

Analysis Of Anabolic Steroids In Urine By LC/MS/MS

MICHAEL C. ZUMWALT¹, JOHN M. HUGHES¹, MATTHEW H. SLAWSON²,
CHAD R BORGES², DENNIS J. CROUCH²,

¹ Agilent Technologies, Englewood, MS, USA

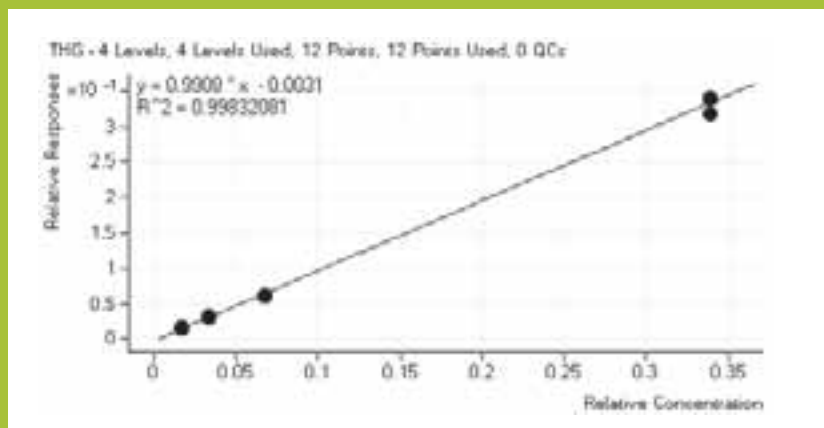
² Center for Human Toxicology, University of Utah, USA

AIMS: This work represents the use of LC/QQQ mass spectrometry for confirmation of performance-enhancing drugs in urine, targeted for sports doping control analysis. LC/MS/MS with high-performance 3.5 μ rapid resolution columns and ionization by APCI on the QQQ instrument, using MRM analysis, is expected to provide a lower-cost alternative to the current de-facto standard in international doping control, which is the EI-GC/MS high-resolution magnetic sector instrument. Additionally, increased throughput as a result of bypassing the necessary sample derivatization step, without sacrificing the sensitivity required to meet the WADA MRPLs, is also considered an advantage. Confirmation is carried out using designated quantitation ions in MRM mode. Samples are obtained from the Center for Human Toxicology (University of Utah) to generate calibration curves for quantitation. The samples are extracts of control urine spiked at specified levels.

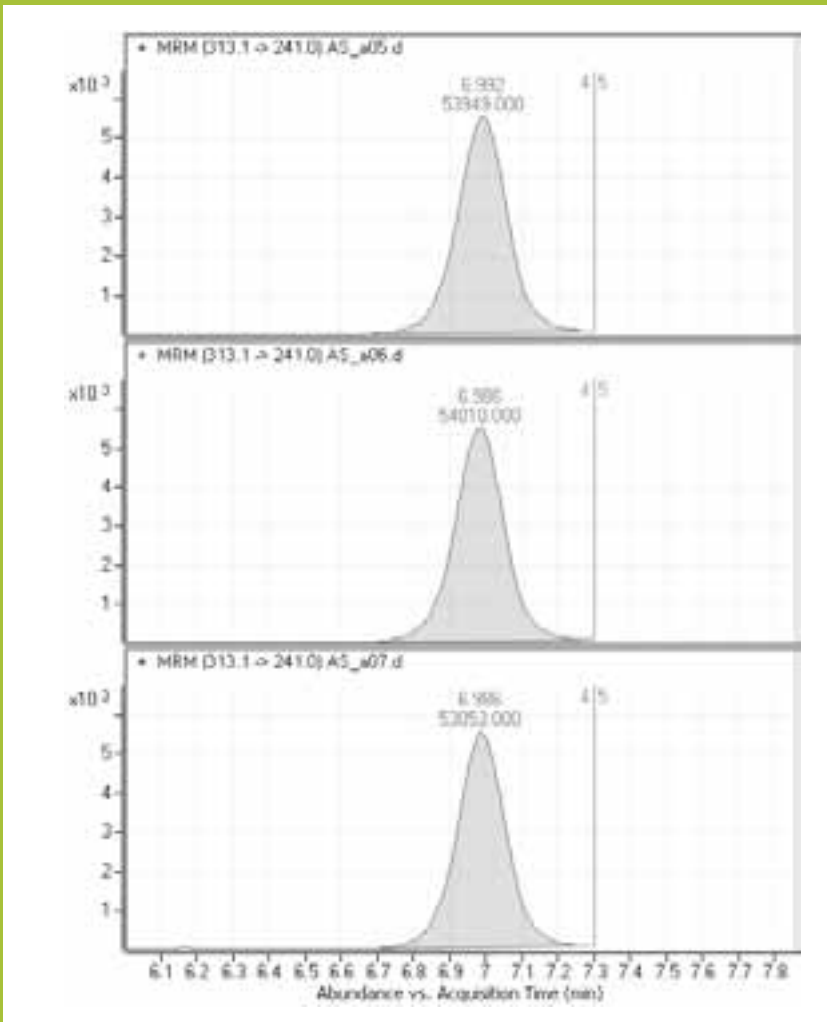
RESULTS: The sensitivity requirements of WADA for compounds such as stanzolol metabolites, 19-norandrosterone and tetrahydrogestrinone (THG) are easily met even in such a difficult matrix. Methyltestosterone and d5-eticholanolone are used as internal standards. Calibration curves are generated for extracted standards at levels of 1/2, 1, 2, and 10x MRPL levels with %RSD values calculated at the 1/2 MRPL levels to show that sensitivity is sufficient to detect limits of quantitation even below the 1/2 MRPL levels.

For example, results for THG are shown below.

Calibration curve



Repeat injections at 1/2 MRPL level



Average area = 53670
StDev = 535.7
%RSD = 0.998

KEYWORDS: Anabolic steroids, Urine, LC/MS/MS

Corresponding author: michael_zumwalt@agilent.com