EVALUATION OF SWEAT AS AN EVIDENTIAL SPECIMEN IN DRUG-FACILITATED SEXUAL ASSAULT

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EVALUATION OF SWEAT AS AN EVIDENTIAL SPECIMEN IN DRUG-FACILITATED SEXUAL ASSAULT

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Abstract: Drug-facilitated sexual assault (DFSA) investigations can be hindered by delayed reporting or victim unwillingness to fully participate in a physical examination. In turn, biological evidence collected to prove incapacitation at the time of the incident can be difficult to obtain. Sweat was evaluated as a specimen type for confirmation of DFSA agents. A methanol extraction paired with qualitative liquid chromatography-tandem mass spectrometry (LC/MS/MS) analysis was developed and validated for the analysis of sweat patches. Eight sedatives that are commonly-encountered in DFSA cases were included in the method panel. In addition, a preliminary study was completed to assess the effectiveness of sweat patches in practical DFSA situations. One subject took 50 mg diphenhydramine and sweat patches were applied upon dosing, after 1 d, and after 3 d to simulate delayed reporting. Sweat patches were compared to urine samples taken at 1 d, 3 d, and 7 d to evaluate differences between specimens. Method validation successfully characterized limit of detection, carryover, interference, matrix effects, and stability. All sweat patch samples from the preliminary study, including those with delayed application, contained detectable diphenhydramine in an amount > 0.75 ng/patch, while only the urine sample taken at 1 d contained > 7.5 ng/mL diphenhydramine. These results suggest that sweat may be more effective than urine for the detection of some DFSA compounds in cases of delayed reporting. As noninvasive and cumulative collection devices, sweat patches may prove to be appealing to both toxicologists and DFSA victims during DFSA investigations.

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CHAPTER I

INTRODUCTION

Drug-facilitated sexual assault (DFSA) is the abuse of drugs or alcohol to perform nonconsensual sexual acts on an incapacitated victim.[1] Legal definitions vary by jurisdiction but generally include a broad spectrum of sexual offenses, ranging from intimate touching to forcible rape. The central characteristic in DFSA is victim incapacitation – a factor which complicates reporting, evidence collection, laboratory analysis, and criminal prosecution. With no universal definition, the term "incapacitation" also lends itself to interpretation, but is typically defined as the inability to express consent due to the influence of drugs or alcohol. DFSA victims describe drug-induced incapacitation as paralysis accompanied by flashes of intermittent consciousness or complete blackout.[1]

Sweat has been identified as a potential sample matrix for the detection of DFSA agents due to its noninvasiveness and potential to extend detection times. This project examines if sweat qualifies as a viable specimen type for toxicological analysis in DFSA investigations. First, a review of literature will give background information about DFSA, describe the drugs to be included in the panel, offer insight about the elution of drugs into sweat, and provide an overview of current DFSA instrumental drug analyses. An extraction procedure and liquid chromatography-tandem mass spectrometry (LC/MS/MS) detection method containing detailed instructions and parameters will then be presented. Data from the method validation and a

preliminary study will be shown. Finally, discussion on the practicality and implementation of sweat patches as a means of DFSA testing are presented.

CHAPTER II

LITERATURE REVIEW

2.1 Background

2.1.1 Drug-Facilitated Crimes

DFSA is just one of many types of drug-facilitated crime (DFC); others include robbery, homicide, kidnapping, mental manipulation, and maltreatment.[2] Although DFSA is believed to comprise of the majority of DFCs in the United States, other DFCs remain prominent in other areas of the world. A French study concluded that only 46% of DFCs were isolated DFSAs and as many as 22% were isolated robberies.[2] Although this research will primarily focus on DFSA, many ideas presented here can be applied to both DFSAs and DFCs due to the inherent similarities of the crimes.

2.1.2 History

Anecdotes of DFCs date to the early twentieth century, most notably with bartender Mickey Finn and the Lone Star Saloon in Chicago, Illinois. Finn was alleged to have spiked customers' drinks with chloral hydrate in order to draw them into an easy theft.[3] DFSAs received little attention until the late 1990s when the notoriety of fast-acting sedatives like flunitrazepam (Rohypnol®) and γ-hydroxybutyric acid (GHB) thrust the crime into the spotlight.

2.1.3 Methods of Administration

Drug administration in DFSA cases can be classified into three categories: surreptitious, voluntary, and misrepresentative.[1] Each method carries unique significance in the laboratory and the courtroom. Surreptitious administration occurs when an offender covertly administers a drug to a victim without the knowledge or consent of the latter. Media and society perpetuate this image as the prototypical scenario of DFSA, yet a U.S. Department of Justice study concluded that only 6 out of 144 sexual assault complainants (4.2%) were given a drug surreptitiously.[4] In contrast, voluntary drug consumption occurs with the intent of intoxication by the victim. The widespread recreational consumption of ethanol has staged the drug to be the most prominent enabler of DFSA and is present in as high as two-thirds of DFSA cases.[5] Finally, misrepresentative administration occurs when an offender lies about a drug that the victim uses voluntarily. Using this technique, an offender can discreetly increase the concentration of an alcoholic drink or falsify the identity of a victim's medication.

2.1.4 Approaches

In combination with the three methods of administration, offenders use one of two general approaches to commit DFSAs: proactive and opportunistic.[6] A DFSA in which the offender has the deliberate intent to incapacitate and assault the victim is considered proactive. Proactive DFSAs are premeditated and more likely to be surreptitious or misrepresentative in nature. In contrast, an opportunistic DFSA occurs when an offender spontaneously takes advantage of a victim's compromised physical or mental state. Opportunistic DFSAs are more commonly associated with voluntary drug use by the victim.

2.1.5 Settings

Parties and nightclubs are publicized as stereotypical settings for DFSAs, but a number of unsuspecting locations can also host the crime. These include offices, homes, schools, health care

facilities, restaurants, and parks.[7] Offenders look to establish and maintain control of the setting, isolate the victim, and ensure drug administration. One law enforcement training manual identified three potential crime scenes for DFSAs: the site where the drug was bought or manufactured, the site where the drug was ingested, and the site where the victim was sexually assaulted.[8]

2.1.6 Prevalence/Reporting

The significance of DFSA lies within the number of incidents – that is, if there exists a proper method of assessment. The prevalence of incapacitation with drugs or alcohol has been cited anywhere from 7 to 35% of all sexual assaults.[4,9] Other experiments claim incidence of drug- or alcohol-positive victim samples as high as 60% in sexual assaults suspected of drug involvement.[10] DFSA is a difficult crime to accurately quantify for several reasons. The lack of standardization among definitions and data collection techniques complicates the application and interpretation of statistical analyses. In addition, it is unclear whether any given surge of cases is due to an actual increase in prevalence or simply a rise in awareness, advances in laboratory research, and/or successful prosecution.[11]

Underreporting also plagues the legitimacy of estimates. This phenomenon, already well-established in traditional sexual assaults, is believed to be even more pervasive in DFSAs. Admitting to voluntary drug use may feel self-incriminating to victims, especially in cases involving illicit drugs or underage users, and discourage reporting. Debilitating residual effects of a drug may endure over time after an assault, preventing the victim from recalling important details about the incident such as the identity of the offender or the timeline of events. These lasting effects may also leave victims in a physical state that renders them unable to report the crime until they have fully recovered.

Psychological sequelae are thought to be large contributors to victims' underreporting of DFSAs. Anterograde amnesia may impede the recollection of critical details about the offender's identity or actions, precluding the ability of the victim to speak out. Victims may feel that their trauma would be re-aggravated by unrelenting interrogation in police interviews, courtroom testimonies, and therapy sessions. The sense of powerlessness experienced during a DFSA may carry over to the hours, days, and weeks following the crime and discourage the victim from pursuing medical treatment or legal action.

In addition to the unique circumstances presented above, DFSA victims share many of the consequences experienced by victims of traditional sexual assaults. Feelings of guilt or self-blame may arise and persuade the victim to second-guess the cause of the assault. Similarly, the victim can downplay the physical or emotional impact of the event. The fear of retaliation or repetition by the offender may persist as a constant threat. Post-traumatic stress disorder, depression, and substance abuse may develop as a result of victimization.[12]

Delayed reporting in DFSA cases presents a multitude of challenges to investigators and forensic scientists. The victim's memory may become unreliable as time elapses after the assault, especially if recollection is impaired by drug effects or emotional trauma. The physical examination becomes less effective as wounds heal or evidence is removed. Toxicological testing loses value if the delay in reporting exceeds a drug's residence within the body.

DFSA victims may also fear accusations of false reporting, a common argument of defense attorneys. False reporting for traditional sexual assaults are estimated to have an incidence rate of 2-8%.[13] The unique circumstances of DFSAs, especially cases involving voluntary drug use, give defense attorneys the opportunity to question a victim's character.

Victim credibility is a major source of debate throughout any sexual assault case. A victim is often the only witness and may carry perceived or actual bias. Drug use can be argued to

link to “risky” behavior, and proof of drug ingestion – even if administered surreptitiously – may taint the victim’s innocence. Cognitive impairment and amnesic effects caused by DFSA drugs may reduce the accuracy of a victim’s statements about the incident in the eyes of a judge or jury.

Even if victims overcome these obstacles, sexual assault cases have infamously low success in the criminal justice system. In fact, two of the main setbacks encountered in sexual assault prosecution are particularly relevant to DFSAs.[14] First, alcohol use by the victim prior to the assault decreases the likelihood that the case will be prosecuted. Second, as the time interval between the assault and the forensic medical examination increases, the likelihood that the case will progress through the legal system decreases. Conclusive evidence of victim incapacitation, such as toxicological evidence, can serve as a strong counterpoint to these attacks.

2.1.7 Sample Collection/Testing

Effective initiatives in forensic nursing are designed to balance comprehensive patient care with proper evidence collection in sexual assault cases. The development of the Sexual Assault Nurse Examiner (SANE) position exemplifies this revolution. SANEs provide sexual assault victims with injury treatment, emotional support, and referral to other aid services. In addition, SANEs supply evidence to police and prosecutors and can testify in court as an expert witness. The implementation of a SANE program within a community is proven to advance sexual assault cases to higher levels of deposition.[14] Still, the absence of information in DFSA cases places a significant weight on evidence that is available and biological evidence can quickly become a high priority.

The use of evidence collection kits is standard in many SANE examinations. The exact content of a kit may vary by jurisdiction, but evidence items generally include blood, oral fluid, head hair, pubic hair, fingernail debris, swabs from genitalia, and clothing. SANEs may also request a urine sample if drug use by the victim is admitted or suspected.

The collection of these evidence types can be invasive and demanding, and victims may refuse some of the samples to be taken. As a result, investigators may be denied a critical piece of information to substantiate the victim's claim. In addition, blood and urine – the traditional sample matrices in which DFSA testing is conducted – are only feasible for drug analysis within hours or days of exposure.[15] If collected at a later time, any remnants of a DFSA dose may be fully metabolized or excreted.

Given the widespread prevalence of DFSA and the relatively low rates of reporting and prosecution, problems like examination invasiveness force SANEs, investigators, and forensic scientists to seek alternative methods for victim support.

2.2 Alternative Sampling

The lack of peer-reviewed research is currently a limitation to alternative specimen assessment. Workplace drug testing, which has many similarities to forensic drug testing, has not yet validated alternative specimen testing to achieve the same level of confidence that a urine test provides. A 2008 publication by the U.S. Department of Health and Human Services stated that additional research is needed for sweat, hair, and oral fluid analysis for the testing to become standalone instead of merely supplemental.[16]

Still, the potential of alternative specimens has caused forensic laboratories to consider developing new methods to utilize their advantages. In fact, the United Nations Office on Drugs and Crime recently published guidelines for sweat, hair, and oral fluid laboratory techniques in an effort to standardize international practices.[17] The restrictions that accompany the analysis of DFSA samples cannot be ignored, but a more detailed investigation into the intricacies of sweat testing reveals that sweat may be a valuable resource for forensic laboratories that conduct DFSA testing.

2.3 Sweat Sampling/Methods

Like other specimens collected for forensic analyses, sweat contains drugs and metabolites that are present in the body. The pathway of a drug's excretion in sweat is not yet completely understood. Factors such as sweat pH, rate of diffusion, and rate of perspiration are thought to affect the volume and composition of excreted sweat.[18] Passive diffusion from capillaries into sweat glands or migration from interstitial fluid to the skin surface are thought to be the primary pathway of drugs' incorporation into sweat.[19,20] The former process should be particularly favorable for lipid-soluble, basic substances due to blood's high pH in relation to sweat. The latter process involves migration across subcutaneous fat and fascia as well as the dermis, epidermis, and stratum corneum, where the compounds would be readily dissolved in eccrine and apocrine secretions. Little is known of many drugs' potential interaction with other components of the skin such as hair follicles, sebaceous glands, nerve fibers, and connective tissue. Overall, as long as the distribution mechanism of drugs in sweat remains a mystery, only limited conclusions can be made on the subject.

Next, sweat must be effectively transferred from the skin to the sweat patch. Sweat patches are typically manufactured with a large adhesive surrounding an absorption pad, similar to a bandage. The area of patch application may affect sweat collection; different areas feature different secretion rates and chemical constituents.[21] Pharmchem Inc., a prominent supplier of sweat patches, suggests application in an area that is free of hair/scars/open wounds, is not exposed to excessive skin flexing or wrinkling, and has minimal contact with clothing.[22] For these reasons, the company advocates the dorsal upper arm as a primary application site and the lower torso or lower back as backup sites.[22] Each of the proposed areas has moderate eccrine sweat gland density and secretion relative to others.[21] Sweat collection with a patch is cumulative during the time of patch wear. Pharmchem recommends a wear time of greater than 24 hours in order to obtain an adequate sample.[22]

The volume of sweat collected with a patch is currently unknown; however, Appenzeller et al used a similar collection device on 25 subjects and collected volumes ranging from 0.45 to 3.8 mL.[23] This study also suggests that sodium may be viable as an internal standard within the sweat sample to accurately quantify other analytes.[23] The possibility of drug transfer from the absorption pad back to the skin has been postulated as a cause for concentration decrease in patches worn for long periods of time.[24] A means to reduce or remove equilibration, such as a one-way absorption mechanism, may prove useful to combat this problem. Uemura et al concluded that toxicological results obtained from sweat patch analysis may be affected length of the collection period, the location of the patch placement on the body, and the potential of drug reabsorption from the patch back to the skin.[25] Despite variable sweat volumes and possible redistribution back onto the skin, potential issues can be reduced with proper use of the patch.

Still, sweat analysis needs to overcome those barriers to become independent of other testing. DFSA victims that wear the patch should expect that the results will hold in court; after all, it may be the only viable item of evidence to prove intoxication at the time of the incident. Therefore, the development of an accurate confirmatory method that boasts detection of several common DFSA-related drugs and metabolites with high sensitivity is vital. To do so, valuable information must be drawn from previously-published methods in sweat analysis.

Research in sweat patch analysis fluctuates considerably in methodology due to the youth of the specialty and the lack of standardization. The first variable is specimen collection. When available, clinical subjects can be recruited to simulate actual patch application, wear, and removal. This approach incorporates many variables of realistic sweat collection but is far less controlled than a pure laboratory experiment. Studies investigating interindividual differences in drug distribution to sweat or potential effects of patch wear would benefit from clinical sampling the most. If subjects are unavailable, researchers may employ the use of artificial sweat that contains an approximate concentration of the most common or most significant compounds in

sweat.[26] In these experiments, drugs are mixed with the artificial sweat and injected into the patch, or the patch is submerged directly into the mixture. Artificial sweat is well-suited for refining method procedures, such as extraction techniques or detection parameters, due to its availability and reproducibility relative to natural sweat.

Patches with either real or artificial sweat then undergo an extraction procedure. This process is completed to isolate the drugs that are to be detected; any extraneous compounds that remain in solution upon analysis may complicate or interfere with the analytes of interest. The most common extraction approach for sweat patches is solid-phase extraction, which separates components of a sample using a column with specific physical and chemical properties.[27] Minor parameters, like the composition of extraction buffer or sequence of column preconditioning, may be modified depending on the goals of the experiment. In a variation on extraction technique, De Martinis et al describe a disk solid phase extraction for amphetamines.[27] Effective extraction is important for a matrix like sweat because samples are typically low in volume and may contain many impurities.

Following isolation of the analytes of interest, gas chromatography-mass spectrometry (GC/MS) and liquid chromatography-tandem mass spectrometry (LC/MS/MS) are the standard methods of analyte separation and detection for confirmatory sweat patch testing. The goal of this portion of the method is to optimize for high sensitivity in anticipation of the low drug amounts that are expected to appear in sweat patches. Quantification of drug concentration in sweat does not currently represent a meaningful correlation to physiological symptoms or concentrations in other biological fluids, but more exact results can potentially lay the foundation for such relationships.

Sweat patch research has been completed for many different classes and types of drugs. Fay et al successfully detected amphetamines in sweat patches by immunoassay and GC/MS.[28] In

this study, one notable finding is that two potential adulterants, tile cleaner and cough syrup, caused a false-positive result by immunoassay. Similar research by Kintz & Samyn and Barnes et al revealed that MDMA and metabolites are present in sweat patches worn by recreational ecstasy users.[29,30] Brunet et al quantified methadone, heroin, cocaine and metabolites in sweat using solid-phase extraction coupled with GC/MS.[31] Huestis et al explored the disposition of Δ^9-tetrahydrocannabinol (THC) in the sweat of cannabis users.[32] Data from this experiment suggested that daily cannabis users remained above 0.4 ng/patch for up to four weeks following cessation and naïve cannabis users administered 14.8 mg THC/day for five consecutive days did not produce a positive sample.

The circumstances of the victim's drug usage may also affect sweat patch analysis. Environmental contamination of drugs present on the skin prior to application of the patch is possible.[33] One-time drug exposure may yield low concentrations in sweat, sometimes below the limit of detection of analytical instruments. Chronic drug exposure, on the other hand, may yield higher concentrations in sweat but complicates the landscape of proving drug use in a specific DFSA incident. Adulteration, another potential source of contamination, is referred only in manufacturer instructions by the visual inspection of the patch upon removal.[22] Perhaps the reason that tampering is not addressed in research is because sweat patch testing is not prevalent; if this specimen type increases in popularity, more attempts will surely to be made to bypass detection.

Regardless of drug type or usage conditions, research that specifically addresses DFSA will provide more practical benefit to toxicologists than interpreting results from general research. Only one method discovered during literature review utilized sweat sampling associated with DFSA. Demoranville & Verkouteren developed a panel including GHB, ketamine, flunitrazepam, cocaine, MDMA, and metabolites.[34] The experiment featured simulated sweat as a specimen

matrix and ion mobility spectroscopy (IMS) for detection. The authors concluded that IMS was a valid initial screen for the analytes under investigation.

Other methods utilize sweat as a specimen type, but do not employ long-term sweat patches as a collection device. Huestis et al evaluated the disposition of cocaine, codeine, and metabolites in sweat using heat-induced stimulation of the palm and torso.[20] Specialized "fast patches", designed for only 30 minutes of wear, were compared against traditional sweat patches. "Fast patches" were found to contain greater concentrations of analytes than traditional sweat patches.

Abanades et al compared a cotton wipe of the armpit and forehead to traditional sweat patch sampling of clinical subjects exposed to GHB.[35] The authors concluded that the variability of GHB concentrations seen in both wipes and patches rendered the analysis unsuitable for practical use. In another study, cotton wipes were used on the forehead to detect MDMA 5 h following administration.[36] Average sweat concentrations remained below 25 ng/wipe. Similarly, Pichini et al administered single doses of MDMA to subjects and compared armpit wipes to sweat patches via immunoassay and GC/MS; the research concluded that both were viable within 24 h of administration, but the authors acknowledged that the results were preliminary for application across a full range of toxicological substances.[24] Sweat wipes have also been explored as a viable sampling technique for drivers under the influence of a drug other than alcohol.[37] Variability and the risk of contamination in uncontrolled wipe studies are believed to be high, especially in cases of repeated dosing or external contact with areas to be wiped.

2.4 DFSA-Related Drugs

To a DFSA offender, an ideal drug to deploy on a victim will be easy to obtain and administer. The drug must sedate the victim quickly and effectively, complete with pharmacological effects to disrupt the victim's ability to resist force or remember the assault.

Many drugs and drug classes meet these qualifications, but only the classes utilized in this research will be described below. Notable omissions include ethanol, marijuana, barbiturates, GHB, ketamine, cocaine, and amphetamines.

Benzodiazepines are a therapeutic drug class that are commonly used as prescription anxiolytics, anticonvulsants, muscle relaxants, pre-anesthetics, and hypnotics.[38,39] Absorption rates of the class are variable; however, activity may be expedited if the drug is already in solution (i.e. mixture into an alcoholic drink).[38] In addition, metabolism is complex and extensive, and the presence or absence of certain metabolites can be different among matrices.[38] Benzodiazepines act as central nervous system (CNS) depressants by binding to receptors on gamma-amino butyric acid ($GABA_A$). Once the drug is bound, the inhibitory effects of $GABA_A$ are potentiated and cause sedation.[38,39] In combination with illicit drugs, benzodiazepines minimize the effects of dangerous highs and withdrawal symptoms.[38,39] This interaction increases abuse potential and establishes the drug class as a popular choice among recreational and chronic users. Rapid onset of action and user symptoms of sedation, amnesia, and impaired judgment are characteristics of benzodiazepines that appeal to DFSA offenders.[39]

Opioids are therapeutic analgesics primarily intended for pain management, but can also serve as surgical anesthetics and cancer treatment medications.[40] Opioids act on opioid receptors in the central and peripheral nervous systems to induce drowsiness, muscle relaxation, and a decreased sensation to pain.[40] Despite strict government regulation, opioids are prevalent and can be obtained from doctor's offices, dental suites, veterinary facilities, hospitals, pharmacies, and the black market. Symptoms of particular interest to DFSA offenders include amnesia, impaired cognitive function, and loss of consciousness.[40] Co-administration of ethanol enhances the sedative effects of many opioids.[41]

Opioids were one of the first drug classes to be tested using sweat patches, done so as early as 1996, and were originally explored to assess practicality in the monitoring of abusers.[42] Huestis et al detected heroin, 6-acetylmorphine, morphine, and codeine in sweat samples from clinical trial subjects at levels of 10 ng/mL using enzyme-linked immunosorbent assay (ELISA) and 5 ng/mL using GC/MS.[43] Similarly, Concheiro et al detected buprenorphine, methadone, heroin, morphine, codeine, and metabolites in sweat as low as 1 ng/patch via LC/MS/MS.[26]

Antihistamines are a class of over-the-counter medications designed to regulate the versatile compound histamine. The role of histamine includes allergic response, fluid secretion in the stomach, regulation of appetite and alertness via the CNS, contraction of muscle cells in pulmonary and gastrointestinal systems, and relaxation of small blood vessels.[44] Drugs in this class are antagonists to histamine receptors located in smooth muscle, cardiac muscle, gastric parietal cells, the CNS, endothelial cells, and hematopoietic cells.[45] An adverse side effect in first-generation histamines is moderate sedation, which has since been corrected in modern formulations. Still, first-generation antihistamines remain available on the open market. From a DFSA perspective, antihistamines are dangerous due to the onset of CNS depression, slowed response, reduced attention, and drowsiness.[44] Anticholinergic effects such as confusion, blurred vision, and lightheadedness can also be demonstrated after antihistamine use.[45] Interaction with ethanol, benzodiazepines, and other depressants is additive and intensifies sedation.[45] The extensive availability, hypnotic pharmacological response, rapid onset of activity (15-60 min), and moderate duration of action (4-8 h) primes antihistamines for use in DFSAs.[45]

Antidepressants are medications prescribed by clinicians to treat mental conditions like depression or panic disorders. Tricyclic antidepressants (TCAs), one of the oldest subgroups of the drug class, are the most relevant to DFSA. TCAs exert their intended effect by inhibiting reuptake of serotonin and norepinephrine but also interact with adrenergic, muscarinic, and histamine receptors to produce unwanted side effects on a variety of physiological systems.[46]

TCAs slow the depolarization of cardiac action potentials, ultimately leading to hypotension and cardiac arrhythmia.[41] Anticholinergic effects such as blurred vision and cognitive impairment can manifest.[41] CNS effects include confusion, hallucinations, and drowsiness.[46] In the presence of ethanol, TCA concentration in blood is found to be markedly increased.[46] TCAs generally have a long onset of action (4-8 h), but DFSA offenders can counteract this phenomenon by deploying a large single dose or exploiting interaction with another sedative.[46]

"Z-drugs" (zolpidem, zopiclone, and zaleplon) are novel sedatives designed to treat short-term insomnia. Z-drugs have pharmacology similar to benzodiazepines but bind more selectively to the $GABA_A$ sites that regulate sleep induction.[47-49] The primary effect of Z-drugs is sedation but hallucinations, indigestion, headache, and nausea have also been reported.[48] The use of some Z-drugs causes significant residual effects such as a hangover.[47] The ability of Z-drugs to induce and maintain a strong sedation along with the amnesic properties of the class make them attractive candidates for DFSA offenders. Z-drugs have been successfully quantified in blood, urine, oral fluid, and hair, but have not yet been explored in sweat.[50] Leloup et al published an LC/MS/MS method for the detection of benzodiazepines and Z-drugs in blood, urine, and hair.[51]

Drug deployment in DFSA is not limited by class or desired effect; in theory, any drug that exerts a significant impact on one's mind or body can qualify. In 2014, the Society of Forensic Toxicologists issued a list of recommended detection limits for as many as 104 drugs and metabolites that were commonly encountered in DFCs.[52] Adamowicz & Kala published a GC/MS method that screens for 128 DFC-related compounds in urine.[53]

2.5 Conclusion

Alternative specimen types are being explored for unique or specific applications. One such application is the deployment of sweat patches to detect drugs of abuse. Drug disposition into sweat is currently under investigation, but recent discoveries lend insight into possible

mechanisms of interaction. Volume variability, drug reabsorption, and environmental contamination remain as possible flaws in an otherwise effective collection device. Common analytical techniques are customized to address the unique characteristics of the matrix. Sweat patches have proven successful for the detection of numerous drug classes thus far, leading to the hypothesis that DFSA sedatives will be no different. An additional element to the current research – a preliminary study with a focus on DFSA – provides a novel and practical application to expand the knowledge base of the subject.

CHAPTER III

METHODOLOGY

3.1 Introduction

This section outlines the validation and preliminary study protocols used in this research. Descriptions of standard preparation, extraction technique, and instrument setup are provided. Validation requirements for a qualitative method are defined and applied to the current project. Management of preliminary study sampling is detailed. Procedures were developed with input from the Federal Bureau of Investigation Laboratory.

3.2 Materials

The materials required for this experiment include reference standards, sweat patches, reagents, and miscellaneous lab supplies. All reference standards were obtained from Cerilliant® (Round Rock, TX). Sweat patches are Pharmchek® Sweat Patches obtained from PharmChem, Inc. (Fort Worth, TX). Chemicals, laboratory-grade reagents and miscellaneous laboratory supplies were obtained from VWR International® (Radnor, PA). SMx™ blank sweat and blank urine were obtained from UTAK Laboratories (Valencia, CA).

3.3 Drug Panel

Eight analytes were chosen by the research team as prominent or emerging drugs in current DFSA casework. The panel contains three benzodiazepines (alprazolam, clonazepam,

diazepam), two opioids (fentanyl, hydrocodone), one antihistamine (diphenhydramine), one antidepressant (nortriptyline), and one Z-drug (zolpidem).

Solutions containing all analyte compounds were produced from certified reference standards in methanol at high, medium, and low concentrations. When used for method validation or comparison for preliminary samples, control solutions were added to the absorbent pad of the sweat patch at a volume of 100 μL. The amount of each analyte following 100 μL addition of control solution to a patch is also displayed in Table 1, listed in ng/patch.

Table 1. Analyte Amounts Per Patch Upon Addition of 100 μL Control Solution

Analyte	**High Control (ng/patch)**	**Medium Control (ng/patch)**	**Low Control (ng/patch)**
Alprazolam	100	10	3
Clonazepam	25	2.5	0.75
Diazepam	100	10	3
Diphenhydramine	25	2.5	0.75
Fentanyl	100	10	3
Hydrocodone	25	2.5	0.75
Nortriptyline	25	2.5	0.75
Zolpidem	25	2.5	0.75

Deuterated internal standards were added to controls and samples to minimize variances in analysis processes. One deuterated internal standard was included for each respective analyte, displayed below in Table 2. A stock internal standard mix was produced in methanol at a concentration of 1 μg/mL for each internal standard.

Table 2. Analyte and Internal Standard Pairings

Analyte	Internal Standard
Alprazolam	Alprazolam-D5
Clonazepam	Clonazepam-D4
Diazepam	Diazepam-D5
Diphenhydramine	Diphenhydramine-D3
Fentanyl	Fentanyl-D5
Hydrocodone	Hydrocodone-D6
Nortriptyline	Nortriptyline-D3
Zolpidem	Zolpidem-D6

3.4 Extraction

Sweat patches were placed in a fume hood to air-dry for 30 min. The absorbent pad was removed from each patch using disposable tweezers, folded, and placed into a 7 mL test tube. 50 μL of internal standard mix was added to each test tube. Four mL of methanol was then added to each test tube. Test tubes were briefly hand-shaken and inverted to ensure complete saturation of all areas of the patch. Using an automated 5 mL glass pipette, 2.5 mL of solution was withdrawn from each test tube and deposited into another test tube. Test tubes containing patches and remaining solutions were discarded.

Withdrawn solutions were placed in a sample concentrator using nitrogen gas at 40°C until solutions had completely evaporated. Samples were then reconstituted with 150 μL of solution containing 65% aqueous mobile phase and 35% organic mobile phase (described in Section 3.5). Reconstituted solutions were mixed, vortexed, and transferred to 1 mL LC/MS/MS injection vials with plastic inserts.

3.5 Instrument Parameters

All samples were run on a Shimadzu 8040 LC/MS/MS system equipped with 2 LC-20AD solvent pumps, an SIL-20AC HT autosampler, a CTO-20A column oven, a CBM-20A control module, an FCV-20AH2 diverter valve, and a triple quadrupole mass spectrometer.. Aqueous mobile phase, consisting of 2 mM ammonium formate and 0.1% formic acid in water, and organic mobile phase, consisting of 2 mM ammonium formate and 0.1% formic acid in methanol, were used in gradient to move the samples through the column at a total flow rate of 0.35 mL/min. The column used was a Restek® 2.7 μm Raptor™ 50 x 2.1 mm column with an attached guard cartridge to increase durability. The mass spectrometer used electrospray ionization and operated in positive ion mode. Injection volume of each sample was 10 μL.

Multiple reaction monitoring (MRM) was used to enhance detection and identification of precursor and product ions. Retention times and two sets of MRM transitions were individually optimized for each analyte and internal standard and are displayed in Table 3.

Table 3. Analyte and Internal Standard Retention Times and MRM Transitions

Analyte/Internal Standard	Retention Time (min)	MRM Transitions (m/z)	
		Primary	Secondary
Alprazolam	3.697	309.30>281.00	309.30>205.00
Clonazepam	3.463	315.80>270.00	315.80>214.00
Diazepam	3.763	284.90>193.00	284.90>153.95
Diphenhydramine	3.066	255.90>167.00	255.90>165.05
Fentanyl	3.156	336.95>105.00	336.95>188.10
Hydrocodone	2.577	299.90>198.90	300.00>171.00
Nortriptyline	3.291	263.85>233.05	263.85>91.10
Zolpidem	3.126	307.90>235.10	307.90>219.00
Alprazolam-D5	3.691	314.10>285.95	314.10>210.00
Clonazepam-D4	3.457	320.10>274.10	320.10>217.90
Diazepam-D5	3.755	289.90>153.95	289.90>227.10
Diphenhydramine-D3	3.080	259.00>167.00	258.90>152.00
Fentanyl-D5	3.152	341.85>105.10	341.85>137.10
Hydrocodone-D6	2.564	306.20>202.00	306.30>173.90
Nortriptyline-D3	3.291	266.95>233.00	266.95>91.00
Zolpidem-D6	3.127	314.20>235.10	314.00>236.10

3.6 Validation

Validation procedures are aligned with the Scientific Working Group on Forensic Toxicology (SWGTOX) Standard Practices for Method Validation in Forensic Toxicology guidelines for qualitative confirmation/identification.[54] SWGTOX guidelines do not establish exact specifications for qualitative methods; instead, the document passes responsibility to the laboratory to determine what is acceptable or significant.[54] Definitions of validation parameters in the following sections were derived from those within the SWGTOX document. Methanol was used in place of sample matrix for all procedures except for matrix effects studies due to lack of availability.

3.6.1 Limit of Detection

The limit of detection (LOD) is defined as is the lowest concentration of an analyte that can be reliably differentiated from blank matrix and identified by the analytical method.[54] SWGTOX guidelines allow for an administratively-defined decision point to be selected for qualitative methods, even if a lower LOD is analytically possible.[54] Low control solution was selected as the decision point for this portion of the validation. Low control solution was added to three separate unused patches at a volume of 100 µL per patch. Patches underwent the full extraction procedure and were injected in triplicate to ensure that all detection and identification criteria were met. Precision was calculated to determine variance among injections.

3.6.2 Carryover

Carryover is defined as the appearance of unintended analyte signal in subsequent samples after the analysis of a positive sample.[54] There was no possible cause of carryover during the extraction procedure as all items throughout the process were disposable. Therefore, only the LC/MS/MS was evaluated for carryover. High control solution at a volume of 100 µL and internal standard mix at a volume of 50 µL were added to a microcentrifuge vial. The concentrated solution was vortexed and transferred to an LC/MS/MS injection vial for analysis. The concentrated solution was injected three times, with each control injection followed immediately by a blank methanol injection.

3.6.3 Matrix Effects

Properties of a matrix itself may interfere with extraction and detection. SMx™ artificial blank sweat, which contains 17 biological compounds in concentrations approximate to those in real sweat, was used to evaluate matrix effects. One mL of artificial blank sweat was added to an unused patch. In addition to the artificial sweat, a sweat patch with a wear time of 24 hr was

obtained from the preliminary study subject before dosing. The patches underwent the full extraction procedure and were analyzed to interpret potential matrix effects.

3.6.4 Stable-Isotope Internal Standard Interference

Isotopically-labeled internal standards may contain non-labeled compounds as an impurity or have equivalent mass-to-charge ratios as ions of non-labeled compounds. In addition, non-labeled compounds may have similar effects on labeled internal standards. Stock internal standard mix was added at a volume of 50 µL to methanol at a volume of 100 µL in a microcentrifuge tube and vortexed. The solution was then transferred to an LC/MS/MS vial and analyzed for the presence of analyte ions. In a different microcentrifuge vial, 100 µL of high control solution was added with 50 µL of methanol and vortexed. The solution was then transferred to an LC/MS/MS vial and analyzed for the presence of internal standard.

3.6.5 Analyte Interference

Interference of other commonly encountered compounds – including over-the-counter, prescription, and illicit drugs – is assessed to ensure that analytes in the method remain unaffected. A mixture of 64 compounds was produced in methanol at a concentration of 1000 ng/mL of each compound. Medium control solution was added to four unused patches at a volume of 100 µL. Interference mix was added to two separate patches at a volume of 100 µL (for a total amount of 100 ng/patch of each interferent); the remaining two patches served as controls free of interference. All patches then underwent a full extraction procedure and LC/MS/MS analysis to interpret analyte interference. Each patch was injected in duplicate. Compounds in the interference mix are listed in Table 4.

Table 4. Interference Compounds

6-acetylmorphine	Doxepin	JWH-200	Norhydrocodone
7-aminoclonazepam	Doxylamine	JWH-250	Norpropoxyphene
Acetaminophen	Duloxetine	MDA	Oxazepam
AM2201	Ethyl glucuronide	Meperidine	Oxycodone
Amitriptyline	Ethyl sulfate	Meprobamate	Pentazocine
Amobarbital	Fluoxetine	Methamphetamine	Peroxetine
Amphetamine	Flurazepam	Methaqualone	Propoxyphene
Benzoylecgonine	Heroin	Methcathinone	R-pseudoephedrine
Brompheniramine	HU-211	Methylphendiate	Salicylic acid
Caffeine	Ibuprofen	Midazolam	Secobarbital
Carbamazapine	Imipramine	Naloxone	Tapentadol
Carisoprodol	JWH-018	Naltrexone	Trazodone
Chlorpheniramine	JWH-019	Naproxen	UR-144
Cocaine	JWH-073	Norbuprenorphine	Venlofaxine
Cotinine	JWH-081	Nordiazepam	Zaleplon
CP 47, 497	JWH-122	Norfluoxetine	Zolpiclone

3.6.6 Storage Stability

The evaluation of sample stability in various conditions simulates what may be encountered in the processing of actual evidence. Medium control solution was added to seven unused patches at a volume of 100 µL per patch. One patch was immediately extracted and injected to serve as a reference. Remaining patches were placed into large test tubes. Two patches were stored in a freezer (-15°C), two patches were stored in a refrigerator (5°C), and two patches were left in the laboratory at room temperature (20°C). One patch from each storage condition was removed, extracted, and analyzed after 3 d. The remaining patches were removed, extracted, and analyzed after 7 d. This process was duplicated for patches containing low control solution.

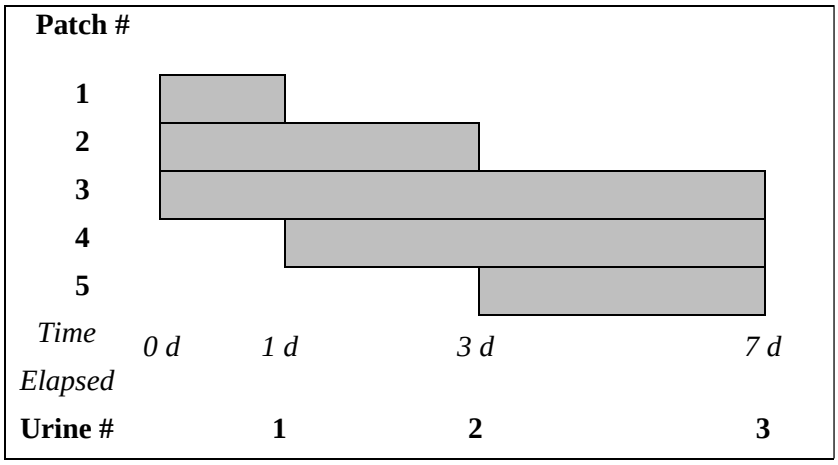
3.6.7 Autosampler Stability

In practice, samples may be reinjected from an autosampler in the hours and days after its initial run; therefore, an evaluation of sample stability in this environment is also practical to forensic laboratories. Medium control solution was added to an unused patch at a volume of 100 µL. The patch underwent a full extraction and was injected immediately to serve as a reference. The sample was left in the autosampler at 4°C after the injection and was reinjected every 24 h for 7 d.

3.7 Preliminary Study

Diphenhydramine at a dose of 50 mg was administered to a naïve subject following the submission of a pre-dose urine and sweat sample. A total of five patches were applied and removed at various times throughout the course of one week to simulate real-world use. Three unused sweat patches were applied to the subject's upper right arm immediately after dosing. After 1 d and 3 d, one of the three original patches was removed and an unused patch was applied. After 7 d, all three remaining patches were removed. Urine samples were taken at 1 d, 3 d, and 7 d after dosing and extracted using a method validated by the Oklahoma State University Forensic Trace & Toxicology Laboratory. Patch application and removal time frames as well as urine sampling times are displayed in Figure 1. All patches were applied and removed using the patch manufacturer's application recommendations. Once patches were removed, they were immediately extracted alongside a low control sample for comparison. Urine samples were compared to a urine control sample containing 7.5 ng/mL diphenhydramine. An exemption was obtained by the Oklahoma State University Institutional Review Board to complete this portion of the research, as a study with only one subject does not meet the generalizable knowledge component under the definition of human subject research.

Figure 1. Subject Patch Application and Removal Time Frames and Urine Sampling Times



3.8 Data Analysis

Post-run data analysis was completed using Shimadzu LabSolutions software in Browser mode. Software was configured to automatically integrate and identify potential positive responses. All data was examined manually for the verification of true positives and negatives. Analyte identity was confirmed using the area ratios of prominent precursor and product ions. Analytes will be reported as positive if the calculated concentration is above the analyte's LOD; a concentration below the analyte's LOD will be reported negative. Identification criteria include the elution of a peak within ± 5 % of expected retention time and the presence of precursor-product ion ratios within ± 20 % of optimized values.

CHAPTER IV

RESULTS/DISCUSSION

4.1 Validation Data

Although lower LOD values were potentially achievable for some analytes, method LODs were set at the amounts present in low control patches to ensure proper detection and identification of all analytes. Retention time and precursor-product ion ratios were met for all nine injections. The imprecision values of each of the eight analytes in the method are displayed in Table 5.

Table 5. Analyte Imprecision in Limit of Detection Controls

Analyte	Imprecision
Alprazolam	4.57%
Clonazepam	5.50%
Diazepam	2.15%
Diphenhydramine	3.66%
Fentanyl	5.48%
Hydrocodone	2.27%
Nortriptyline	2.51%
Zolpidem	2.90%

Potential carryover responses were subject to identification criteria and were compared to LOD responses to assess the potential for a false positive. No carryover responses met identification criteria for any analyte in any of the three blank methanol injections.

Matrix effects were evaluated using artificial sweat and blank sweat from the preliminary study subject. The patch with artificial sweat did not behave in a similar manner to patches with real sweat during extraction and displayed response suppression for some internal standards during detection. In addition, matrix effect responses from artificial sweat exceeded 20% of the LOD for only one analyte, clonazepam, but did not meet identification criteria for any analyte. Matrix effects of blank sweat obtained from the preliminary trial did not exceed 20% of the LOD or meet identification criteria for any analyte.

Potential interferent responses of deuterated internal standard precursor or product ions into analyte precursor and product ions, or vice versa, were subject to identification criteria. Interferent areas were then compared to areas of the expected analyte or internal standard. Only one analyte (clonazepam) displayed interference with its respective internal standard, generating an internal standard area that was 0.3% of the analyte area. Only one internal standard (fentanyl-D5) displayed interference with its respective analyte, generating an analyte area that was <0.1% of the internal standard area. All other potential interferent peaks of deuterated internal standard ions into analyte ions, or vice versa, did not meet identification criteria.

Potential interferent responses from patches fortified with other commonly-encountered toxicological compounds (see Table 4) were subject to identification criteria and compared to those of unfortified controls. Ratios of analyte area to internal standard area were then compared among interferent and non-interferent patches. In patches fortified with interference mix, retention time and precursor-product ion ratio identification criteria for all analytes and internal standards were met. However, area ratios varied from those at the same control concentration without interference mix. Area ratio interference is displayed in Table 6 as a percentage change from the unfortified control. In Table 6, a positive value indicates suppression of the target analyte and a negative value indicates enhancement.

Table 6. Analyte Interference from 64 Commonly-Encountered Toxicological Compounds

Analyte	Interference
Alprazolam	25 %
Clonazepam	19 %
Diazepam	20 %
Diphenhydramine	23 %
Fentanyl	25 %
Hydrocodone	17 %
Nortriptyline	-40 %
Zolpidem	22 %

Storage stability was assessed by first ensuring that analyte responses in each sample met identification criteria. Ratios of analyte area to internal standard area in controls under the various storage conditions were then compared to the reference control. Identification criteria were met for each analyte in all stability samples. Area ratios of controls under various storage conditions are displayed in Table 7 for medium control patches and Table 8 for low control patches as percentages compared to the respective reference patch. The largest decay indicated is 30% but most are below 25%.

Table 7. Storage Stability of Medium Control Sweat Patches at -15°C, 5°C, and 20°C

Storage Temperature	**Storage Time**	**Alprazolam**	**Clonazepam**	**Diazepam**	**Diphenhydramine**
-15°C	3 d	112 %	101 %	112 %	105 %
	7 d	110 %	104 %	107 %	103 %
5°C	3 d	120 %	105 %	114 %	107 %
	7 d	114 %	104 %	111 %	104 %
20°C	3 d	101 %	87 %	97 %	90 %
	7 d	115 %	99 %	113 %	100 %
Storage Temperature	**Storage Time**	**Fentanyl**	**Hydrocodone**	**Nortriptyline**	**Zolpidem**
-15°C	3 d	100 %	108 %	111 %	108 %
	7 d	108 %	107 %	99 %	100 %
5°C	3 d	109 %	114 %	113 %	113 %
	7 d	111 %	110 %	101 %	100 %
20°C	3 d	92 %	93 %	91 %	93 %
	7 d	113 %	111 %	92 %	98 %

Table 8. Storage Stability of Low Control Sweat Patches at -15°C, 5°C, and 20°C

Storage Temperature	**Storage Time**	**Alprazolam**	**Clonazepam**	**Diazepam**	**Diphenhydramine**
-15°C	3 d	89 %	86 %	89 %	86 %
	7 d	98 %	98 %	95 %	96 %
5°C	3 d	93 %	84 %	91 %	86 %
	7 d	86 %	87 %	84 %	80 %
20°C	3 d	98 %	89 %	93 %	86 %
	7 d	88 %	81 %	85 %	78 %
Storage Temperature	**Storage Time**	**Fentanyl**	**Hydrocodone**	**Nortriptyline**	**Zolpidem**
-15°C	3 d	91 %	89 %	89 %	88 %
	7 d	103 %	98 %	94 %	90 %
5°C	3 d	93 %	88 %	87 %	90 %
	7 d	90 %	84 %	78 %	77 %
20°C	3 d	97 %	91 %	84 %	89 %
	7 d	92 %	85 %	70 %	74 %

Autosampler stability responses were subject to identification criteria. Ratios of analyte area to internal standard area in each of the daily autosampler injections were then compared to that of the initial injection. Responses for each analyte in daily injections are displayed in Table 9 as percentages of the initial injection area ratio. These data show that the analytes are stable in the autosampler.

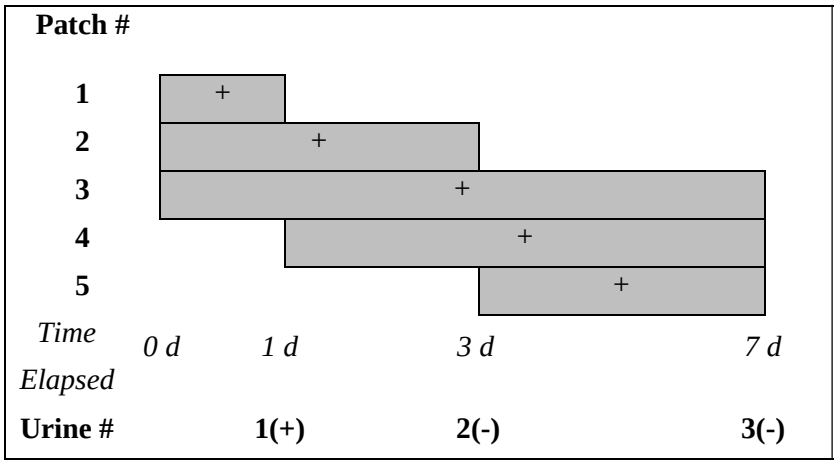
Table 9. Autosampler Stability of Medium Control Sweat Patches at 4°C

Storage Time	Alprazolam	Clonazepam	Diazepam	Diphenhydramine
1 d	2%	-8%	0%	-3%
2 d	1%	-6%	-2%	0%
3 d	-3%	1%	-4%	-5%
4 d	2%	1%	-3%	-7%
5 d	0%	-6%	-2%	-3%
6 d	1%	-3%	-4%	-3%
7 d	-2%	-5%	-4%	4%
Storage Time	**Fentanyl**	**Hydrocodone**	**Nortriptyline**	**Zolpidem**
1 d	8%	4%	-6%	-5%
2 d	3%	2%	-5%	-2%
3 d	8%	0%	-7%	-9%
4 d	8%	1%	-9%	-14%
5 d	-3%	3%	-5%	-3%
6 d	6%	3%	-7%	-6%
7 d	-11%	1%	-3%	-3%

4.2 Preliminary Study Data

The urine and sweat samples obtained from the subject prior to dosing were negative for all eight analytes in the panel. All five preliminary study sweat samples met identification criteria for diphenhydramine and registered an area ratio response greater than that of the LOD of 0.75 ng/patch. Only urine sample 1, taken 24 hr after dosing, met identification criteria for diphenhydramine and registered an area ratio response greater than that of the 7.5 ng/mL control sample; subsequent samples were negative. Results are displayed in Figure 2; a plus sign (+) indicates a detected response above the LOD or control sample and a minus sign (-) indicates a detected response below the LOD or control sample.

Figure 2. Urine and Sweat Preliminary Study Data



4.3 Discussion

The extraction method described here is fairly straightforward and would be simple for an equipped forensic laboratory to implement. Few solutions are required for the extraction, but control solutions may be a challenge to make; blank sweat is difficult to obtain and artificial sweat may not accurately represent the physical and chemical properties of real sweat. Large batches are possible without much extra work, time, or resources per sample. The procedure does consume a moderate amount of methanol, but produces little waste for disposal due to the evaporation step. The destructive nature of the extraction process does not allow for a preliminary presumptive test unless part of the patch is cut or removed beforehand.

The qualitative LC/MS/MS method demonstrates high precision, sensitivity, and specificity. The administratively-defined cutoffs of 0.75 ng/patch for clonazepam, diphenhydramine, hydrocodone, nortriptyline, and zolpidem, and 3 ng/patch for alprazolam, diazepam, and fentanyl demonstrated identifiable responses and good precision. Carryover was not present in blank samples following the injection of concentrated controls.

There was no prominent interference between deuterated internal standards and the respective analytes; however, interference was indicated with other commonly-encountered analytes. Although identification criteria were still met for all analytes, seven of the eight analytes experienced response suppression; the remaining compound, nortriptyline, experienced enhancement. These response changes can be attributed to increased or decreased ionization of target analytes caused by the interferent compounds. Specificity against common toxicological compounds was sufficient, but further method optimization to address interference should be conducted before the method is introduced in a forensic setting – especially if results are quantified.

Formal statistical comparison of stability effects and post-extraction degradation were not completed for these data sets due to the absence of explicit standards within the SWGTOX guidelines, but the variance displayed among controls in both studies are approximate to normal laboratory variance. Provided that samples are promptly stored in a refrigerator or freezer within a reasonable time frame and tested within days after extraction, stability of the analytes in the method does not present an issue. Overall, the methanol extraction and qualitative LC/MS/MS detection were successful. A quantitative method would be preferred if more definitive results are desired in future studies.

The outcomes from the preliminary study samples are perhaps the most important findings of this research. The positive detection of diphenhydramine in patches applied immediately after dosing was somewhat expected; however, positive detection in patches that were applied after a time delay was surprising. The cumulative sampling effect of patches appeared to influence presence of the analyte. Noting the substantial role that elapsed time plays in toxicological sample collection, this result warrants a meaningful conclusion: a noninvasive sampling technique was successful in determining the presence of a DFSA agent in a scenario similar to a delayed reporting.

The comparison of diphenhydramine presence in sweat and urine samples further maintains the use of sweat as a specimen type for DFSA compound analysis. The urine sample collected three days after dosing displayed a detector response that met identification criteria, but did not exceed the LOD of 7.5 ng/mL. In contrast, the sweat patch that was applied three days after dosing was positive for diphenhydramine. One important factor to note is that a hydrolysis step was included in the urine extraction to convert phase II metabolites back to the parent compound, but no other diphenhydramine analogues or metabolites were included in this study. Still, these data suggest that sweat is actually more effective than urine at indicating the presence of diphenhydramine after some time has elapsed. Once again, the results generate the meaningful conclusion that noninvasive sweat patches are successful indicators of a DFSA sedative.

CHAPTER V

CONCLUSION

DFSA prevalence cannot be accurately gauged and is likely underestimated. A clear definition of DFSA usable by all jurisdictions would establish a strong foundation for assessment. The approaches and methods of administration used by offenders can be examined to discover trends in drug acquisition and deployment. Similarly, studying DFSA behavior in traditional and unexpected settings can shed light on measures for prevention. Victim advocacy should be supported to make reporting as easy as possible and ensure that proper treatment is being given. Valuable evidence must be collected without exhaustive invasion to make allowances for the unique vulnerabilities of DFSA victims.

Currently, sweat patches are best suited as supplemental to other forms of confirmation testing, such as urine testing, in a DFSA setting. Although research has proved the emergence of drugs in sweat and manufacturers have designed a fairly efficient mechanism of sample collection, the nuances of the patch – such as external contamination, variability in sample volume, difficulty of quantitation, and lack of procedure standardization – preclude the device from being reliably standalone.

However, research can be completed to improve the utility of the patch. The mechanisms and pathways of drug distribution from the bloodstream to sweat glands and exterior layers of the skin must be investigated to better understand the various cofactors behind drug and metabolite

migration. Patches must be designed to facilitate movement and retention of sweat without reabsorption of analytes back onto the skin. Method development and optimization could expand the analyte panel to include more drug and metabolite compounds. Finally, research establishing a correlation of sweat concentration to blood concentration, urine concentration, or physiological symptoms must be conducted to give significant meaning to quantitation.

As this specimen type builds momentum with future research, standardization of methods and practices will be vital in maintaining consistency across industry and academia. In addition to the collection procedures mentioned earlier, other areas of laboratory analysis – such as sample extraction, instrument settings, and data interpretation – should be optimized specifically for sweat patches in order to obtain the highest level of accuracy, precision, and sensitivity for analyte detection. Steps taken by manufacturers like Pharmchem and international organizations like the U.N. Office on Drugs and Crime serve as solid foundations to establish more uniformity in the testing of these devices. If laboratories can prove to handle sweat patches effectively from sample collection to data analysis, the device will gain significant credibility toward independence.

This research demonstrated an effective qualitative method for the extraction and detection of eight common drug-facilitated sexual assault agents from sweat patches. In addition, one of the drug compounds – diphenhydramine – was detected in sweat after patch application in a preliminary study. As noninvasive and cumulative collection devices, sweat patches demonstrate characteristics that appeal to toxicologists and DFSA victims alike. Using trusted laboratory techniques, toxicologists should continue to work towards innovative applications in solving complex challenges to better serve law enforcement, the research community, and society as a whole.

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