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Hydrogen Isotope Ratio Mass Spectrometry and High Resolution/High Accuracy Mass Spectrometry in Metabolite Identification Studies: Detecting Target Compounds for Sports Drug Testing

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Abstract

In sports drug testing, comprehensive studies on the metabolism of therapeutic agents with misuse potential are necessary to identify metabolites that provide utmost retrospectivity and specificity. By commonly employed approaches minor and/or long-term metabolites in urine might remain undetected. Hence, an alternative strategy to unambiguously identify the majority of urinary metabolites including low abundance representatives is desirable.
Urine samples were collected for 20 days during an elimination study with an oral dose of 5 mg of 17α-C^2H_3-metandienone. The specimens were processed according to established sample preparation procedures (including fractionation and deconjugation) and subjected to gas chromatography-hydrogen isotope ratio mass spectrometry (GC/IRMS) analysis. Due to the deuteration of the administered drug, urinary metabolites bearing the deuterium label yield abundant and specific signals on the GC/IRMS instrument resulting from the substantially altered $^2H/^1H$ ratio. The sample aliquots were measured on a gas chromatography-time of flight (GC/Q-TOF) mass spectrometer using identical GC conditions, allowing high resolution/high accuracy mass data to be obtained on all urinary metabolites previously identified by IRMS.
Within the IRMS chromatograms, labeled metabolites were identified up to 20 days after administration at urinary concentration down to 0.25 ng/mL. More than 50 metabolites were observed with the earlier described long-term metabolite of metandienone, 18-nor-17β-hydroxymethyl,17α-methyl-androst-1,4,13-trien-3-one, being the most prominent glucuronidated metabolite in the studied time window. In the sulfoconjugated steroids fraction, a yet unknown metabolite was observed at m/z 283.1997 comprising the experimentally determined elemental composition of $C_{20}H_{21}{}^2H_3O$.
Combining IRMS with high-resolution mass spectrometry considerably facilitates and accelerates metabolite identification of deuterium-labeled compounds in urine. Of particular relevance in doping control, the principle is applicable also to other arenas of drug research allowing the preparation and administration of e.g. radioactively labeled substances, to be omitted.

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