December 9, 2021

Participation in NIST Study (Quarterly Environmental Sampling) – Narcotics Background Quantitation & Screening Summary Report

The Toxicology Laboratory continues its collaboration with NIST in a current study, the goal of which is to establish drug background levels present in a forensic science facility. NIST provides the laboratory with test kits for the collection of samples and Toxicology Laboratory personnel collect and send the samples to NIST for analysis. An initial set of 100 samples was collected in June 2021, with results from testing performed by NIST summarized in the report dated 7/7/21. Results from the Toxicology Laboratory will contribute to the NIST study.

The Laboratory has implemented a quarterly environmental sampling schedule, with the first round of quarterly sampling performed 10/28/21 using test kits provided by NIST. A total of 25 sample sites included the 5 areas with positive results and the 3 areas with presumptive results (not confirmed) listed in the report from the initial NIST analysis (NIST report dated 7/7/21), and 17 additional locations. A summary of testing performed by NIST is attached, with test results listed on page 4 of the report.

The second round of quarterly environmental sampling is planned for January 2022. The on-site visit by NIOSH, originally scheduled for November 2021, has been rescheduled to January 2022, due to delays experienced by NIOSH as a result of the pandemic.

December 2^{nd}, 2021

Brian Capron
Acting Laboratory Manager
Washington State Patrol
2203 Airport Way South
Seattle, WA 98134

Brian,

Thank you for participating in our study. The goal of this project was to establish the narcotics background present in a forensic science laboratory. The following report contains the results from the analysis of 25 samples collected from the Washington State Toxicology Laboratory. The analysis scheme involved a broad screening of over 800 drugs and common excipients and a targeted quantification of 29 drugs.

We would be happy to discuss these results in further detail with you at any time, and hope to continue collaborative efforts in the future. If we can be of any assistance to you, please don't hesitate to ask.

Sincerely,

Edward Sisco

Research Chemist
Materials Measurement Science Division
National Institute of Standards & Technology
100 Bureau Dr. Gaithersburg, MD 20899
Phone: (301)975-2093
E-mail: edward.sisco@nist.gov

Narcotics Background Quantitation & Screening Summary

Introduction

The recent spike in forensic cases containing highly toxic fentanyl analogues highlights the critical need to safeguard analysts from inadvertently encountering these, or other, compounds through skin adsorption and/or inhalation.[1] Establishing background levels of compounds of interest in a forensic laboratory can provide drug analysts and laboratory quality managers with valuable information to make informed decisions on a range of topics such as: workflow processes, adequate PPE, cleaning protocols, and occupational safety hazards.

Given that trace amounts of narcotics have been reported in a variety of environments including public spaces,[2] and that instruments continue to improve in sensitivity, it is important to monitor the environmental background levels of these compounds. For field and/or screening applications, establishing the background is key to setting instrument detection thresholds and preventing false positives.[3] This is especially critical in environments where there is an expected higher background level such as prisons or border crossings. In a laboratory setting, high environmental background levels can suggest a need to monitor background for quality and health purposes.

Finally, since forensic laboratories continue to struggle with a high number of emerging drug cases and rising backlogs, opportunities for rapid screening / presumptive testing are desired. The ability to screen evidence in a high throughput manner with little to no sample preparation is currently being investigated. To ensure the results from such analysis are from the evidence and not from possible background within the laboratory, a baseline of the environment must be known.

Experimental

Samples were collected with manual Nomex wipes (Part No. DSW1210P) (DSA Detection, North Andover, MA) which are commonly used for particle collection in trace contraband detection. The particle collection efficiency of this material has been previously measured by our laboratory and results demonstrate that it is an adequate substrate for the collection of trace residues off a variety of surfaces.[4] A total of 25 samples were provided to us for analysis. Upon receipt samples were stored, at -10 °C, until they were processed.

Prior to analysis, the Nomex wipes were trimmed in size to remove the unused area of the wipe. The trimmed wipe was placed in a 10 mL amber glass vial and extracted with 4.0 mL of methanol (Chromasolv Grade, Sigma-Aldrich). The 4.0 mL extract was subsequently split into two 2.0 mL aliquots – one for the presumptive screening analysis and one for the quantitative analysis. Both aliquots were then evaporated to dryness under a stream of nitrogen. The aliquot for the screening analysis was reconstituted in 200 µL of methanol, to concentrate the sample, while the aliquot for quantitation was reconstituted in 500 µL of methanol containing 5 internal standards. 5 µL of the screening aliquot was pipetted onto a Teflon-coated fiberglass wipe for analysis by TD-DART-MS. The quantitation aliquot was directly loaded onto the LC-MS/MS system.

Chemicals & Materials

Analytes for the screening and quantitation studies were obtained from either Cayman Chemical (Ann Arbor, MI), Cerilliant (Round Rock, TX), or Sigma-Aldrich (St. Louis, MO) as 1 mg/mL standards (when possible) or as pure crystalline material. Solvents for extraction and the LC mobile phase were Chromasolv-grade solvents purchased from Sigma-Aldrich. For quantitation, the 5 deuterated internal standards were: methamphetamine-d_5, heroin-d_9, cocaine-d_3, fentanyl-d_5, and THC-d_9. They were added to 1 L of methanol, providing an internal standard concentration of approximately 1 µg/mL, to be used for the reconstitution of the quantitation aliquot. Wipe materials, both Nomex and Teflon-coated fiberglass, were purchased from DSA Detection and used as-is.

Quantitation of Drugs by LC-MS/MS

In order to have the highest level of sensitivity and specificity for the quantitation runs, a LC triple quadrupole MS operating in multiple reaction monitoring (MRM) mode was used. The system consisted of a Thermo Ulti-Mate 3000 LC system coupled to a ABSciex Q-Trap 4000 mass spectrometer. Separation was achieved using a Restek Raptor Biphenyl column (150 mm x 4.6 mm x 2.7 µm). The analysis time was 15 minutes with a flow rate of 0.75 mL/min and an injection volume of 15 µL. During the run, a 12-minute solvent gradient was used (95 % water / 5 % methanol + 0.1 % formic acid to 100 % methanol with 0.1 % formic) followed by a 3-minute isocratic period (100 % methanol + 0.1 % formic acid). The MS utilized zero-air nitrogen as both the desolvating and nebulizing gases. An electrospray ionization (ESI) source was used with a temperature of 550 °C and a spray voltage of +5500 V. A timed MRM was used to monitor two transitions for all drugs (one for quantitation and one for confirmatory identification) and one transition for each of the 5 internal standards. The MRM detection window was set to 120 s and the target scan time was set to 0.1 s.

Quantitation was calculated by taking the ratio of the peak areas of a drug to the appropriate internal standard and comparing that ratio to a 13-point calibration curve. Absolute concentrations reported in the summary account for the various dilution and sample splitting steps in the extraction process. They do not, however, account for the extraction efficiency of the Nomex wipe, which is typically in the range of 30 % - 40 %.

Presumptive Screening of Drugs and Excipients by TD-DART-MS

The aliquot prepared for the screening analysis was pipetted (10 µL) onto a Teflon-coated fiberglass wipe and analyzed by TD-DART-MS. The TD-DART-MS system used a JEOL AccuTOF JMS T100-LP time-of-flight mass spectrometer (JEOL USA) coupled with a DART ion source (IonSense) and an in-house built thermal desorption unit.[6] A thermal desorber temperature of 270 °C was utilized with a 400 °C DART gas temperature, a +100 V DART exit grid voltage, and nitrogen as the ionization gas. Mass spectrometer settings included operation in positive ionization mode, a +400 V peaks voltage, a +5 V orifice 2 and ring lens voltage, and a mass scan range of 60 *m/z* – 700 *m/z* at 1 s/scan. To obtain characteristic molecular and fragmentation spectra, the orifice 1 voltage was cycled between +30 V and +60 V.

Samples were analyzed through direct insertion of Teflon-coated fiberglass wipe into the thermal desorber. Blank wipes were also analyzed in between samples to allow for mass spectra to be background subtracted. PEG-600 was used as a mass calibrant and was analyzed with each batch of samples. The resulting mass spectra were searched against an in-house created library of over 800 drugs and excipients for both the characteristic molecular ions (in the +30 V spectra) and fragment ions (in the +60 V spectra). The screening results reported met the following identification criteria: the protonated molecular ion peak of the compound was present at greater than 5 % relative abundance and within ±5 amu of the calculated accurate mass.

Results

From the 25 samples provided, none were found to have quantifiable levels of any of the 29 drugs included in the LC-MS/MS panel.

From the presumptive screening analysis, no compounds of interest were detected in any of the samples provided, when analyzed by TD-DART-MS utilizing a 5 % relative intensity identification threshold.

As stated in the opening letter, we would be more than happy to discuss these results with you and other interested members of your lab. If you would like us to analyze samples from additional areas, re-sample after any operational changes, or re-sample to monitor trends, we would be happy to do so. If there is any other way which we could be of assistance or form a stronger collaboration, please let us know.

Disclaimer

Certain commercial equipment, instruments, or materials are identified in this document. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the products identified are necessarily the best available for the purpose.

References

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