Applied Research and Development in Forensic Science for Criminal Justice Purposes

Analysis of Drugs of Abuse in Human Hair: Surface Contamination and Localization of Analysis

Final Summary Overview

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RTI INTERNATIONAL

Purpose

For more than two decades, researchers and scientists have investigated and employed hair testing for drugs of abuse as a complementary and alternate matrix to blood and urine. The utility of hair testing is founded on the hair's ability to reflect long-term drug use and to incorporate drug analyte securely, as well as on the ease of testing agencies to collect and store hair samples. Testing for drugs in hair has evolved to the point that determining the identity of the drug found is less an issue than the explanation of the route of deposition. Despite considerable research, given current analytical technologies and interpretive methods, environmental contamination remains an unresolved issue for hair, and controversy exists over the source of drug residues found in hair and the potential for environmental contamination to cause false-positive test results.

Methamphetamine has been confirmed as a major contaminant associated with clandestine methamphetamine laboratories. The persistence of surface contamination of methamphetamine in clandestine laboratories presents a non-trivial possibility for environmental contamination to persons entering those spaces, including law enforcement first responders. The purpose of this study was to examine the effects of environmental contamination of human hair leading to external deposition of methamphetamine and heroin on drug tests designed to identify drug use. The goals of this project were to determine 1) the likelihood of whether methamphetamine and heroin can be adequately removed by an extended aqueous phosphate buffer decontamination procedure, 2) if, over time and with normal hygienic treatment of the hair, the drug will be removed from the hair or prove resistant to removal and 3) the extent to which normal hygienic treatment and the extended aqueous phosphate buffer decontamination procedure affect measurable levels of methamphetamine and heroin in hair from drug users who have ingested those compounds and 4) whether several imaging techniques could be used to localize the site of incorporation of the drugs into hair as an indicator of the route of incorporation (e.g., contamination versus consumption).

Project Design

Blank hair samples from non-drug users (E1-E5) and hair samples from known drug users (U1-U6) were acquired through IRB approved protocols. Blank hair samples were contaminated with methamphetamine and heroin using an in vitro contamination process at a concentration of 8mg/drug for every10g of hair. The project was divided into two parts: one involving an LC-MS analysis of the hair samples under different hygienic and decontamination treatments, and one involving imaging of the hair samples.

For the LC-MS portion of the project, each sample was divided into two portions, one was shampooed every weekday for 8 weeks, the other was left on the bench top with no further treatment over the same 8 week period. Two aliquots were taken from each portion of each sample at 8 time points over the course of the study, according to the sampling schedule depicted in Figure 1.

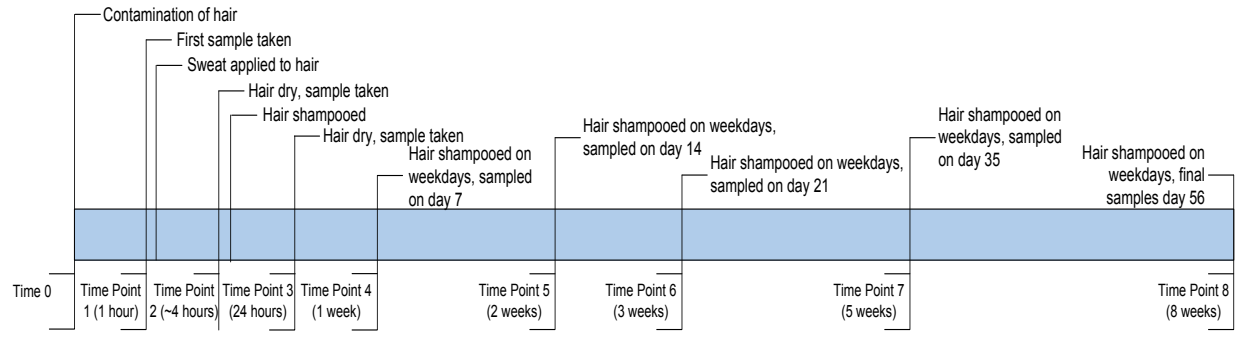


Figure 1 Hair sampling schedule with time point descriptions

One aliquot was decontaminated using an extended phosphate buffer decontamination procedure and one received no additional decontamination prior to extraction and LC-MS analysis. The sampling scheme is shown in Figure 2. Samples were analyzed for the presence of methamphetamine (MAMP), amphetamine (AMP), 6-acetylmorphine (6-AM) and morphine.

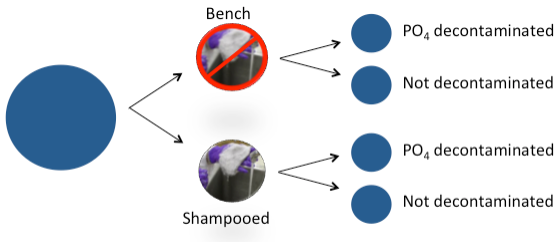


Figure 2 Diagram of the hair sampling scheme

For the imaging portion of the project, unless otherwise noted in the imaging results section below, blank hair, externally contaminated hair, and drug user hair were analyzed, and the externally contaminated hair samples underwent 1 shampooing prior to analysis.

Results

LC-MS

An LC-MS/MS method for the extraction and quantification of MAMP, AMP, heroin, 6-AM, and morphine from human hair was developed and validated according to SWGTOX guidelines. Explicit details of the methods and validation will be provided in future publications. Briefly, hair samples were extracted by gentle heating and shaking in phosphate buffer for 18 hours. Matrix matched calibrators and QCs were prepared by spiking blank hair with known amounts of analytes prior to extraction. Stable isotope labeled analogs of each analyte of interest were used as internal standards. Validated calibration ranges for each analyte are as follows: MAMP 25-10000 pg/mg, AMP 50-2000 pg/mg, Heroin 25-10,000 pg/mg, 6-AM 2.5-75 pg/mg, morphine 2.5-200 pg/mg. Society of Hair Testing (SOHT) guidelines for drug testing in hair (Cooper, Kronstrand, and Kintz, *FSI* vol. 218 Issues 1-3, pages 20-24 2012) recommended cut-offs for MAMP, AMP, 6-AM and morphine are 200 pg/mg. No cut-off recommendations are made for heroin.

MAMP

There was significant individual variation in how much MAMP was absorbed by the hair samples, despite all the samples being dark brown or black. After the contamination and a single shampooing (TP3), non-decontaminated hair concentrations ranged from 13,175 (E5) to 49,675 (E2) pg/mg with a relative standard deviation of 47%. At the same time point, samples that underwent PO_4 decontamination prior to extraction ranged from 2,935 (E5) to 15,275 (E2) pg/mg with a relative standard deviation of 59%.

Decontamination was highly effective immediately after the contamination and much less effective once synthetic sweat was applied. With PO_4 decontamination, 4/5 samples were below the

cut-off one hour after contamination. After synthetic sweat was applied 0/5 samples were below the cut-off. Before synthetic sweat was applied (TP1), PO_4 decontamination removed on average 99.8% of the added MAMP, 4 hours after synthetic sweat was applied (TP2) the PO_4 decontamination only removed 89% of the MAMP.

Shampooing steadily decreased the levels of MAMP present in the externally contaminated hair samples. However, of the 5/5 samples above cut-off at TP3, 4/5 were still above 200 pg/mg after 8 weeks of shampooing, and including the PO_4 decontamination prior to extraction. The one sample that was below the cut-off at TP8, was above the cut-off at TP7. Continued shampooing also steadily decreased the levels of MAMP present in the user hair samples.

AMP

Initial testing of the blank hair samples determined that the concentration of AMP in all samples was 2-3 pg/mg, which is below the LOQ of our methods. Despite contaminating only with MAMP measurable levels of the MAMP metabolite AMP were detected in all samples. After the contamination and a single shampooing (TP3), samples that underwent PO_4 decontamination prior to extraction ranged from 9 (E5) to 82 (E2) pg/mg. Without the decontamination step concentrations ranged from 22 (E5) to 465 (E2) pg/mg. For externally contaminated hair that was not shampooed, the level of AMP increased over time. This trend was not observed in the user hair samples, but in all cases the levels were below the validated LOQ.

Heroin

Shampooing effectively removed parent heroin from the contaminated hair. 3/5 samples that underwent PO_4 decontamination and 5/5 non-decontaminated samples were over 200 pg/mg at TP3. At TP3 samples that weren't decontaminated prior to analysis ranged from 7078 (E1) to 23,050 (E2) pg/mg. By TP7 the highest level of heroin measured in any shampooed hair sample was 85 pg/mg, by TP8 the highest level measured was 48 pg/mg. At TP3 the decontamination procedure removed on average 97% of heroin from externally contaminated hair. Upon initial testing only two of the

user hair samples had levels of heroin above our limit of quantification. The decontamination procedure removed 97% of the heroin from one sample and 74% of the heroin from the other.

6-AM

Initial testing of the blank hair samples determined that the concentration of 6-AM in all samples was below the LOD of our methods. After contamination with MAMP and heroin, significant amounts of the heroin metabolite 6-AM were detected in all samples. After the contamination and a single shampooing (TP3) samples that underwent PO_4 decontamination prior to extraction ranged from 28 (E1) to 214 (E3) pg/mg. 3/5 of the samples were above the SOHT suggested cut-off. Without the decontamination step 5/5 of the samples were above the SOHT suggested cut-off with concentrations ranging from 920 (E1) to 3153 (E2) pg/mg. After 8 weeks of shampooing and including the PO_4 decontamination, one of the samples was still above the SOHT suggested cut-off.

Morphine

Initial testing of the blank hair samples determined that the concentration of morphine in all samples was below the LOD of our methods. After contamination with MAMP and heroin, morphine was detected in all samples. After the contamination and a single shampooing (TP3) samples that underwent PO_4 decontamination prior to extraction ranged from 5 (E1) to 42 (E2) pg/mg. Without the decontamination step concentrations ranged from 152 (E1) to 471 (E2) pg/mg.

Imaging

The original proposal included imaging the analytes of interest in user and contaminated hair by matrix assisted laser desorption ionization mass spectrometry (MALDI-MS). Through a GAN, the scope of the imaging portion of the project was expanded to include atomic force microscopy coupled with infrared spectrometry (AFM-IR), scanning electron microscopy (SEM) both with and without electron dispersive x-ray spectroscopy (EDS), and time of flight secondary ion mass spectrometry (TOF-SIMS).

MALDI-TOF

Initial MALDI-TOF imaging experiments were performed by scientists at JEOL Inc. Samples of hair from 2 individual users (U1 and U3) were extracted in MeOH (~35ug/uL). The methanolic extracts were mixed 1:1 with Cyano-4-hydroxycinnamic acid (CHCA) as a MALDI matrix then spotted for analysis. The CHCA matrix contained interferents that obscured the signals for methamphetamine and amphetamine. Heroin, morphine, and 6-acetylmorphine (6-AM) were detected at low levels in U3, but not in U1. MALDI-TOF imaging of 6-AM on intact strands of U3 was performed and is shown in Figure 3.

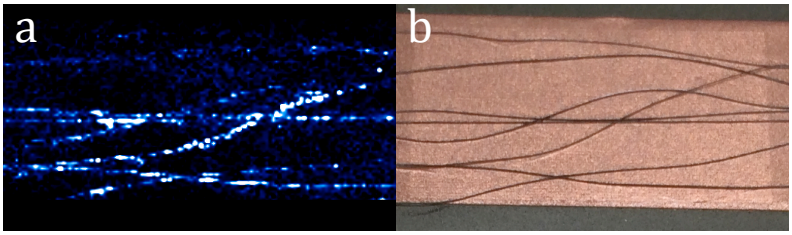


Figure 3 MALDI-TOF image of 6-AM distribution on user hair strands (a) and an optical image of the strands post analysis (b)

As shown in Figure 3, MALDI-TOF has the required sensitivity to detect 6-AM on intact strands of user hair. However, the spatial resolution of the technique is not sufficient to localize the position of the analytes to the cuticle or cortex of a cross section of a hair sample. Therefore work to find a MALDI matrix with fewer interferents was not pursued and instead alternate imaging techniques were investigated.

AFM-IR

AFM-IR imaging experiments were performed by scientists at Anasys Instruments. Hair strands were embedded in epoxy and cross sections ~ 400 nm thick were prepared using a

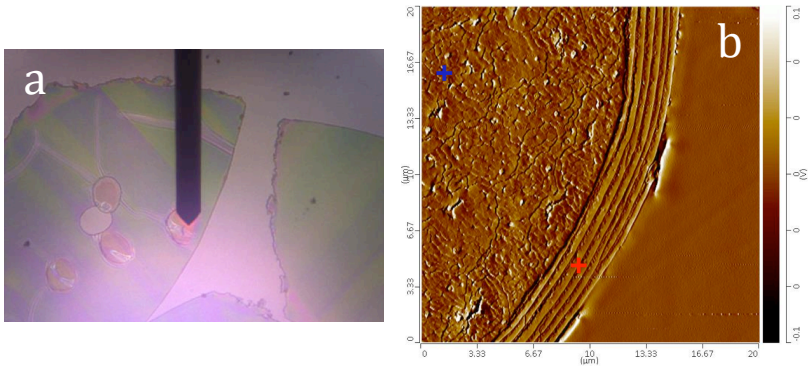


Figure 4 Optical image of hair cross sections embedded in epoxy for AFM-IR analysis (a) and an AFM deflection image showing the cuticle and cortex (interior) of a cross section of an externally contaminated hair sample (b).

microtome. Cross sections of blank hair and externally contaminated hair were analyzed. Figure 4a shows an optical image of the cross sections embedded in epoxy positioned for analysis. An AFM deflection image of one of the externally contaminated hair cross sections is shown in Figure 4b.

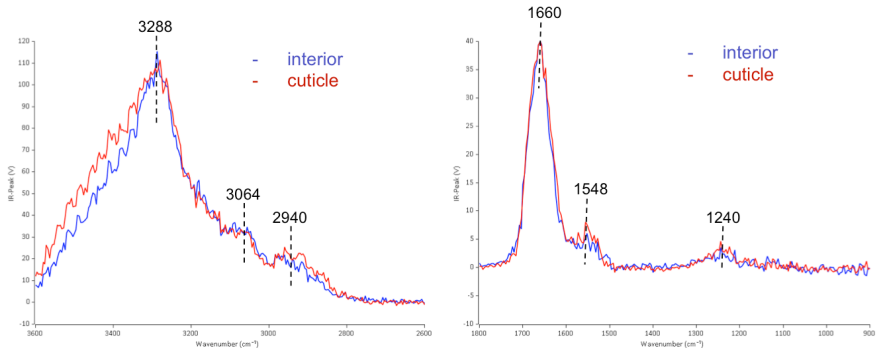


Figure 5 IR spectra taken from the interior and cuticle regions of the cross section shown in Figure 4 Optical image of hair cross sections embedded in epoxy for AFM-IR analysis (a) and an AFM deflection image showing the cuticle and cortex (interior) of a cross section of an externally contaminated hair sample (b).Figure 4b.

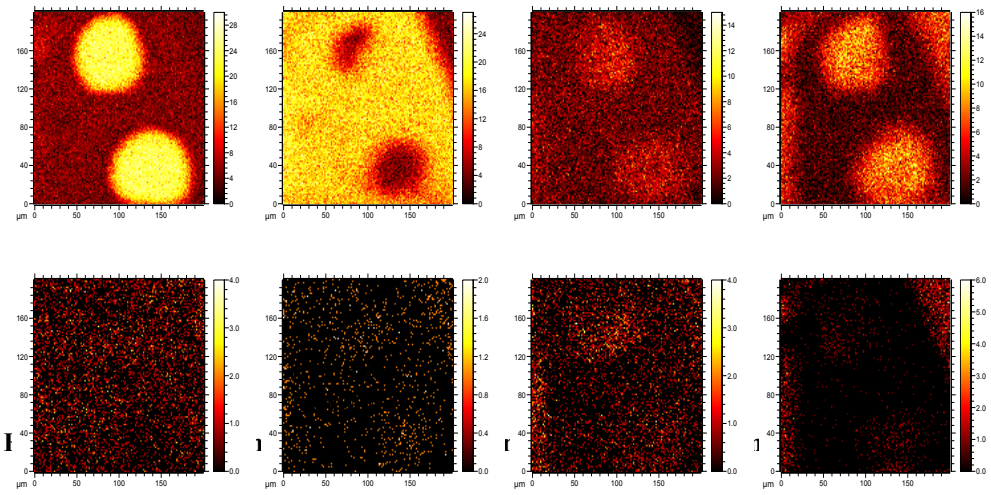
The spectra in Figure 5 were acquired from the positions marked with red and blue plus signs in Figure 4b. The red trace is from the cuticle, and the blue trace is from the interior of the hair. No clear differences between multiple spectra from these two regions were detected, and no heroin, methamhetamine, or their metabolites were detected in any of the spectra from either region. Spectra from an externally contaminated hair sample were indistinguishable from spectra from a blank hair sample. The exterior surface of intact strands of both blank and externally contaminated hair samples were also analyzed, with no clear differences detected between them and no heroin, methamphetamine or metabolites detected.

As shown in Figure 4b, AFM-IR has sufficient spatial resolution to collect distinct spectra from the cuticle and the interior, however, our methods lack the required sensitivity to detect analytes at physiologically relevant concentrations.

TOF-SIMS

TOF-SIMS analysis was performed at the Advanced Instrument Facility at North Carolina State University. Good signal was obtained for standards of the analytes of interest when spotted at 1000 ng/mL directly onto plates for analysis. Intact hair strands were analyzed by placing the hair

on conductive carbon tape. Hair cross sections were prepared by embedding the hair in epoxy then

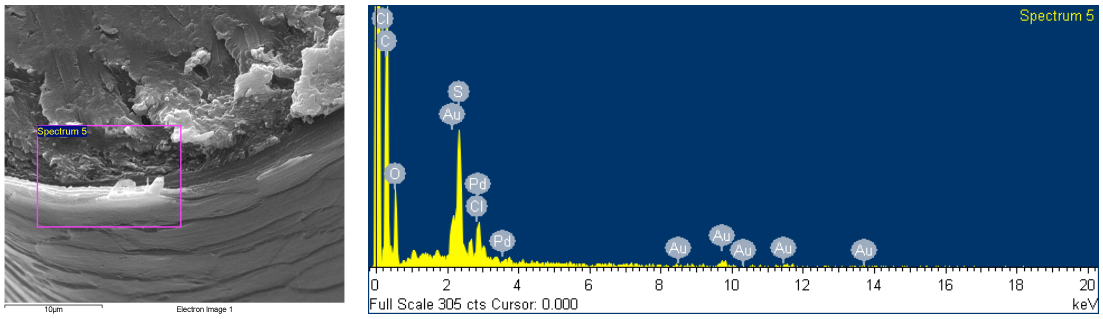


microtoming. Despite confirmed presence of the molecules by LC-MS analysis, no molecular ions of methamphetamine, heroin, or any metabolites were detected on the surface or cross sections of contaminated hair. An additional analysis was performed on a cross section of externally contaminated hair that had not undergone the initial shampooing, but no analytes of interest were detected on that sample either. Similarly to AFM-IR, TOF-SIMS has the required spatial resolution to distinguish ions located in the cortex vs. interior of a hair cross sections, but with the methods employed in this study lacked to required sensitivity to detect the ions of interest at concentrations that would be relevant to improve the reliability of hair testing.

SEM-EDS

The addition of an EDS detector to an SEM microscope allows for elemental analysis and can show elemental distribution on a sample surface. Since the analytes used in the original contaminations contain the same elements (C, H, N, O) that are naturally found in hair a separate contamination was performed using 4-bromoamphetamine, specifically for the SEM-EDS analysis.

This introduced Br into the hair as a contaminant. Hair was externally contaminated at the same analyte to hair ratio as in the previous contamination (8 mg:10 g) then after one hour synthetic sweat was applied. Once the hair was dry, approximately 4 hours later, several strands were removed for analysis and the remaining hair was shampooed. The following morning, several strands of the



shampooed hair were also removed for analysis. SEM-EDS was unable to detect a signal for Br from either set of samples. The SEM image of the non-shampooed sample is shown in Figure 7a and a summed spectrum from the region enclosed in the pink box in Figure 7a is shown in Figure 7b. This region contains a contribution from the external surface of the hair, and yet no Br is detected in the region. Similar to AFM-IR and TOF-SIMS, SEM-EDS has the necessary spatial resolution to localize analytes to either the cortex or cuticle of hair, but does not have sufficient sensitivity to detect the analytes at physiologically relevant concentrations.

Scholarly Products

Planned Publication

External contamination with heroin and methamphetamine in human hair
Megan Grabenauer, Breda Munoz, Katherine N. Moore, Nichole D. Bynum
Manuscript in preparation

Presentations

Can drug use be distinguished from external contamination in hair drug testing?
Megan Grabenauer, Jeri D. Ropero-Miller, Peter R. Stout, Katherine N. Moore, Nichole D. Bynum
Presented at the 2015 Triangle Chromatography Discussion Group Symposium, May 21, 2015
Raleigh, NC

Analysis of heroin and methamphetamine in human hair: surface contamination and localization of analytes
Megan Grabenauer, Breda Munoz, Katherine N. Moore, Jeri D. Ropero-Miller, Nichole D. Bynum
Abstract submitted to the 2015 Society of Forensic Toxicologists Meeting, October 18-23, 2015 Atlanta, GA

Implications for Policy and Practice

Prior studies have raised significant concerns about the potential for contamination to confound hair testing results, which could have direct consequences, either supporting or refuting, claims of contamination being the source of positive hair results. Our results continue to raise such concerns. Despite contaminating blank hair with only methamphetamine and heroin, measurable levels of their respective metabolites amphetamine, 6-acetylmorphine and morphine were detected. In some instances, the levels of metabolites detected were above the SOHT recommended cut-offs, indicating that basing positive results solely on the presence of metabolites is not sufficient to rule out possible contributions from external contamination. Many hair testing laboratories institute decontamination procedures prior to analysis in an attempt to remove any contribution from external contamination. Our studies show that a decontamination step alone is likely not sufficient to remove contributions from external contamination.

Setting positive call criteria based on parent/metabolite ratios has been proposed as a method to distinguish between use and contamination for some analytes. Given the large individual variation in drug absorption during the contamination phase that we observed, further studies using larger samples sizes are needed to determine if that is a valid route for identifying methamphetamine and heroin contamination. However, our initial results indicate that this route may be problematic as the concentrations of metabolites present in externally contaminated hair can vary depending on how long the hair sits prior to analysis.

Appendix to Final Summary Overview for 2013-DN-BX-K021

Materials and Methods

Drug standards for preparing calibrators were purchased from Cerilliant (Round Rock, Texas). Heroin HCl and MAMP HCl used in hair contamination were purchased from Lipomed (Cambridge, MA). All reagents were high-performance liquid chromatograph (HPLC) grade. Acetonitrile, water, methanol, and isopropanol were purchased from Fisher Scientific (Fair Lawn, NJ). Ammonium hydroxide and urea were purchased from EMD (Gibbstown, NJ). Potassium phosphate monobasic, potassium phosphate dibasic, sodium lactate, and bovine serum albumin (BSA) were purchased from Sigma Aldrich (St. Louis, MO). Sodium chloride and potassium chloride were purchased from BDH (VWR West Chester, PA), and Research Organics (Cleveland OH), respectively. Ammonium formate, formic acid and extra virgin olive oil were purchased from Alfa Aesar (Ward Hill, MA), Electron Microscopy Sciences (Hatfield, PA), and a local supermarket, respectively. Oasis HLB 3cc (60 mg) extraction cartridges and Orbital Shaker were purchased from Waters (Milford, MA), and Thermo Fisher Scientific (Waltham, MA), respectively.

Synthetic Sweat Preparation

Synthetic sweat was prepared by mixing. 22 mM urea, 65 mM sodium chloride, 5 mM potassium chloride, 9 mM sodium lactate, and 150 µL of extra virgin olive oil (Ropero-Miller *et al).* The mixture was refrigerated until use.

Time point samples

RTI collected non-chemically treated head hair from 10 drug-free donors and 20 drug-user donors under approved IRB protocol. Head hair samples that ranged in color from blond to dark brown were shaved using electric clippers or cut with clean scissors. All of the hair samples were analyzed by LC-MS/MS to determine if they were negative (drug-free) or positive for the analytes of interest. This analyses represented time point 0 (TP0). Five separate lots of dark brown to black drug-free hair at 10 g each were used for the contamination studies and one separate dark brown lot was used for preparation of calibration curves. For external contamination, 8 mg of heroin and 8 mg of methamphetamine were mixed together in gloved hands and distributed on the palms for approximately 5 minutes until no solid was visible. After 5 minutes, one lot of hair was placed into the gloved hands and rubbed for 5 minutes to evenly transfer the heroin and methamphetamine. The contaminated hair was then placed between two pieces of filter paper for one hour. The procedure was repeated for each hair lot, using a new pair of gloves each time.

After an hour, time point 1 samples were removed from each lot. The remaining hair samples were sprayed with the synthetic sweat solution until saturated. The samples were then placed between two pieces of filter paper to dry for 3-4 hours before time point 2 samples were removed. After time point 2 samples were removed, each sample was shampooed, then placed between two new pieces of filter paper to dry. The following morning time point 3 samples were taken. At that point the remaining samples were split into approximately 5 g portions. The first portion (i.e. shampooed samples) was shampooed daily (Monday-Friday) over 8 weeks. The second portion (i.e. bench samples) remained on the lab bench for 8 weeks. The shampooed hair samples were washed with Finesse 2-in-1 shampoo for approximately 5 minutes and rinsed until

all shampoo was removed. The samples were placed between two pieces of filter paper and dried overnight. Each of the five externally contaminated hair samples were washed each week day and samples were taken from the bench and shampooed samples at 5 additional time points: TP4 at 1 week, TP5 at 2 weeks, TP6 at 3 weeks, TP7 at 5 weeks, and TP8 at 8 weeks post contamination.

Six different lots of drug-user hair with the highest measured levels of the analytes of interest at TP0 were used for the study. The same procedure was followed as described previously for the externally contaminated hair specimens, with the exception of the contamination part.

Decontamination Process

At each time point for both drug-user hair and externally contaminated hair, two aliquots of approximately 75-80 mg of each hair lot (bench and shampooed for both drug-user and externally contaminated hair (n=8)) were removed and placed into separate labelled scintillation vials. One aliquot underwent the decontamination process, while the second aliquot was not decontaminated. The samples for decontamination were decontaminated using 0.01 M phosphate buffer containing 0.01% BSA. The decontamination procedure consisted of an initial 15-minute isopropanol wash, followed by three 30-minute and two 60-minute phosphate buffer containing BSA washes. Washes were replaced after each interval and the last 60-minute wash was kept in the freezer for future analysis.

After the decontamination process, each sample was placed between two pieces of filter paper until dry. Once dried, both the PO_4 decontaminated samples and the non-decontaminated samples were placed into labeled scintillation vials and the hair strands were cut and weighed into four separate test tubes containing approximately 10 mg each.

Extraction Process

Following the decontamination process, internal standard was added to each 10 mg sample, drug spiked calibrator, and drug spiked QC, and vortexed. An aliquot of 1.5 mL of 100 mM potassium phosphate monobasic (pH 5) was added to each sample. Samples were vortexed and centrifuged for 5 min at 3500 rcf followed by incubation at 37 °C for 18 hrs in an orbital shaker at 100 rpm. After incubation, the samples were vortexed and centrifuged at 3500 rcf for 5 minutes followed by solid phase extraction (SPE) using Oasis HLB cartridges. SPE cartridges were conditioned with 1 mL of methanol and 100 mM potassium phosphate monobasic (pH 5), each. The samples were loaded onto the cartridges and allowed to flow by gravity. Samples were washed with 1 mL of 5% methanol in water. Cartridges dried for 5 minutes under nitrogen. The samples were eluted in 1 mL of methanol containing 2% ammonium hydroxide. Samples were evaporated to dryness under nitrogen at room temperature (25°C) and reconstituted in 100 µL of ammonium formate with 0.1% formic acid and acetonitrile with 0.1% formic acid (95:5).

LC-MS/MS Methods

Samples were acquired on an AB Sciex 4000 QTrap (MS/MS) (Framingham, MA) mass spectrometer coupled to a Waters Acquity ultra performance liquid chromatography (UPLC) system (Waters, Milford, MA) with a turbo spray source operating in positive mode. Samples were injected (10 µL) onto an Agilent Zorbax RRHD Eclipse Plus C18 (1.8 µm 2.1x50 mm, Santa Clara, CA) column held at 30 °C. A gradient elution was used consisting of 5 mM ammonium formate with 0.1% formic acid (mobile phase A) and acetonitrile with 0.1% formic acid (mobile phase B). The flow rate was 0.4 ml/min. Beginning conditions were 5% B; this was increased linearly to 22% B by 4 min; increased linearly to 50% B by 5 min; then increased

linearly to 95% B from 5.1 to 6 min; followed by returning to initial conditions and equilibrating from 6.2 to 7.5 min.

Validation Methods

Calibration Curve

The calibration curve spanned the range of biologically relevant concentrations expected in hair using non-zero calibrators for 6-acetylmorphine (6 calibrators), amphetamine (5 calibrators), heroin (8 calibrators), morphine (7 calibrators), and methamphetamine (8 calibrators). The calibration curve was established, extracted and analyzed five times (n=5 at each concentration level). Each analyte's respective stable isotope labeled compound was used as an internal standard.

Precision and Accuracy

Precision and accuracy were determined by analyzing two quality control (QC) samples for amphetamine and morphine and four QCs for heroin, methamphetamine, and 6-acetylmorphine each fortified with analyte concentrations at the lower, middle, and upper portion of the calibration curve. Each QC sample was analyzed in triplicate within each linearity run over the course of five runs.

Limit of Quantification (LOQ)

The LOQ was defined as the concentration of the lowest calibrator, which was administratively set during establishment of the calibration curve.

Carryover

Carryover was determined by analyzing blank sample matrix immediately after a high concentration sample in each calibration curve (n=5). A sample was considered to have

carryover if the average peak area in the blank after the carryover sample was greater than 20% of the peak area of the established LOQ.

Interference

Ten different blank lots of drug-free donor hair samples were run by LC-MS/MS without addition of internal standard (ISTD) to evaluate interference from the matrix. Hair matrix was considered to interfere with an analyte if the average area of the blank samples (n=10) was greater than 20% of the average LOQ peak area of that analyte from the five calibration curves (n=5). In addition, five blank matrix samples containing ISTD were analyzed to demonstrate the absence of interferences originating from ISTD. A sample was considered to have interference if the average peak area of the blank + ISTD samples (n=5) was greater than 20% of the average LOQ peak area from the five calibration curves (n=5).

Matrix Effect

Matrix effects were evaluated using a modified version of the method described by Matuszewski and colleagues (Matuszewski, 2003). Three sets of samples were created for each target analyte. As described by Matuszewski and colleagues, comparative calculations were used to evaluate the data:

ME (%) = B/A x 100

RE (%) = C/B x 100

PE (%) = C/A x 100

where A, B, and C = the mean responses as represented by the area under the peaks for target and internal standard quantitative ions, ME = matrix effect, RE = recovery efficiency, and PE = process efficiency. Type A samples are target analytes and ISTD in mobile phase. Type B samples are hair matrix extract post extraction spiked with target analytes and ISTD. Type C

samples are hair matrix spiked with ISTD and target analytes prior to extraction. The mean responses for A, B, and C were determined across these 10 hair lots. The assessment of a relative matrix effect was determined by comparing the MEs between the 10 lots. The variability (%CV) in the MEs between lots is considered to be a measure of the relative matrix effect.

Validation Results

Two fragment ions were monitored for each drug analyte as a quantitative ion and qualifying ion. One fragment ion was monitored for the ISTD. Fragment ions and optimized parameters for each analyte and ISTD are shown in Table A-1.

Analyte	**Transitions Monitored**	**DP**	**CE**	**CxP**
Heroin	370.18>165.3*	101	68	6
	370.18>211.3	101	39	12
Heroin-D_9	379.27>212.1	121	45	10
6-AM	328.2>165.3*	100	54	9
	328.2>211.2	100	37	11
6-AM-D_6	334.2>165.2	121	53	8
Morphine	286.2>152.2*	91	80	12
	286.1>128.2	91	79	20
Morphine-D_6	292.2>152.1	116	91	24
MAMP	150.2>91.3*	45	27	20
	150.2>119.3	45	16	10
MAMP-D_5	155.1>121.1	46	15	4
AMP	136.2>119.2	45	12	19
	136.2>91.2*	45	23	10
AMP-D_6	142.1>125.1	66	13	6

Table A-1. Transitions monitored and optimized parameters for each drug analyte.

*Fragment ion used for quantification

Calibration Curve

Analyst software (version 1.4.2) was used for data reduction. Summary of calibration ranges, correlation coefficients (r^2), ISTD and QC concentrations of each drug analyte in hair are shown in Table A-2. A linear regression (weighted 1/x) calibration model was established for all analytes except MAMP, which was quadratic (weighted 1/x).

Analyte	r^2	Calibration points (pg/mg)	ISTD Conc. (pg/mg)	QC Conc. (pg/mg)
Heroin	0.9997	25, 50, 100, 200, 500, 1000, 2000, 10000	50	75, 300, 1200, 8000
MAMP	0.9992	25, 50, 100, 200, 500, 1000, 2000, 10000	50	75, 300, 1200, 8000
6-AM	0.9995	2.5, 5, 10, 20, 50, 75	5	7.5, 30
Morphine	0.9945	2.5, 5, 10, 20, 50, 75, 200	5	7.5, 30, 120
AMP	0.9992	50, 75, 200, 500, 2000	5	120, 1600

Table A-2. Summary of r^2, calibration, ISTD, and QC sample concentration for each analyte.

Precision and Accuracy

The precision and accuracy results for QC samples of each analyte are shown in Table A-3. Each QC level was analyzed in triplicate over five runs. The average overall within-run precision was represented by a %CV ≤ 7.1 for all compounds. The average overall between-run precision was represented by a %CV ≤ 11.4 for all compounds. The % accuracy and its associated %CV for all analytes in hair were 98.1-110.7 with %CV of accuracy ≤ 11.2.

	Precision Evaluation		Accuracy Evaluation	
Analyte	**Average Overall Within-Run %CV**	**Average Overall Between-Run %CV**	**Average Overall Accuracy**	**Accuracy %CV**
Heroin	2.5	3.6	98.1	4.7
MAMP	2.5	5.7	107.0	9.2
6-AM	2.0	2.5	98.2	4.2
Morphine	7.1	11.4	110.7	11.2
AMP	4.2	3.3	108.0	5.1

Table A-3. Evaluation of precision and accuracy of each analyte by LC-MS/MS.

Limit of Quantification (LOQ)/Limit of Detection (LOD)

Table A-4 presents the LOQ results for all analytes in hair. All LOQs were acceptable, with %CVs ranging from 12.6-19, with the exception of heroin with a %CV of 22.7. Accuracies ranged from 86.9-117.2% with accuracy %CV for all analytes ≤ 9.9.

Analyte	N	Target Conc. (pg/mg)	Average overall %CV	Average overall %Accuracy	Accuracy %CV
Heroin	5	25	22.7	106.0	4.4
MAMP	5	25	12.6	117.2	2.5
6-AM	5	2.5	14.2	107.6	2.5
Morphine	5	2.5	19.0	100.6	9.9
AMP	5	50	18.9	86.9	2.9

Table A-4. LOQ target concentrations, average overall %CV and %Accuracy and Accuracy %CV.

Carryover

Carryover was not observed for heroin and MAMP at 10,000 pg/mg or for 6-AM, AMP, and morphine at 2,000 pg/mg.

Interference

The average peak area of the ten drug-free hair lots without addition of ISTD were all less than 20% of the average peak areas of the LOQ for each analyte, indicating no interference from the hair matrix. One of the drug-free hair samples was positive for amphetamine and was not used in the interference calculation, therefore the amphetamine interference calculations are based on an n=9. No interference from ISTD was detected either.

Matrix Effect

The concentration used for matrix effects for 6-AM, AMP, and morphine was 50 pg/mg. MAMP and heroin concentration used for matrix effects was 500 pg/mg. Table A-5 shows the matrix effect, recovery efficiency, and process efficiency for each analyte. Heroin, MAMP, and AMP experienced suppression with %ME less than 100% while 6-AM and morphine experienced enhancement with matrix effects greater than 100%. The high and low %RE and %PE for heroin and 6-AM respectively indicate likely conversion of heroin to 6-AM during the extraction process.

	Target Ion Response			ISTD Ion Response			Relative Matrix Effect*
Analyte	%ME	%RE	%PE	%ME	%RE	%PE	%CV for B/A
Heroin	68.1	16.9	11.5	67.0	15.8	10.6	10.8
MAMP	84.9	76.8	65.3	83.7	78.2	65.5	10.0
6-AM	154.5	332.4	513.7	143.7	333.2	478.8	20.8
Morphine	110.2	170.2	187.6	100.5	37.3	37.5	18.0
AMP	83.3	55.7	46.5	102.0	57.1	58.3	12.0

* Heroin, MAMP, 6-AM, Morphine n=10 AMP n=9

Table A-5. Summary of matrix effects for each analyte.

References

Ropero-Miller, J., Stout, P. (January 2009) Analysis of cocaine and analytes in human hair: evaluation of concentration ratios in different hair types, cocaine surfaces, drug-user populations, and surface-contaminated specimens. NIJ Final Report. Retrieved on 5/28/2015 from https://www.ncjrs.gov/pdffiles1/nij/grants/225531.pdf.

Matuszewski BK, Constanzer ML, Chavez-Eng CM. Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC-MS/MS. Anal Chem. 2003 75(13):3019-30.

E1	Not Decontaminated		Decontaminated	
	Bench	Shampoo	Bench	Shampoo
TP0	3.6		3.4	
TP1	127750.0		80.5	
TP2	83250.0		5547.5	
TP3	20525.0		4355.0	
TP4	22500.0	16525.0	5570.0	4997.5
TP5	23375.0	10600.0	6602.5	6002.5
TP6	26050.0	11325.0	8340.0	6392.5
TP7	19550.0	6945.0	7722.5	4500.0
TP8	15100.0	4247.5	4015.0	3155.0

E2	Not Decontaminated		Decontaminated	
	Bench	Shampoo	Bench	Shampoo
TP0	3.9		3.6	
TP1	214750.0		638.5	
TP2	110750.0		18950.0	
TP3	49675.0		15275.0	
TP4	74575.0	52275.0	22925.0	19900.0
TP5	63925.0	33575.0	17025.0	18050.0
TP6	60675.0	36075.0	26550.0	17550.0
TP7	51050.0	13275.0	22500.0	8265.0
TP8	36825.0	9985.0	16775.0	5850.0

E3	Not Decontaminated		Decontaminated	
	Bench	Shampoo	Bench	Shampoo
TP0	4.6		3.6	
TP1	113500.0		79.1	
TP2	70750.0		9990.0	
TP3	41775.0		11025.0	
TP4	46100.0	34475.0	7765.0	9402.5
TP5	46850.0	26400.0	9087.5	9275.0
TP6	51800.0	27875.0	14550.0	11575.0
TP7	46875.0	12175.0	11125.0	5837.5
TP8	29200.0	6395.0	9755.0	3550.0

E4	Not Decontaminated		Decontaminated	
	Bench	Shampoo	Bench	Shampoo
TP0	4.1		3.6	
TP1	60675.0		33.8	
TP2	51550.0		2930.0	
TP3	21700.0		5407.5	
TP4	42000.0	14100.0	5995.0	4040.0
TP5	34275.0	11340.0	6622.5	4252.5
TP6	34775.0	9127.5	10600.0	3607.5
TP7	32525.0	2832.5	6765.0	1262.5
TP8	21900.0	1130.0	6300.0	541.0

E5	Not Decontaminated		Decontaminated	
	Bench	Shampoo	Bench	Shampoo
TP0	3.8		3.6	
TP1	45175.0		46.1	
TP2	35350.0		2135.0	
TP3	13175.0		2935.0	
TP4	15675.0	10525.0	2592.5	2785.0
TP5	16625.0	7437.5	2510.0	2462.5
TP6	18350.0	6170.0	4125.0	2825.0
TP7	16500.0	1985.0	3105.0	656.0
TP8	12225.0	0.0	2822.5	142.3

MAMP
pg/mg hair

U1	Not Decontaminated		Decontaminated	
	Bench	Shampoo	Bench	Shampoo
TP0	21.8		8.1	
TP1	22.4		5.7	
TP2	n/a		n/a	
TP3	31.2		10.0	
TP4	24.7	20.1	9.1	44.1
TP5	22.1	11.7	5.3	2.7
TP6	27.5	9.6	6.9	70.6
TP7	24.3	1.5	2.1	0.0
TP8	31.8	6.7	12.0	7.0

U2	Not Decontaminated		Decontaminated	
	Bench	Shampoo	Bench	Shampoo
TP0	3.5		3.5	
TP1	0.0		0.0	
TP2	n/a		n/a	
TP3	5.7		3.2	
TP4	5.0	4.6	5.3	5.8
TP5	0.0	0.0	0.0	0.0
TP6	0.0	0.0	0.1	1.4
TP7	0.0	0.0	0.0	0.0
TP8	6.1	6.1	5.9	6.1

U3	Not Decontaminated		Decontaminated	
	Bench	Shampoo	Bench	Shampoo
TP0	343.0		218.8	
TP1	321.0		122.8	
TP2	n/a		n/a	
TP3	342.8		160.5	
TP4	270.5	199.5	140.3	135.3
TP5	317.0	189.3	183.3	108.5
TP6	334.5	157.3	192.0	83.7
TP7	333.3	33.3	149.8	13.7
TP8	339.3	19.8	167.0	10.7

U4	Not Decontaminated		Decontaminated	
	Bench	Shampoo	Bench	Shampoo
TP0	122.8		192.0	
TP1	91.3		29.7	
TP2	n/a		n/a	
TP3	147.8		67.1	
TP4	86.8	60.9	67.1	41.9
TP5	75.7	59.1	38.4	41.5
TP6	101.5	71.8	55.2	30.0
TP7	62.3	37.2	36.7	20.7
TP8	68.6	23.1	54.0	13.3

U5	Not Decontaminated		Decontaminated	
	Bench	Shampoo	Bench	Shampoo
TP0	88.4		75.6	
TP1	60.2		49.3	
TP2	n/a		n/a	
TP3	82.8		49.8	
TP4	83.2	80.5	53.2	37.3
TP5	117.5	45.3	50.3	25.2
TP6	98.2	34.3	47.8	24.8
TP7	78.9	36.8	44.9	12.8
TP8	86.9	20.9	54.1	14.5

U6	Not Decontaminated		Decontaminated	
	Bench	Shampoo	Bench	Shampoo
TP0	16.5		6.2	
TP1	9.5		0.1	
TP2	n/a		n/a	
TP3	12.7		4.9	
TP4	17.8	10.9	7.1	6.3
TP5	10.7	2.9	0.8	0.1
TP6	12.4	3.1	2.9	0.6
TP7	10.4	0.0	0.0	0.0
TP8	18.7	6.4	8.2	6.2

E1	Not Decontaminated		Decontaminated	
	Bench	Shampoo	Bench	Shampoo
TP0	1.9		2.5	
TP1	128.8		12.9	
TP2	72.1		18.7	
TP3	75.7		14.8	
TP4	297.8	50.6	21.7	12.9
TP5	403.0	24.5	47.0	7.8
TP6	304.0	33.7	63.4	29.3
TP7	196.0	21.0	58.2	13.0
TP8	255.0	26.0	45.3	18.9

E2	Not Decontaminated		Decontaminated	
	Bench	Shampoo	Bench	Shampoo
TP0	3.0		2.9	
TP1	103.5		13.0	
TP2	122.3		22.4	
TP3	464.3		82.2	
TP4	492.8	237.0	127.0	87.6
TP5	599.0	162.8	120.5	56.9
TP6	1180.0	124.8	275.5	77.7
TP7	1357.5	60.3	296.3	34.0
TP8	1157.5	56.4	390.8	36.3

E3	Not Decontaminated		Decontaminated	
	Bench	Shampoo	Bench	Shampoo
TP0	2.3		2.7	
TP1	102.3		12.5	
TP2	176.8		31.5	
TP3	153.5		44.2	
TP4	164.0	139.3	27.1	35.0
TP5	201.8	98.8	29.5	19.6
TP6	301.0	80.6	107.0	52.4
TP7	310.0	47.7	101.2	20.0
TP8	453.8	35.4	104.5	23.0

E4	Not Decontaminated		Decontaminated	
	Bench	Shampoo	Bench	Shampoo
TP0	3.3		2.5	
TP1	62.2		12.1	
TP2	42.5		14.7	
TP3	25.9		12.2	
TP4	311.3	27.1	16.8	5.7
TP5	108.5	9.3	10.8	0.0
TP6	153.8	24.4	41.6	18.6
TP7	135.3	6.3	32.4	3.1
TP8	181.0	13.1	49.4	11.2

E5	Not Decontaminated		Decontaminated	
	Bench	Shampoo	Bench	Shampoo
TP0	3.3		2.6	
TP1	42.2		12.4	
TP2	26.4		13.1	
TP3	22.4		9.1	
TP4	88.8	16.6	2.7	1.7
TP5	111.5	2.5	3.4	0.0
TP6	157.0	22.6	53.1	18.4
TP7	92.0	7.1	31.3	2.5
TP8	154.0	11.4	40.2	10.8

AMP
pg/mg hair

U1	Not Decontaminated		Decontaminated	
	Bench	Shampoo	Bench	Shampoo
TP0	10.8		6.7	
TP1	8.5		3.1	
TP2	n/a		n/a	
TP3	17.5		11.7	
TP4	13.4	8.4	6.6	6.6
TP5	9.6	5.2	2.8	2.4
TP6	15.5	9.8	8.9	8.5
TP7	18.5	11.0	10.8	9.4
TP8	12.7	5.7	8.3	6.1

U2	Not Decontaminated		Decontaminated	
	Bench	Shampoo	Bench	Shampoo
TP0	2.5		3.0	
TP1	0.5		0.4	
TP2	n/a		n/a	
TP3	9.0		8.6	
TP4	2.6	2.6	2.9	3.2
TP5	0.0	#DIV/0!	0.0	0.0
TP6	5.8	5.5	5.7	5.7
TP7	8.2	8.1	8.0	7.9
TP8	4.0	3.8	4.1	4.1

U3	Not Decontaminated		Decontaminated	
	Bench	Shampoo	Bench	Shampoo
TP0	23.8		16.7	
TP1	27.1		10.3	
TP2	n/a		n/a	
TP3	33.2		21.4	
TP4	25.7	19.7	14.2	13.1
TP5	27.9	15.8	15.8	7.8
TP6	31.2	16.6	19.4	11.4
TP7	37.8	10.8	19.5	9.3
TP8	30.4	4.7	17.3	4.8

U4	Not Decontaminated		Decontaminated	
	Bench	Shampoo	Bench	Shampoo
TP0	24.2		42.7	
TP1	15.0		7.1	
TP2	n/a		n/a	
TP3	38.6		18.6	
TP4	17.3	12.7	14.0	11.2
TP5	14.2	11.4	7.4	7.6
TP6	25.1	16.8	13.9	10.1
TP7	21.6	13.6	15.8	12.0
TP8	15.3	6.4	15.8	5.6

U5	Not Decontaminated		Decontaminated	
	Bench	Shampoo	Bench	Shampoo
TP0	12.6		9.2	
TP1	9.3		5.8	
TP2	n/a		n/a	
TP3	18.1		13.1	
TP4	13.0	9.0	7.7	6.4
TP5	12.5	4.5	4.7	2.1
TP6	17.6	9.2	10.5	8.1
TP7	19.3	10.1	12.9	9.3
TP8	13.4	5.3	9.5	5.2

U6	Not Decontaminated		Decontaminated	
	Bench	Shampoo	Bench	Shampoo
TP0	5.7		3.4	
TP1	3.1		1.1	
TP2	n/a		n/a	
TP3	10.4		9.3	
TP4	5.4	4.3	3.9	3.7
TP5	2.0	0.5	0.0	0.0
TP6	8.0	6.6	6.4	5.9
TP7	10.9	9.0	8.8	8.3
TP8	6.8	4.4	4.9	4.5

E1	Not Decontaminated		Decontaminated	
	Bench	Shampoo	Bench	Shampoo
TP0	0.0		0.0	
TP1	122250.0		10.5	
TP2	73625.0		89.6	
TP3	7077.5		60.4	
TP4	6540.0	1687.5	78.1	56.8
TP5	6680.0	172.0	79.7	31.5
TP6	6025.0	77.6	82.8	25.7
TP7	5302.5	20.7	99.1	4.8
TP8	5617.5	10.0	49.8	9.0

E2	Not Decontaminated		Decontaminated	
	Bench	Shampoo	Bench	Shampoo
TP0	0.0		0.0	
TP1	205250.0		37.9	
TP2	97200.0		990.5	
TP3	23050.0		694.8	
TP4	27450.0	7617.5	1205.0	752.8
TP5	26300.0	1200.0	636.0	279.8
TP6	21000.0	586.0	1052.5	188.8
TP7	17625.0	83.7	1095.0	54.4
TP8	19550.0	37.8	1059.8	47.9

E3	Not Decontaminated		Decontaminated	
	Bench	Shampoo	Bench	Shampoo
TP0	0.0		0.0	
TP1	94875.0		9.3	
TP2	43575.0		408.0	
TP3	12075.0		500.3	
TP4	14875.0	2765.0	422.3	320.8
TP5	13350.0	457.8	405.5	167.3
TP6	14825.0	259.8	577.3	114.5
TP7	14675.0	85.5	495.5	43.3
TP8	9952.5	22.4	460.0	27.7

E4	Not Decontaminated		Decontaminated	
	Bench	Shampoo	Bench	Shampoo
TP0	0.0		0.0	
TP1	63700.0		8.2	
TP2	47625.0		101.7	
TP3	8225.0		185.8	
TP4	21625.0	1120.0	206.0	86.5
TP5	10407.5	110.4	214.8	45.9
TP6	10850.0	51.6	286.3	28.7
TP7	9927.5	3.7	182.8	1.0
TP8	9530.0	8.8	163.3	8.8

E5	Not Decontaminated		Decontaminated	
	Bench	Shampoo	Bench	Shampoo
TP0	0.0		0.0	
TP1	41975.0		10.8	
TP2	31875.0		194.3	
TP3	7227.5		246.5	
TP4	9852.5	1672.5	202.5	181.5
TP5	6372.5	215.8	157.0	50.6
TP6	7450.0	129.8	295.3	37.3
TP7	8852.5	0.6	234.0	0.2
TP8	4745.0	6.9	170.8	7.5

Heroin
pg/mg hair

U1	Not Decontaminated		Decontaminated	
	Bench	Shampoo	Bench	Shampoo
TP0	0.0		0.0	
TP1	2.2		2.2	
TP2	n/a		n/a	
TP3	3.1		3.3	
TP4	10.8	10.8	10.8	11.3
TP5	0.0	0.0	0.0	0.0
TP6	0.0	0.0	0.0	0.0
TP7	3.9	3.9	3.9	3.9
TP8	7.8	7.5	7.2	8.2

U2	Not Decontaminated		Decontaminated	
	Bench	Shampoo	Bench	Shampoo
TP0	104.5		19.6	
TP1	156.5		17.9	
TP2	n/a		n/a	
TP3	251.5		7.2	
TP4	86.4	31.0	15.0	16.2
TP5	102.6	9.5	6.0	2.8
TP6	109.5	0.6	1.1	0.0
TP7	91.8	5.4	11.3	4.6
TP8	102.6	7.5	11.7	8.1

U3	Not Decontaminated		Decontaminated	
	Bench	Shampoo	Bench	Shampoo
TP0	2605.0		1172.5	
TP1	1982.5		565.0	
TP2	n/a		n/a	
TP3	3217.5		846.3	
TP4	1765.0	668.8	1497.5	415.5
TP5	1940.0	486.5	566.8	273.8
TP6	1967.5	429.0	652.8	189.3
TP7	1720.0	150.3	624.5	85.0
TP8	1712.5	38.9	627.0	28.4

U4	Not Decontaminated		Decontaminated	
	Bench	Shampoo	Bench	Shampoo
TP0	25.8		17.6	
TP1	16.8		8.0	
TP2	n/a		n/a	
TP3	23.1		8.7	
TP4	27.2	17.2	18.9	13.4
TP5	22.9	1.8	8.9	1.0
TP6	6.0	0.0	0.0	0.0
TP7	17.9	4.5	7.4	4.4
TP8	22.6	7.3	10.6	9.8

U5	Not Decontaminated		Decontaminated	
	Bench	Shampoo	Bench	Shampoo
TP0	14.3		14.3	
TP1	3.2		3.0	
TP2	n/a		n/a	
TP3	4.4		3.4	
TP4	11.7	11.8	11.1	11.0
TP5	0.6	0.0	0.0	0.0
TP6	0.0	0.0	0.0	0.0
TP7	4.7	3.9	4.1	3.9
TP8	7.7	7.3	7.4	7.4

U6	Not Decontaminated		Decontaminated	
	Bench	Shampoo	Bench	Shampoo
TP0	19.1		15.4	
TP1	6.9		4.1	
TP2	n/a		n/a	
TP3	4.8		3.7	
TP4	12.0	12.5	11.3	11.5
TP5	0.0	0.0	0.0	0.0
TP6	0.0	0.0	0.0	0.0
TP7	4.9	4.5	4.4	4.2
TP8	8.5	7.7	7.8	7.5

E1	Not Decontaminated		Decontaminated	
	Bench	Shampoo	Bench	Shampoo
TP0	0.0		0.0	
TP1	10525.0		0.8	
TP2	7312.5		41.2	
TP3	919.5		28.1	
TP4	1076.5	556.5	50.1	53.7
TP5	1532.5	261.8	49.9	53.5
TP6	1330.0	198.8	74.4	76.5
TP7	979.8	135.8	62.7	44.3
TP8	1052.0	33.7	28.2	26.7

E2	Not Decontaminated		Decontaminated	
	Bench	Shampoo	Bench	Shampoo
TP0	0.0		0.0	
TP1	18575.0		5.6	
TP2	10462.5		348.8	
TP3	3152.5		201.8	
TP4	4665.0	2345.0	465.0	502.5
TP5	5445.0	1450.0	213.3	322.8
TP6	4215.0	1270.0	528.0	450.8
TP7	3645.0	443.8	426.5	216.8
TP8	3817.5	259.8	474.0	201.0

E3	Not Decontaminated		Decontaminated	
	Bench	Shampoo	Bench	Shampoo
TP0	0.0		0.0	
TP1	7862.5		0.9	
TP2	4745.0		189.5	
TP3	2275.0		214.0	
TP4	2232.5	1016.3	232.5	266.0
TP5	3122.5	817.0	234.3	215.5
TP6	3642.5	712.8	484.8	381.3
TP7	3362.5	377.0	241.5	166.5
TP8	2140.0	207.3	226.5	138.8

E4	Not Decontaminated		Decontaminated	
	Bench	Shampoo	Bench	Shampoo
TP0	0.0		0.0	
TP1	4910.0		0.6	
TP2	4942.5		64.4	
TP3	1715.0		135.0	
TP4	5050.0	785.5	206.0	148.8
TP5	3690.0	510.0	227.8	137.0
TP6	2815.0	368.3	355.3	186.8
TP7	2975.0	159.3	140.8	72.8
TP8	2480.0	66.7	161.5	49.8

E5	Not Decontaminated		Decontaminated	
	Bench	Shampoo	Bench	Shampoo
TP0	0.0		0.0	
TP1	3127.5		1.4	
TP2	2985.0		60.6	
TP3	923.8		92.2	
TP4	1108.8	490.0	88.3	133.0
TP5	1165.0	301.5	65.6	90.7
TP6	1222.5	269.5	172.0	141.0
TP7	1230.0	130.9	80.4	58.4
TP8	826.8	69.9	73.3	37.7

6-AM
pg/mg hair

U1	Not Decontaminated		Decontaminated	
	Bench	Shampoo	Bench	Shampoo
TP0	0.0		0.0	
TP1	0.0		0.0	
TP2	n/a		n/a	
TP3	0.2		0.3	
TP4	0.0	0.0	0.1	0.1
TP5	0.0	0.0	0.0	0.0
TP6	0.3	0.3	0.3	1.3
TP7	0.2	0.1	0.1	0.1
TP8	0.9	0.4	0.4	0.4

U2	Not Decontaminated		Decontaminated	
	Bench	Shampoo	Bench	Shampoo
TP0	58.3		16.9	
TP1	125.3		27.7	
TP2	n/a		n/a	
TP3	157.8		8.2	
TP4	55.8	23.9	10.8	15.4
TP5	104.4	27.8	13.0	11.8
TP6	106.6	24.5	19.3	13.0
TP7	69.3	17.8	19.7	10.4
TP8	119.0	7.3	7.5	4.2

U3	Not Decontaminated		Decontaminated	
	Bench	Shampoo	Bench	Shampoo
TP0	4162.5		3782.5	
TP1	3907.5		1940.0	
TP2	n/a		n/a	
TP3	5032.5		1840.0	
TP4	3047.5	1557.5	3375.0	1665.0
TP5	4107.5	2265.0	1705.0	1082.8
TP6	4200.0	2517.5	2067.5	1305.0
TP7	3667.5	1847.5	2365.0	1367.5
TP8	3435.0	1605.0	1737.5	567.3

U4	Not Decontaminated		Decontaminated	
	Bench	Shampoo	Bench	Shampoo
TP0	78.4		70.2	
TP1	104.5		72.3	
TP2	n/a		n/a	
TP3	122.5		64.3	
TP4	107.6	71.2	109.1	52.1
TP5	215.5	83.5	108.0	55.4
TP6	107.6	78.0	65.1	60.5
TP7	125.5	35.1	61.5	22.5
TP8	117.0	15.0	35.5	9.5

U5	Not Decontaminated		Decontaminated	
	Bench	Shampoo	Bench	Shampoo
TP0	32.7		57.6	
TP1	27.1		25.2	
TP2	n/a		n/a	
TP3	31.8		13.5	
TP4	27.0	17.8	19.9	16.3
TP5	51.9	23.7	17.2	11.0
TP6	38.0	18.2	18.9	13.6
TP7	31.8	14.2	20.8	9.8
TP8	18.7	6.9	8.4	4.9

U6	Not Decontaminated		Decontaminated	
	Bench	Shampoo	Bench	Shampoo
TP0	300.3		283.5	
TP1	325.8		226.5	
TP2	n/a		n/a	
TP3	125.0		81.6	
TP4	91.4	157.3	87.9	166.5
TP5	157.5	198.3	74.1	119.8
TP6	147.0	203.0	104.9	144.3
TP7	144.0	217.8	109.8	103.7
TP8	108.3	120.0	54.8	65.9

E1	Not Decontaminated		Decontaminated	
	Bench	Shampoo	Bench	Shampoo
TP0	0.0		0.0	
TP1	1136.0		0.0	
TP2	1415.0		5.0	
TP3	151.5		5.5	
TP4	335.8	178.0	17.0	22.3
TP5	235.0	47.7	23.6	22.3
TP6	213.8	50.3	15.2	25.2
TP7	224.0	44.1	26.6	26.5
TP8	383.0	25.0	9.8	21.4

E2	Not Decontaminated		Decontaminated	
	Bench	Shampoo	Bench	Shampoo
TP0	0.0		0.0	
TP1	2050.0		1.7	
TP2	1930.0		31.1	
TP3	470.8		41.8	
TP4	1795.0	828.0	137.5	187.5
TP5	931.3	261.0	94.2	165.3
TP6	682.5	268.8	95.5	121.0
TP7	869.5	149.5	175.8	131.3
TP8	1477.5	175.5	158.0	121.5

E3	Not Decontaminated		Decontaminated	
	Bench	Shampoo	Bench	Shampoo
TP0	0.0		0.0	
TP1	640.8		0.0	
TP2	942.3		18.5	
TP3	301.5		40.2	
TP4	808.8	357.3	71.2	97.8
TP5	452.5	150.8	102.8	111.5
TP6	711.8	204.8	73.2	89.3
TP7	779.5	134.8	100.6	114.2
TP8	843.3	127.8	96.9	83.9

E4	Not Decontaminated		Decontaminated	
	Bench	Shampoo	Bench	Shampoo
TP0	0.0		0.0	
TP1	375.8		0.0	
TP2	913.5		6.3	
TP3	292.8		27.7	
TP4	1995.0	232.0	84.9	55.4
TP5	675.0	107.5	99.6	77.4
TP6	737.5	125.0	86.9	68.9
TP7	740.3	86.7	72.9	60.6
TP8	1127.3	64.4	66.0	40.7

E5	Not Decontaminated		Decontaminated	
	Bench	Shampoo	Bench	Shampoo
TP0	0.0		0.0	
TP1	281.8		0.0	
TP2	608.3		6.9	
TP3	97.7		15.2	
TP4	393.5	137.0	30.4	47.8
TP5	178.3	58.1	27.1	48.5
TP6	261.8	78.3	34.5	43.6
TP7	260.5	62.0	41.5	47.0
TP8	376.3	67.4	30.9	32.4

U1	Not Decontaminated		Decontaminated	
	Bench	Shampoo	Bench	Shampoo
TP0	0.2		0.0	
TP1	0.9		0.9	
TP2	n/a		n/a	
TP3	1.0		1.2	
TP4	0.0	0.0	0.0	0.0
TP5	0.7	0.6	0.5	0.6
TP6	1.2	1.2	1.2	1.5
TP7	0.2	0.2	0.2	0.2
TP8	2.6	2.3	2.2	2.3

U2	Not Decontaminated		Decontaminated	
	Bench	Shampoo	Bench	Shampoo
TP0	71.5		34.6	
TP1	98.0		49.8	
TP2	#DIV/0!		n/a	
TP3	131.5		38.0	
TP4	97.1	55.3	39.5	37.4
TP5	102.4	55.4	42.6	37.6
TP6	105.3	51.8	41.5	32.4
TP7	76.1	36.0	32.9	23.5
TP8	140.0	22.2	27.6	18.2

U3	Not Decontaminated		Decontaminated	
	Bench	Shampoo	Bench	Shampoo
TP0	1875.0		912.0	
TP1	2110.0		946.8	
TP2	#DIV/0!		n/a	
TP3	3062.5		1350.0	
TP4	2595.0	1370.0	1317.5	1039.3
TP5	2120.0	1402.5	1255.0	1107.5
TP6	2385.0	1615.0	1272.5	1012.5
TP7	2135.0	1055.0	1127.5	672.8
TP8	2517.5	1365.0	1377.5	783.5

U4	Not Decontaminated		Decontaminated	
	Bench	Shampoo	Bench	Shampoo
TP0	159.0		68.1	
TP1	187.3		143.5	
TP2	#DIV/0!		n/a	
TP3	216.8		157.3	
TP4	248.5	172.5	174.3	140.8
TP5	268.3	188.3	215.8	150.3
TP6	256.3	195.3	179.8	142.7
TP7	221.0	96.1	149.3	68.8
TP8	211.0	49.7	140.8	37.2

U5	Not Decontaminated		Decontaminated	
	Bench	Shampoo	Bench	Shampoo
TP0	93.5		93.1	
TP1	93.3		73.0	
TP2	#DIV/0!		n/a	
TP3	116.8		74.3	
TP4	110.5	74.3	80.4	64.2
TP5	139.8	90.9	93.7	66.2
TP6	126.8	82.0	88.6	63.1
TP7	108.0	56.9	73.6	38.9
TP8	86.8	41.5	57.0	40.8

U6	Not Decontaminated		Decontaminated	
	Bench	Shampoo	Bench	Shampoo
TP0	199.8		124.0	
TP1	198.0		138.8	
TP2	#DIV/0!		n/a	
TP3	148.5		94.4	
TP4	105.5	169.8	75.5	111.3
TP5	128.8	155.8	97.5	137.3
TP6	135.8	153.3	107.5	116.3
TP7	118.3	147.3	82.1	78.9
TP8	123.0	161.0	90.2	106.8

Morphine
pg/mg hair