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Analysis of Androgenic Steroids, β_2-agonists and other Substances by GC-MS-ITD

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Introduction

The Medical Commission of the International Olympic Committee bans the use of anabolic androgenic steroids and β_2-agonists to improve athletic performance [1]. Due to their slow excretion and extensive metabolization the analytical methods must be very sensitive and specific. CG-MS-MS has shown these qualities becoming an interesting option for screening and confirmation of exogenous substances in biological matrix [2]. In 1998 the IOC stated that each IOC laboratory should confirm the presence of five "key" anabolic agents (clenbuterol, epimethendiol, nandrolone-M1, methyltestosterone-M2 and 3'OH-stanozolol) down to a concentration level of at least 2 ng/mL[3]. In this work we have optimized the chromatographic and spectrometric conditions for a screening method for steroids, β_2-agonists (including clenbuterol, mabuterol, orciprenaline) and doping agents at concentration levels ranging from 2 – 15 ng/mL.

Experimental

Sample pretreatment and purification

Urine samples were processed as described by Geyer *et al.* (1998)[4] for screening analysis of anabolic substances.

GC-ITD conditions

Column:Crosslinked methyl silicone gum, 17m x 0.2mm x 0.11mm film. Flow rate: 1mL/min / Pulse pressure 25 psi (0.85 min). Injection temperature 280°C. Transfer line temperature, 280°C. Injection mode split, 1:20. Injection volume 3μL. Oven program "1": Temperature program of 140°C / 40°C/min / 180°C // 3°C/min / 230°C / 40°C/min / 300 °C (total time 21

min). Oven program "2": The chromatographic conditions were changed to 180°C followed by 15°C/min to 320°C with the aim to reduce the analysi's time. ITD condition: Target tic 20000 counts, Max ionization time 25000 ms, background mass 90 m/z, ion trap temp. 210°C, fragmentation collision-induce dissociation (CID). Other spectrometric parameters are described in table 1.

Results and discussion

The same spectrometric parameters were employed for both chromatographic conditions (Table 1). The oven program "1" allows the detection from clenbuterol as well as late eluting analytes such as the 3'OH-stanozolol. Analytes were monitored including steroids, β_2-agonists and other doping agents as THC-M1, probenecide and trianterene [5]. The fragmentation of clenbuterol was otimizated in $MS^{\wedge n}$ mode. Clenbuterol and 3'OH-stanozolol showed better fragmentation with resonant waveform. Due to the similar structure, orciprenaline, isoprenaline and terbutaline were optimized with the same CID parameters. The oven program "2" allows fast analysis of the five "key" anabolic analites at low concentration (2 ppb) with a suitable signal / noise ratio (S/N) (Figures 1, 2 and 3). The detection of the late eluting analytes for GC-MS-MS-ITD is extremely associated to the low bleeding column conditions. To this respect, additional care must be taken with 3'OH-stanozolol detection. The GC-MS-MS-ITD allows the decrease of the lower level of detection comparated with the GC-MS.

Conclusion

These results show the potential of Ion Trap GC-MS-MS in screening and confirmation of anabolic steroids, β_2-agonists and other drugs monitored in screening IV. The oven program "2" allows time saving confirmation of the five "key" anabolic agents with great sensitivity and specificity.

Acknowledgement

CNPq, FUJB, FINEP, CAPES.

References

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5.Huenerbein, A., Marques, M.A.S., Pereira, A.S., Aquino Neto, F.R. J. Chromatogr. A, 985 (2003) 375–386.

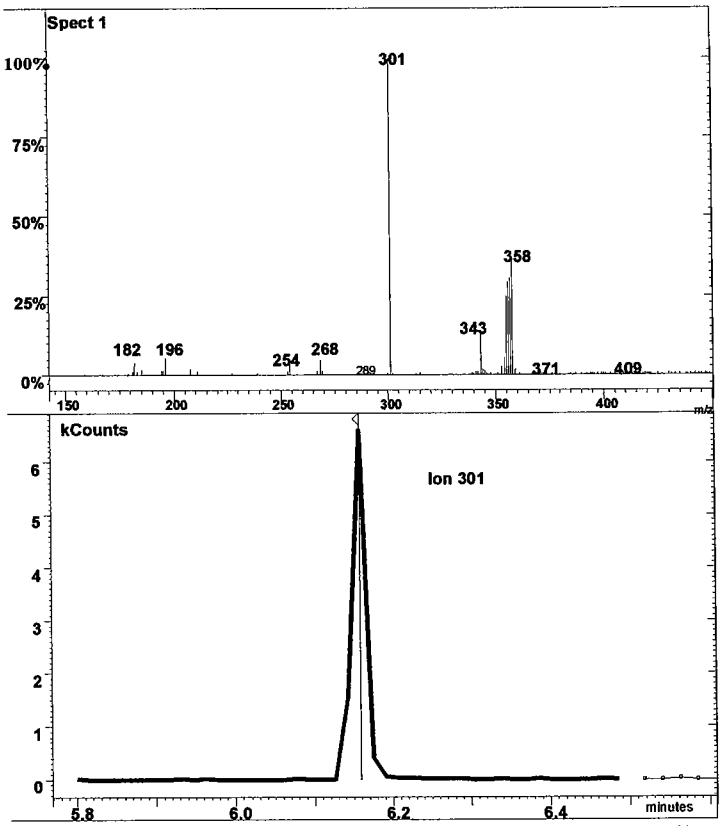

Figure 1. Mass spectra and fragmentation of epimethendiol in sample spiked at 2ng/mL from oven program "2".

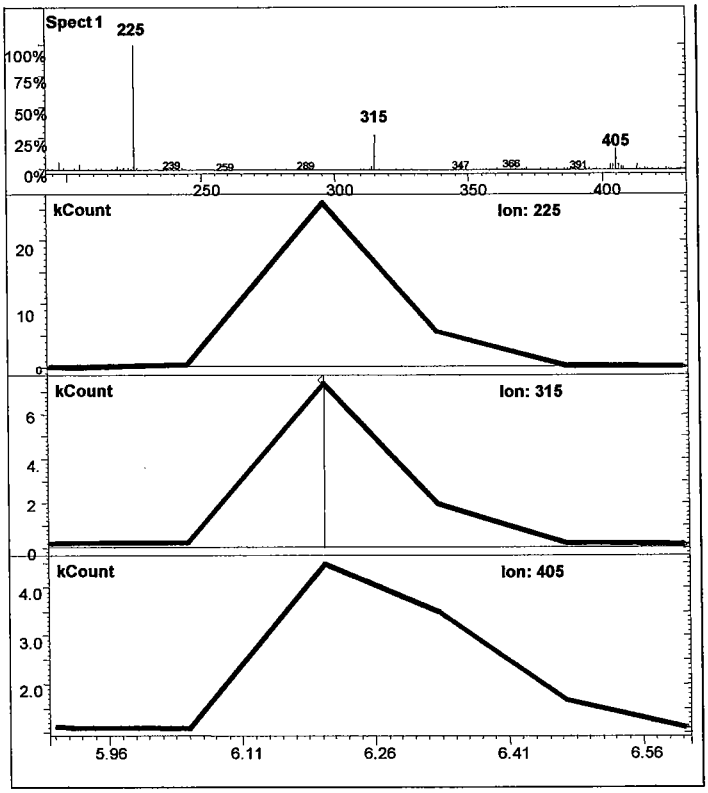

Figure 2. Mass spectra and fragmentation of nandrolone-M1 in sample spiked at 2ng/mL from oven program "2".

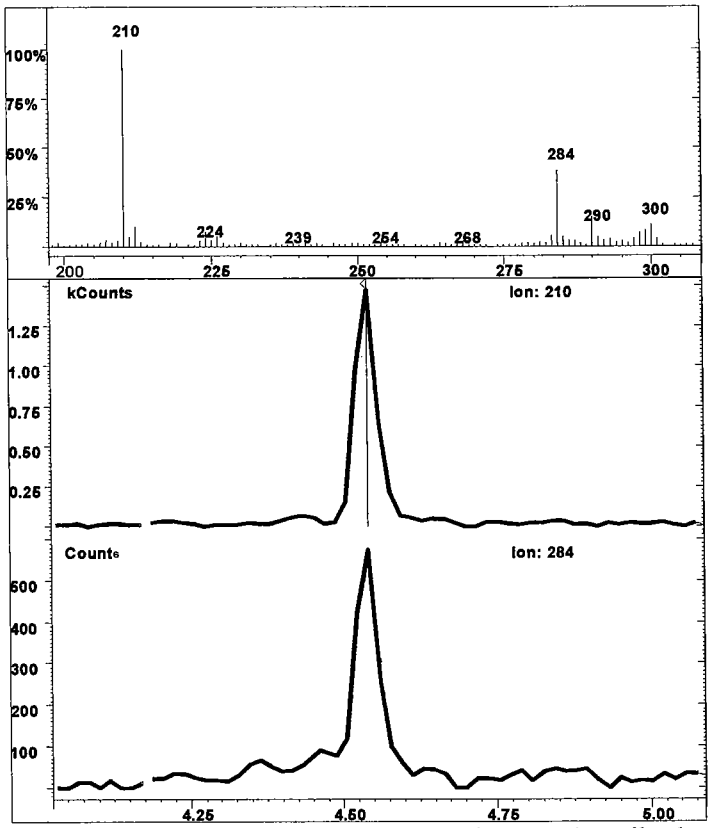

Figure 3. Mass spectra and fragmentation of clenbuterol in sample spiked at 2ng/mL from oven program "2".

Table 1. Parent ion, monitorated ions, retention time and CID parameters for the analytes.

Target Analytes	Parent Ion	Monitored Ion	t_R*** (min)	t_R**** (min)	CID parameters		
					RF (m/z)	Wave form**	Amplitude (volts)
Mabuterol	369	**334, 276, 184**	3.77	-	100	NR	60
Orciprenaline	**355**	281, 267, 193	3.94	-	100	NR	70
Isoprenaline	355	281, 267, 149	4.12	-	100	NR	70
Terbutaline	355	281, 267, 149	4.30	-	100	NR	70
Ethamivan	294	294, 278, 234	4.34	-	65	NR	45
Salbutamol	369	207, 191, 163	5.36	-	100	NR	60
Pemoline	392	392, 177, 163	5.08	-	85	NR	52
Clenbuterol	335 300	300, 284, 210	6.51	4.52	110 110	R NR	0.45 77
Brombuterol	407	351, 335, 279	9.31	-	150	NR	100
Probenecide	329	329, 301, 269	9.61	-	72	NR	34
Nandrolone-M1	405	315, 225, 155	13.00	6.26	110	NR	70
Nandrolone-M2	405	315, 225, 155	14.15	-	110	NR	70
Boldenone-M1	432	417, 342, 194	13.39	-	160	NR	68
epimethendiol	358	301, 268, 253	14.15	6.30	130	NR	70
Drostanolone-M1	433	343, 253, 181	15.53	-	100	NR	65
Metenolone-M1	431	431, 341, 251	16.16	-	103	NR	62
Fluoxymesterone-M1	552	552, 462, 486	19.79	-	150	NR	80
Fluoxymesterone-M2	462	372, 357, 337	16.57	-	102	NR	52
Methyl-M1	435	435, 345, 255	16.77	-	150	NR	65
Methyl-M2	345	345, 255	16.89	6.77	150	NR	65
Calusterone-M	374	374, 284, 269	17.92	-	180	NR	65
Bolasterone-M1	374	284, 269	18.45	-	180	NR	65
Epioxandrolone	363	273, 213, 161	18.46	-	130	NR	75
Clostebol-M1	451	415, 361, 325	18.57	-	130	NR	80
Noretandrolone-M1	421	336, 241	18.61	-	150	NR	80
Fenoterol	322	322, 305, 293	19.17	-	100	NR	65
THC-M1	473	473, 355, 297	19.27	-	82	NR	50
Methyltestosterone	446	356, 251	19.29		150	NR	80
Protokylol-P	322	322, 305, 281	19.50	-	100	NR	65
Fenoterol	322	305, 289, 281	19.17	-	100	NR	65
Oxandrolone-P	363	273, 243, 161	19.65	-	130	NR	75
Oxymesterone	534	445, 389, 355	20.30	-	150	NR	95
DHCL-M	315	241, 227	20.60	-	115	NR	80
Canrenone	412	412, 397, 383	21.25	-	91	NR	54
3'OH-stanozolol	545	546, 456	21.33	8.43	120	R	0.8

*Isolation window: 3.0 m/z. **NR: CID Non-resonant excitation form; R: CID resonant. Optimized for MS^n.*** t_R were obtained with temperature program 1. **** t_R were obtained with temperature program 2. Methyl-M1: Methyltestosterone – M1. Methyl-M2: Methyltestosterone – M2.