Identification and Quantitation of Compounds in the Blood of Illicit Drug Users Presenting to an Emergency Department

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Abstract

This study examines blood drug concentrations (BDC) in a population of individuals with substance use disorder that presented to the UC Davis Health emergency department (ED) in Sacramento, California. 116 remnant whole blood samples in BD Vacutainer REF 367861 lavender top tubes with K_2EDTA 7.2 mg from routine medical testing were collected. The data accounts for higher BDC and drug combinations found in active recreational drug users, which are not often addressed in clinical settings. Using liquid chromatography-tandem mass spectrometry to quantify for 37 drugs in whole blood, the study compares analytical drug detection with self-reported drug use. Methamphetamine, fentanyl, and cocaine use were the most frequently detected drugs, with methamphetamine having the greatest agreement between analytical results and self-reported use. The role of pharmacokinetic variability and drug tolerance in complicating BDC interpretation is highlighted in this study. The findings represent an effort to improve the interpretation of BDC in the context of forensic toxicology by providing data on age, sex, observations on ED admission, self-reported drug use, and detected drug concentrations.

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1. Introduction

Forensic toxicologists play a pivotal role in criminal and non-criminal investigations by determining blood drug concentrations (BDC) in post-mortem samples collected during death inquiries and in blood samples obtained from living individuals, such as for impaired driving or drug-facilitated offenses. BDC are evaluated to determine if the concentration of drug(s) present plays a role in the investigation.[1]

The interpretation of BDC is complex. Difficulty in interpretation originates from interindividual variability in pharmacokinetics, pharmacodynamics, the presence or absence of tolerance, as well as observed trends in polydrug use.[2] The establishment of universal thresholds for drug toxicity is likely impossible. This necessitates the study of pharmacokinetic and pharmacodynamic models to understand the general relationship between drug concentration over time and tolerance.[3,4] Forensic toxicologists rely on such studies to gain insight into concentrations of drugs observed in a variety of scenarios. Clinical observations are useful in comparing the BDC of patients receiving similar doses, but they are not always directly relevant. These studies do not address scenarios where higher doses are administered or abused and the use of unregulated drugs. Consequently, there is a lack of sufficient data on the BDC observed in individuals who are tolerant and experience significantly higher concentrations that are ethically not attainable in clinical settings.[2] There is a lack of dosing studies for illicit drugs and therefore there exists insufficient data and research on the typical concentrations detected in drug users. Moreover, the presence of multiple drugs in drug users, whether intentional or unintentional, must be taken into consideration.[5] This thesis aims to address these challenges, providing accurate data on the BDC observed in drug users to better assist forensic toxicologists.

According to the Substance Abuse and Mental Health Services Administration, in 2022, the landscape of drug abuse and related medical emergencies highlighted the prevalence of opioids and other substances. Opioids, a class of drugs widely used for their analgesic properties with potential for chronic abuse and

tolerance, were the second most commonly reported drugs in drug-related Emergency Department (ED) visits across the United States. This class of drugs includes prescription opioids, namely morphine, fentanyl, oxycodone, codeine, hydrocodone, as well as heroin and synthetic opioids. Along with opioids, methamphetamine, cocaine, and benzodiazepines are among the top six drugs associated within drug-related ED visits. Cocaine was the third most frequently used drug (behind alcohol and cannabis) in polysubstance ED visits.[6] These substances further contribute to the complexity of forensic toxicology cases due to their abuse and tolerance potential.[7–9]

A surge of counterfeit tablets in the unregulated drug market further complicates forensic toxicology casework.[10] For instance, unregulated oxycodone or alprazolam pills may contain fentanyl that can be lethal even in small quantities.[11] In addition, drug users who believe to be consuming other substances such as cocaine, heroin, or methamphetamine, have been inadvertently exposed to fentanyl.[12-15] It is not always clear if the presence of fentanyl exists intentionally or if there is accidental cross-contamination in a drug supply.[16] These changes in the drug market introduce a heightened risk of overdose and death, as individuals unknowingly ingest substances far more potent than anticipated.[17,18] According to the Centers for Disease Control and Prevention, fentanyl remained the top drug involved in overdose fatalities in the US in 2022 and 2023.[19] The emergence of fentanyl analogs, such as acetyl fentanyl and carfentanil, are also a public health threat because they have been found as adulterants. The emergence of analogs is difficult to predict, the potency of novel analogs are unknown, and forensic laboratories often do not have the resources to immediately detect them.[20] Besides fentanyl analogs, other substances found in counterfeit pills or used as a filler in drugs are synthetic opioids like isotonitazene.[21,22] Many analogs and novel psychoactive substances (NPS) exist, but there is limited information for these emerging compounds so new drug standards and methods need to be continually developed.[23-26] All in all, the development of drug trends escalates the complexity of forensic toxicological analysis by introducing unpredictability in the types and quantities of substances present in an individual's system at the time of intoxication or death.

When evaluating the clinical severity of chronic drug intoxication in an individual, BDC are more reliable than making assessments based on the estimated quantity consumed and the time of ingestion.[2] The combination of blood-drug analysis with the clinical features on presentation provides more reliable judgment of an individual's condition.[27] This context is something that biological testing alone cannot provide.[28]

2. Background

2.1 Biological Specimens for Drug Testing

Drug testing can be conducted using a variety of biological specimens, each having advantages or disadvantages depending on the drug(s) in question, the time frame of its use (window of detection), invasiveness, and cost. The two most common specimens for drug testing are urine and blood.

A positive result for a urine drug test only confirms that the drug has been used at some prior time and cannot provide information on the dose used, the time of consumption, or provide information on effects.[28] Moreover, a positive drug result in urine does not indicate its presence in blood. Detection of drugs in urine depends on the pattern of drug use, where heavy or persistent drug use can cause a positive drug test for longer periods of time, but a single dose would have a shorter detection window. The presence of a drug in urine does not demonstrate that the individual is under the influence of that drug at or near the time of urine sample collection.[29] Urine testing is not as invasive as blood testing, but there is risk for adulteration, substitution or urine dilution leading to false-negatives.[30] Other difficulties with urine samples are related to the inability to collect a sample due to decreased urine output from dehydration and intoxication, for example.[29]

Blood testing provides valuable insight into recent drug exposure. Although BDC determination plays a crucial role in the broader context of understanding impairment, the correlation between blood concentrations and actual impairment is complicated by individual differences that can affect the pharmacokinetics and pharmacodynamics of substances.[31] Blood testing is only part of a comprehensive evaluation that includes clinical observations and other contextual information to interpret the potential impact on cognitive and motor functions accurately. Further, the detection windows for drugs in blood can vary significantly based on the substance, frequency of use, metabolic rate, and other individual factors, and is generally shorter than urine.[28] Blood samples may capture the presence of the drug, enabling an association with observed impairment or intoxication. Likewise, determining BDC is effective in therapeutic drug monitoring. The measurement of BDC can potentially provide insight into an individual's drug use behavior and compliance.[32]

Overall, the selection of the appropriate biological specimen for drug testing is critical and should be aligned with the type of drug, period of time, and the specific objectives of the analysis, whether it is to determine recent use, monitor compliance, or assess potential impairment.

2.2 Blood Drug Concentrations and its Role in Forensic Investigations

Therapeutic drug monitoring, which compares BDC to therapeutic drug ranges and can help determine if an effective or supra-therapeutic dose has been consumed, involves the assumption that there is a determinate relationship between dose and BDC and between BDC and pharmacodynamic effects.[28,33,34] It is important to note that BDC changes within a dosage interval and for best interpretation, the time in which the blood sample was drawn in relation to when the drug was administered should be taken into account. Such variables and understanding of pharmacokinetic and pharmacodynamic concepts are essential for interpretation of BDC in forensic casework.

In forensic toxicology, determining BDC is significant in studying drug toxicity. Supplemental information that can aid the investigative aspect of forensic toxicology includes observations and drug history information found in medical reports, behaviour of the individual during the incident, and the analysis of drug paraphernalia.[35] The analysis and interpretation of BDC benefits legal investigations involving drugs such as driving incidents, death investigation and sexual assault. For death investigations in particular, BDC are interpreted with consideration of therapeutic and toxic concentrations found in literature.[1] Forensic toxicologists may be asked to testify about the significance of the BDC as it relates to potential drug effects including impairment and toxicity. This necessitates evaluating various literature to reach a conclusion.

2.3 Challenges in Interpreting Blood Drug Concentrations

The misinterpretation of BDC can occur if the circumstances or contextual information of each case are not considered.[5,36] The age, sex, weight, physical and clinical status, rates of drug absorption, distribution, metabolism, excretion, plasma and tissue protein binding and drug tolerance varies between each person and affects drug response, drug handling and therefore interpretation.[34] As a result, the context of drug dose, route, and frequency of use, can provide more clarity in the interpretation of BDC, but such information is generally unknown in the context of forensic investigations. Interpretation is further complicated by polydrug use. When multiple drugs are co-administered, the toxicological response observed is the result of the combined actions of each drug. The observed status of each individual is dependent on the drugs present in their polydrug use.[5]

There are challenges in the blood-drug interpretation of certain drugs. The interpretation of morphine in blood can be a challenge since it can be used on its own for pain relief but is also a common active metabolite of both heroin and codeine. Without context, it is difficult to determine if the presence of morphine is from the consumption of morphine, heroin, or codeine. It may be beneficial to perform a supplementary urine test to confirm heroin use because 6-monoacetylmorphine (6-MAM), the unique

metabolite of heroin, is present in urine longer than in blood.[37] Often in toxicological interpretation, the presence of drug metabolites in the absence of the parent drug can offer information regarding an individual's use of the parent drug. The ratio between parent drug to metabolite(s) can offer some insight on chronic versus acute use.[35] Additionally, it can be a challenge to analyze drugs with a small detection window and is limited by the capabilities of the laboratory.

Another challenge for blood-drug interpretation is the presence of drug tolerance. The threshold between therapeutic and toxic drug concentration for each person varies and is more complicated when tolerance exists.[32] The interpretation of effects and behavior is difficult in cases of living individuals with high BDC observed. An example is in opioid-tolerant individuals, where they are able to function with opioid concentrations that may be considered toxic or lethal for naive users. From a clinical perspective, an individual exhibiting normal vital signs and physical function despite elevated opioid concentrations indicates tolerance.[38] In death investigation, the death of an individual could be attributed to high opioid concentration if there is a lack of evidence of the individual being opioid-tolerant. So, caution is needed to interpret the BDC present in an individual depending on their drug history.[1]

When reviewing the literature, forensic toxicologists must be cognizant of study subjects' histories and the study design, e.g., sample collection procedures, sample storage conditions and methods of analyses to make appropriate comparisons with their own casework. These factors can contribute to the difference between suspected lethal BDC in a death investigation and those found in the literature.[35]

2.4 Development of Drug Tolerance

Interindividual genetic variability affects the development of tolerance. For example, the pharmacogenetics of opioid analgesia and tolerance are studied to characterize the thousands of single-nucleotide polymorphisms present in opioid receptors, proteins, and enzymes that alter pain perception, metabolism and transport.[39] Such research improves healthcare outcomes by providing safer and more effective drug dosing with the focus on tolerance and withdrawal prevention.

Drug tolerance can be acquired as a consequence of repeated drug use. In the context of chronic drug use, it exists in three forms: pharmacokinetic, pharmacodynamic, and learned tolerance.[42] Pharmacokinetic tolerance emerges when the body's ability to metabolize a drug changes over time and leads to altered drug distribution within the body. Pharmacodynamic tolerance occurs as the responsiveness of the receptor system to the drug diminishes with continuous exposure. This form of tolerance can also lead to cross-tolerance, where tolerance to one drug results in a reduced response to structurally similar drugs within the same pharmacologic class.[3] To prevent the possibility of overdose, physicians prescribing a new opioid take into consideration that cross-tolerance is incomplete and that the new opioid is more potent than anticipated, so only 50% of the calculated equianalgesic dose is prescribed.[40] Lastly, learned tolerance is when a drug user learns to function despite drug use or when the removal of environmental cues leads to an increase in pharmacologic response.[3] On the whole, tolerance is dynamic as it can be both acquired and lost over time, with loss of tolerance increasing sensitivity to drugs after a period of abstinence or reduced drug use. Such fluctuations in tolerance make it a challenge to predict how an individual may respond to a drug dose as their tolerance may be unknown or different from previous usage patterns.[41] Consequently, the development of tolerance and its potential loss add complexity to the interpretation of BDC which warrants the consideration of drug history and case-specific factors.

The development of tolerance to opioids is particularly complex. While pharmacokinetic variability plays a relatively minor role in the development of opioid tolerance, pharmacodynamic factors such as genetic predisposition, cross-tolerance, receptor affinity, and others significantly contribute to its development.[3,42] As a result, patients who have developed tolerance to opioids require higher doses to achieve the same level of analgesic effect.[1] Or in the case of recreational drug use, higher doses are needed to achieve the same level of euphoria. It is impossible to determine the threshold between therapeutic and toxic BDC for an individual.[2]

Individuals with Substance Use Disorder (SUD) can have drug tolerance. In the diagnostic criteria for SUD, the development of tolerance is identified as one of the contributing factors. This pharmacological

indicator, while part of the criteria set, is not mandatory for a diagnosis but can be a significant element in the overall assessment.[43]

2.5 Substance Use Navigators and Substance Use Intervention Team

The Substance Use Navigator (SUN) program, which focuses on patients with SUD, provides treatment and assistance for the University of California, Davis ED and inpatient units. Primary medical treatment teams identify patients with evidence of SUD for SUN consult. SUN evaluates these patients and recommends medication assisted treatment (MAT). If the patient is not ready for MAT, the SUN team provides substance use counseling, safety resources, and harm reduction kits. For patient cases deemed too complicated for the SUN team, the Substance Use Intervention Team (SUIT) is available for consultation. SUIT is comprised of a physician trained in addiction medicine and provides assessment and treatment recommendations for complex cases. The SUIT and SUN teams work together to provide support and MAT both in the hospital and after discharge, resulting in the best possible care for patients with substance use disorder.[44]

2.6 Benefits and Limitations of Self-Reported Drug Use

Studies that rely exclusively on self-reported drug use can be more cost-effective compared to those that use laboratory testing, allowing for a larger sample size or more frequent data collection. However, in many studies, drug use is found to be under-reported or unreliably reported.[45-48] Self-reported frequency and magnitude of drug use can vary from substance to substance and be impacted by memory, comprehension, reluctance in disclosing illegal drug use, clinical factors including intoxication, confusion, mental illness, or other symptoms and factors.[29,45,49] The accuracy of self-reported drug use improves when there are no consequences for the individual reporting. Additionally, there is good agreement between self-reported and biological measures of drug use.[50] But NPS or adulterants present in illicit drugs may be consumed unintentionally or replace illicit drugs without the knowledge of the user, which complicates self-report reliability. Despite this limitation, self-reported drug use provides

information on drug use patterns like the frequency, dose and the route of administration which would otherwise be unavailable with testing alone. Therefore, self-reported drug use should be used to supplement analytical testing.[28,51]

2.7 Methodologies and Gaps in Current Literature

Some studies rely on preliminary testing methods like immunoassays, for instance, enzyme-linked immunosorbent assay, to verify self-reported drug use because it is quick and cost efficient.[29,52,53] Positive immunoassay results should be verified with confirmatory methods (GC-MS or LC-MS/MS) for the most accurate results, or other direct confirmatory methods can be used.[45,49,52] While LC-MS/MS can be costly, time consuming, and require extensive validation, it has the ability to detect a large range of substances with high sensitivity and selectivity and is regarded as a top choice for applications in fields such as toxicology.[30,54,55]

To aid forensic toxicologists in the interpretation of BDC, there are compilations of data for therapeutic, toxic and fatal concentrations for various drugs published in the literature.[5,32,36,56,57] While these data can be useful, they do not necessarily provide insight into an individual's drug use patterns and the dosage of drug(s) that led to intoxication.[58] It is crucial to recognize that toxic and fatal drug concentrations are not static values; they vary considerably among individuals, influenced by their unique history of drug consumption. This variability introduces challenges when attempting to align an individual's BDC, particularly in cases of developed tolerance, with the established benchmarks in the literature. As a consequence, forensic toxicologists exercise caution when relying on literature to interpret specific cases.

Studies involving drug impaired drivers are beneficial in identifying BDC in living individuals with crossover into drug tolerance.[59-62] Other studies that have investigated populations such as chronic pain patients have encountered tolerance in the context of supratherapeutic drug thresholds.[38] However, a limitation of these types of studies is the lack of comprehensive drug use history. Although some research incorporates self-reported drug histories, they may not corroborate these histories with tests on biological

specimens.[28,63,52] Studies that do attempt to validate self-reported drug usage often rely on preliminary testing methods, do not examine BDC, and may not include detailed information about therapeutic drug use in the self-reports.[49-53] Especially in extracting medical data for a study, it is usual for physicians to evaluate drug use based on the qualitative analysis of blood or other matrices, e.g. urine, therefore BDC must be specifically analysed.[64]

Drug trends have an impact on the BDC observed in living individuals and require up-to-date research. With fentanyl, for example, past studies have shown that popular modes of administration were transmucosal, transdermal, and intravenous.[65] In these studies, death occurred in fentanyl concentrations as low as 3.0 ng/mL.[66,67] Overtime, smoking became a more frequent method of administration.[68,69] Blood-fentanyl concentrations in 153,234 DUID cases from 25 states across all geographical regions also rose, suggesting that there is an increase in tolerance to fentanyl among this population.[60] This trend has impacted driving under the influence of drugs (DUID) cases in the US. Between 2014-2019, Rohrig et al. found that the range of blood fentanyl concentrations was 0.1 to 157 ng/mL with a 466% to 524% increase in fentanyl-positive DUID cases in New Hampshire, Palm Beach County, FL, and Sedgwick County, KS.[70] Further, Chan-Hosokawa and Bierly's study of DUID cases between 2010 and 2020 found that the mean and median blood fentanyl concentrations tripled by the end of the study period, where the mean increased from 3.2 to 9.6 ng/mL and the median increased from 1.9 to 5.3 ng/mL.[60] Both these studies demonstrate the increased incidence of fentanyl with high blood fentanyl concentrations detected in living individuals. Consequently, to accurately assess drug trends, it is important to identify drug prevalence and obtain recent data on drug users' BDC.

3. Research Objectives

This study aims to determine BDC for a subset of recreational and therapeutic substances in individuals who use drugs recreationally. It addresses a gap in the literature as clinical studies typically do not encompass the higher doses or combinations of drugs that are commonly sought or used by recreational

drug users. The data collected will provide real-world data on BDC in recreational users alongside demographics, observations on admission to the ED, and self-reported drug use.

To achieve this objective, remnant whole blood samples originating from patients in the ED on the SUN and SUIT consult list in the UCD Hospital system were collected for analysis. BDC for a variety of prescription drugs, metabolites, and drugs of abuse were quantified in these samples and were compared with BDC found in existing literature. Demographic information and key observed symptoms are presented. A secondary objective was to determine how analytically detected drugs compare with drug use that were self-reported.

4. Method

4.1 Patient Selection

The UCD Institutional Review Board (IRB) approved this study, and a waiver of informed consent was granted (IRB ID: 2047006-2).

Patients who were on the SUN and SUIT consult list at the UCD Hospital from October 2023 to January 2024 were initially selected. Patient history including age, sex, self-reported drug use and observations on arrival were collected via electronic medical record review. Patients were excluded if they presented in an unconscious state, were intubated or required respiratory support at the time of admission. Pregnant mothers, prisoners, children and patients with an insufficient amount of remnant blood for further testing were excluded.

4.2 Sample Collection

Remnant whole blood collected in BD Vacutainer REF 367861 lavender top tubes with K_2EDTA 7.2 mg used for routine clinical testing were analyzed in this study. Sample tubes were deidentified by the UCD Health Biorepository either by removal of medical labels or by transfer into new EDTA tubes of the same

type. Patient history was linked to the samples and toxicology analysis by unique study IDs. Samples were stored at 4°C prior to analysis.

4.3 Analytical Method

The analytical method consisted of 37 analytes (Table I and Table II). Flubromazolam and its deuterated internal standard were obtained from Cayman Chemical Company (Ann Arbor, MI). Xylazine and its deuterated internal standard were obtained from Sigma-Aldrich (St. Louis, MO). All other substances and deuterated internal standards used for quantification were obtained from Cerilliant (Round Rock, TX).

Table I. Calibration standard concentrations and calculated <LOQ values for analytes (ng/mL).

Analyte	LOD	<LOQ	STD 1	STD 2	STD 3	STD 4	STD 5	STD 6	LPC	HPC
7-aminoclonazepam	6.25	9.38	12.50	25	50	100	200	400	25	300
acetyl fentanyl	0.31	0.78	1.25	2.5	5	10	20	40	2.5	30
alprazolam	6.25	9.38	12.50	25	50	100	200	400	25	300
amphetamine	6.25	9.38	12.50	25	50	100	200	400	25	300
benzoylecgonine	6.25	9.38	12.50	25	50	100	200	400	25	300
bromazolam	0.63	0.94	1.25	2.5	5	10	20	40	2.5	30
carfentanil	0.019	0.028	0.038	0.075	0.15	0.3	0.6	1.2	0.075	0.9
clonazepam	6.25	9.38	12.50	25	50	100	200	400	25	300
clonazolam	0.63	0.94	1.25	2.5	5	10	20	40	2.5	30
cocaethylene	3.13	4.69	6.25	12.5	25	50	100	200	12.5	150
cocaine	3.13	4.69	6.25	12.5	25	50	100	200	12.5	150
codeine	25.00	37.50	50.00	100	200	400	800	1600	100	1200
diazepam	25.00	37.50	50.00	100	200	400	800	1600	100	1200
etizolam	2.50	3.75	5.00	10	20	40	80	160	10	120
fentanyl	0.31	0.78	1.25	2.5	5	10	20	40	2.5	30
flualprazolam	0.63	0.94	1.25	2.5	5	10	20	40	2.5	30
flubromazolam	0.63	0.94	1.25	2.5	5	10	20	40	2.5	30
hydrocodone	12.50	18.75	25.00	50	100	200	400	800	50	600
hydromorphone	6.25	9.38	12.50	25	50	100	200	400	25	300
isotonitazene	0.63	0.94	1.25	2.5	5	10	20	40	2.5	30
ketamine	25.00	37.50	50.00	100	200	400	800	1600	100	1200
lorazepam	6.25	9.38	12.50	25	50	100	200	400	25	300
mephedrone	12.50	18.75	25.00	50	100	200	400	800	50	600
methadone	12.50	18.75	25.00	50	100	200	400	800	50	600
methamphetamine	6.25	9.38	12.50	25	50	100	200	400	25	300
methylenedioxyamphetamine	6.25	9.38	12.50	25	50	100	200	400	25	300
methylenedioxyethylamphetamine	6.25	9.38	12.50	25	50	100	200	400	25	300
methylenedioxymethamphetamine	6.25	9.38	12.50	25	50	100	200	400	25	300

morphine	12.50	18.75	25.00	50	100	200	400	800	50	600
nordiazepam	25.00	37.50	50.00	100	200	400	800	1600	100	1200
norketamine	25.00	37.50	50.00	100	200	400	800	1600	100	1200
oxazepam	25.00	37.50	50.00	100	200	400	800	1600	100	1200
oxycodone	12.50	18.75	25.00	50	100	200	400	800	50	600
oxymorphone	12.50	18.75	25.00	50	100	200	400	800	50	600
temazepam	25.00	37.50	50.00	100	200	400	800	1600	100	1200
cis-tramadol	25.00	37.50	50.00	100	200	400	800	1600	100	1200
xylazine	6.25	9.38	12.50	25	50	100	200	400	25	300

Standards were prepared in methanol and stored at -15°C. Calibrators were prepared by spiking 0.25 mL of drug-free blood with standard solutions at six levels across the quantitation ranges presented in Table I. The limit of detection (LOD) for each substance ranged from 0.019 to 25 ng/mL, both low positive controls (LPC) and high positive controls (HPC) were included for each analyte. Drugs and metabolites were quantified using a previously validated LC-MS/MS method.

Table II. Multiple Reaction Monitoring (MRM) transitions for analytes and internal standard (ISTD).

Analyte	MRM 1	MRM 2	ISTD	MRM	ISTD Conc. (ng/mL)
7-aminoclonazepam	286.0 / 121.1	286.0 / 222.1	7-Aminoclonazepam-D_4	290.0 / 226.1	50
acetyl fentanyl	323.2 / 202.1	323.2 / 188.1	Acetylfentanyl-$^{13}C_6$	329.0 / 188.2	5
alprazolam	309.0 / 281.1	309.0 / 205.1	Alprazolam-D_5	314.0 / 286.1	50
amphetamine	136.0 / 91.1	136.0 / 119.3	Amphetamine-D_5	141.0 / 124.2	50
benzoylecgonine	290.5 / 168.1	290.5 / 105.1	Benzoylecgonine-D_3	293.0 / 171.0	50
bromazolam	353.0 / 325.0	353.0 / 274.1	Flubromazolam-D_4	376.9 / 296.0	5
carfentanil	395.04 / 335.2	395.04 / 113	Carfentanil-D_5	400.1 / 340.2	0.3
clonazepam	316.0 / 270.0	316.0 / 214.1	Clonazepam-D_4	320.0 / 274.1	50
clonazolam	354.1 / 308.1	354.1 / 280.1	Clonazolam-D_4	358.1 / 312.1	5
cocaethylene	318.0 / 196.0	318.0 / 82.2	Cocaethylene-D_3	321.0 / 85.1	25
cocaine	304.1 / 182.1	304.1 / 105.0	Cocaine-D_3	307.1 / 185.2	25
codeine	300.1 / 152.1	300.1 / 165.1	Codeine-D_6	306.1 / 152.1	200
diazepam	285.0 / 193.1	285.0 / 154.0	Diazepam-D_5	290.0 / 198.2	200
etizolam	343.0 / 314.3	343.0 /206.2	Etizolam-D_3	346.0 / 138.1	20
fentanyl	337.1 / 188.2	337.1 / 105.1	Fentanyl-D_5	342.2 / 188.2	5
flualprazolam	327.0 / 223.0	327.0 / 299.0	Flualprazolam-D_4	331.0 / 227.1	5
flubromazolam	372.9 / 223.0	372.9 / 292.1	Flubromazolam-D_4	376.9 / 296.0	5
hydrocodone	300.1 / 199.2	300.1 / 141.0	Hydrocodone-D_6	306.1 / 202.2	100
hydromorphone	286.2 / 185.1	286.2 / 157.0	Hydromorphone-D_6	292.1 / 185.1	50
isotonitazene	411.2 / 100.1	411.2 / 72.0	Isotonitazene-$^{13}C_6$	417.2 / 100.1	5
ketamine	238.0 / 125.0	238.0 / 179.1	Ketamine-D_4	242.1 / 129.1	200
lorazepam	320.9 / 275.1	320.9 / 229.0	Lorazepam-D_4	327.0 / 281.0	50
mephedrone	178.1 / 160.2	178.1 / 91.0	Mephedrone-D_3	181.1 / 90.9	100
methadone	310.1 / 265.2	310.1 / 105.1	Methadone-D_9	319.1 / 268.1	100
methamphetamine	150.0 / 91.1	150.0 / 119.1	Methamphetamine-D_5	155.1 / 121.1	50
methylenedioxyamphetamine	180.0 / 163.1	180.0 / 79.1	Methylenedioxyamphetamine-D_5	185.1 / 110.2	50

methylenedioxyethylamphetamine	208.1 / 163.1	208.1 / 105.1	Methylenedioxyethylamphetamine-D_5	213.1 / 163.1	50
methylenedioxymethamphetamine	194.0 / 163.1	194.0 / 104.9	Methylenedioxymethamphetamine-D_5	199.1 / 165.1	50
morphine	286.0 / 152.1	286.0 / 165.1	Morphine-D_6	292.1 / 152.0	100
nordiazepam	271.0 / 165.1	271.0 / 140.2	Nordiazepam-D_5	276.0 / 213.1	200
norketamine	224.0 / 125.1	224.0 / 89.0	Norketamine-D_4	228.1 / 129.1	200
oxazepam	287.0 / 241.2	287.0 / 104.1	Oxazepam-D_5	292.0 / 246.1	200
oxycodone	316.1 / 241.1	316.1 / 256.2	Oxycodone-D_6	322.1 / 247.0	100
oxymorphone	302.1 / 284.1	302.1 / 227.2	Oxymorphone-D_3	305.1 / 230.2	100
temazepam	301.1 / 255.1	301.1 / 177.1	Temazepam-D_5	306.0 / 260.0	200
cis-tramadol	264.1 / 58.1	264.1 / 246.0	Cis-Tramadol-C^{13}-D_3	268.1 / 58.0	200
xylazine	221.0 / 163.8	221.0 / 89.7	Xylazine-D_6	227.0 / 170.1	50

25 µL of internal standard was added to each matrix-matched calibrator, positive and negative controls, quality controls, and 250 µL of each sample. Samples were also prepared with a dilution factor of 10. Samples were vortexed in new tubes with the addition of 750 µL acetonitrile. They were left to stand for 5 minutes and then centrifuged at 4000 rpm for 20 minutes. The supernatant was decanted into Agilent Captiva EMR-Lipid 1 mL tubes and centrifuged at 1500 g for 10 minutes at 10 ℃. The samples were centrifuged in 2-minute increments until all acetonitrile was filtered. Samples were then dried for 10 minutes in the TurboVap dryer at 40 ℃ and 4 psi. The samples were reconstituted with 1 mL of mobile phase A (90:10 v/v 10 mM ammonium formate in 0.2% formic acid:0.2% formic acid in acetonitrile) and vortexed. Samples were then injected at a volume of 5 µL and analyzed using LC-MS/MS (Waters Acquity I-Class UPLC, SCIEX QTrap 5500 MS). Data was acquired in multiple reaction monitoring mode where MRM 1 and MRM 2 for each drug is presented in Table II. Quantitation was completed using SciexOS software with results fitted to a quadratic curve and to a 1/x weighting. Samples requiring further dilution were analyzed using an appropriate dilution factor as required and was matrix-matched (maximum dilution factor of 20).

4.4 Data Interpretation

The data generated from analytical testing was interpreted with and without the cases where drug use was confounded by the medical administration of drugs in the ED.

While the method includes 6-MAM, it is unlikely that 6-MAM is detected in blood due to its rapid metabolism. Therefore, heroin use, when reported by a study participant, is inferred by the presence of morphine in blood, unless morphine was administered in hospital.

While amphetamine can be administered as a drug on its own, it is also an active metabolite of methamphetamine; the presence of amphetamine in a patient sample was assumed to be from previous use of methamphetamine and these analytes are grouped together for statistical purposes.

Cocaine is known to degrade in stored blood samples over time. The presence of benzoylecgonine, its major metabolite, indicates previous use of cocaine. Cocaine and benzoylecgonine were grouped together for statistical purposes.

For inclusion in the calculation of statistical values, where the detected drug concentration was between the limit of detection and the limit of quantitation (LOQ), the reported drug concentration was set to the average of the LOD and the LOQ. These values, denoted as <LOQ, are detailed in Table I.

The Glasgow Coma Scale (GCS) and the Alert and Oriented (AO) assessment are included in a few study participants' observations on arrival. GCS and AO tests are used by physicians in the ED to assess consciousness and mental status, respectively. A greater score on the GCS indicates a greater ability in eye, verbal, and motor response. AO is followed by x1, x2, x3, or x4 to indicate that someone is alert and oriented to person, time, place, and event. A patient that is AOx3 is alert and oriented to person, time, and place, for example.[71] Clinical Institute Withdrawal Assessment (CIWA) scores under 8 to 10 indicate minimal to mild alcohol withdrawal.[72]

Each case was categorized into “possibly drug-related” (PDR) or “not drug-related” (NDR) based on the observations on arrival to the ED and self-reported drug use. Cases were classified into NDR when the symptoms on presentation were not indicative of recent drug use or withdrawal, particularly if the self-reported drug use was not recent. In cases where patients presented to the ED with swelling in an

extremity and reported IV drug use, but it was not recent, it was classified as NDR. Cases were classified as PDR if the observed symptoms were typical of withdrawal, an acute drug-related event, or toxicity, and if the patient reported recent drug use. Additionally, if the stated reason for admission was related to withdrawal from alcohol or drugs, the case was also classified as PDR. Two members of the study team independently categorized each case, and there was agreement for 85 out of 116 cases (73.3%). For the remaining cases where there was disagreement, a third member of the study team provided a final classification.

One-way analysis of variance (ANOVA) was performed to determine if there was statistically significant difference in drug concentrations (fentanyl, methamphetamine, amphetamine, benzoylecgonine) between PDR and NDR cases. ANOVA was also performed to determine if there was a statistical difference for the number of drugs detected between PDR and NDR cases. A significance value of 0.05 was used and statistical analysis was completed using Excel.

The following formula was used to calculate agreement between self-reported drug use and analytical detection for each drug:

$$Percent\ agreement\ = \frac{Self\ reported\ and\ detected}{Self\ reported} \times 100$$

5. Results

One hundred and sixteen samples were included in the study. Of these patients, 70 were male and 46 were female with a range in age from 18 to 76 years (average, 44). See Table III.

Of the 37 substances in the LC-MS/MS panel, only 14 substances were detected and quantified. The most frequently detected substances were methamphetamine (n=82), amphetamine (n=74), fentanyl (n=39), and benzoylecgonine (n=12), as seen in Figure I. Fentanyl and amphetamine/methamphetamine was the

most frequent drug combination observed (n=25). Seven participants were not positive for any drugs. See Table III.

Table III. Summary of Demographics, Number of Drugs Detected, and Drug Concentrations

	Female n =46	Male n =70	Total n =116
Age			
Mean	38.8	47.4	44
Range	18-76	20-69	18-76
Median	37	47.5	42.5
Number of drugs detected			
Mean	2.2	2.0	2.1
Range	0-5	0-4	0-5
Median	2	2	2
Drugs detected			
Methamphetamine, n	32	50	82
Average Concentration (ng/mL)	245.1	313.4	286.7
Median Concentration (ng/mL)	170.7	152.4	158.0
Range (ng/mL)	9.4-1062.6	9.4-2340.6	9.4-2340.6
Amphetamine, n	29	45	74
Average Concentration (ng/mL)	42.2	42.0	42.1
Median Concentration (ng/mL)	32.1	23.7	29.1
Range (ng/mL)	9.4-131.5	9.4-236.1	9.4-236.1
Fentanyl, n	17	22	39
Average Concentration (ng/mL)	10	17.9	14.5
Median Concentration (ng/mL)	4.2	5.5	4.2
Range (ng/mL)	0.78-45.6	0.8-113.7	0.8-113.7
Benzoylecgonine, n	6	6	12
Average Concentration (ng/mL)	240.2	818.2	529.2
Median Concentration (ng/mL)	204.8	145.3	154.2
Range (ng/mL)	37.2-557.6	42.7-3876.8	37.2-3876.8
Diazepam, n	5	3	8
Average Concentration (ng/mL)	96.5	272.3	162.4
Median Concentration (ng/mL)	65.3	317.3	104.8
Range (ng/mL)	63.9-206.0	126.8-372.7	63.9-372.7
Nordiazepam, n	2	4	6
Average Concentration (ng/mL)	38.6	119.0	92.2
Median Concentration (ng/mL)	38.6	54.7	41.9
Range (ng/mL)	30.8-46.3	37.5-329.2	30.8-329.2
Oxycodone, n	1	4	5
Average Concentration (ng/mL)	52.6	19.9	26.4
Median Concentration (ng/mL)		18.8	18.8

Range (ng/mL)		18.8-23.3	18.8-52.6
Lorazepam, n	2	2	4
Average Concentration (ng/mL)	11.1	9.4	10.2
Median Concentration (ng/mL)	11.1		9.4
Range (ng/mL)	9.4-12.8		9.4-12.8
Bromazolam, n	2	2	4
Average Concentration (ng/mL)	8.4	2.0	5.2
Median Concentration (ng/mL)	8.4	2.0	4.9
Range (ng/mL)	6.8-9.9	0.9-3.0	0.9-9.9
Morphine, n	1	2	3
Average Concentration (ng/mL)	18.75	24.0	22.3
Median Concentration (ng/mL)		24.0	18.8
Range (ng/mL)		18.8-29.3	18.8-29.3
Norketamine, n	1	1	2
Average Concentration (ng/mL)	802.9	37.5	420.2
Median Concentration (ng/mL)			420.2
Range (ng/mL)			37.5-802.9
Ketamine, n	1	0	1
Average Concentration (ng/mL)	338.1		338.1
Median Concentration (ng/mL)			
Range (ng/mL)			
Hydromorphone, n	0	1	1
Average Concentration (ng/mL)		9.4	9.4
Median Concentration (ng/mL)			
Range (ng/mL)			
Alprazolam, n	1	0	1
Average Concentration (ng/mL)	163.4		163.4
Median Concentration (ng/mL)			
Range (ng/mL)			

A compiled data table for each case number with age, sex, observation notes on ED admission, self-reported drug use, PDR or NDR classification, and detected drug concentrations are presented in Appendix A.

In 17 cases, drugs were administered in the ED before blood draw and were detected analytically. ID 152, 160, 239, 280, 317, 324 were administered fentanyl, ID 305 self-reported heroin use but morphine was also administered, ID 326 was given hydromorphone, ID 137,153,198, 248 were given lorazepam and ID

165,175,176,302, 319 were given oxycodone in the ED. The method used in this study does not distinguish between self–administered and hospital administered drugs. The BDC of each participant was unknown prior to drug administration in the hospital. Therefore, these cases are excluded from statistical analyses and Figure I.

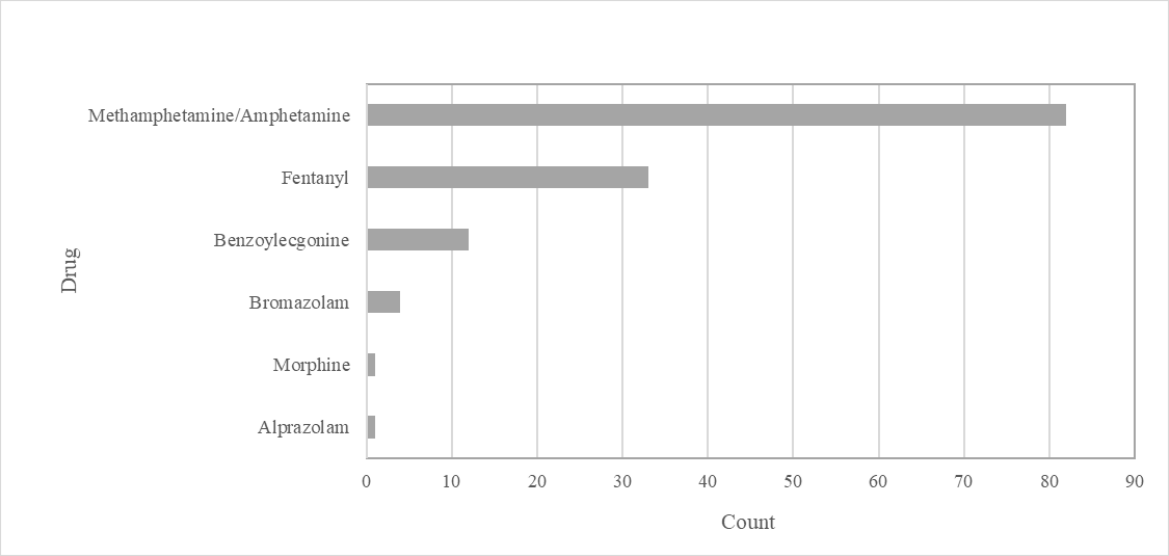

Figure I. Total Count of Drugs Detected that Were Not Administered in the ED.

The reasons for admission were classified as 62 possibly drug-related (PDR) and 54 not drug-related (NDR). ANOVA was used to determine if there were statistical differences between the PDR and NDR cases, number of drugs detected, and specific drug concentrations. There was no significant difference between PDR and NDR groups and the number of drugs detected. There was also no significant difference between fentanyl, methamphetamine, amphetamine, and benzoylecgonine concentrations and the PDR and NDR groups. See Table IV. The full results of ANOVA are presented in Appendix B.

Table IV. Between groups P-values for the number of drugs detected and drug concentrations in PDR and NDR cases.

	PDR Count	NDR Count	Between Groups P-Value
Number of drugs detected, n	62	54	0.41
Fentanyl	24	15	0.32
Methamphetamine	35	47	0.52
Amphetamine	33	41	0.85
Benzoylecgonine	8	4	0.14

Five participants, ID 155, 162, 167, 241, and 248 had either unknown drug use history, denied drug ingestion, or reported consumption of drugs that were unknown. All five participants were positive for methamphetamine.

Four cases, ID 89, 245, 324, 325 reported the use of street “Percocets”. All cases except ID 245 were positive for fentanyl. ID 89 self-reported oxycodone and “Percocet” use but the only drug detected in blood was fentanyl. ID 324 self-reported fentanyl and “Percocet” use and was only positive for fentanyl. However, this case is confounded by fentanyl administration in the ED. ID 325 self-reported “Percocet” use and fentanyl was the only drug detected.

ID 323 describes the use of what they believe to be Xanax from a Mexican pharmacy, and alprazolam was the only drug detected.

Not including patients that were administered drugs in the ED, methamphetamine (n=66), fentanyl (n=28), and cocaine (n=10) had the greatest concordance between self-report and toxicological analysis. The highest self-reported but not detected drug use was heroin (n=13), fentanyl (n=10), and methamphetamine (n=7). The top drugs that were detected but not self-reported were methamphetamine (n=16), fentanyl (n=5), and cocaine (n=2). This data is represented in Figure II.

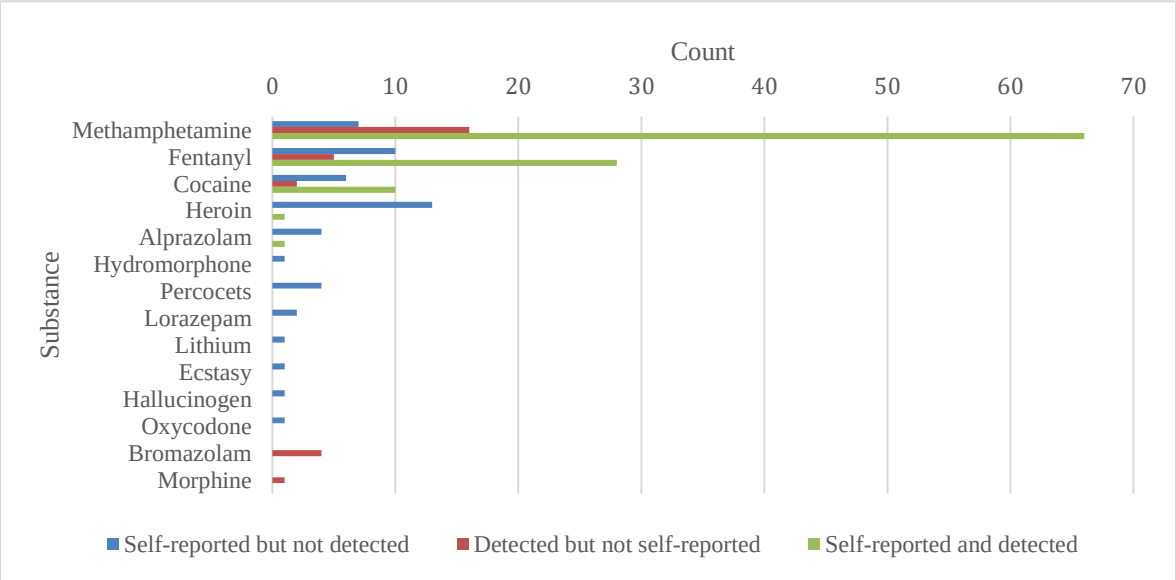

Figure II. Self-reported drug use and detected drug counts.

Based on this data, percent agreement was calculated for methamphetamine (90.4%), fentanyl (73.7%), cocaine (62.5%) heroin (7.14%), and alprazolam (20.0%).

6. Discussion

There was no significant difference between PDR and NDR cases and the number of drugs detected. There was also no significant difference between methamphetamine, amphetamine, fentanyl, and benzoylecgonine concentrations and the PDR and NDR cases. The results of this analysis are similar to research on methamphetamine-related deaths in San Francisco where blood concentrations of methamphetamine and amphetamine were indistinguishable between methamphetamine-related deaths and non-methamphetamine-related deaths.[73] The results of the present study indicate that both the number of drugs and its concentrations do not vary substantially between patients classified as PDR and NDR. This suggests that while BDC are similar across cases, factors such as individual tolerance and other pharmacokinetic variabilities influence the intensity of effects in response to drug exposure. The variability in symptomatology observed emphasizes the challenges in using BDC alone to interpret clinical or forensic cases.

There is overlap in the range of fentanyl concentrations detected in this study with therapeutic and fatal concentrations found in literature. The average fentanyl concentration detected was 14.5 ng/mL (range, 0.8-113.7 ng/mL), with ten cases representing concentrations <LOQ. The average is comparable to a study by Thompson et al. that described two hospitalized patients who were administered 100 and 300 µg/h of transdermal fentanyl for more than three months with blood fentanyl concentrations of 8.5 and 9.9 ng/mL, respectively. Furthermore, Thompson et al. describe three cases in which 25-50 µg/h of transdermal fentanyl was administered but the blood fentanyl concentrations were less than the LOD of 2 ng/mL.[74] A larger study involving transdermal fentanyl administration to 27 participants ranging from 25 to 200 µg/h found fentanyl concentrations in 14 cases below the LOD of 0.9 ng/mL, and the 13 other cases ranged from 1.06 to 3.64 ng/mL.[75] These lower blood-fentanyl concentrations detected in therapeutic settings are reflected in the present study.

With respect to postmortem fentanyl concentrations, Martin et al. describes cases where cause of death was fentanyl overdose (n=54) and natural (n=11) and reported average blood fentanyl concentrations of 25 ng/mL (range, 3.0-383 ng/mL) and 12 ng/mL (range, 2.7-33 ng/mL), respectively.[66] While the patients in this study did not reach blood fentanyl concentrations as high as 383 ng/mL, the highest observed concentration was greater than the average concentrations reported in fentanyl overdose fatalities and the maximum concentration observed in natural deaths. The fentanyl concentrations detected in the living patients of the present study intersect with those reported in therapeutic cases, fentanyl overdose, and natural death. It remains difficult to determine a lethal concentration of fentanyl due to pharmacokinetic variability, especially when tolerance exists. Blood-fentanyl concentrations associated with therapeutic use can approach fatal cases and it complicates differentiation between therapeutic use, misuse, and overdose in clinical and forensic settings. In such cases, it is helpful to consider self-reported drug history and ED observations alongside BDC when a similar case history has been encountered.

Additionally, in the cases where patients were administered fentanyl in the ED but did not self-report fentanyl use, none were positive for fentanyl. It is possible that the doses administered in the ED may have been low and consistent with the lack of self-reported fentanyl use in these cases.

Although there were cases where fentanyl was administered in the ED, self-reported, detected analytically, and excluded from statistical analysis, it should be recognized that some of these cases represent recreational fentanyl use. For example, ID 280 self-reported as a frequent fentanyl user and was administered fentanyl in the ED. 45.6 ng/mL fentanyl, 140.4 ng/mL methamphetamine, and 75.1 ng/mL amphetamine were detected. The supratherapeutic fentanyl concentration is not a result of the fentanyl administered in the ED, but due to the methods of this study, BDC before and after ED drug administration are indistinguishable.

Between 1996 and 2017, UC Davis Health saw an increase in methamphetamine-positive cases and it was predicted that this trend will not reverse in the near future.[76] Consistent with these findings, the present study identifies the majority of its cases as methamphetamine-positive (n=82), which indicates the ongoing prevalence of methamphetamine use. In a study examining methamphetamine overdose deaths in 13 cases, the average blood methamphetamine concentration was 1000 ng/mL (range, 90-18000 ng/mL).[77] Previously reported in 8496 DUID cases, average blood methamphetamine concentrations were 410 and as high as 5600 ng/mL. These same cases had average amphetamine concentrations of 540 ng/mL (max, 10400).[78] The current study detected an average blood methamphetamine concentration of 286.7 ng/mL (range, 9.4-2340.6 ng/mL) and average amphetamine concentration of 42.1 ng/mL (range, 9.4-236.1 ng/mL). Each case that was positive for amphetamine was also positive for methamphetamine and methamphetamine was always in a greater concentration. There were 8 cases that were only positive for methamphetamine. This may reflect very recent intake of methamphetamine before amphetamine, the product of *N*-demethylation, has been produced.[78] It is also possible that the user administered a very low dose of methamphetamine and its metabolite is below the LOD of the method and therefore not detected.

The highest fentanyl concentration was observed in ID 285, a 37 year old male. This patient had a fentanyl concentration at 113.7 ng/mL and methamphetamine and amphetamine concentrations at 707.9 ng/mL and 61.7 ng/mL, respectively. This case is similar to a case described by Adamo et al., where a 27 year old male who had died from a gunshot wound had a femoral blood fentanyl concentration of 400 ng/mL, concentrations of 160 ng/mL methamphetamine, 16 ng/mL amphetamine, and 33 ng/mL etizolam.[79] Similarly, Mata and Coleman identified the BDC of a 30 year old male in a DUID case as fentanyl at 303 ng/mL, methamphetamine at 119 ng/mL, and the detection of fentanyl metabolites, amphetamine and xylazine.[80] These studies describe cases with the highest fentanyl concentrations detected but it is noteworthy that these cases also contain high blood methamphetamine concentrations. The current study's dataset represents a number of methamphetamine and fentanyl co-users (n=25), corresponding with broader drug trends in the US. The co-use of stimulants, such as methamphetamine, with opioids like fentanyl, has become a hallmark of the fourth wave of the opioid epidemic, which has been characterized by an increase in stimulant-opioid-related deaths.[81–83] According to co-users of opioids and stimulants, the combined use is motivated by the increased access to stimulants such as methamphetamine, stronger effects, better control of the effects of opioid withdrawal, and providing energizing effects for employment purposes.[84]

Excluding the cases involving fentanyl administration in the ED, five patients were positive for fentanyl and methamphetamine, but only reported fentanyl use. It is unknown if the methamphetamine use was omitted purposefully or unintentionally, or if its use was unrecognized.

Seven of the 12 cases that were benzoylecgonine-positive had only one drug detected (unless another drug was expected to be present due to its administration in the ED), suggesting that the majority of cocaine users in this study were only using cocaine. Methamphetamine was the most frequently detected drug with benzoylecgonine cases (n=3) and there was one case that was positive for fentanyl and benzoylecgonine. Concentrations of benzoylecgonine have been detected at 5389 ng/mL when ascending cocaine doses, with a maximum of 2 g, were orally administered over 16 dosing sessions.[85,86] Another

study that reviewed 37 cases involving cocaine-related deaths found a mean benzoylecgonine concentration of 7800 ng/mL (range, 740-31000 ng/mL).[87] This current study detected benzoylecgonine concentrations (range, 37.2-3876.8 ng/mL) that fall in both therapeutic and fatal ranges. However, cocaine was not detected in any cases of this study and is attributed to degradation during extended storage of samples. Overall, there is significant variability in benzoylecgonine concentrations found in living individuals and cocaine-related fatalities but because benzoylecgonine is an inactive metabolite, interpretation of its concentrations is limited.

In 16 cases, heroin was self-reported to have been used recently or in the past. ID 260, a 69 year old male, was the only case of these 16 that was morphine-positive (without morphine administered in the ED), and so heroin use was identified even though 6-AM was not detected. ID 260 was considered a PDR case in which injection of heroin into the right hand causing an abscess also led to an infection. The patient reported daily heroin use with his last use 12 hours prior to ED arrival. The detected drug concentrations were 18.6 ng/mL morphine, 125.8 ng/mL methamphetamine, and 18.8 ng/mL amphetamine. The other cases where heroin use was self-reported were positive for drugs such as methamphetamine, fentanyl and/or benzoylecgonine, but it is uncertain if the detected drug use reflects the absence of heroin consumption in the recent past or if it is the result of unintentional drug substitution. Eight of the self-identified heroin users also identified fentanyl use, so in these cases, it is impossible to distinguish if fentanyl-substituted heroin was used or if they only used fentanyl. There is evidence of fentanyl-substituted and fentanyl-adulterated heroin in the US and this could reflect these cases where heroin use was documented and fentanyl was detected.[81,88,89]

Bromazolam was detected in four cases, ID 246, 263, 284, 302, and the average concentration was 5.2 ng/mL (range, 0.9-9.9 ng/mL). Toxicological analysis revealed that the presence of bromazolam was detected with methamphetamine, fentanyl, or both methamphetamine and fentanyl. It is unknown if the four patients that were positive for bromazolam ingested it knowingly and did not report its use, or if it was present as an adulterant and ingested unknowingly. Similar findings have been detailed in research

involving post-mortem cases from British Columbia where the average bromazolam concentration was 11.4 ng/mL (range, 0.5-319.3 ng/mL) and was detected with fentanyl or methamphetamine, and to a lesser extent, cocaine. Although the sample size positive for bromazolam is small in this study, it is interesting to note the findings are similar to those of British Columbia, given that both were conducted on the west coast.[90] In comparison to five DUID cases, bromazolam concentrations have been reported at an average of 41 ng/mL (range, 4.3-68 ng/mL) which encompasses the average bromazolam concentration found in the current study.[91] The bromazolam concentrations detected in this study overlap with both fatal and survivable concentrations described in the literature. Nonetheless, bromazolam is a relatively novel benzodiazepine, and additional research is needed to characterize its blood concentrations in human populations.

ID 323 reported to have taken "200 mg doses of Xanax" from a pharmacy in Mexico and alprazolam was detected at 163.4 ng/mL in this case. A therapeutic drug study found that patients on 9 mg alprazolam per day had steady-state serum concentrations of 102 ng/mL (blood/plasma ratio of 0.8).[92,93] Jones et al. found that in 773 impaired drivers, the average alprazolam concentration was 80 ng/mL (range, 20–3900 ng/mL).[94] There is a wide range in which alprazolam concentrations exist in the living population and in reviewing the observed symptoms in this case, the patient was likely experiencing adverse reactions to alprazolam.[95]

Although methamphetamine, fentanyl, and cocaine had the greatest concordance between self-reported use and detection, there were patients that reported using these drugs but none were detected, and cases where these drugs were reported without being detected. Other substances that were frequently self-reported but not detected were heroin, Percocets, and alprazolam. Further, bromazolam was detected in four cases but was not self-reported. Previous research that compared self-reported drug use with immunoassay and available mass spectrometry results across EDs in Europe found the greatest agreement for heroin (86.1%) and cocaine (74.1%).[53] Another study in London, UK found good agreement between methamphetamine and opiate self-report and immunoassay results.[29] The current study finds similar

results to these two studies with agreement at methamphetamine (90.4%), fentanyl (73.7%), and cocaine (62.5%). In contrast, however, heroin was found to have 6.25% agreement as only one case out of a total of 16 was found to be heroin-positive. It should be highlighted that lower self-reported drug use can be attributed to clinical matters such as memory loss and reluctance to report drug use, which can affect the statistics described. Furthermore, patients that have self-reported drug use but are beyond the detectable window due to a long interval since their last use, or low drug dosages, yields no detection by this method and can affect these statistics as well.

The limitations of this research involve the long storage time of blood samples prior to toxicological testing. The time between blood collection and toxicological analysis ranged from approximately 230-320 days. Cocaine is the most affected because the prolonged storage time, despite refrigeration and preservative, leads to degradation and greatly contributes to the absence of cocaine found in the blood of cocaine users.[96] As a result, this study is not able to link self-reported cocaine use to cocaine concentrations in blood.

Heroin use is difficult to verify because it is unlikely that 6-MAM is detected in blood and morphine-positive blood could be attributed to heroin, codeine, or morphine consumption. The study identified one morphine-positive case, no 6-MAM-positive cases, and there were instances of ED administration of morphine. The source could not be determined in the one morphine-positive case, ID 326, since there was no indication of heroin, codeine or morphine use in the self-reported history. Thus, this case could be an indication of unreliable self-report.

Self-report presents inherent limitations as it can be inaccurate. The mental status of the patient at the time, reluctance to share drug history, or a lapse in memory could contribute to discrepancies between self-reported drug use and analytical testing. For the purpose of this study, self-reported drug use is used to contextualize BDC, but readers should recognize that self-reported drug history can be fallible. For example, there were cases where no drug use was reported but analytical results reveal the presence of

methamphetamine. Drugs prescribed or administered in the ED were identified to eliminate drug use that was not recreational. However, it is possible that drugs detected in blood were administered at other hospitals or previous prescriptions were not identified in the ED and therefore not noted in the medical record. Furthermore, while this study provides valuable data on the BDC of recreational drug users in an ED setting, these findings may not be generalizable to all recreational drug users, especially those in other environments with different patterns of drug use.

An additional limitation to this study is that the method used is a targeted analysis for 37 drugs and metabolites, which may not encompass all substances that could have contributed to observed symptoms. The symptomatology described for each case may be linked to medical causes, but it is also possible that other undetected substances were responsible for those effects. Drawing conclusions between drug concentration and observed symptoms, particularly in cases involving polydrug use or the presence of unidentified drugs, remains a challenge.

7. Conclusion

This study provides insight into the BDC of drug users given the observations at the ED and self-reported drug use. The results reveal variability in the BDC detected, where concentrations are associated with reported therapeutic to fatal BDC in the literature. PDR and NDR cases were found to have no statistical difference between each other for the number of drugs detected and for methamphetamine, amphetamine, fentanyl, and benzoylecgonine concentrations. Additionally, methamphetamine, fentanyl, and cocaine had the greatest concordance between self-reported drug use and analytical results.

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Appendix A.

Age, sex, observations on ED admission, self-reported drug use, PDR or NDR classification, drugs administered at the ED, and detected drug concentrations for 116 patients

ID	Age/ Sex	Observations on ED Admission	Reported Drug Use	Admission Classification	Number of Drugs Detected	Drug Concentration (ng/mL)
76	34F	Burn with boiling water	Methamphetamine use. Last drug use was yesterday.	NDR	2	Amphetamine: 9.4 Methamphetamine: 9.4
84	50M	Suicidal ideation	Methamphetamine use the day before. Recreational use, no IVDU.	NDR	2	Amphetamine: 16.1 Methamphetamine: 134.6
85	48M	Chest pain	Methamphetamine last used 4-5 days prior. No IVDU.	NDR	2	Amphetamine: 9.4 Methamphetamine: 100.1
86	27F	Fentanyl withdrawal: chills, diarrhea, shortness of breath, rhinorrhea, agitation, and restlessness	Last used fentanyl the day before. Has been using fentanyl daily since approximately 4 months ago. Started to taper off of fentanyl use one week ago. No parenteral use.	PDR	1	Fentanyl: 1.4
88	60M	Slurred speech, facial droop, and balance issues	Regular methamphetamine use (one time per day), last used today.	NDR	2	Amphetamine: 69.8 Methamphetamine: 650.3
89	22F	Cramping abdominal pain and nausea (acute withdrawal symptoms)	Daily oxycodone snorts for two years. Using street Percocets 30s for three years (90mg/day). Uses three tablets three times a day. No IVDU. Last opioid use was four days prior. Will go 6-8 hours before feeling withdrawal.	PDR	1	Fentanyl: 3.7
97	48F	Generalized pain throughout, hallucinations	Last used cocaine this morning and methamphetamine yesterday.	PDR	3	Amphetamine: 9.4 Methamphetamine: 63.8 Benzoylecgonine: 126.7
99	32F	Alcohol withdrawal and seizures. Initial CIWA 6	Daily intranasal cocaine use since 2020, last used ~1g today prior to arrival.	PDR	2	Benzoylecgonine: 282.8 Nordiazepam: 46.3

100	63M	Dizziness, states withdrawal from heroin	>20 years intranasal heroin use. Recently uses ~1.5-2g daily. Last used heroin 12 hours ago. Had a small piece of friend's Suboxone tablet four hours ago.	PDR	1	Benzoylecgonine: 592.0
104	64M	Right leg pain	Daily smoking of ~¼ gram of methamphetamine. Last used four days ago. No IVDU.	NDR	2	Amphetamine: 32.6 Methamphetamine: 201.7
105	31F	Slept for two days after injecting methamphetamine. Upon waking up, felt pain on right lower extremity (erythema) but could ambulate. Was no longer able to ambulate due to pain today. AOx4	Injected methamphetamine four days ago and suspects fentanyl because she felt sick. Uses IV methamphetamine daily with last known use prior to arrival. Site of injection primarily limited to bilateral axilla.	PDR	2	Amphetamine: 87.7 Methamphetamine: 1062.6
109	58M	Suicidal ideation	Methamphetamine use.	NDR	2	Amphetamine: 23.5 Methamphetamine: 369.5
110	25M	Chest pain and trouble breathing	Methamphetamine use 11 days ago, possibly via inhalation. History of cocaine use.	NDR	0	
111	40M	Abscess and leg pain, fever, chills, dizziness, vomiting	Prior history of IVDU but has not injected in several years. Fentanyl inhalation two days ago. Methamphetamine use.	NDR	3	Amphetamine: 33.7 Methamphetamine: 265.7 Fentanyl: 40.0
112	30M	Left hand infection, mild withdrawal symptoms	Smokes several grams of fentanyl a day and occasional IV use. Past history of heroin use. Fentanyl used approximately 32 hours prior. Methamphetamine use.	PDR	3	Amphetamine: 9.4 Methamphetamine: 14.5 Fentanyl: 0.78
113	20F	Altered level of consciousness. Audiovisual hallucinations	Took a bad ecstasy pill that someone gave her	PDR	2	Amphetamine: 90.4 Methamphetamine: 713.1
114	58M	Suicidal ideation	Cocaine and methamphetamine use.	NDR	1	Benzoylecgonine: 181.7

117	33M	Suicide attempt	Fentanyl use 48 hours prior and methamphetamine use today.	NDR	3	Amphetamine: 9.4 Methamphetamine: 253.8 Fentanyl: 2.9
118	54M	Suicidal ideation	Methamphetamine use. Last use today by smoking.	NDR	2	Amphetamine: 32.6 Methamphetamine: 286.3
121	60M	Leg pain, chest pain and shortness of breath	History of cocaine and heroin use. Last used methamphetamine and fentanyl today.	PDR	2	Amphetamine: 20.4 Methamphetamine: 553.7
122	43M	Stomach pain	Smokes methamphetamine, last used two days ago.	NDR	2	Amphetamine: 15.1 Methamphetamine: 91.9
128	62M	Mental status change (off psychological baseline and confused). Tachycardia	IV methamphetamine last used today.	PDR	0	
133	35F	Foot pain, suicidal ideation	Methamphetamine, heroin, and hallucinogen use. Smoked methamphetamine three days ago.	NDR	1	Methamphetamine: 9.4
134	22F	Wants to start Suboxone	Reports to have overdosed so self-administered Narcan three times the previous day. After release at a hospital, smoked fentanyl (a few hours ago). Has been using fentanyl consistently since age 15 and uses every few hours.	PDR	2	Fentanyl: 8.0 Diazepam: 82.8
135	39M	Suicidal ideation, alcohol withdrawal	Last used cocaine prior to arrival.	PDR	0	
137	35F	Anxiety, suicidal ideation, hallucinations, diarrhea, subjective fever, pain everywhere. Not compliant with psychiatric medication	Last used crystal meth 2-3days ago and has been "trying to avoid that stuff." Recent cocaine use. Smokes fentanyl and occasionally snorts it. Uses fentanyl a few times a month and last used it two weeks ago.	PDR	3	Amphetamine: 35.7 Methamphetamine: 278.1 Lorazepam: 9.4
139	63M	Shortness of breath, toe infection, feeling withdrawal	Daily heroin use. Typical routine is to inject 30u of heroin subcutaneously once a day. Reports daily use of ~½ gram of heroin	PDR	2	Diazepam: 126.8 Nordiazepam: 71.9

			via skin popping. Last used today.			
140	28M	Back pain	IV methamphetamine, last used three weeks ago.	NDR	2	Amphetamine: 112.3 Methamphetamine: 1242.9
144	44M	Neurosyphilis	Smoked meth prior to arrival.	NDR	2	Amphetamine: 57.3 Methamphetamine: 242.7
149	30M	Altered mental status, rib fracture	Couple of months ago: 1g of fentanyl a day. Last use was 2-3 days ago.	NDR	3	Amphetamine: 33.4 Methamphetamine: 142.4 Fentanyl: 0.78
152	49F	Altered mental status, withdrawal symptoms: body ache, sweats, chills	Uses fentanyl “all day every day” for years. States "I took some fetty's then Suboxone then a bunch of NyQuil”. Prescribed 2mg Suboxone from an outpatient clinic and was told to take tablets throughout the day yesterday while continuing to use fentanyl, with a plan to start Sublocade. Took a full strip of Suboxone and went into precipitated withdrawal. She “chugged” a bottle of NyQuil and took ibuprofen. Also reports ingesting NyQuil and methamphetamine at a similar time.	PDR	3	Amphetamine: 9.4 Methamphetamine: 32.3 Fentanyl: 2.4
153	65F	Opioid withdrawal, diarrhea, headache, anxiety	Uses ~ 1g fentanyl daily for the past 1.5 years. Last use around 2 hours prior to arrival. Previously used norco for chronic neck and shoulder pain for several years.	PDR	4	Fentanyl: 2.5 Lorazepam: 12.8 Diazepam: 64.5 Nordiazepam: 30.8
154	54M	Abdominal pain, appears uncomfortable	History of meth use with last use 2-3 days ago. Only by smoking, no IVDU.	NDR	2	Amphetamine: 9.4 Methamphetamine: 30
155	35M	Found down on a sidewalk with	Unknown	PDR	2	Amphetamine: 175.8

		GCS 12. Found awake with eyes open, pinpoint pupils, not following commands, not answering any questions. Was making purposeful movements and withdrawing to pain but unable to tell staff his name or any pertinent information. Was restless, agitated, flailing his arms, and was making sounds but did not speak coherent words.				Methamphetamine: 843.6
156	69M	Intermittent abdominal pain, coughing up blood	Last drug use prior to presenting, IVDU, is a lifelong user. Uses fentanyl, heroin, meth, and cocaine.	PDR	1	Fentanyl: 10.2
160	38F	Nausea, voices in her head, irritability, pain in lower back, withdrawal from fentanyl	Fentanyl use (smoking) four days ago. Reports daily use of "2 grams" of fentanyl.	PDR	3	Amphetamine: 78.5 Methamphetamine: 708.5 Fentanyl: 9.3
162	61F	Lower back pain, moves all extremities, tingling to both lower extremities, unable to ambulate safely. GCS 15	Smoking lithium and does not answer when last stimulant use	NDR	2	Amphetamine: 32.1 Methamphetamine: 182.8
165	38M	Burns to face and back	Methamphetamine use the day before. History of IVDU. Possible fentanyl use.	NDR	3	Amphetamine: 39.3 Methamphetamine: 449.1 Oxycodone: 18.8
167	41F	Finger pain, itching, psychiatric disorder	Frequent use of a recreational substance called "bitter sweet" which is a crystal but she is unsure of what it is. Uses it as much as possible, possibly once per day. When unable to	PDR	2	Amphetamine: 17.9 Methamphetamine: 43.5

			find "bitter sweet", she uses methamphetamine and sometimes fentanyl.			
170	65M	Nausea, vomiting, body pain, need refill of fentanyl patches. Withdrawal since out of patches for two days	Uses fentanyl 50 mcg patches but used his last one last week, which he is still using.	PDR	1	Fentanyl: 0.78
171	40M	Altered mental state, hallucinations, ataxic	Used prescription Xanax 1mg daily for 10 years. But he was not prescribed this for more than 5 days this year. Possible illicit benzo use.	PDR	0	
172	23F	Dehydration, has not been able to eat or drink	Detoxing from Xanax for five days. Briefly relapsed on methamphetamine and fentanyl. Uses opioids. Last drug use was 5 days ago.	PDR	0	
173	38F	Constipation for two weeks, headache, dizziness, chills, shortness of breath, poor oral intake, states her brain hurts. Arrived tachycardic, remained afebrile, and hemodynamicall y stable	Last used methamphetamine yesterday, stopped using fentanyl three weeks ago. Had opiate withdrawal last month and was discharged with buprenorphine that she stopped taking a few days ago due to it making her stomach feel ill. Last used methamphetamine yesterday.	PDR	2	Amphetamine: 57.0 Methamphetamine: 448.1
174	37M	Suboxone initiation	Relapsed with smoking fentanyl about a year and a half ago, wants to stop street use of opiates. Attempted to get initiated with Suboxone approximately 1 month ago but had precipitated withdrawal. Then relapsed and continued using some fentanyl for the last month. Last use was four hours ago. Has been smoking fentanyl for approximately a year to a year and a half, multiple times a day. Uses amphetamines.	PDR	3	Amphetamine: 23.4 Methamphetamine: 250.8 Fentanyl: 42.0

175	52M	Painful and increased swelling of arm abscess since last night. Fever, nausea	States his home oxycodone is wearing off quickly. Oxycodone use is for pain management only: taking three to four pills daily at 30 mg each. Does not use cocaine, methamphetamine, or other drugs. No IVDU. Oxycodone was used approximately 6 hours prior, and hydromorphone was used approximately 12 hours prior.	NDR	1	Oxycodone: 23.3
176	39M	Management of left arm fracture. Mild tachycardia, hypertension. GCS 15 and vitals within normal limits.	Last fentanyl use was today. History of daily fentanyl use and intermittent methamphetamine use.	NDR	4	Amphetamine: 36.4 Methamphetamine: 139.3 Fentanyl: 0.78 Oxycodone: 18.8
177	19F	Abdominal pain, nausea, vomiting since yesterday. Chest tightness, shortness of breath since today. Fatigue, chest pain, cough, shortness of breath, and diaphoresis present. AOx4. Developed body aches, shaking, and diffuse abdominal pain yesterday. Felt "lightheaded and saw stars" when she stood up and then fell back down on the couch today. History of seizures	Last use was two days ago. Has used intranasal fentanyl daily for about one year. Was prescribed Suboxone six weeks ago for accidental overdose. Took Suboxone for 3 days before quitting and resuming fentanyl use because it "didn't work." No other drug use.	PDR	1	Fentanyl: 0.78
181	65M	Shortness of breath for two weeks. AOx4. History of congestive heart failure, hypertension	Daily marijuana use and methamphetamine, Last used two to three days ago. No opioid use.	NDR	1	Methamphetamine: 14.7

182	65M	AOx4. Fell off bike about three days ago, hit left side ribs on either bike handle or street, 10/10 pain on that side	Last methamphetamine was three days ago.	NDR	2	Methamphetamine: 30 Benzoylecgonine: 108.8
194	69M	Diffuse abdominal pain today, dark colored discharge from nose: was seen with episodes of coffee ground emesis. Severe esophagitis with recurrent admissions for upper gastrointestinal bleeding	Last used fentanyl today. History of cocaine, methamphetamine, and heroin use. Takes $100 worth of fentanyl per day intranasally. Uses IV heroin, last used today. Has used heroin for 53 years (reports since the age of 13).	PDR	3	Amphetamine: 9.4 Methamphetamine: 79.4 Fentanyl: 8.9
195	32F	Alcohol withdrawal symptoms. AOx4	Used intranasal cocaine 1 hour ago and thinks there is fentanyl in it. Chronic cocaine use, almost daily for the last couple years. Testing at another hospital yesterday, was positive for cocaine and fentanyl. Patient unaware that cocaine was laced with fentanyl.	PDR	1	Benzoylecgonine: 330.7
196	57M	Paranoid delusions, auditory and visual hallucinations	Last used methamphetamine today, but possible that it was yesterday.	PDR	2	Amphetamine: 24.2 Methamphetamine: 309.1
197	36M	Suicidal ideation for one week, intensifying today. Occasional shortness of breath	Used methamphetamine yesterday. Injected into right arm muscle and belly and is now having pain there. Also injected into left arm at similar time.	PDR	2	Amphetamine: 236.1 Methamphetamine: 2111.4
198	60M	Shortness of breath for a few days, fatigue, weakness	Methamphetamine use is 1.5 grams a day for 30 years.	PDR	2	Amphetamine: Methamphetamine: 9.4 Lorazepam: 9.4
202	39F	Fell and hit face on counter, swelling on left side of face, tender with	Methamphetamine use most days. IV in distant past. Methamphetamine use is for maintenance,	PDR	2	Amphetamine: 37.6 Methamphetamine: 601.0

		palpation. Intermittent chest pain, shortness of breath and body aches. When coughing, she gets dizzy and blurred vision. Purple hands and feet with delayed cap refill. Congestive heart failure.	taking three to four puffs daily.			
203	38M	Brought in after IV drug use. Subjective chills and skin lesions throughout entire body. Was recently evaluated for skin wounds and was diagnosed with a soft tissue infection. Falls asleep quickly.	Was using fentanyl and methamphetamine, has a history of IV drug use, and relapsed yesterday to use heroin and speed. Recent replace using heroin and speed via injection and used fentanyl prior to arrival.	PDR	3	Amphetamine: 9.4 Methamphetamine: 59.9 Fentanyl: 1.5
204	20M	Suboxone initiation	Last used fentanyl two to four hours ago. Uses fentanyl multiple times per day by insufflation.	PDR	1	Fentanyl: 6.9
205	64M	Eye swelling, hypothermic, tachycardia and history concerned for sepsis. GCS 15	Methamphetamine use prior to arrival. Prior history of fentanyl use, possibly used before presenting.	PDR	2	Amphetamine: 12.7 Methamphetamine: 79.3
206	37M	Alcohol withdrawal. Felt poorly and started having the shakes. Vomiting, but denied any abdominal pain or bowel issues.	Used to be on methadone some time ago for fentanyl use disorder, but has gone back to using fentanyl. Last use was one day ago.	PDR	1	Fentanyl: 24.8
207	60M	Benzodiazepine withdrawal. Irritable with general body pain, left knee pain, unable to take care of self. Unable to tolerate pain	Reports taking more doses of his benzodiazepines. Last took valium yesterday. Takes 20-25 valiums a day. Has been using diazepam almost daily since his 20’s due to anxiety. Typically takes around 2-4 mg daily. Has	PDR	2	Diazepam: 372.7 Nordiazepam: 329.2

			been taking increased doses of diazepam since five months ago. Currently taking about 10 mg in the morning and then 12-20 mg at night. Currently taking Ativan two mg at least two times daily and Valium 10mg two times daily, though he reports taking 20mg valium last night. Also states he took a "small" pain pill yesterday, but generally does not take opioid medications. Last drug use was prior to arrival.			
212	41F	Alcohol and drug withdrawal. Needs to detox from methamphetamine. Mild fasciculations to tongue, sweating, shaking	Last methamphetamine was six hours prior to arrival. Ongoing use, last used yesterday.	PDR	3	Amphetamine: 86.5 Methamphetamine: 400.0 Diazepam: 206.0
213	22F	Coughing since two months ago after overdosing. AOx4, GCS 15, drowsy	Last smoked fentanyl three hours ago or yesterday. Smokes fentanyl twice a day. Has been smoking fentanyl since 15 years old. Initially snorted fentanyl but has recently switched to smoking. Occasionally uses methamphetamine and cocaine.	PDR	2	Fentanyl: 8.2 Diazepam: 65.3
217	47M	Difficult to arouse, EMS gave oral glucose and he improved. Hypoglycemia. AOx4, diaphoretic, and slow to respond.	Smoked methamphetamine within the last 12 hours.	NDR	2	Amphetamine: 20.0 Methamphetamine: 107.5
218	23M	Anxiety in setting of cocaine use. Appears intoxicated. Was drinking and used some cocaine, then	Just used cocaine. Acute cocaine toxicity.	PDR	1	Benzoylecgonine: 107.4

		started to feel anxious and hot.				
224	54F	Problem with speech lasted for 60 seconds. Slurred speech and aphasia lasting approximately a minute, two days ago.	Smokes and inject methamphetamine.	NDR	2	Amphetamine: 24.2 Methamphetamine: 188.3
225	62F	Two days of lower left extremity redness and swelling.	Smokes cocaine. Last used prior to arrival.	NDR	2	Methamphetamine: 20.7 Benzoylecgonine: 557.6
227	35F	History of methamphetamine-related heart failure. Needs medication refill for uncontrolled heart failure symptoms including orthopnea, leg swelling, non-productive cough.	Last drug use was two days ago.	NDR	2	Amphetamine: 29.0 Methamphetamine: 207.0
228	23M	Alcohol problem. Abdominal pain, nausea and vomiting four times since yesterday.	1-2 g of fentanyl per day for three years, last use was 2.5 days to approximately 60 hours prior. Usually smokes, has used needles in the past, however has not done this in months.	PDR	4	Amphetamine: 9.4 Methamphetamine: 14.6 Fentanyl: 0.78 Nordiazepam: 37.5
231	50F	Altered mental status. Confused, agitated and in pain for three days. Is alert to person but confused to place and time situation. Abdominal pain.	Denies recreational drug use on admission. But has documented history of active heroin use, last used a few days ago, possibly one day ago.	PDR	5	Amphetamine: 67.3 Methamphetamine: 162.6 Fentanyl: 0.78 Ketamine: 338.1 Norketamine: 802.9

232	43F	Head trauma after physical assault that occurred prior to arrival.	Daily fentanyl use, last used it either yesterday or today. Usually uses 3g a day by smoking. Has been using fentanyl for one to one and half years. Used IV two days prior to admission, but primarily smokes. Was using heroin for about six months prior to fentanyl use. Has overdosed about six times in the past year. IV and smoked methamphetamine for about 10 years, but stopped after starting opioids.	NDR	3	Amphetamine: 131.5 Methamphetamine: 664.0 Fentanyl: 22.1
233	48M	Presents with shortness of breath, acute heart failure exacerbation, likely methamphetamine use and medication non-adherence. Increased abdominal swelling. GCS 15, no chest pain.	Last used methamphetamine one week ago. No IVDU	PDR	1	Methamphetamine: 21.3
234	43M	Feeling hot and nauseous. Tried to overdose on fentanyl last night and went to another hospital. Suicidal ideation.	History of methamphetamine and opioid use. Recent fentanyl use. Last drug use was one day ago.	PDR	3	Amphetamine: 9.4 Methamphetamine: 37.7 Fentanyl: 4.0
239	37F	Subjective fever for three days and has not been speaking much since yesterday. Altered mental status, severe sepsis, encephalopathy and opioid withdrawal.	Daily IV heroin user per groin, last used yesterday. States that she injects it "everywhere" and uses "a lot". Tries to avoid fentanyl. Heroin and fentanyl last used approximately > 48 hours prior.	PDR	4	Amphetamine: 21.0 Methamphetamine: 128.0 Fentanyl: 0.78 Diazepam: 63.9
241	44F	Altered mental status and suicidal ideation.	Denies ingestion of substances and medications.	NDR	3	Amphetamine: 35.3 Methamphetamine:

		AAOx3, disorientated.				178.8 Fentanyl: 24.6
243	59M	Lower leg swelling for four days.	Smokes cocaine twice daily.	NDR	2	Benzoylecgonine: 3876.8 Nordiazepam: 37.5
244	59M	Shortness of breath, chest pain intermittently for several weeks. Congestive heart failure.	Smokes methamphetamine daily, no IVDU. Last use was prior to admission. Used medication and stimulants since he was a child, and discovered methamphetamine later in his life.	PDR	2	Amphetamine: 9.4 Methamphetamine: 99.6
245	18F	Opioid withdrawal: nausea and vomiting with yellow vomitus, has not eaten for the past three days. Difficulty sleeping, waking up every 10-20 minutes to vomit or diaphoretic. Scleral icterus, intermittent diarrhea, constipation, chills, difficulty sitting still, some lower extremity pain.	Uses fentanyl recreationally, has not used for two days. Has been taking 4-6 pills of Percocet (unknown dosage) daily but stopped three days ago. Has never tested her medications for fentanyl. Took 6mg of her sister's Suboxone yesterday and 4mg three hours prior to admission. No IVDU.	PDR	0	
246	56M	Flu-like symptoms, fever started today. All over body pain, chills, cough.	Used methamphetamine immediately prior to being admitted.	PDR	3	Amphetamine: 29.1 Methamphetamine: 150.5 Bromazolam: 0.94
248	43M	Hypoxia on room air, shortness of breath	No cocaine, fentanyl, or methamphetamine use.	NDR	2	Methamphetamine: 29.6 Lorazepam: 9.4
260	69M	Abscess to the right hand for three days. Injected heroin there and developed an infection after. GCS 15	Last used heroin in right hand IVDU. Daily heroin use, last used 12 hours prior.	PDR	3	Amphetamine: 18.8 Methamphetamine: 125.8 Morphine: 18.8

261	38M	18% total body surface area wounds after rubbing alcohol exploded on him pouring over a candle. Burns to face, arms, and chest.	Presented with methamphetamine use. Used for over seven years, started 'shooting' it the past two months. Last drug use yesterday, uses methamphetamine daily. Regular fentanyl use and feels like he is withdrawing, estimated last use a few days ago.	NDR	3	Amphetamine: 178.3 Methamphetamine: 2340.6 Norketamine: 37.5
262	48M	Found on the street. History of diabetes. Generalized body pain, most severe in bilateral feet.	Lat used crystal methamphetamine three days ago. Uses five days a week. No IVDU.	NDR	0	
263	20M	Intermittent episodes of shaking. Calms down with lower heart rate then goes back up. Nauseated, hallucinating/hearing music	Xanax and fentanyl use. Last used both (smokes and injects) two days ago. 4mg a day of Xanax.	PDR	2	Fentanyl: 22.9 Bromazolam: 3.0
269	45F	Two days of shortness of breath, chest pain that is sharp, midsternal, non-radiating.	Methamphetamine use 2-3 days ago, then later states 4-5 days ago.	NDR	1	Methamphetamine: 9.4
270	47M	Uncomfortable, no acute distress. Abscess on neck	Last used fentanyl today. History of methamphetamine, cannabis, and fentanyl use. Has been taking fentanyl intermittently for pain control. Recent methamphetamine use and cannabis use. No IVDU.	NDR	3	Amphetamine: 9.4 Methamphetamine: 46.0 Fentanyl: 13.2

271	40F	Patient discharged from here this morning after presenting with bilateral buttock wounds after intimate partner violence. Found by shelter staff to be altered so was brought back. No new trauma or falls. Was lethargic, arouses to stimulation and follows simple commands. Left lower laceration and bilateral gluteal lacerations covered by dressings. Vital signs were stable with no signs of hypoxia and maintaining an airway. Normal cardio and pulmonary abdominal exams. Found to have drug paraphernalia, suspected to be intoxicated.	Smokes crack cocaine, no IVDU.	PDR	1	Benzoylecgonine: 37.2
280	24F	Right hand swelling, tachycardic.	Frequent fentanyl use. Smokes and injects some methamphetamine but mostly fentanyl. Primarily uses opioids now. Fentanyl last used <24 hours prior. Last IV use was a few months ago.	NDR	3	Amphetamine: 75.1 Methamphetamine: 140.4 Fentanyl: 45.6
281	54M	In wheelchair due to wound on bilateral lower extremity.	Last methamphetamine use was three days ago.	NDR	2	Amphetamine: 46.2 Methamphetamine: 258.0

283	47F	Headache, brain tumor.	IV methamphetamine use. Has been using IVDU to help manage headaches without relief. States “ I have a high pain tolerance so if the meth isn't working you know I'm in pain"	NDR	2	Amphetamine: 15.1 Methamphetamine: 242.8
284	56F	Injected heroin into left upper extremity and subsequently developed erythema and edema two weeks ago. Abscess to left arm, sore throat w/ recent strep exposure, recent fevers.	Heroin use seven days ago.	PDR	3	Amphetamine: 54.6 Methamphetamine: 338.6 Bromazolam: 6.8
285	37M	Abdominal "blockage" and constipation for three weeks.	Daily fentanyl use (smoking) for the past year and three months. History of methamphetamine and heroin use.	PDR	3	Amphetamine: 61.7 Methamphetamine: 707.9 Fentanyl: 113.7
297	37M	Lockjaw started about one hour prior to arrival, causing difficulty breathing, pain, trouble speaking. NAD noted, AOx4, VSS, GCS 15.	Treated for cocaine toxicity. Took Latuda and started to have this reaction. Then used cocaine to help with the symptoms since it has helped in the past.	PDR	2	Fentanyl: 0.78 Benzoylecgonine: 42.7
298	29F	Fever, cough for three days. Today woke up with abdominal pain, bright red blood vomiting.	IVDU and smoking methamphetamine, last used prior to arrival.	PDR	2	Amphetamine: 57.6 Methamphetamine: 191.4
299	51M	Left foot infection, Medication nonadherence.	Methamphetamine use for past three years. Last used either 1-3 or 3-4 days ago.	NDR	2	Amphetamine: 29.8 Methamphetamine: 161.8
300	32F	Headache after being hit in head with rock three days prior, scattered thinking but oriented.	Last drug use was two days ago. Methamphetamine use for past 13 years.	NDR	2	Amphetamine: 21.4 Methamphetamine: 50.7

302	37F	One week of cough, significant chest pain. Fevers, chills, productive cough with green phlegm and mild sore throat. AOx4.	Daily, multiple times a day use of fentanyl and occasional methamphetamine use. No recent IVDU. Last heroin use unknown. Fentanyl last used approximately 7-8 hours prior. Benzodiazepines used occasionally, last used Xanax yesterday.	NDR	5	Amphetamine: 16.5 Methamphetamine: 105.1 Fentanyl: 4.2 Oxycodone: 52.6 Bromazolam: 9.9
303	36M	Fentanyl withdrawal. AOx4. Moderate Clinical Opiate Withdrawal Score. Feeling anxious, tremors, fever, chills, nausea, vomiting, abdominal discomfort, sweating, feeling hot and cold.	Smokes methamphetamine and fentanyl, smokes fentanyl every day, 1-2g per day. Last used four days ago. Withdrawal symptoms usually precipitate within 24 hours from stopping opioid use.	PDR	3	Amphetamine: 14.3 Methamphetamine: 17.3 Fentanyl: 0.78
304	35F	Akathisia, chest pressure in the setting of recent cocaine and daily alcohol use. AOx4, GCS 15, vital signs stable. Since 6 hours ago, has intermittent dizziness, tremors, tactile hallucinations.	Snorted cocaine approximately seven hours ago due to tiredness after not sleeping last night. Also snorted cocaine two days ago, used some alcohol, had difficulty sleeping two nights ago, felt off one day ago in the morning.	PDR	1	Benzoylecgonine: 106.4
305	37F	Right sided abdominal pain for two days. Nausea, vomiting, appears uncomfortable. Has cancer (chronic pain). Signs of active withdrawal.	Smoking and snorting heroin almost every waking hour for the past several months. History of methamphetamine use. Currently taking morphine (30mg) PO BID daily at home for pain management. Heroin, last used 96 hours prior or within the last week.	PDR	3	Amphetamine: 12.8 Methamphetamine: 113.5 Morphine: 18.8
310	50F	Vaginal discharge, appears sleepy.	IV heroin use. Last heroin use was two days ago. Struggling with drug use including fentanyl and methamphetamine.	NDR	3	Amphetamine: 14.6 Methamphetamine: 52.6 Fentanyl: 2.7

			Intermittent heroin and methamphetamine use and is not taking any medications currently.			
316	49M	Methamphetamine-associated heart failure history. Walking and inhaled something in the air, now coughing and feels like there is dust in his lungs and lips are numb.Feels off. For the past 2-3 weeks, having more and more shortness of breath.	Methamphetamine use.	NDR	2	Amphetamine: 9.6 Methamphetamine: 37.2
317	55F	11% total body surface area burn.	Uses fentanyl and currently experiencing withdrawals. History of IVDU. Smokes fentanyl multiple times a day, last used approximately 12 hours ago.	NDR	3	Amphetamine: 16.4 Methamphetamine: 140.8 Fentanyl: 15.0
318	54F	Bilateral lower extremities edema with history of congestive heart failure.	Has been using methamphetamine for a long time, last used two days ago.	NDR	2	Amphetamine: 31.3 Methamphetamine: 245.1
319	41M	Swollen finger.	Frequent methamphetamine use and has used at least once in the past two days.	NDR	3	Amphetamine: 36.7 Methamphetamine: 265.0 Oxycodone: 18.8
323	76F	Suspected unwitnessed ground level fall. Somnolent, AAOx3, able to answer questions appropriately, but persistently falling asleep.	Sister expressed concern for Ativan abuse. Patient states her friends obtained some medications from a pharmacy in Mexico and believes she has been taking "200mg" doses of "Xanax". She notes taking Xanax TID (100 mg in the AM, 100 mg in the afternoon, and 50 mg in the evening) but doses are too high and likely incorrect. Notes that the	PDR	1	Alprazolam: 163.4

			Xanax is not prescribed. Last dose was about 10 hours ago.			
324	28M	Brought in from scene for motor vehicle accident >40 mph. Back pain, left shoulder and arm pain. GCS 15	Daily fentanyl use for the past three years, last used fentanyl 22 hours prior, prior to accident. Possible drug use seven hours ago. Uses "street Percocets". Takes percocet/fentanyl 30 mg (approximately 15-30 tabs daily for the last three years).	NDR	2	Fentanyl: 3.0 Diazepam: 317.3
325	22F	Brought in from scene, motorcycle vehicle accident at 40mph into wall. Right elbow and right hip pain, AOx4, GCS 15.	Takes street Percocets, possibly M30s, multiple times daily. Last use was today.	NDR	1	Fentanyl: 18.0
326	68M	Opioid cessation.	Normally uses Dilaudid three times daily, started due to cancer. Last used today.	PDR	2	Morphine: 29.3 Hydromorphone: 9.4
327	45M	Needs diabetes medications. Suicidal ideation, sleepy.	Last drug use was two days ago. Methamphetamine use.	NDR	2	Amphetamine: 46.6 Methamphetamine: 273.1
328	34M	Wrist swelling.	Last use was <24 hours. IV fentanyl use, normally injects in femoral vein region.	NDR	3	Amphetamine: 29.7 Methamphetamine: 203.9 Fentanyl: 81.8
329	53M	Shortness of breath, higher heart rate that keeps persisting for a few weeks especially on exertion, anemia	Remote history of IV drug use, but now reports that he smokes his methamphetamine with last use prior to arrival or two days ago.	PDR	2	Amphetamine: 9.4 Methamphetamine: 21.7
330	42M	Altered and agitated. Tachyc ardic, paranoid.	Has been having altered mental status since smoking methamphetamine (that he thinks is laced with fentanyl) yesterday. Cocaine, alcohol, and methamphetamine use.	PDR	2	Amphetamine: 19.1 Methamphetamine: 387.8
332	32M	Facial skin problem.	Fentanyl and methamphetamine use. Last drug use was today.	NDR	3	Amphetamine: 166.8 Methamphetamine:

						717.0 Fentanyl: 13.0
333	31F	Psychosis, schizoaffective history. Abdominal pain, diarrhea, threatening to kill herself.	Smoked methamphetamine yesterday.	NDR	2	Amphetamine: 47.4 Methamphetamine: 109.4
334	68M	Shortness of breath on and off for a couple of weeks, worse today. Bilateral lower extremities swelling up to calf, nonproductive cough, diarrhea for one month, "grumbling" stomach for one month	Long history of methamphetamine use, last used either three or seven days ago.	NDR	2	Amphetamine: 23.7 Methamphetamine: 154.2
335	67M	On and off dizziness for past year with episodes of blurred vision and blacking out.	Methamphetamine use three times weekly for about 40 years. Last used yesterday.	NDR	2	Amphetamine: 60.9 Methamphetamine: 534.9

Appendix B.

ANOVA for PDR and NDR cases and the number of drugs detected and methamphetamine, amphetamine, fentanyl, and benzoylecgonine concentrations.

Number of drugs detected

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
PDR	62	125	2.016129	1.16367
NDR	54	117	2.166667	0.745283

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.65406	1	0.65406	0.674875	0.413072	3.92433
Within Groups	110.4839	114	0.969157			
Total	111.1379	115				

Methamphetamine concentrations

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
PDR	35	11180.38	319.4393	174571.3
NDR	47	12330.43	262.3495	148598.3

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	65383.68	1	65383.68	0.409578	0.524012611	3.960352421
Within Groups	12770946	80	159636.8			
Total	12836330	81				

Amphetamine concentrations

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
NDR	41	1687.175	41.15060976	1579.112078
PDR	33	1424.575	43.16893939	2513.381993

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	74.48181858	1	74.48181858	0.037346541	0.847305325	3.973896992
Within Groups	143592.7069	72	1994.343151			
Total	143667.1887	73				

Fentanyl concentrations

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
PDR	24	276.65	11.52708333	571.3719656
NDR	15	287.6625	19.1775	491.6659364

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	540.2665401	1	540.2665401	0.998251358	0.324223704	4.105455897
Within Groups	20024.87832	37	541.2129275			
Total	20565.14486	38				

Benzoylecgonine concentrations

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	8	1625.9	203.2375	35924.88839
Column 2	4	4724.9	1181.225	3268059.443

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2550558.8	1	2550558.8	2.53644285	0.142329457	4.964602744
Within Groups	10055652.55	10	1005565.255			
Total	12606211.35	11				