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**Mass spectrometric characterization of the hypoxia-inducible factor (HIF) stabilizer drug candidate BAY 85-3934 (molidustat) and its glucuronidated metabolite BAY-348, and their implementation into routine doping controls.**

[Dib J](http://www.ncbi.nlm.nih.gov/pubmed/?term=Dib%20J%5BAuthor%5D&cauthor=true&cauthor_uid=27346747)1, [Mongongu C](http://www.ncbi.nlm.nih.gov/pubmed/?term=Mongongu%20C%5BAuthor%5D&cauthor=true&cauthor_uid=27346747)2, [Buisson C](http://www.ncbi.nlm.nih.gov/pubmed/?term=Buisson%20C%5BAuthor%5D&cauthor=true&cauthor_uid=27346747)2, [Molina A](http://www.ncbi.nlm.nih.gov/pubmed/?term=Molina%20A%5BAuthor%5D&cauthor=true&cauthor_uid=27346747)2, [Schänzer W](http://www.ncbi.nlm.nih.gov/pubmed/?term=Sch%C3%A4nzer%20W%5BAuthor%5D&cauthor=true&cauthor_uid=27346747)1, [Thuss U](http://www.ncbi.nlm.nih.gov/pubmed/?term=Thuss%20U%5BAuthor%5D&cauthor=true&cauthor_uid=27346747)3, [Thevis M](http://www.ncbi.nlm.nih.gov/pubmed/?term=Thevis%20M%5BAuthor%5D&cauthor=true&cauthor_uid=27346747)1,4.

**[Author information](http://www.ncbi.nlm.nih.gov/pubmed/27346747" \o "Open/close author information list)**

* 1Center for Preventive Doping Research/Institute of Biochemistry, German Sport University Cologne, Am Sportpark Müngersdorf 6, 50933, Cologne, Germany.
* 2Agence Française de Lutte contre le Dopage (AFLD), 143 avenue Roger Salengro, 92290, Châtenay-Malabry, France.
* 3Bayer Pharma AG, Aprather Weg, Aprather Weg, 42096, Wuppertal, Germany.
* 4European Monitoring Center for Emerging Doping Agents (EuMoCEDA), Cologne/Bonn, Germany.

**Abstract**

The development of new therapeutics potentially exhibiting performance-enhancing properties implicates the risk of their misuse by athletes in amateur and elite sports. Such drugs necessitate preventive anti-doping research for consideration in sports drug testing programmes. Hypoxia-inducible factor (HIF) stabilizers represent an emerging class of therapeutics that allows for increasing erythropoiesis in patients. BAY 85-3934 is a novel HIF stabilizer, which is currently undergoing phase-2 clinical trials. Consequently, the comprehensive characterization of BAY 85-3934 and human urinary metabolites as well as the implementation of these analytes into routine doping controls is of great importance. The mass spectrometric behaviour of the HIF stabilizer drug candidate BAY 85-3934 and a glucuronidated metabolite (BAY-348) were characterized by electrospray ionization-(tandem) mass spectrometry (ESI-MS(/MS)) and multiple-stage mass spectrometry (MSn ). Subsequently, two different laboratories established different analytical approaches (one each) enabling urine sample analyses by employing either direct urine injection or solid-phase extraction. The methods were cross-validated for the metabolite BAY-348 that is expected to represent an appropriate target analyte for human urine analysis. Two test methods allowing for the detection of BAY-348 in human urine were applied and cross-validated concerning the validation parameters specificity, linearity, lower limit of detection (LLOD; 1-5 ng/mL), ion suppression/enhancement (up to 78%), intra- and inter-day precision (3-21%), recovery (29-48%), and carryover. By means of ten spiked test urine samples sent blinded to one of the participating laboratories, the fitness-for-purpose of both assays was provided as all specimens were correctly identified applying both testing methods. As no post-administration study samples were available, analyses of authentic urine specimens remain desirable. Copyright © 2016 John Wiley & Sons, Ltd.