

A rapid screening assay for measuring urinary androsterone and etiocholanolone $\delta^{13}\text{C}$ (‰) values by gas chromatography/combustion/isotope ratio mass spectrometry

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A gas chromatography/combustion/isotope ratio mass spectrometry (GC/C/IRMS) method is described and validated for measurement of $\delta^{13}\text{C}$ values of the acetate derivatives of urinary etiocholanolone and androsterone. The analysis was performed with only 2 mL of urine. The sample preparation consisted of deconjugation with β -glucuronidase, solid phase extraction, and derivatization with acetic anhydride and pyridine. The within-assay precision of two quality control (QC) urine samples ranged from 0.5 to 2.1 CV%. The between-assay precision in the same QC urines ranged from 1.7 to 3.4 CV%. Administration of testosterone enanthate to a subject resulted in a 6‰ decrease in $\delta^{13}\text{C}$ values from -25‰ (baseline) to -31‰. Two weeks after testosterone administration was discontinued, the $\delta^{13}\text{C}$ values remained abnormally low while the urine testosterone/epitestosterone (T/E) ratio returned to less than 6. This relatively simple method is useful for rapidly screening a large number of urine samples, including those with T/E < 6. Copyright © 2000 John Wiley & Sons, Ltd.

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Detecting doping with endogenous androgens has long been an analytical challenge because the main technique in use, gas chromatography/mass spectrometry (GC/MS), cannot distinguish pharmaceutical from endogenous androgens. For this reason, testosterone doping is detected by measuring the testosterone/epitestosterone (T/E) ratio by GC/MS in the ~100 000 urine samples collected annually by the doping authorities.¹ In less than 1% of all cases, the screen finds T/E ratios > 6. Some of these cases are T users and others have naturally, chronically elevated T/E.² The current procedure for determining which are users and which are non-users is costly and time consuming. Typically the authorities conduct a T/E versus time profile of past samples from the same athlete or obtain additional samples for T/E analysis. The authorities may commission or recommend an endocrine evaluation of the athlete. These steps are needed before a doping infraction is declared, because rare individuals will have naturally elevated T/E, and other drugs and conditions may influence the urine T/E.³

In the last six years, isotope ratio mass spectrometry (IRMS) methods have helped enormously with the complex problem of discerning androgen users from non-users. The methods capitalize on the difference in $^{13}\text{C}/^{12}\text{C}$ ratio

between pharmaceutical T and endogenous human T and, therefore, between its precursors and metabolites.^{4–8} GC/C/IRMS methods have been used successfully to detect doping with T,^{5–7} dehydroepiandrosterone (DHEA),⁸ and dihydrotestosterone (DHT).^{9,10}

While IRMS methods have proved extremely useful in detecting the administration of exogenous androgens, one disadvantage is their limited capacity to process a large number of samples in a short time. Current GC/C/IRMS methods are not optimized for rapid screening as they are labor-intensive, require large sample volumes, and often require preliminary sample clean-up by high performance liquid chromatography. This paper describes a new rapid GC/C/IRMS screening method based on determining the $\delta^{13}\text{C}$ values of the acetate derivatives of the etiocholanolone and androsterone extracted from 2 mL of urine.

EXPERIMENTAL

Urine samples

Urine samples were obtained from two healthy male subjects, age 29 and 52, from a 24-week study of the effects of T on behavior. Both subjects received weekly injections: placebo in weeks 1–13 and 20–24, and T enanthate in weeks 14–15 (150 mg), in weeks 16–17 (300 mg), and in weeks 18–19 (600 mg). One urine from one of the two subjects was selected to be the positive quality control urine (QC-Pos). Nine urines from the other subject were analyzed. The protocol was approved by the Harvard Medical School institutional review board and has been previously described.¹¹ The negative quality control urine (QC-Neg)

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