valid alternative to the GC–MS method for analysing anabolic compounds.

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