## B21 Metabolism of Furanylfentanyl in Fresh Human Hepatocytes: Detection and Confirmation of Furan Ring-Opened Carboxylic Acid Metabolite

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**Learning Overview:** After attending this presentation, attendees will have gained a deeper knowledge regarding the metabolism of furanylfentanyl, an analog of fentanyl that has a furan-2-carbonyl group instead of the propionyl group found in fentanyl. Furanylfentanyl has been available in the United States and Europe since at least 2015 and has been detected in more than 18 countries.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing information on the metabolic pathways of furanylfentanyl definitively determined using authentic standards of its metabolites.

In the present study, the metabolism of furanylfentanyl was investigated using fresh human hepatocytes. Four metabolites of furanylfentanyl were definitively identified in the culture medium of hepatocytes incubated with furanylfentanyl by comparing the analytical data with chemically synthesized authentic standards.

**Methods:** The authentic standards of furanylfentanyl and its five putative metabolites (nor-furanylfentanyl, 4′-hydroxy-furanylfentanyl, β-hydroxy-furanylfentanyl, 4′-hydroxy-3′-methoxy-furanylfentanyl, and furan ring-opened carboxylic acid metabolite) were synthesized in the laboratory. Fresh human hepatocytes (seeded in a 24-well plate at $2.1 \times 10^5$ cells/cm$^2$) were incubated with 10μM furanylfentanyl at 37°C and 5% $CO_2$, and the culture medium was sampled 48h after addition of the drug. The medium was treated with β-glucuronidase/aryl sulfatase, followed by deproteinization by the addition of acetonitrile. After centrifugation, the supernatant was dried under a stream of nitrogen and reconstituted with the initial mobile phase used in liquid chromatography. Metabolites in the samples were analyzed via liquid chromatography-triple stage quadrupole mass spectrometry.

**Results:** 4′-Hydroxy-furanylfentanyl ($[M+H]^+$; *m/z* 391), β-hydroxy-furanylfentanyl (*m/z* 391), 4′-hydroxy-3′-methoxy-furanylfentanyl (*m/z* 421), and furan ring-opened carboxylic acid metabolites (*m/z* 409) were detected on the Extracted Ion Chromatograms (EICs) of their protonated molecules. Furan ring-opened carboxylic acid metabolite was considered the main metabolite of furanylfentanyl owing to its extremely strong peak intensity. The other three metabolite peaks were minimally detected in the EICs. In the case of fentanyl, the desphenethylated metabolite, nor-fentanyl, was the main metabolite found *in vivo* and *in vitro* in the previous study; however, the desphenethylated metabolite of furanylfentanyl, nor-furanylfentanyl, was not detected in the culture media at all. To date, two studies on the metabolism of furanylfentanyl have been published.[1,2] Both studies reported that the dihydrodiol metabolite ($[M+H]^+$; *m/z* 409, identical to the furan ring-opened carboxylic acid metabolite) is formed from furanylfentanyl and the presence of the furan ring-opened carboxylic acid metabolite was ruled out. However, the dihydrodiol metabolite was not identified with authentic standards in those studies. In the present study, the metabolite with a molecular weight of 408 ($[M+H]^+$; *m/z* 409) was definitively identified as a furan ring-opened carboxylic acid metabolite by comparing the analytical data with authentic standards. Several studies have reported the formation of furan ring-opened carboxylic acid metabolites. For example, loop diuretic furosemide and $\alpha_1$-blocker prazosin are known to form furan ring-opened carboxylic acid metabolites. Therefore, these facts support the results obtained in this study.

**Reference(s):**

1. Goggin M.M., Nguyen A., Janis G.C. Identification of unique metabolites of the designer opioid furanyl fentanyl. *J Anal Toxicol* 2017;41:367-75.
2. Watanabe S., Vikingsson S., Roman M., Green H., Kronstrand R., Wohlfarth A. *In vitro* and *in vivo* metabolite identification studies for the new synthetic opioids acetylfentanyl, acrylfentanyl, furanylfentanyl, and 4-fluoro-isobutyrylfentanyl. *AAPS J* 2017;19:1102-22.

**Furanylfentanyl, Metabolism, Hepatocyte**



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