



K1 The Analysis of PM Oral Swabs by SPE and LC-MSMS for Fentanyl as an Indicator of Administration

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After attending this presentation, attendees will learn about the usefulness and versatility of the oral swabs as a means of extracting fentanyl/norfentanyl from postmortem oral cavities. Attendees will also learn about the efficiency of solid phase extraction LC-MSMS methods in confirming these drugs in this matrix.

This presentation will impact the forensic science community by showing how fentanyl/ norfentanyl can be extracted/isolated and analyzed from swabs taken from the oral cavities in postmortem cases. This methodology will assist forensic toxicologists and pathologists when samples of blood and urine are limited. The goal of the presentation is to show how useful oral swabs taken at postmortem examination can be for the analysis of fentanyl/norfentanyl in cases when limited samples are available to analysts, forensic toxicologists, and forensic pathologists. The levels of the fentanyl (and norfentanyl) found on the swabs are referenced against the values obtained by toxicological analysis of postmortem blood for the same cases. The data presented should add another method of analysis for facilities providing toxicological services.

In 2007-2008, oral swabs were taken from 72 post mortem cases by the pathology staff at Erie Co. Medical Examiner's Office New York where fentanyl was related to the case. In each of the cases, two swabs were employed simultaneously to extract samples from the oral cavities. The swabs were forensically sealed and submitted to Northern Tier Research (NTR). The swabs from each case were split and half of the samples were sent to Massachusetts State Police Crime Laboratory (MSPCL). These samples were used to confirm Northern Tier Research findings. Following submission to the respective laboratories, the oral swabs were extracted with 200 μ L of methanol for 30 minutes in a sample tube containing fentanyl-d5/norfentanyl-d5, the swabs were washed further with a 100 μ L of methanol. The swabs were removed from the sample tube before 2 mL of phosphate buffer (pH 6) were added. This solution was extracted by solid phase extraction using a mixed mode (C₈/SCX) column (200 mg, 6 mL). The SPE columns were conditioned with methanol, DI water, and pH 6 phosphate buffer (3 mL, 3 mL, 1 mL, respectively). After washing with DI water, 0.1 M acetic acid, and methanol (3 mL of each), the SPE columns were dried and eluted with: (NTR): 3 mL of ethyl acetate/acetonitrile/ammonia (78:20:2) and (MSPCL): 3 mL of dichloromethane/ isopropanol/ ammonia (78:20:2). The eluates were evaporated to dryness and reconstituted in methanol for analysis by LC-MSMS using 5 μ L for injection.

At NTR, tandem mass spectrometry was performed in MRM (Fentanyl: 337.2-> 188.1/105.7, Norfentanyl: 233.0->84.0/55.1) with a 50 x 2.1mm (3 μ m) phenyl column. At MSPCL tandem mass spectrometry was employed in the same MRM mode using a 50 x 2.1mm (5 μ m) C₁₈ column. Chromatography was performed with a gradient program of acetonitrile and 0.1% aqueous formic acid at each laboratory.

Calibrators and controls were set up by extracting 0, 1, 2, 5, 10, and 7 ng of fentanyl/norfentanyl from aqueous buffer samples (2 mL) by the individual procedures. From the analysis of the calibrators and controls: r² value > 0.995, recoveries > 90% (NTR/ MSPCL), and a limit of detection of 0.1 ng/ mL, respectively were achieved.

Of the 72 postmortem cases where oral swabs were taken, six were confirmed to be positive for fentanyl. In two of the six cases, both fentanyl and norfentanyl levels greater than 1 ng were confirmed by LC- MSMS in both forensic laboratories (NTR/ MSPCL). These six cases were shown to have fentanyl and norfentanyl levels in blood ranging from 0.8 ng/ mL to 10.5 ng/ mL for fentanyl and 0.8 ng/ mL to 30.8 ng/ mL for norfentanyl, respectively. This data was obtained by the forensic toxicology laboratory, Erie Co. NY.

Based on data presented, analysts, forensic toxicologists and pathologists involved in post mortem cases where fentanyl and norfentanyl is suspected may wish to consider the usefulness of oral swabs in their analytical protocols. Although no direct correlation between the concentration of the drugs found in blood and those obtained from oral swabs can be drawn, this study has shown that in those cases where fentanyl was positive in oral swabs, it was confirmed in the corresponding blood samples. This relationship may be very useful in postmortem fentanyl cases.

Fentanyl, SPE, LCMSMS