Fast GC/MS/MS of Androgenic Anabolic Steroids in Urine Using an Agilent J&W FactorFour VF-5ms Column

Application Note

Forensic Toxicology

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Introduction

The use of anabolic steroids is prohibited in sport, where athletes are subject to continuous screening for these banned substances. The analysis of large numbers of samples in a short time with a high degree of specificity is an important requirement for any screening program. The key factor is the use of a rapid gas chromatographic method in combination with a sensitive detector. This note describes a fast and sensitive method to screen 13 anabolic androgenic steroids within 12 minutes, based on a short VF-5ms GC column and multiple reaction monitor (MRM) detection. This method is approximately twice as fast than a classical steroids' method analysis.

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Sample Preparation

Urine (2 mL) was prepared by adding 17a-methyltestosterone as an internal standard, and the 13 compounds at concentrations of 2, 5 ng/mL. The urine sample was then buffered to pH 6 and incubated at 55 ºC for one hour after the addition of 50 µL of ß-glucuronidase. The hydrolyzed urine was passed through an SPE cartridge, which was conditioned successively with methanol and water. The column was rinsed with water, 10% methanol in water, and hexane. The steroids were then eluted with methylterbutyl ether. The eluate was evaporated to dryness and subsequently derivatized with 50 µL of MSTFA/NH4I/dithioerythritol at 60 ºC for 20 minutes.

Conditions

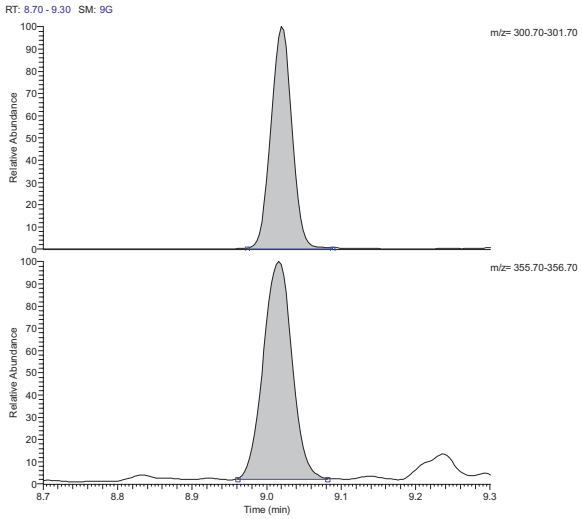
Column:	VF-5ms, 10 m x 0.15 mm x 0.15 µm (part number CP9034)
Cartridge:	BondElut C18, 200 mg
Sample Vol:	3 µL
Carrier Gas:	0.5 mL/min Helium, constant flow
Injector:	250 ºC, split ratio 1:10
Temp Gradient:	170 ºC for 0.5 min, 10 ºC/min to 260 ºC, 50 ºC/min to 320 ºC (1 min)
Detector:	Triple quadripole GC, 70 eV EI Mode, ion source 250 ºC

Results

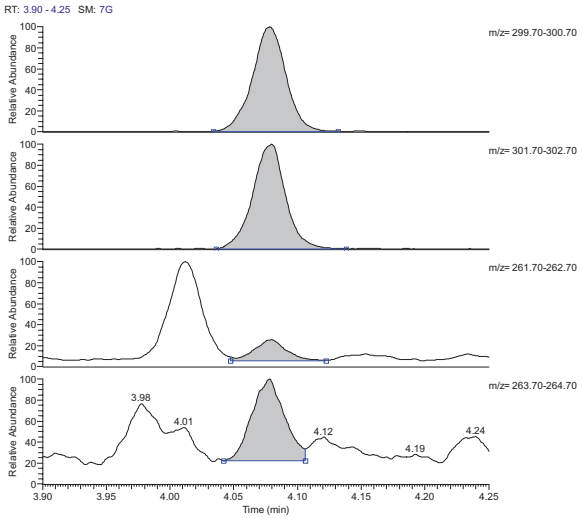
Table 1 shows the characteristics of the 13 steroids. Figure 1 shows the mass spectra obtained using the method described.

Table 1. Anabolic steroids, detection level in sample, retention time, associated precursors and daughter ions

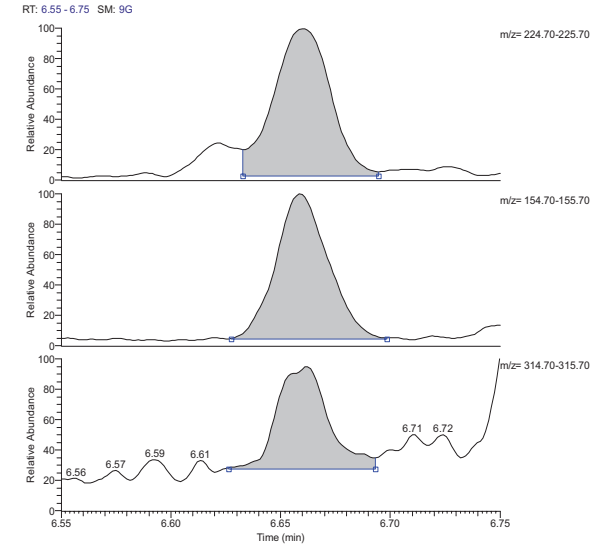
Compounds	Detection level (ng/mL)	Retention time (min)	Relative retention time	Precursor ion	Daughter ions		
17a-Methyltestosterone (ISTD)	200	9.02	-	446	301	356	
Clenbuterol	2	4.08	0.452	335	300	262	
				337	302	264	
19-Norandrosterone	2	6.66	0.738	405	225	155	315
Epimethenediol	2	6.92	0.767	358	301		
19-Noretiocholanolone	2	7.12	0.789	405	155	225	315
17-Epimethanedienone	2	8.26	0.916	444	206	339	
5a-Methyltestosterone	2	8.01	0.888	435	255	345	
5b-Methyltestostérone	2	8.07	0.895	435	255	345	
Norethandrolone metabolite	5	8.67	0.961	421	331	241	
Ethisterone	2	9.17	1.017	456	316	301	208
Bolasterone	5	9.05	1.003	460	445	355	315
Calusterone	5	9.14	1.013	460	445	355	315
6ß-Hydroxymethanedienone	2	9.74	1.080	517	229	317	281
Fluoxymesterone metabolite	5	9.33	1.034	552	495	319	
				462	337		



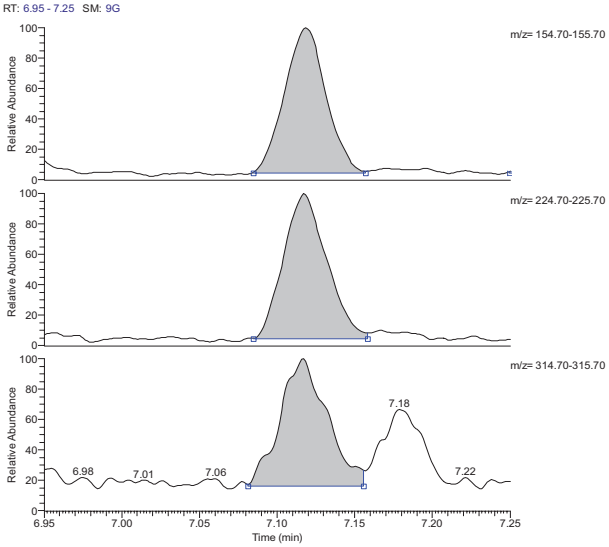
17a-Methyltestosterone (ISTD)



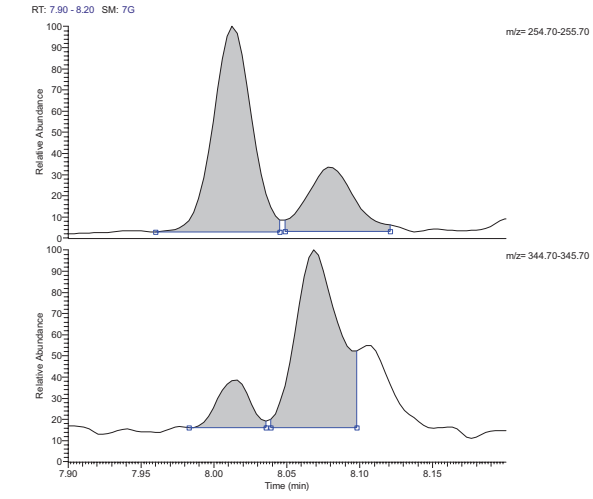
Clenbuterol



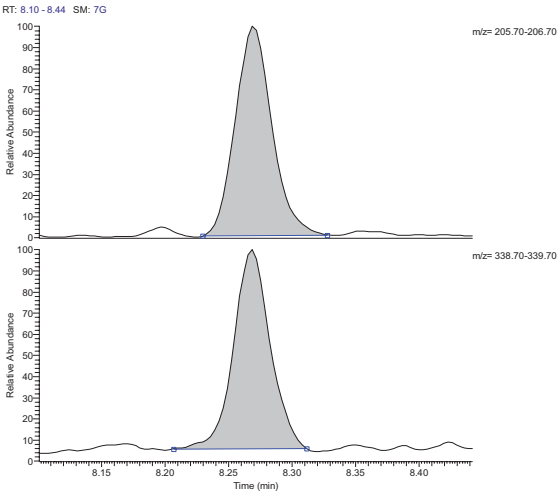
19-Norandrosterone



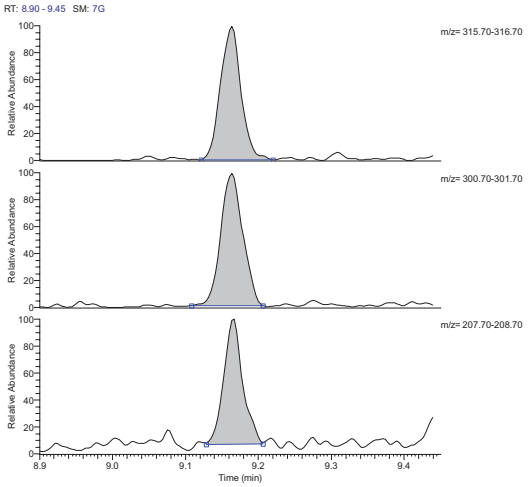
19-Noretiocholanolone



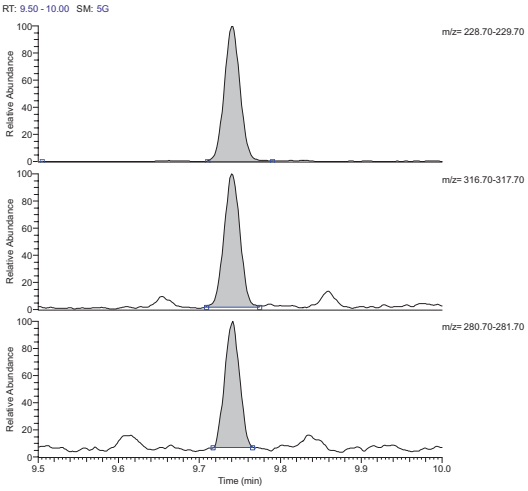
5a-Methyltestosterone, 5b-Methyltestosterone



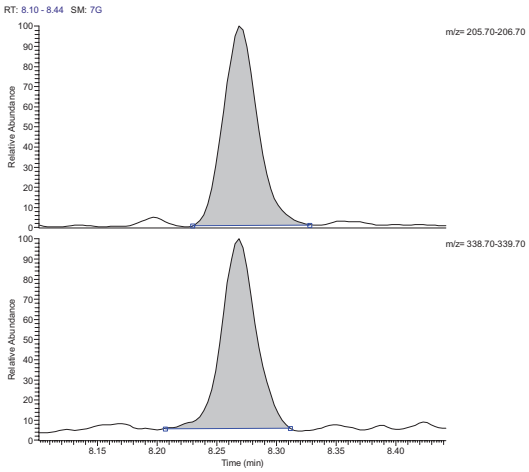
17-Epimethanedienone



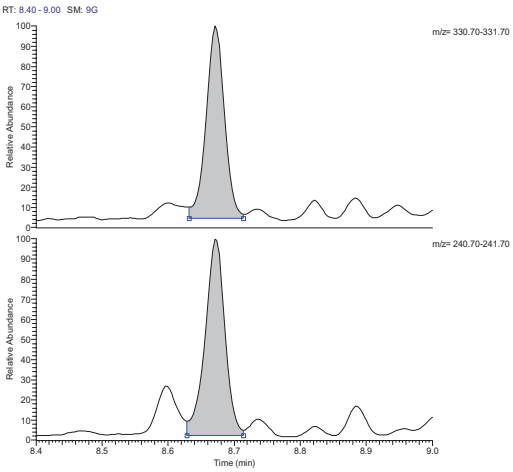
Ethisterone



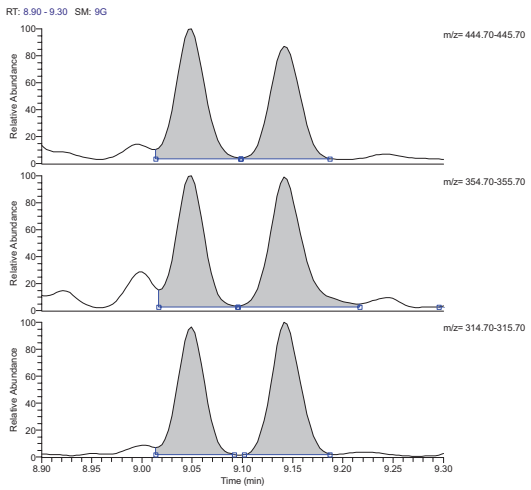
6ß-Hydroxymethanedienone



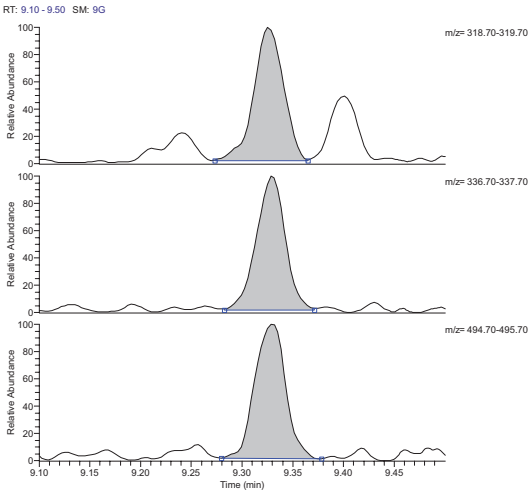
Epimethenediol



Norethandrolone metabolite



Bolasterone and Calusterone



Fluoxymesterone metabolite

Figure 1. Mass spectral information of the anabolic steroids

Conclusion

The GC/MS/MS analytical method described here detected ten anabolic steroids commonly tracked as banned substances using a VF-5ms capillary column. The method was optimized for a fast analysis speed, while maintaining important chromatographic separations of structurally related steroids that exhibited identical MRM fragmentation patterns. This approach permitted rapid detection of prohibited substances and delivered specific information on the compound detected.

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This information is subject to change without notice.

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