

## ***In vitro* human metabolism of designer cathinones: LC-MS(/MS) metabolites identification and characterization for doping control purposes**

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Stimulant agents are in the 2015 World Anti-Doping Agency (WADA) List of Prohibited Substances and Methods, among the classes of drugs banned “in competition”, due to their psychotropic effects that can lead to an enhancement of sport performance during competition. Among these compounds, designer cathinones are synthetic molecules obtained through the modification of the chemical structure of cathinone (e.g. methylone, benzedrone, nafirone). This variety of new stimulants is specifically intended to “bypass” the analytical controls and is becoming increasingly available on the market. For these reasons it is essential the constant monitoring of the emergence of any previously unknown substance, and, at the same time, the study of their pharmacokinetic properties, with the aim to develop effective analytical methods to be applied by the anti-doping laboratories for the detection of these substances.

We have specifically investigated, using *in vitro* model systems, the human phase I and phase II metabolism of designer cathinones with the aim to evaluate the most appropriate analytical markers for their intake. Incubations were performed using human tissue fractions (human microsomes, S9 fraction) and single recombinant enzyme isoforms (CYP450s, UGTs). Different substrate and enzyme concentrations, solvent ratios, pH values and incubation times were tested in order to optimize the incubation conditions. Metabolic pathways characterization and metabolites identification were carried out using LC-MS(/MS)-based techniques.

Our results show that (1) based on the results of specifically carried out validation assays, the *in vitro* protocol set up for this study is able to effectively reproduce the human phase I and II metabolism of the substances under investigation, (2) cathinone derivatives undergo extensive phase I and phase II metabolism, and the mayor metabolic pathways detected include hydroxylation, demethylation, dehydrogenation and subsequent conjugation with glucuronic acid, (3) the hydroxylated and demethylated metabolites represent the best analytical markers to detect the intake of designer cathinones.