

April 18, 2023

Participation in NIST Study (Supplemental 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, which the Laboratory uses to collect environmental samples, and the samples are sent to NIST for testing.

Laboratory personnel conducted environmental sampling from multiple fume hood areas following work performed by an external vendor. The sampling was performed 2/24/23, with a total of 18 samples collected. The samples were sent to NIST for analysis. A summary of testing performed by NIST is attached (report dated 3/27/23), with test results listed on page 4 of the report. The samples had no compounds of interest detected when screened by DART-MS and no quantifiable levels of any compound in the LC-MSMS panel.

This round of sampling is supplemental to the Laboratory's quarterly environmental sampling plan. The next round of regularly scheduled quarterly sampling will take place in second quarter 2023.

March 27th, 2023

Eric Lo
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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 18 sample collected from the Washington State Toxicology Laboratory on February 24th, 2023. The analysis scheme involved a broad screening of over 1100 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

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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 1 sample was 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 screening analysis and one for the quantitation 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 acetonitrile, to concentrate the sample, while the aliquot for quantitation was reconstituted in 500 µL of methanol containing 5 internal standards. 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-d5, heroin-d9, cocaine-d3, fentanyl-d5, and THC-d9. 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 were purchased from Smiths 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 %.

Screening of Drugs by DART-MS

The aliquot prepared for the screening analysis was sampled using glass microcapillary rods. The DART-MS system used a JEOL AccuTOF JMS T100-LP time-of-flight mass spectrometer (JEOL USA) coupled with a DART ion source (IonSense). A 400 °C DART gas temperature, a +50 V DART exit grid voltage, and helium as the ionization gas was used. Mass spectrometer settings included operation in positive ionization mode, a +800 V peaks voltage, a +5 V orifice 2 and ring lens voltage, and a mass scan range of 80 m/z – 800 m/z. To obtain characteristic molecular and fragmentation spectra, the orifice 1 voltage was cycled between +30 V and +60 V.

PEG-600 was used as a mass calibrant and AB-FUBINACA was used as a mass drift compensation compound. The resulting mass spectra were searched against an in-house created library of over 1,100 compounds using the NIST DART-MS Data Interpretation Tool. 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

There were no quantifiable levels of any compound in the LC-MS/MS panel found in any of the 18 provided samples (Table 1).

No compounds of interest were detected in any of the 18 samples when screened by DART-MS using 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.

Table 1. Locations of samples collected. All samples were noted to have been collected on February 24th, 2023.

Sample #	Location	Sample #	Location
1	Hood 1 Inside	10	Hood 5 Outside
2	Hood 1 Outside	11	Hood 6 Inside
3	Hood 2 Inside	12	Hood 6 Outside
4	Hood 2 Outside	13	Hood 7 Inside
5	Hood 3 Inside	14	Hood 7 Outside
6	Hood 3 Outside	15	Hood 8 Inside
7	Hood 4 Inside	16	Hood 8 Outside
8	Hood 4 Outside	17	Hood 9 Inside
9	Hood 5 Inside	18	Hood 9 Outside

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

1. Daughton, C. G. Illicit Drugs and the Environment. in *Illicit Drugs in the Environment* (eds. Castiglioni, S., Zuccato, E. & Fanelli, R.) 1–27 (John Wiley & Sons, Inc., 2011).
2. Forbes, T. P. & Najarro, M. Ion mobility spectrometry nuisance alarm threshold analysis for illicit narcotics based on environmental background and a ROC-curve approach. *Analyst* **141**, 4438–4446 (2016).
3. Sisco, E. *et al.* Rapid detection of fentanyl, fentanyl analogues, and opioids for on-site or laboratory based drug seizure screening using thermal desorption DART-MS and ion mobility spectrometry. *Forensic Chem.* **4**, 108–115 (2017).
4. Verkouteren, J. R. *et al.* A method to determine collection efficiency of particles by swipe sampling. *Meas. Sci. Technol.* **19**, 115101 (2008).