High-resolution mass spectrometric *in vitro* and *in vivo* metabolite profiling of novel  synthetic cannabinoids, APINAC and its fluorined analogue

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**Background:** The data are reported for an *in vivo* and *in vitro* metabolism study of two novel synthetic cannabinoids, N-(1-adamantyl)-1-pentyl-1H-indazole- 3-carboxylate  (APINAC) and its fluorinated analog N-(1-adamantyl)-1-(5-fluoropentyl)-1H-indazole-3-carboxylate (5F-APINAC), which are active ingredients of smoking mixtures sold in Russia since 2016. Despite  increasing prevalence, no human metabolism data are currently available, making it challenging for forensic laboratories to confirm intake of  APINAC or 5F-APINAC.

**Methods:** The cannabinoids were incubated with human liver microsomes (HLMs). The formed metabolites were characterized by liquid chromatography – triple quadrupole mass spectrometry and high-resolution mass spectrometry with electrospray ionization in positive ion mode.

**Results:** It was found that HLMs produce mono-, di-, and trihydroxylated metabolites, as well as N-desalkyl metabolites, which can be further hydroxylated; the amide bond resisted the metabolic cleavage. For 5F-APICA, a series of oxidative defluorination products formed as well. For in vivo confirmation of the formed in vitro metabolites, spot urine samples from drug users were analyzed with the created method. It was shown that for the detection of APICA abuse, the preferred metabolites are the di- and tri-hydroxylated species, while in case of 5F-APICA, a monohydroxy metabolite is a better target. The N-despentyl (desfluoropentyl) hydroxyadamantyl metabolite also provides good retrospectivity to confirm the administra- tion of any of these cannabinoids.

**Conclusions:**  We have characterized APINAC and 5F-APINAC *in vitro* metabolism with human hepatocytes and *in vivo* metabolism using of rat urine and high- resolution mass spectrometry, and we recommend characteristic major metabolites for clinical and forensic laboratories to identify APINAC and 5F-APINAC intake and link observed adverse events to these novel synthetic cannabinoids

Introduction

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# Acknowledgements

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