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Application News

No. M243A

Gas Chromatograph Mass Spectrometer

Screening Techniques in Doping Analysis by GC/MS

■ Introduction

Sports doping is not only contradictory to the concept of fair play, but it has a negative impact on the health of athletes as well as society in general. For these reasons, drug doping testing is conducted based on regulations imposed by the World Anti-Doping Agency (WADA).

Table 1 lists the sports doping screening techniques. The quadrupole GC/MS is used for analysis of difficult-to-volatilize drugs (Screening Method No.2), diuretics (No.5), and β-blocker agents (No.7).

This Application News introduces an example of the analysis of a difficult-to-volatilize drug (Screening Method No.2) obtained with the cooperation of MITSUBISHI KAGAKU BIO-CLINICAL LABORATORIES, INC., officially recognized as a WADA testing agency.

Table 1: Classification of Screening Methods in Sport Doping Analysis

Screening No.	Classification	Drug Example	Analytical Instrument
1	Volatile drugs	Amphetamine	GC-NPD
2	Difficult to volatilize drugs	Cocaine metabolites	GC/MS (Scan)
3	Thermally decomposed substances	Dexamethasone	Q-TOF LC/MS
4	Designer steroids	Testosterone	GC/MS (SIM)
	Anabolic steroids	Stanozolol	GC/HRMS (SIM)
5	Diuretics	Furosemide	GC/MS (SIM)
6	Steroid hormones	Androstenedione	GC/C/IRMS
7	β-blocker agents	Metoprolol	GC/MS (Scan)
8	Peptide hormones	EPO, hCG	EIA, immunoblotting

■ Analytical Procedures

The pretreatment flow chart and GC/MS analytical conditions for Screening Method No.2 are shown in Figure 1 and Table 2, respectively. In the pretreatment procedure, 6 M of hydrochloric acid was added to 5 mL of urine, and this was heated for 30 minutes at 105 °C to conduct hydrolysis. After washing with diethyl ether, 2-methyl-2-propanol and internal standards were added to the liquid phase, and after adjusting the pH to 9.6+/-0.1, extraction was conducted with diethyl ether. The extract was dried under a stream of nitrogen gas, and after adding methyl orange/acetonitrile/TFA solution, MSTFA was added until the solution turned yellow, after which the solution was heated for 5 minutes at 80 °C. Then, MBTFA was added, and the solution was heated for 10 minutes at 80 °C to conduct N-TFA-O-TMS derivatization.

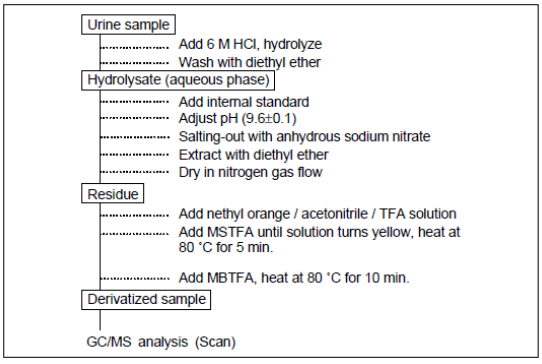

Figure 1: Pretreatment Flow for Screening Method No. 2

Table 2: Analytical Conditions

Model	GCMS-QP2010		
Workstation	GCMSsolution Ver2.5		
Column	DB-5 (15 m × 0.25 mm I.D. df=0.25 (um)		
-GC-		**-MS-**	
Inj. Temp.	280°C	**Interface Temp.**	300°C
Column Temp.	100°C (1 min)-16 °C/min-300°C (2 min)	**Ion Source Temp.**	200°C
Carrier Gas	He (Constant Linear Velocity Mode)	**Scan Range**	m/z 50-550
Linear Velocity	51.8 cm/sec	**Scan Interval**	0.5 sec
Injection Method	Split		
Split Ratio	11:1		

■ Sports Doping Test Report Format

In order to present test results in the most effective manner, the results of each analyte must be arranged in an easy-to-view format. For instance, the report must be as compact as possible, displaying chromatograms of the drug and its metabolites side-by-side for easy viewing.

GCMSsolution allows the reporting items to be pasted to the screen and freely positioned to easily generate highly effective doping test reports (Figure 2)

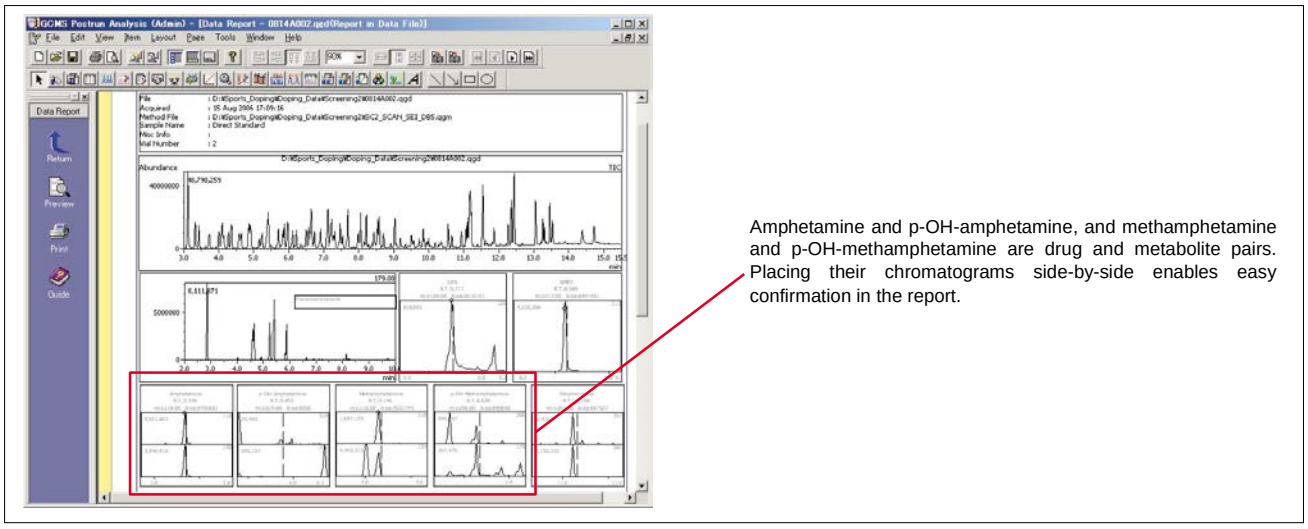

Figure 2: Report Creation Screen

To ensure data reliability, WADA requires various confirmation tests. In the case of Screening Method No. 2, a Minimum Required Performance Limit (MRPL) of 0.5 ug/mL (strychnine only, 0.2 ug/mL) is set to verify the GC/MS sensitivity.[1] In addition, analysis of a control sample, consisting of drug-free urine, and a blank sample is required to ensure the reliability of the pretreatment procedure and system blank.

Figure 3 shows these testing results in a report formatted using GCMSsolution. The chromatograms of the analyte target ions and their identifying ions are positioned one above the other, enabling convenient judgment of the presence or absence of the compound at a glance.

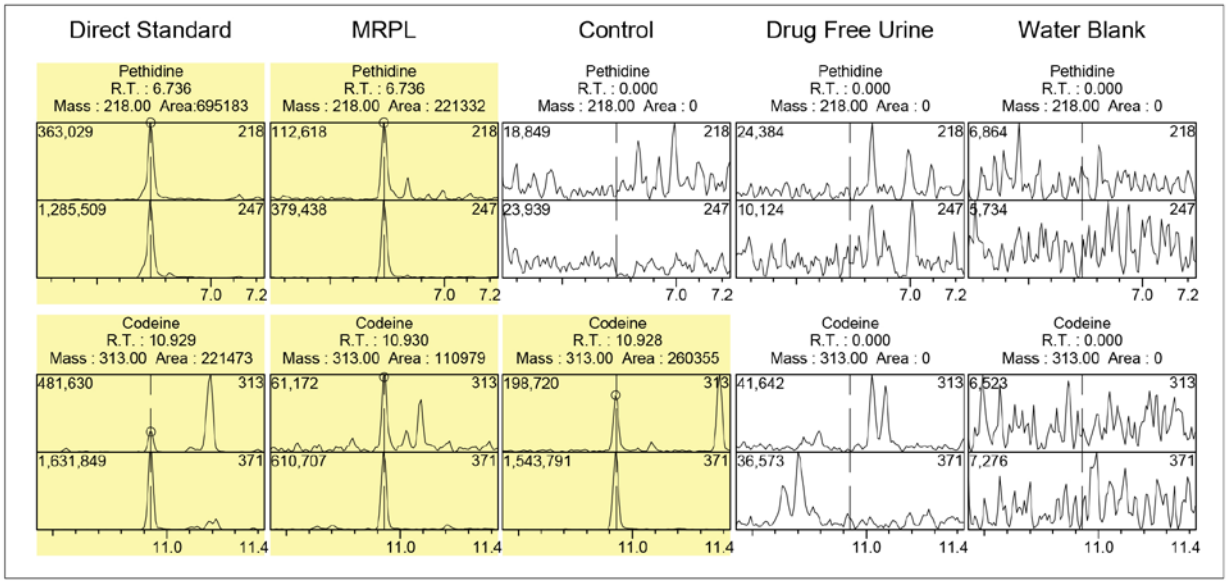

Figure 3: Example of Report Format for Sports Doping Test

Reference

1) MINIMUM REQUIRED PERFORMANCE LIMITS FOR DETECTION OF PROHIBITED SUBSTANCES - WADA Technical Document TD2004MRPL

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