



Houston Forensic Science Center

INTEROFFICE MEMO

To: Toxicology Analytical Manual v3.8

From: Dayong Lee, Ph.D., F-ABFT, Manager - Toxicology

CC: Erika Ziemak, Director - Quality
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Date: January 6, 2023

Re: Interference to the HFSC Alprazolam Confirmation Analysis by 4'-chloro-deschloroalprazolam

This memo is to explain how HFSC Toxicology section addresses the possible interference from 4'-chloro-deschloroalprazolam with its alprazolam confirmatory analysis using liquid chromatography-tandem mass spectrometry.

4'-chloro-deschloroalprazolam is an emerging designer benzodiazepine that has mass spectra extremely similar to those of alprazolam, which can potentially lead to misidentification. The first case report of seized drug capsules containing the drug was published in Australia in June 2022 (Trigg et al. Drug Test Anal 2022;14:1672-1680). 4'-chloro-deschloroalprazolam in seized drug materials was reported to National Forensic Laboratory Information System for the first time between July 1, 2022 and September 30, 2022 (https://www.nflis.deadiversion.usdoj.gov/nflisdata/docs//NFLIS_Snapshot_Sept_2022.pdf). HFSC became aware of this interference on October 31, 2022 when the outsource laboratory informed HFSC of the possible presence of 4'-chloro-deschloroalprazolam in six case samples outsourced to the laboratory. The presence could not be confirmed because the outsource laboratory did not have a method for the novel drug.

In response, HFSC Toxicology section performed an interference study in which blood samples were fortified at increasing concentrations of 4'-chloro-deschloroalprazolam covering the calibration range of alprazolam analysis along with low quality control concentrations of alprazolam. The study design and evaluation followed the exogenous interference validation procedure as described in the Analytical Manual v3.8, section 24.5.5. The data showed significant interference from 4'-chloro-deschloroalprazolam starting at 100 ng/mL with alprazolam as this interfering peak merged with the alprazolam peak. 4'-chloro-deschloroalprazolam showed the same ion transitions with ion ratios within the acceptable range established for alprazolam identification. However, the retention times of 4'-chloro-deschloroalprazolam and alprazolam are sufficiently different that the HFSC method can differentiate between the two drugs: for

all injected samples, 4'-chloro-deschloroalprazolam eluted before alprazolam-d5 (internal standard for alprazolam) whereas alprazolam eluted after alprazolam-d5. This retention time difference was further supported by additional data with different concentrations of alprazolam and 4'-chloro-deschloroalprazolam. The data also demonstrated that 4'-chloro-deschloroalprazolam does not elute in the retention time window of alpha-hydroxyalprazolam, a major metabolite of alprazolam.

Based on the interference study data, HFSC Toxicology section will report positive alprazolam results only if the analyte elutes after alprazolam-d5 because if the compound elutes before alprazolam-d5, this can potentially be 4'-chloro-deschloroalprazolam. Any positive alprazolam findings with a retention time before alprazolam-d5 will be reported as not suitable for the HFSC alprazolam analysis due to a possible interference. This data analysis criterion will be added to the next Analytical Manual v3.9.

HFSC Toxicology section reviewed all 2022 reports issued as of December 13, 2022 that were positive for alprazolam (n=8). None of the reports had alprazolam eluted before alprazolam-d5, confirming the presence of alprazolam rather than 4'-chloro-deschloroalprazolam. If evaluation of 4'-chloro-deschloroalprazolam is needed for any 2021 reports issued before December 13, 2022 is needed, please contact the HFSC Toxicology section at toxicology@hfsctx.gov.