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The Evaluation of Alternative Sample Preparation Techniques for Use in Forensic Laboratories

Final Summary Overview

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Purpose

In forensic toxicology, analysis of drugs of abuse is performed to determine cause of death, drug use, and whether someone was driving under the influence of drugs. The Census of Publicly Funded Crime Laboratories compiled by the U.S. Department of Justice's Bureau of Justice Statistics (BJS) classifies a case not completed within 30 days as backlogged. High sample request volumes, along with the need to identify and quantify a wide range of analytes and their metabolites at low concentrations, make sample preparation and instrumentation two of the most important factors in method development. During sample preparation, potential interferences from the matrix are removed and the analyte of interest is concentrated to enhance selectivity and reproducibility. Determining low concentrations of analyte in biological matrices can be complex, especially when dealing with signal suppression or enhancement due to matrix effects encountered during instrumental analysis. Issues such as these increase the need for optimal sample preparation.

The purpose of this study was to evaluate alternative sample preparation techniques for use in forensic laboratories. Specifically, we compared supported liquid extraction (SLE), dispersive solid-phase extraction (dSPE) and modified solid phase extraction (SPE B) techniques, which are designed to require fewer steps, to traditionally used multi-step SPE (SPE A) and liquid-liquid extraction (LLE). We determined which techniques resulted in more efficient sample analysis (i.e., greater productivity in less or equivalent time) and how easily they can be implemented into existing laboratory workflows. We also performed cost and solvent usage analyses.

Project Design

Twenty-six drugs and metabolites (Table 1) were extracted in blood and urine, in small volumes ($\leq 500 \mu\text{L}$) for this study using four different techniques (SPE, dSPE, LLE, SLE). Each technique and matrix were evaluated at RTI International (Lab A) and Boston University (Lab B), except for dSPE, which was only evaluated at Lab A. The purpose for this interlaboratory comparison was to examine if changes in the work environment affect the reliability of the methods and result in a bias between laboratories.

Materials

All compounds and their respective deuterated internal standards were purchased from Cerilliant Corporation (Round Rock, TX) and Cayman Chemical Company (Ann Arbor, MI). Reagents were LC-MS or ACS grade. Blood was purchased from UCT Laboratories (Valencia, CA), Bio Specialty (Colmar, PA), and Equitech Enterprises (Kerrville, TX). Urine was collected from anonymous donors under an IRB protocol. For SPE evaluation, Clean Screen® DAU (UCT, Lewistown, PA), Evolute® Express CX (Biotage, Charlotte, NC), and Oasis Prime HLB (Waters, Milford, MA) were used. For SLE, Isolute® SLE+ (Biotage) was used. Universal Wax and Universal SCX tips (DPX Technologies, Columbia, SC) were used for the evaluation of dSPE.

Method Development and Validation

Methods for each drug class, matrix, and sample preparation technique were developed with guidance from product vendors to ensure optimal extraction and are shown in Tables [A1](#) and [A2](#) in the appendix. After completion, each method was validated at both laboratories to assess the efficacy and

Table 1: 26 compounds analyzed in blood and urine

Drug Class	Compound
Barbiturate	Amobarbital
	Butalbital
	Phenobarbital
Amphetamine	Amphetamine
	Methamphetamine
	MDA
	MDEA
	MDEA
Miscellaneous	Benzoylcegonine
	Cocaine
	Lidocaine
Opiate/Opioid	Codeine
	Methadone
	Morphine
	Fentanyl
	Oxycodone
	6-AM
Hallucinogens	LSD
	PCP
Antidepressant	Amitriptyline
	Citalopram
	Fluoxetine
	Trazodone
Synthetic	Ethylone
	Alpha-PVP
	25I-NBOMe

reliability of the developed methods. For this study, the primary validation parameters used for comparison of the techniques were bias, precision, limit of detection (LOD) and quantitation (LOQ), ion suppression/enhancement, and recovery.

Instrumental and Data Analysis

Instrumental analysis performed at Lab A was conducted using an Agilent 1290 LC, with a Poroshell 120 SB-C18 analytical column, coupled to an Agilent 6490 triple quadrupole MS. At Lab B, samples were analyzed using a Shimadzu ultra-fast LC, with a Phenomenex Kintex F5 analytical column, coupled to an SCIEX 4000 QTrap MS. Validation parameters were calculated using SWGTOX Standard Practices for Method Validation in Forensic Toxicology.

Results/Findings

Validation Parameters

Urine dynamic range, LOQ, bias, and precision

The dynamic ranges were 0.5-500 ng/mL, 0.5-1000 ng/mL, 10-1,000 ng/mL, and 20-2000 ng/mL (Table [A3](#)) at both laboratories. LOQs were set at the lowest calibrator for validation purposes, as these values are representative of typical limits needed by laboratories, however further experiments showed that many analytes could be reliably quantified below the validated LOQ (Table [A4](#)). Calibration curves at both laboratories were weighted 1/x and were linear at Lab A, except for amphetamine and benzoylecgonine which were quadratic. All curves were quadratic at Lab B. The average correlation coefficient (r^2) was greater than 0.990. The concentrations of the samples used for bias and precision analysis are shown in Table [A5](#). All analytes were within $\pm 20\%$ for bias for all sample preparation methods at both laboratories, except for trazodone which had -22% and -23% bias for SLE and LLE respectively at Lab A. All analytes had within-run and between-run precisions within 20% for all sample preparation methods. The results are displayed in Tables [A6](#), [A7](#), and [A8](#). Lab A encountered an interference for MDA (SPE B and dSPE) in blood and urine and oxycodone (dSPE) in urine. Future

method development can be performed to choose alternative qualitative ion transitions for both compounds to eliminate this issue. Lab B did not assess 6-AM by LLE due to inadequate extraction.

SPE A

Bias ranged from -18.06% (trazodone) to 8.46% (butalbital) (Lab A) and -5.21% (MDMA) to 4.40% (butalbital) (Lab B). Within-run precision ranged from 4.30% for morphine to 10.22% for 25I-NBOMe (Lab A) and from 2.45% for fentanyl to 7.81% for morphine (Lab B). Between-run precision ranged from 5.13% for MDMA to 17.65% for fentanyl (Lab A) and from 3.38% for fentanyl to 8.55% for butalbital (Lab B).

SPE B

Bias ranged from -5.21% (trazodone) to 11.62% (butalbital) (Lab A) and -7.56% (25I-NBOMe) to 5.54% (citalopram) (Lab B). Within-run precision ranged from 3.66% for benzoylecgonine to 7.88% for citalopram (Lab A) and 3.49% for amphetamine to 9.03% for PCP (Lab B). Between-run precision ranged from 7.04% for amobarbital to 14.87% for trazodone (Lab A) and 4.26% for MDMA to 12.18% for PCP (Lab B).

SLE

Bias ranged from -21.97% (trazodone) to 13.13% (butalbital) (Lab A) and -1.73% (amobarbital) to 7.88% (methadone) (Lab B). Within-run precision ranged from 4.45% for codeine to 8.81% for LSD (Lab A) and 3.66% for cocaine to 6.31% for LSD (Lab B). Between-run precision ranged from 7.08% for amobarbital to 13.53% for trazodone (Lab A) and 4.31% for lidocaine to 11.98% for butalbital (Lab B).

LLE

Bias ranged from -23.10% (trazodone) to 6.95% (butalbital) (Lab A) and -3.74 (25I-NBOMe) to 4.45% (citalopram) (Lab B). Within-run precision ranged from 2.96% for butalbital to 7.28% for amitriptyline and citalopram (Lab A) and 2.34% for fentanyl to 5.82% for codeine (Lab B). Between-run precision ranged from 6.04% for oxycodone to 15.03% for fentanyl (Lab A) and 3.85% for 25I-NBOMe to

9.62% for 6-AM (Lab B). Benzoylecgonine and morphine are zwitterions and were poorly extracted using LLE.

dSPE

Bias ranged from -1.54% (citalopram) to 16.79% (fluoxetine). Within-run precision ranged from 3.46% for methadone to 10.37% for 25I-NBOMe. Between-run precision ranged from 7.48% for morphine to 18.65% for methadone.

Urine LOD, ion suppression/enhancement, and overall recovery

In general, the LODs at Lab A were equal to or lower than those at Lab B (Table [A9](#)). Citalopram and amphetamine were the most variable across methods ranging from 0.5 to 5 ng/mL. Trazodone, methamphetamine, codeine, PCP, 6-AM, and fluoxetine had a LOD of 5 ng/mL for Lab B using SPE B and/or LLE compared to Lab A and other methods that were lower. Overall barbiturates had the highest LODs regardless of laboratory or extraction method ranging from 2 (Lab A, SLE) to 20 ng/ml (Lab B, SPE B). The lowest LODs were 0.05 ng/mL for cocaine using dPE and oxycodone for LLE at Lab A.

Ion enhancement was evaluated at two concentrations (low and high) within the dynamic range. At the low concentration was observed for 25I-NBOMe (Lab B, SPE A), 6-AM (Lab A, SPE B), and amphetamine (Lab A, SLE) (Table [A10](#)). There was more ion suppression observed across the two laboratories and extraction methods. For both high and low concentrations for Lab B, SPE B exhibited ion suppression ranging from -83.88% for phenobarbital at the low concentration to -31.28% for alpha-PVP at the high concentration. Across all methods, barbiturates displayed ion suppression except the low concentration for Lab A using SPE A, and Lab B using SLE for both high and low concentrations. Excluding barbiturates, SPE A, SLE, and LLE had the least analytes exhibiting ion suppression for both laboratories.

Recoveries were evaluated at two concentrations (low and high) within the dynamic range. SLE recoveries ranged from 75 to 110% for most analytes at each concentration for both laboratories (Table

[A11](#)). Recoveries for benzoylecgonine and morphine at both laboratories for LLE ranged from 0.17 to 2.93%, which was to be expected since they are zwitterions. 6-AM had low recovery for both laboratories using LLE (29.76 to 37.12%). Across both laboratories and all extraction methods (except SLE) most barbiturates ranged from 46.7% to 72.61%, except Lab A using SPE B. When comparing Lab A across all extraction methods, the greatest recoveries were for SLE, similar recoveries for SPE A and SPE B, and the least recoveries for LLE and dSPE. The pneumatic extractor used for dSPE required several transfer steps between test tubes and well plates and this likely resulted in lower recoveries. When comparing Lab B across all extraction methods, the greatest and most consistent recoveries were for SLE and LLE. Recovery of amphetamines were less using dSPE than other methods, but most opiates/opioids were similar across all methods and laboratories.

Blood dynamic range, LOQ, bias, and precision

The dynamic ranges were 0.5-500 ng/mL, 0.5-1000 ng/mL, 10-1,000 ng/mL, and 20-2000 ng/mL (Table [A3](#)) at both laboratories. LOQs were set at the lowest calibrator for validation purposes, as these values are representative of typical limits needed by laboratories, however further experiments showed that many analytes could be reliably quantified below the validated LOQ (Table [A12](#)). Analytes at both laboratories were weighted 1/x and linear at Lab A, except for benzoylecgonine and methadone which were quadratic. All curves were quadratic at Lab B. The average correlation coefficients (r^2) was greater than 0.990 at both laboratories. For all sample preparation methods at both laboratories, all analytes were within $\pm 20\%$ for bias, within-run precision and between-run precision except benzoylecgonine, which had a bias of 21.8% using SPEA at Lab A. These results are displayed in Tables [A13](#), [A14](#), and [A15](#).

SPE A

Bias ranged from -10.18% (amobarbital) to 21.82% (benzoylecgonine) (Lab A) and -4.53% (amobarbital) to 5.57% (amphetamine) (Lab B). Within-run precision ranged from 2.43% for morphine to 7.53% for 25I-NBOMe (Lab A) and 2.73% for lidocaine to 7.36% for phenobarbital (Lab B). Between-run

precision ranged from 3.51% for morphine to 10.50% for amitriptyline (Lab A) and 4.70% for lidocaine to 8.88% for phenobarbital (Lab B).

SPE B

Bias ranged from -10.57% (MDMA) to 9.32% (amphetamine) (Lab A) and -2.57% (25I-NBOMe) to 8.48% (6-AM) (Lab B). Within-run precision ranged from 4.41% for phenobarbital to 10.36% for trazodone (Lab A) and 2.92% for phenobarbital to 14.66% for 6-AM (Lab B). Between-run precision ranged from 1.83% for oxycodone to 16.10% for LSD (Lab A) and 2.92% for phenobarbital to 18.14% for 6-AM (Lab B)

SLE

Bias ranged from -0.01% (methadone) to 15.75% (amitriptyline) (Lab A) and -5.61% (25I-NBOMe) to 4.93% (fluoxetine) (Lab B). Within-run precision ranged from 3.40% for phenobarbital to 9.38% for citalopram (Lab A) and 4.49% for alpha-PVP to 8.69% for 6-AM (Lab B). Between-run precision ranged from 4.61% for butalbital to 17.48% for 25I-NBOMe (Lab A) and 6.04% for amphetamine to 12.59% for butalbital (Lab B).

LLE

Bias ranged from -18.44% (trazodone) to 7.85% (citalopram) (Lab A) and -2.03% (amobarbital) to 6.69% (MDEA) (Lab B). Within-run precision ranged from 3.29% for lidocaine to 9.22% for citalopram (Lab A) and 1.45% for butalbital to 6.71% for PCP (Lab B). Between-run precision ranged from 5.47% for lidocaine to 17.75% for 25I-NBOMe (Lab A), and 2.07% for butalbital to 9.73% for amitriptyline (Lab B).

dSPE

Bias ranged from -15.16% (fentanyl) to 12.16% (ethylone). Within-run precision ranged from 4.36% for morphine to 10.99% for 25I-NBOMe. Between-run precision ranged from 7.31% for lidocaine to 16.78% for 25I-NBOMe.

Blood LOD, ion suppression/enhancement, and overall recovery

In general, the LODs at Lab A were equal to or lower than those at Lab B (Table [A16](#)). MDA LOD was higher in Lab A for all methods while MDEA was only greater for SPE B. In general, barbiturates had the highest LODs ranging from 2 to 20 ng/mL.

Ion enhancement was observed for benzoylecgonine (Lab B, SPE B, high concentration), PCP (Lab A, SLE, low concentration), and fluoxetine (Lab A, SPE B, low concentration) (Table [A17](#)). No ion suppression was observed using SLE and only PCP displayed ion suppression for Lab A using dSPE at the high concentration. In general, the highest levels of ion suppression were observed with Lab B using SPE A and SPE B.

As expected, recoveries for benzoylecgonine and morphine at both laboratories for LLE were low (Table [A18](#)). SPE B (Lab B) produced the highest recoveries for methamphetamine, MDA, MDEA, MDMA, codeine, oxycodone, and ethylone. Recoveries for these analytes at Lab A using SPE B ranged from 55.75% for methamphetamine at the low concentration to 75.67% for MDMA at the high concentration. Recoveries for barbiturates were lower than 70% for all Lab B extractions, but between 70.14% to 81.54% for Lab A using SPE B and SLE. Besides barbiturates, most analytes were greater than 60% recovery. In general, Lab A recoveries were greater than 60% for the high concentrations for most analytes compared to the low concentration for dSPE and SPE B.

Cost, Time, and Solvent Usage

Extraction Time

The time needed to perform the extraction of drugs and metabolites in blood for 48 samples was recorded (Table [A19](#)). It must be noted that extraction times are staff dependent. The amount of staff experience performing extractions prior to the start of this project ranged from 0 to 15 years. All extractions were performed manually, using 48-sample vacuum (Lab B) and pressure (Lab-A) extraction manifolds for SPE and SLE and a pneumatic extractor for dSPE. For high throughput laboratories, the use

of an automated extractor will greatly increase productivity. Sample loading for SPE A and SPE B included pouring the samples onto the columns and allowing the blood to flow through. Sample load for dSPE involved removing the acetonitrile layer and placing in into well plates. The extraction of urine using dSPE required several additional steps; however, the total extraction time was similar to blood because it did not require the acetonitrile crash. For both blood and urine dSPE, a pneumatic extractor was used, which required an additional transfer steps between test tubes and well plates. Overall, dSPE was completed in the least amount of time while LLE took the longest. SPE A, SPE B, and SLE took similar lengths of time.

Since most forensic laboratories are familiar with multi-step SPE, SLE and modified SPE can be easily implemented. dSPE required the longest amount of training due to time needed to become familiar with the pneumatic extractor. Although most laboratories use an automated extractor when using the dSPE tips chosen for this evaluation, staff were able to carry-out extractions within 2 days of training and the technique was easily implemented into the workflow.

Extraction Solvent/Reagent Use and Disposal

Solvent/reagent usage was calculated only for those used during the extraction (condition, wash, elution, and protein precipitation) (Figure [A1](#)). SPE A required the most solvent/reagent because of the three conditioning and wash steps. The SPE B required the second largest volume because of the two conditioning and wash steps, while SLE required 6 mL of elution solution resulting in the third highest solvent use. dSPE required the least amount of solvent use (62.4 mL urine and 24 mL blood). For LLE, SLE, and dSPE blood all solvents used were dried down prior to reconstitution, therefore there was no disposal of solvent waste. Total amount of waste for SPE A, SPE B, and dSPE urine was 480, 192, and 43.2 mL, respectively.

Extraction Cost

Cost was calculated only for solvents and reagents used at Lab A for extraction (condition, wash, elution, or protein precipitation) with the assumption of no cost for D.I. water (Figure [A2](#)). Although the

amount of solvents used for LLE was the highest, overall LLE was least expensive because no extraction columns were needed, followed by dSPE. The SPE B method was the most expensive, while SLE and SPE A were similar in overall costs. Summary of time, solvent usage and cost is shown in Table 2.

Table 2: Cost and solvent usage summary for the extraction of 48 samples

	LLE	SPE A	SPE B	SLE	dSPE
Time for 48 blood samples	73 min	49 min	47 min	53 min	36 min
Total solvent for 48 blood samples	192 mL	624 mL	336 mL	192 mL	1 mL
Cost for 48 blood samples	\$19	\$120	\$160	\$114	\$75

Implications for Criminal Justice Policy and Practice

Laboratories are often faced with an influx of samples requiring testing and continue to benefit from ways to increase sample throughput, including novel sample preparation techniques, that result in more efficient ways to test samples. Providing forensic laboratories with information that supports novel sample preparation techniques with higher sample throughput while providing the same, if not better, quality of data can positively impact the number of samples laboratories are able to analyze. This can have a significant impact on laboratory efficiency. It will also allow for better utilization of forensic laboratories by reducing the frequency of outsourcing samples to other laboratories for testing.

Dissemination

SOFT Meeting October 2018 (Minneapolis, MN): McGowan (presenter): *The Simultaneous Analysis Twenty-Three Amphetamines, Antidepressants, Opioids, and Hallucinogenic Drugs by UFLC-MS/MS*

TIAFT Meeting August 2018 (Ghent, Belgium): Jenquine (presenter): *Method validation for the detection and quantitation of barbiturates in whole blood using liquid chromatography-tandem mass spectrometry following solid phase extraction*

AAFS February 2019 Meeting (Baltimore, MD): McGowan (presenter): *Detection and Quantification of Twenty-Three Drugs in Blood and Urine following SPE using UPLC-MS/MS*

AAFS February 2020 Meeting (Anaheim, CA): Chan (presenter) Validation and Comparison of Three Sample Preparation Techniques for Quantitation of Amobarbital, Butalbital and Phenobarbital in Blood and Urine Using UFLC-MS/MS

AAFS February 2020 Meeting (Anaheim, CA): Stewart (presenter) Evaluation of Sample Preparation Techniques for the Detection and Quantitation of Benzodiazepines in Human Urine and Whole Blood Using High-Performance Liquid Chromatography/Tandem Mass Spectrometry (HPLC-MS/MS)

Appendix

[Table A1 Extraction of amphetamine, opiate/opioid, hallucinogen, antidepressant, synthetic and miscellaneous drug classes mix](#)

PREPARE SAMPLE:

- Aliquot 200 µL of urine or blood (exception of LLE blood-500µL) into the appropriate number of test tubes for calibrators, QCs, blank, and double blank samples
- Add appropriate amount of ISTD to all samples, except the double blank
- Add appropriate amount of working standards to designated calibrators and QCs samples

Extraction	Steps
SLE	<ul style="list-style-type: none"> • Add 200 µL of 1% ammonium hydroxide to each sample • Mix/vortex • LOAD SAMPLES • Apply pressure for 3-5 seconds • Allow to absorb for 5 min • ELUTE: • Apply 3 mL of dichloromethane (DCM)/isopropyl alcohol (IPA) 95/5 (v/v). Allow to flow by gravity for 5 min • Apply a second 3 mL aliquot of DCM/IPA. Allow to flow by gravity for 5 min • Apply pressure to pull through remaining solvent
SPE A	<ul style="list-style-type: none"> • Add 200 µL of 100 mM phosphate buffer (pH 6.0) to each sample • Mix/vortex • Centrifuge 4000 rpm for 5 min (blood) • CONDITION: <ul style="list-style-type: none"> ○ 2 mL methanol ○ 2 mL D.I. H₂O ○ 2 mL 100 mM phosphate buffer (pH 6.0) • LOAD SAMPLES • WASH: <ul style="list-style-type: none"> ○ 2 mL D.I. H₂O ○ 1 mL 100 mM acetic acid ○ 1 mL methanol ○ Dry column (5 minutes at full vacuum or pressure) • ELUTE: <ul style="list-style-type: none"> ○ 3 mL DCM/IPA/ammonium hydroxide (NH₄OH) 78/20/2 (v/v)
SPE B	<ul style="list-style-type: none"> • Add 200 µL of 1% formic acid to each sample • Mix/vortex • Centrifuge 4000 rpm for 5 min • CONDITION: <ul style="list-style-type: none"> ○ 1 mL methanol ○ 1 mL 1% formic acid • LOAD SAMPLE • WASH: <ul style="list-style-type: none"> ○ 1 mL 1% formic acid ○ 1 mL 50:50 methanol:water ○ Dry column (1 minute at full vacuum or pressure)

	<ul style="list-style-type: none"> • ELUTE: <ul style="list-style-type: none"> ○ 2 x 1.5 mL DCM/IPA/NH₄OH 78/20/2 (v/v)
LLE	<ul style="list-style-type: none"> • Add 500 µL of 0.2 M sodium carbonate solution (blood samples) • Add 4 mL of n-butyl chloride • Mix for 10 minutes • Centrifuge samples for 5 min at 3500 rpm • Transfer organic layer to test tube
dSPE Urine	<ul style="list-style-type: none"> • Add 20 µL of 1.0 M HCl • Vortex mix • CONDITION: • Condition with 500 µL 50% methanol in water by aspirating and then waiting 30 seconds before dispensing • Perform an air gap • LOAD SAMPLE • Load the samples by aspirating and then waiting 30 seconds before dispensing. Do this three times. • Perform an air gap • WASH: • Wash with 400 µL 10% methanol in water by aspirating and then waiting 30 seconds before dispensing • Perform an air gap • ELUTE: • Elute analytes with 400 µL 4% ammonium hydroxide in methanol by aspirating and then waiting 30 seconds before dispensing
dSPE Blood	<ul style="list-style-type: none"> • Add 500 µL acetonitrile to blood • Vortex mix • Centrifuge for 5 min at 4000 rpm • Remove organic layer into well plate • Load samples by aspirating and then waiting 30 seconds before dispensing. Repeat three times.

EVAPORATION AND RECONSTITUTION:

- Add 100 µL of HCl (1%) (10 µL for DPX urine and blood) to each sample to prevent volatilization of amphetamines and vortex
- Evaporate samples to dryness under nitrogen at 38 °C
- Reconstitute samples in 400 µL mobile phase, except LLE was 500 µL for blood
 - Lab A mobile phase=90:10, 5 mM ammonium formate (0.1% formic acid): acetonitrile (0.1% formic acid)
 - Lab B mobile phase=95:5, water (0.1% formic acid): acetonitrile (0.1% formic acid)

Table A2 Extraction of barbiturates

PREPARE SAMPLE:

- Aliquot 200 µL of urine or blood into the appropriate number of test tubes for calibrators, QCs, blank, and double blank samples
- Add appropriate amount of ISTD to all samples, except the double blank

- Add appropriate amount of working standards to designated calibrators and QC samples

Extraction	Steps
SLE	<ul style="list-style-type: none"> • Add 200 µL of 100 mM ammonium acetate (pH 5) to each sample • Mix/vortex • LOAD SAMPLES • Apply pressure for 3-5 seconds • Allow to absorb for 5 min • ELUTE: • Apply 1.5 mL of DCM. Allow to flow by gravity for 5 min • Apply a second 1.5 mL aliquot of DCM. Allow to flow by gravity for 5 min • Apply pressure to pull through remaining solvent
SPE A	<ul style="list-style-type: none"> • Add 200 µL of 100 mM phosphate buffer (pH 6.0) to each sample • Mix/vortex • Centrifuge 4000 rpm for 5 min (blood) • CONDITION: <ul style="list-style-type: none"> ○ 2 mL methanol ○ 2 mL D.I. H₂O ○ 2 mL 100 mM phosphate buffer (pH 6.0) • LOAD SAMPLES • WASH: <ul style="list-style-type: none"> ○ 2 mL D.I. H₂O ○ 1 mL 100 mM acetic acid ○ Dry column (5 minutes at full vacuum or pressure) ○ 1 mL hexane • ELUTE: <ul style="list-style-type: none"> ○ 3 mL ethyl acetate:hexane (50:50) at 1 to 2 mL/min
SPE B	<ul style="list-style-type: none"> • Add 1 mL acetonitrile to each sample (800 µL for urine at Lab A) • Mix/vortex • Centrifuge 4000 rpm for 5 min • CONDITION (performed at Lab A for blood and urine and at Lab B only for blood) <ul style="list-style-type: none"> ○ 2 mL methanol ○ 2 mL D.I. H₂O • LOAD and ELUTE SAMPLE <ul style="list-style-type: none"> ○ Load the sample and allow to flow through the column into collection test tubes
LLE	<ul style="list-style-type: none"> • Add 500 µL of phosphate buffer (pH 5) • Add 1.5 mL of 50:50 (hexane: ethyl acetate) • Mix for 5 minutes • Centrifuge samples for 5 min at 3500 rpm • Transfer organic layer to test tube

EVAPORATION AND RECONSTITUTION:

- Evaporate samples to dryness under nitrogen at 35 °C
- Reconstitute samples in 100 µL mobile phase

Table A3: Dynamic range of drugs and metabolites in blood and urine

Compound	Calibration Range (ng/mL)	Compound	Calibration Range (ng/mL)	Compound	Calibration Range (ng/mL)
Amphetamine	10-1000	Codeine	10-1000	Amitriptyline	10-1000
Methamphetamine	10-1000	Methadone	10-1000	Citalopram	10-1000
MDA	10-1000	Morphine	10-1000	Fluoxetine	10-1000
MDEA	10-1000	Fentanyl	0.5-500	Trazodone	10-1000
MDMA	10-1000	Oxycodone	10-1000	Ethylone	0.5-1000
Benzoyllecgonine	10-1000	6-MAM	10-1000	Alpha-PVP	0.5-1000
Cocaine	10-1000	LSD	0.5-1000	25I-NBOMe	0.5-500
Lidocaine	10-1000	PCP	10-1000	Amobarbital	20-2000
Butalbital	20-2000	Phenobarbital	20-2000		

Table A4: Experimental determination of lowest quantifiable values for drugs and metabolites extracted in urine

Compound	Concentration (ng/mL)								
	Urine								
	SPE A		SPE B		SLE		LLE		dSPE
	Lab A	Lab B	Lab A	Lab B	Lab A	Lab B	Lab A	Lab B	Lab A
Amphetamine	1	5	5	10	5	10	5	5	10
Methamphetamine	5	5	5	10	1	10	1	5	5
MDA	5	5	NR	10	5	10	5	10	NR
MDEA	1	5	1	10	1	10	0.5	5	1
MDMA	1	5	5	5	1	10	1	5	10
Benzoyllecgonine	2	10	5	5	5	10	NR	NR	10
Cocaine	5	10	1	10	1	10	1	5	1
Lidocaine	1	5	5	5	1	10	1	10	1
Codeine	5	10	5	5	1	10	1	5	10
Methadone	1	5	5	5	1	10	1	5	5
Morphine	5	5	1	10	1	10	NR	NR	10
Fentanyl	0.5	0.5	0.5	0.5	1	0.5	1	0.5	1
Oxycodone	0.5	5	5.0	10	10	10	1	5	NR
6-AM	0.5	10	5	10	0.5	10	1	NR	5
LSD	1	1	0.5	0.5	0.5	0.5	0.5	0.5	0.5
PCP	5	5	0.5	10	1	10	1	10	5
Amitriptyline	5	5	5	5	1	10	10	5	5
Citalopram	5	10	5	5	5	10	5	5	5
Fluoxetine	5	10	5	5	1	10	1	10	5
Trazodone	5	5	5	1	5	10	10	10	5
Ethylone	0.5	0.5	1	0.5	1	0.5	1	0.5	0.5
Alpha-PVP	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
25I-NBOMe	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Amobarbital	15	15	15	20	10	20	15	20	TBD
Butalbital	15	20	10	20	10	15	15	15	TBD
Phenobarbital	15	20	15	20	10	15	15	20	TBD

Table A5: Bias and precision sample concentrations for all methods except where noted

Compound	Low (ng/m)	Mid (ng/mL)	High (ng/mL)
Amphetamine	20	450	950
Methamphetamine	20	450	950
MDA	20	450	950
MDEA	20	450	950
MDMA	20	450	950
Benzoylcegonine	20	450	950
Cocaine	20	450	950
Lidocaine	20	450	950
Codeine	20	450	950
Methadone	20	450	950
Morphine	20	450	950
Fentanyl	1.5 (Lab B=20 for SPE)	225 (Lab B=125 for SPE)	450
Oxycodone	20	450	950
6-MAM	20	450	950
LSD	1.5 Lab B=20 for SPE)	450	950
PCP	20	450	950
Amitriptyline	20	450	950
Citalopram	20	450	950
Fluoxetine	20	450	950
Trazodone	20	450	950
Ethylone	1.5 Lab B=20 for SPE)	450	950
Alpha-PVP	1.5 Lab B=20 for SPE)	450	950
25I-NBOMe	1.5 Lab B=20 for SPE)	225 Lab B=125 for SPE)	450
Amobarbital	60	950	1800
Butalbital	60	950	1800

Phenobarbital	60	950	1800
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Table A6: Average bias for drugs and metabolites in urine

Average Bias (%)									
Compound	Urine								
	SPE A		SPE B		SLE		LLE		dSPE
	Lab A	Lab B	Lab A	Lab B	Lab A	Lab B	Lab A	Lab B	Lab A
Amphetamine	-1.70	-1.95	3.56	3.73	-1.43	5.76	-3.18	2.61	3.32
Methamphetamine	-6.39	-1.11	1.13	3.42	-5.57	4.03	-5.42	2.54	8.12
MDA	-6.42	-1.86	NR	3.82	-6.25	4.85	-6.13	0.90	NR
MDEA	4.48	-4.12	3.60	4.33	5.28	5.11	3.26	2.26	5.34
MDMA	-3.26	-5.21	1.54	3.65	-1.59	5.51	-6.17	4.39	3.75
Benzoyllecgonine	1.63	1.34	10.33	1.82	0.50	6.60	NR	NR	15.33
Cocaine	-3.14	-1.08	4.58	0.25	2.98	6.71	-0.35	1.25	7.15
Lidocaine	-3.30	-2.90	3.42	0.57	-1.31	6.24	-3.30	2.15	9.76
Codeine	2.32	2.90	-1.15	0.04	4.77	7.79	1.47	1.02	6.18
Methadone	-1.88	1.08	-1.15	1.25	2.21	7.88	-1.28	2.15	4.49
Morphine	-3.14	-0.27	5.00	2.79	-0.64	7.87	NR	NR	6.37
Fentanyl	3.78	1.16	-1.15	-2.55	5.42	4.23	1.30	-0.38	3.90
Oxycodone	-6.79	-0.01	-0.30	0.47	-4.69	7.48	-6.25	3.50	NR
6-AM	1.09	2.22	6.14	0.32	3.57	6.05	4.30	NR	10.44
LSD	2.10	-0.63	7.56	-2.91	2.56	3.33	-1.83	-0.13	6.24
PCP	-3.23	1.90	5.28	0.51	-2.62	6.84	-3.56	1.72	10.93
Amitriptyline	4.40	-0.10	7.07	3.55	7.11	6.23	1.14	2.64	-1.47
Citalopram	5.93	0.56	3.70	5.54	10.50	5.50	3.25	4.45	-1.54
Fluoxetine	-5.70	-2.23	-2.41	2.01	-0.87	7.05	-8.43	1.46	16.79
Trazodone	-18.06	0.24	-5.21	4.19	-21.97	4.99	-23.10	1.36	0.87
Ethylone	1.61	-1.23	1.99	-0.81	-0.82	3.11	5.13	-1.98	3.40
Alpha-PVP	-1.89	-1.06	2.29	-2.97	-1.14	4.69	-2.47	-0.79	6.61
25I-NBOMe	1.64	1.13	0.03	-7.56	-1.75	1.21	-2.46	-3.74	2.38
Amobarbital	6.66	1.80	5.80	-5.80	5.70	-1.73	-1.27	-0.64	TBD
Butalbital	8.46	4.40	11.62	-2.58	13.13	0.50	6.95	-0.97	TBD
Phenobarbital	1.44	0.40	9.06	-3.63	8.56	0.95	2.98	-0.58	TBD

Table A7: Average within-run precision for drugs and metabolites in urine

Average Within-Run Precision (%)									
Compound	Urine								
	SPE A		SPE B		SLE		LLE		dSPE
	Lab A	Lab B	Lab A	Lab B	Lab A	Lab B	Lab A	Lab B	Lab A
Amphetamine	4.53	5.40	5.16	3.49	4.93	4.35	6.01	3.73	6.21
Methamphetamine	5.39	5.79	5.99	5.01	6.09	4.07	5.38	4.53	5.58
MDA	5.59	4.46	NR	4.15	6.43	4.04	5.51	3.38	NR
MDEA	5.61	4.21	7.50	3.87	5.62	4.31	4.27	3.33	5.89
MDMA	5.78	3.86	7.03	3.52	5.43	4.12	5.55	4.78	6.89
Benzoylcegonine	5.18	5.15	3.66	4.81	5.84	4.22	NR	NR	5.99
Cocaine	5.31	4.06	5.77	4.10	5.26	3.66	6.19	3.52	6.16
Lidocaine	6.47	4.68	5.72	4.05	5.43	4.34	5.15	3.90	4.85
Codeine	5.44	5.75	6.50	5.67	4.45	5.94	5.02	5.82	4.21
Methadone	5.67	3.42	6.66	4.38	4.82	4.32	5.10	3.46	3.46
Morphine	4.30	7.81	4.40	4.91	5.57	4.64	NR	NR	3.62
Fentanyl	8.62	2.45	7.65	4.83	6.46	4.72	5.68	2.34	8.12
Oxycodone	7.75	4.66	6.09	4.34	7.85	4.72	4.52	3.72	NR
6-AM	6.64	6.50	6.96	5.95	6.52	5.15	5.46	NR	5.02
LSD	7.55	4.29	6.38	5.22	8.81	6.31	5.86	5.32	7.92
PCP	5.14	4.09	7.61	9.03	5.11	5.00	6.41	3.93	6.55
Amitriptyline	6.74	3.97	7.32	4.40	5.71	4.85	7.28	3.65	5.93
Citalopram	5.94	3.70	7.88	4.66	6.80	4.08	7.28	5.05	6.79
Fluoxetine	5.92	3.95	6.85	4.20	7.86	4.73	7.27	3.62	9.16
Trazodone	5.25	3.46	7.65	4.36	7.69	4.66	6.99	3.89	7.78
Ethylone	5.82	4.44	6.01	4.13	6.06	6.07	5.11	4.32	5.83
Alpha-PVP	5.75	5.19	6.61	4.68	5.57	5.09	5.76	3.91	6.19
25I-NBOMe	10.22	4.20	6.82	3.73	5.79	5.74	6.53	3.17	10.37
Amobarbital	6.69	4.35	5.86	5.13	4.75	4.70	4.03	2.44	TBD
Butalbital	8.21	5.27	6.10	3.83	5.21	4.07	2.96	2.44	TBD
Phenobarbital	7.59	4.22	6.21	4.65	5.34	5.83	3.75	3.59	TBD

Table A8: Average between-run precision for drugs and metabolites in urine

Average Between-Run Precision (%)									
Compound	Urine								
	SPE A		SPE B		SLE		LLE		dSPE
	Lab A	Lab B	Lab A	Lab B	Lab A	Lab B	Lab A	Lab B	Lab A
Amphetamine	7.48	6.07	10.15	5.45	8.64	4.89	8.11	6.06	10.86
Methamphetamine	8.24	7.43	11.90	7.34	11.19	5.77	8.52	5.52	9.32
MDA	7.86	5.31	NR	5.00	11.78	5.24	9.01	5.25	NR
MDEA	8.46	5.13	14.01	5.06	11.07	5.89	7.13	4.96	10.17
MDMA	5.13	4.84	10.05	4.26	11.92	4.36	8.04	4.92	10.91
Benzoylcegonine	8.30	5.89	7.12	5.83	9.70	6.14	NR	NR	7.68
Cocaine	9.77	5.32	10.22	5.06	11.03	5.64	8.43	5.92	8.35
Lidocaine	9.56	5.84	9.97	4.79	9.50	4.31	8.13	5.40	7.96
Codeine	7.64	6.47	11.88	9.82	9.61	6.20	7.00	6.48	9.54
Methadone	9.04	4.83	10.03	4.94	10.68	4.99	9.37	5.10	18.65
Morphine	7.40	7.80	8.87	6.57	8.91	4.83	NR	NR	7.48
Fentanyl	17.65	3.38	10.52	6.00	11.77	6.09	15.03	4.24	13.53
Oxycodone	10.98	5.93	8.94	7.19	12.01	6.19	6.04	4.29	NR
6-AM	9.13	7.24	10.23	7.08	11.84	7.17	8.61	NR	10.10
LSD	10.75	4.99	11.57	5.49	11.54	6.95	8.73	5.09	17.16
PCP	8.33	4.87	13.01	12.18	8.69	5.31	10.51	6.97	9.34
Amitriptyline	9.12	6.58	12.42	5.28	11.15	5.33	11.18	4.54	14.83
Citalopram	10.75	5.24	13.52	6.01	10.68	5.50	9.19	6.16	13.30
Fluoxetine	9.13	5.63	12.21	6.66	12.29	5.37	10.18	5.68	16.81
Trazodone	9.17	5.09	14.87	4.73	13.53	5.48	8.90	5.13	11.48
Ethylone	8.59	6.33	8.14	4.97	10.71	6.82	7.67	6.24	10.02
Alpha-PVP	8.84	5.73	11.71	7.03	8.76	5.87	9.04	7.41	10.37
25I-NBOMe	16.98	6.07	13.35	5.30	9.88	5.98	10.01	3.85	18.21
Amobarbital	11.46	8.39	7.04	8.70	7.08	6.84	9.02	4.96	TBD
Butalbital	11.83	8.55	7.29	9.62	7.31	11.98	8.30	4.90	TBD
Phenobarbital	11.83	7.70	8.02	9.95	7.14	10.68	10.15	5.65	TBD

Table A9: LOD values for drugs and metabolites extracted in urine

Compound	LOD (ng/mL)								
	Urine								
	SPE A		SPE B		SLE		LLE		dSPE
	Lab A	Lab B	Lab A	Lab B	Lab A	Lab B	Lab A	Lab B	Lab A
Amphetamine	0.5	1	5.0	1	1.0	5	5.0	5	5
Methamphetamine	0.5	0.5	0.5	5	1.0	0.5	0.5	5	1
MDA	1	1	NR	5	5.0	0.1	0.5	10	NR
MDEA	0.5	0.5	0.5	1	0.5	0.5	0.1	0.5	0.5
MDMA	0.5	0.5	0.5	1	0.5	0.5	0.1	1	1
Benzoylecgonine	0.5	1	0.1	5.0	0.5	0.5	NR	NR	0.5
Cocaine	0.1	0.5	0.1	0.5	0.1	0.5	0.1	1	0.05
Lidocaine	0.5	0.5	0.1	1.0	0.1	0.5	0.1	1	0.1
Codeine	0.5	0.5	0.5	5.0	0.5	0.5	0.5	5	1
Methadone	0.5	0.5	0.5	5.0	0.5	0.5	0.5	1	1
Morphine	1.0	1	0.5	5.0	1.0	0.5	NR	NR	1
Fentanyl	0.5	0.5	0.5	0.5	0.5	0.1	0.1	0.5	1
Oxycodone	0.5	1	0.5	1.0	0.5	0.5	0.05	1	NR
6-AM	0.5	1	0.5	5.0	0.5	1	0.5	NR	1
LSD	0.5	0.5	0.1	0.1	0.1	0.1	0.1	0.5	0.5
PCP	0.5	0.5	0.5	5.0	0.5	0.5	0.1	5	1
Amitriptyline	0.5	1	0.5	1.0	0.5	0.5	0.5	1	0.5
Citalopram	0.5	0.5	5.0	1.0	5.0	0.5	5.0	5	5
Fluoxetine	0.5	1	0.5	1.0	1.0	0.5	1.0	5	0.5
Trazodone	0.5	0.5	0.1	1.0	0.5	0.1	0.1	5	1
Ethylone	0.1	0.5	0.1	0.5	0.5	0.5	0.1	0.5	0.5
Alpha-PVP	0.1	0.5	0.1	0.5	0.1	0.1	0.1	0.5	0.1
25I-NBOMe	0.1	0.5	0.1	0.5	0.1	0.5	0.1	0.5	0.5
Amobarbital	5	15	5	15	5	10	5	15	TBD
Butalbital	5	15	5	20	2	10	5	15	TBD
Phenobarbital	5	15	10	20	2	10	5	15	TBD

A10: Ion suppression/enhancement of drugs and metabolites in urine

Compound	Ion Suppression/Enhancement (%)																	
	Urine																	
	SPE A				SPE B				SLE				LLE				dSPE	
	Low		High		Low		High		Low		High		Low		High		Low	High
	Lab A	Lab B	Lab A	Lab B	Lab A	Lab B	Lab A	Lab B	Lab A	Lab B	Lab A	Lab B	Lab A	Lab B	Lab A	Lab B	Lab A	Lab A
Amphetamine	0.92	-5.91	-2.10	0.21	-12.2	-46.99	-6.55	-35.04	31.0	-0.96	6.98	-2.08	5.60	-7.48	4.46	-2.24	-29.03	-26.04
Methamphetamine	-7.33	-4.80	-5.02	-0.13	-8.69	-56.36	-6.14	-53.94	-7.07	-4.72	-5.51	-2.30	0.78	-8.85	2.47	-3.31	-31.32	-25.70
MDA	0.80	-2.75	-1.15	3.66	NR	-51.08	NR	-42.58	6.24	-8.54	2.63	-0.16	5.70	-8.23	4.45	-2.66	NR	NR
MDEA	-2.41	-2.22	2.12	-3.10	-2.58	-48.69	-3.59	-48.31	-6.27	-4.01	-4.07	-1.50	1.11	-8.87	3.56	-3.96	-12.74	-12.87
MDMA	0.54	-0.32	1.18	-3.31	-7.80	-53.61	-3.47	-51.15	-5.62	-5.27	-3.38	-4.48	-0.60	-11.37	2.15	-5.07	-21.76	-15.90
Benzoylcegonine	-0.69	-9.15	1.75	-5.40	7.75	-36.38	4.36	-38.4	-3.07	6.85	-1.13	9.30	2.37	-13.15	2.83	-7.61	-1.65	-1.89
Cocaine	-2.89	-10.79	-1.19	-5.13	-1.26	-46.07	-1.70	-47.88	-4.22	2.64	-2.31	-0.07	5.40	-12.40	4.10	-8.96	0.57	1.19
Lidocaine	-0.49	-3.43	1.95	-1.56	-2.37	-42.33	-1.88	-41.57	-7.15	-4.09	-1.55	-2.33	3.35	-10.90	2.75	-5.41	-14.42	-11.61
Codeine	4.32	-14.32	0.93	-7.16	-3.43	-48.17	-6.51	-45.86	-4.11	-9.50	-3.36	0.54	4.46	-12.14	5.30	-6.66	-13.77	-13.93
Methadone	-2.84	-27.26	-4.29	-8.42	-7.30	-54.90	-3.75	-51.92	-4.84	-6.02	2.24	-2.91	-2.98	-12.36	-0.18	2.73	-2.74	-3.84
Morphine	-22.50	-24.29	-15.54	-17.11	-35.91	-63.56	-25.79	-60.46	-22.11	-30.01	-18.70	-17.02	-9.97	-11.68	-2.85	-4.95	-68.63	-59.10
Fentanyl	-5.37	-17.17	-4.01	-9.51	-36.19	-53.01	-24.04	-52.25	-10.45	-3.26	-4.35	-1.07	-0.92	-10.54	0.09	-5.48	-45.99	-40.92
Oxycodone	0.43	-11.43	-1.54	-11.76	-64.73	-44.16	-56.94	-37.06	-0.85	-5.07	3.38	-5.49	-6.48	-9.06	1.67	-3.36	NR	NR
6-AM	6.96	-12.29	6.48	-9.27	26.52	-52.43	-23.95	-51.89	-11.26	-1.83	-7.93	1.09	2.05	-14.81	3.20	-7.66	-49.85	-46.35
LSD	-8.12	-16.42	-7.78	-9.28	-26.11	-41.01	-16.87	-38.96	-9.26	-8.06	0.13	-5.56	-2.85	-7.39	0.59	-4.34	-58.06	-46.23
PCP	-6.77	-18.12	-8.04	-5.58	-46.03	-64.13	-38.45	-46.03	-15.48	-0.05	-8.21	-2.30	-6.17	-12.11	1.16	-6.90	-63.96	-55.01
Amitriptyline	-16.81	-41.10	-12.03	-19.01	-20.44	-51.52	-15.48	-45.14	-7.90	-6.27	-4.53	-3.93	-4.84	-11.41	-1.93	2.28	-26.44	-20.97
Citalopram	-7.55	-23.99	-3.22	-12.19	-38.38	-59.51	-27.06	-55.47	-10.22	-12.26	-5.61	-5.78	-7.85	-13.78	3.54	-7.27	-47.74	-36.69
Fluoxetine	-7.91	-37.44	-4.50	-15.23	-21.01	-48.70	-10.80	-41.50	-10.57	-3.07	-2.85	-3.76	-2.44	-9.14	0.72	6.71	-14.44	-4.82
Trazodone	-8.65	-17.85	-5.74	-10.13	-36.02	-51.69	-25.46	-52.20	-12.05	-14.33	-3.89	-9.83	-1.55	-13.33	0.62	-8.41	-59.68	-47.40
Ethylone	-1.65	-8.48	-0.29	-4.68	-4.33	-43.11	-5.60	-38.23	-4.24	-1.33	-5.25	-0.66	-0.25	-12.28	-0.92	-5.90	-26.50	-18.91
Alpha-PVP	-1.27	-6.10	0.37	-1.44	-3.31	-38.58	0.61	-31.28	-3.95	-1.15	-0.71	0.26	3.69	-5.88	4.13	-2.09	0.56	-0.60
25I-NBOMe	-17.56	34.80	-13.81	-14.88	-9.65	-49.98	-2.43	-46.16	-9.40	-2.64	-7.11	-3.00	-1.95	-10.14	2.59	7.05	-3.67	-1.73
Amobarbital	-10.53	-40.17	-36.18	-37.99	-57.64	-71.24	-56.76	-68.45	-28.54	4.73	-30.82	4.06	-9.27	-25.97	-6.86	-17.64	TBD	TBD
Butalbital	-11.17	-35.11	-37.88	-26.42	-65.52	-79.92	-64.55	-76.77	-32.82	9.63	-36.17	6.70	-5.59	-25.89	-1.93	-15.81	TBD	TBD
Phenobarbital	-7.33	-25.27	-27.55	-27.65	-63.71	-83.88	-64.82	-80.82	-25.78	10.00	-27.93	7.74	-3.90	-20.00	-1.05	-15.77	TBD	TBD

A11: Recovery of drugs and metabolites in urine

Compound	Recovery (%)																	
	Urine																	
	SPE A				SPE B				SLE				LLE				dSPE	
	Low		High		Low		High		Low		High		Low		High		Low	High
	Lab A	Lab B	Lab A	Lab B	Lab A	Lab B	Lab A	Lab B	Lab A	Lab B	Lab A	Lab B	Lab A	Lab B	Lab A	Lab B	Lab A	Lab A
Amphetamine	79.58	63.61	89.50	64.60	82.90	114.43	85.70	104.43	66.88	89.88	74.94	91.42	93.56	96.21	92.39	91.30	47.39	54.48
Methamphetamine	78.36	65.59	87.95	68.12	79.50	127.62	86.90	113.47	82.35	90.36	83.45	94.83	85.07	99.87	76.07	91.83	42.41	52.96
MDA	79.55	77.05	NR	85.07	NR	120.05	NR	108.50	84.21	90.77	86.92	91.02	93.40	93.69	92.40	89.66	NR	NR
MDEA	78.61	72.67	85.17	82.58	75.84	120.40	84.86	110.03	83.63	91.90	82.81	94.16	81.91	100.32	71.91	93.00	39.45	48.04
MDMA	76.52	76.16	83.64	83.38	77.13	126.15	84.19	111.83	82.64	90.00	82.08	93.22	82.16	99.92	69.44	90.90	36.94	44.04
Benzoylcegonine	77.64	73.24	81.92	76.59	76.16	118.08	86.29	109.38	88.90	94.46	82.82	95.57	0.17	0.35	0.38	0.30	63.63	67.98
Cocaine	77.30	74.65	84.40	84.37	78.00	123.11	89.94	113.33	83.95	89.53	76.97	90.74	77.31	97.41	72.30	89.08	62.82	67.09
Lidocaine	81.70	71.81	84.98	84.78	77.56	118.74	89.17	111.74	89.96	94.32	84.80	95.56	80.68	102.26	78.50	95.82	66.32	75.45
Codeine	77.55	73.92	81.55	78.02	73.88	122.36	87.47	112.44	84.80	89.25	82.32	89.87	76.80	86.64	65.96	81.71	61.07	65.70
Methadone	81.39	54.02	91.96	70.67	80.34	120.12	91.54	107.00	88.19	85.74	83.00	87.48	69.23	100.89	79.30	92.16	62.63	85.33
Morphine	76.16	72.86	81.30	71.18	83.07	120.63	83.07	113.53	97.40	92.60	97.14	93.24	2.10	1.66	2.93	1.74	72.82	70.96
Fentanyl	79.37	65.48	81.27	73.18	75.22	127.82	109.71	109.41	84.43	84.41	78.36	90.10	75.01	102.43	64.95	97.28	59.99	73.15
Oxycodone	77.13	77.38	79.47	81.75	128.57	120.32	89.31	113.63	81.65	72.49	77.58	75.66	97.84	95.51	69.22	89.78	NR	NR
6-AM	79.20	73.58	82.29	77.00	101.79	122.47	121.79	105.82	82.79	88.61	80.42	91.98	32.30	37.12	29.76	30.32	63.15	71.23
LSD	88.80	66.58	79.92	75.65	81.64	115.27	89.07	105.47	65.42	86.68	65.34	90.34	64.43	97.00	75.50	91.50	73.73	78.12
PCP	77.21	57.02	88.11	67.45	81.20	114.42	93.74	112.01	84.64	86.72	78.78	89.49	70.06	100.51	66.41	96.44	57.85	55.35
Amitriptyline	91.60	44.22	84.43	55.20	74.80	116.07	84.79	106.36	78.74	76.81	83.76	81.97	67.24	99.25	68.96	91.86	56.03	70.48
Citalopram	73.30	60.42	88.94	72.62	81.01	124.43	92.17	110.94	77.96	81.70	83.27	95.31	70.68	102.02	67.59	92.75	64.11	65.18
Fluoxetine	76.56	39.42	91.28	52.27	79.27	102.1	90.10	94.74	74.49	71.79	84.24	76.57	71.36	98.64	77.91	90.91	54.79	64.58
Trazodone	71.03	65.17	87.63	76.30	87.67	125.44	95.52	112.59	81.81	85.94	83.34	88.37	79.06	103.09	75.11	95.88	57.22	62.72
Ethylone	80.61	74.28	83.20	78.34	87.12	118.77	87.34	108.91	85.34	90.63	83.25	93.76	89.80	94.83	67.21	90.10	70.25	68.75
Alpha-PVP	73.94	54.26	83.93	61.56	72.79	111.79	77.47	103.75	81.29	90.06	78.77	94.11	69.40	99.38	62.60	96.79	57.98	64.05
25I-NBOMe	68.42	36.23	82.70	46.65	70.52	115.48	85.32	101.22	79.41	78.67	75.25	83.70	70.44	100.07	67.76	90.65	51.15	68.80
Amobarbital	55.10	52.36	59.49	54.90	73.90	70.30	77.14	63.93	72.88	77.03	84.48	86.07	63.37	57.41	68.80	62.03	TBD	TBD
Butalbital	51.15	63.12	55.73	62.64	93.92	72.61	90.39	57.96	88.07	74.55	97.31	80.09	77.04	60.75	78.97	55.17	TBD	TBD
Phenobarbital	46.70	63.93	46.03	62.92	87.23	60.07	91.71	54.15	81.16	82.37	87.92	81.93	71.91	67.89	72.43	53.64	TBD	TBD

Table A12: Experimental determination of lowest quantifiable values for drugs and metabolites extracted in blood

LOQ (ng/mL)									
Compound	Blood								
	SPE A		SPE B		SLE		LLE		dSPE
	Lab A	Lab B	Lab A	Lab B	Lab A	Lab B	Lab A	Lab B	Lab A
Amphetamine	5	5	10	10	5	5	1	10	5
Methamphetamine	5	5	1	10	10	5	5	10	5
MDA	10	10	NR	10	10	1	10	10	NR
MDEA	5	5	5	10	5	5	10	10	5
MDMA	5	10	5	10	5	5	5	10	5
Benzoylecgonine	10	10	5	10	5	5	NR	NR	5
Cocaine	5	1	5	5	5	5	10	10	1
Lidocaine	5	5	1	10	5	5	1	10	5
Codeine	1	10	5	10	5	10	1	10	5
Methadone	1	5	5	10	5	5	10	10	1
Morphine	5	5	10	5	5	10	NR	NR	5
Fentanyl	0.5	0.5	0.5	0.5	0.5	0.5	0.1	0.5	0.5
Oxycodone	1	10	5	10	1	5	0.5	10	5
6-AM	5	10	1	10	1	5	0.5	NR	5
LSD	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
PCP	1	0.5	1	10	1	5	1	10	1
Amitriptyline	5	10	5	10	5	1	1	10	5
Citalopram	5	10	5	10	10	0.5	0.5	10	10
Fluoxetine	5	10	5	10	5	10	0.5	10	5
Trazodone	5	10	5	10	10	10	1	10	1
Ethylone	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Alpha-PVP	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
25I-NBOMe	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Amobarbital	10	10	15	15	15	15	10	15	TBD
Butalbital	10	20	15	15	15	10	10	20	TBD
Phenobarbital	10	20	15	20	5	20	10	20	TBD

Table A13: Average bias for drugs and metabolites in blood

Average Bias (%)									
Compound	Blood								
	SPE A		SPE B		SLE		LLE		dSPE
	Lab A	Lab B	Lab A	Lab B	Lab A	Lab B	Lab A	Lab B	Lab A
Amphetamine	-2.23	5.57	9.32	4.12	2.40	2.95	-3.91	4.64	2.98
Methamphetamine	6.46	4.15	4.99	3.09	7.64	3.33	-5.32	4.09	6.66
MDA	3.94	-0.67	NR	3.10	4.89	3.26	-6.39	5.35	NR
MDEA	-1.17	0.60	-8.00	4.01	3.04	3.64	6.92	6.69	-1.13
MDMA	-1.78	0.46	-10.57	2.05	2.17	2.98	-6.21	5.10	0.88
Benzoylecgonine	21.82	-1.38	9.27	4.45	12.77	2.01	NR	NR	10.16
Cocaine	0.71	0.57	1.70	3.2	3.31	1.07	-1.58	3.84	6.62
Lidocaine	6.95	2.66	0.72	4.79	7.39	4.02	-2.53	2.56	11.02
Codeine	0.45	-0.74	-0.20	1.18	5.15	3.47	2.99	1.82	-0.59
Methadone	-6.81	2.28	-7.91	3.86	-0.01	4.79	1.68	2.49	-6.44
Morphine	3.77	-1.71	2.99	3.38	8.36	4.14	NR	NR	3.12
Fentanyl	5.29	-0.40	2.21	-1.22	10.32	-3.76	4.16	1.18	-15.16
Oxycodone	1.98	-0.04	1.83	4.17	2.70	2.70	-6.19	2.74	1.38
6-AM	5.08	-1.21	5.18	8.46	10.97	2.69	5.08	NR	8.98
LSD	6.03	1.35	-2.45	1.84	9.31	-0.31	2.06	-1.20	10.49
PCP	1.70	1.12	0.30	5.1	7.16	1.30	-0.46	2.04	2.11
Amitriptyline	6.19	2.58	5.73	1.24	15.75	3.77	4.88	5.13	9.68
Citalopram	-4.78	3.65	-6.03	4.24	0.25	2.59	7.85	5.28	-6.16
Fluoxetine	-6.55	2.20	-9.37	2.42	2.22	4.93	-4.15	6.59	-0.59
Trazodone	-3.32	1.74	-5.83	3.29	0.03	3.32	-18.44	4.53	-1.11
Ethylone	1.22	0.12	-4.68	3.39	5.65	1.03	-1.18	0.92	12.16
Alpha-PVP	-0.16	0.0008	-7.58	3.27	5.54	-0.47	-1.17	1.71	5.36
25I-NBOMe	2.55	-3.56	-2.48	-2.57	5.89	-5.61	5.20	-0.05	12.13
Amobarbital	-10.18	-4.53	2.77	-1.02	1.22	2.77	1.78	-2.03	TBD
Butalbital	-5.7	-4.43	-0.25	1.19	0.98	0.21	2.40	-1.04	TBD
Phenobarbital	-9.14	-4.22	-1.14	0.39	0.20	-3.82	2.13	-1.13	TBD

Table A14: Average within-run precision for drugs and metabolites in blood

Average Within-Run Precision (%)									
Compound	Blood								
	SPE A		SPE B		SLE		LLE		dSPE
	Lab A	Lab B	Lab A	Lab B	Lab A	Lab B	Lab A	Lab B	Lab A
Amphetamine	3.67	3.30	5.15	4.65	5.15	4.73	3.48	3.19	5.34
Methamphetamine	6.10	3.52	5.85	3.04	5.41	5.49	4.25	3.86	6.71
MDA	3.53	3.92	NR	4.39	6.28	5.38	4.27	3.46	NR
MDEA	4.46	4.80	6.15	4.08	5.62	5.91	3.89	3.83	4.90
MDMA	3.95	4.74	6.56	4.71	5.83	5.86	3.72	4.07	5.92
Benzoylecgonine	3.35	4.67	4.60	4.10	4.61	6.89	NR	NR	5.20
Cocaine	3.78	4.61	5.63	3.80	5.99	6.27	3.29	3.64	5.46
Lidocaine	3.70	2.73	5.01	3.32	5.45	6.08	3.29	3.45	5.17
Codeine	5.00	3.11	6.65	4.69	6.44	6.06	4.48	5.70	6.97
Methadone	5.80	4.26	9.01	5.03	6.11	7.25	6.50	4.75	7.62
Morphine	2.43	4.84	4.89	12.51	5.24	6.40	NR	NR	4.36
Fentanyl	5.83	3.18	8.54	4.32	6.02	5.14	8.23	4.73	8.15
Oxycodone	3.63	3.99	6.54	4.06	5.49	5.93	4.10	3.76	5.76
6-AM	4.34	5.02	6.91	14.66	5.88	8.69	4.61	NR	7.66
LSD	5.04	5.29	8.31	6.84	7.76	6.67	5.07	6.47	7.97
PCP	4.06	5.52	6.93	7.58	6.94	6.25	7.63	6.71	5.88
Amitriptyline	6.98	4.11	8.27	5.94	4.88	7.05	6.31	4.51	9.04
Citalopram	6.05	4.14	7.95	4.30	9.38	7.02	9.22	3.39	6.43
Fluoxetine	6.34	3.89	8.04	5.00	6.53	6.25	6.37	5.83	8.52
Trazodone	6.69	4.05	10.36	4.16	7.43	6.52	6.17	4.12	8.85
Ethylone	4.82	3.97	5.46	4.33	4.34	6.48	3.37	3.80	6.87
Alpha-PVP	3.88	3.54	5.83	4.82	5.88	4.49	4.95	3.83	5.39
25I-NBOMe	7.53	3.32	7.69	4.78	8.46	6.55	8.67	6.09	10.99
Amobarbital	3.82	4.97	4.92	3.09	3.61	6.61	5.56	1.78	TBD
Butalbital	5.20	5.26	4.78	2.94	3.46	5.65	4.80	1.45	TBD
Phenobarbital	4.27	7.36	4.41	2.92	3.40	6.23	5.22	2.50	TBD

Table A15: Average between-run precision for drugs and metabolites in blood

Average Between-Run Precision (%)									
Compound	Blood								
	SPE A		SPE B		SLE		LLE		dSPE
	Lab A	Lab B	Lab A	Lab B	Lab A	Lab B	Lab A	Lab B	Lab A
Amphetamine	6.47	5.33	10.79	7.5	8.98	6.04	8.40	6.89	11.57
Methamphetamine	6.10	5.99	12.83	6.78	9.99	7.44	5.70	6.10	11.80
MDA	-5.23	6.09	NR	7.24	11.87	6.66	7.38	6.17	NR
MDEA	7.43	7.50	10.83	6.48	8.87	7.84	6.90	6.70	10.17
MDMA	6.69	6.67	10.47	6.94	9.84	8.44	6.48	6.59	11.44
Benzoyllecgonine	8.74	6.53	7.30	5.67	7.86	8.63	NR	NR	10.08
Cocaine	6.28	6.65	10.84	5.83	10.45	8.50	5.59	4.68	8.50
Lidocaine	5.35	4.70	7.79	6.24	10.67	8.73	5.47	5.44	7.31
Codeine	7.54	7.28	11.74	9.02	9.22	8.41	6.81	8.09	11.04
Methadone	8.76	6.48	12.09	6.92	11.13	8.09	9.94	6.88	11.61
Morphine	3.51	7.27	10.67	17.3	11.09	8.10	NR	NR	7.73
Fentanyl	9.31	5.78	13.67	8.58	15.15	6.64	11.64	6.83	13.04
Oxycodone	5.01	6.30	1.83	6.03	9.79	7.59	5.78	4.62	12.01
6-AM	7.08	6.57	12.89	18.14	10.89	11.68	8.11	NR	12.00
LSD	8.93	8.29	16.10	9.88	14.53	9.33	8.48	6.65	10.50
PCP	8.23	7.76	10.88	11.68	11.30	8.45	11.73	9.43	10.01
Amitriptyline	10.50	5.76	11.75	7.42	11.54	9.41	13.04	9.73	13.01
Citalopram	7.89	6.29	13.60	8.65	14.64	9.30	13.16	7.39	12.76
Fluoxetine	10.43	6.28	10.81	6.95	13.30	9.18	12.47	7.98	16.76
Trazodone	9.09	7.40	13.43	6.71	15.08	8.84	8.91	6.45	11.34
Ethylone	7.51	5.06	11.62	7.36	10.32	8.15	5.53	5.51	10.87
Alpha-PVP	6.82	4.99	9.37	5.88	11.39	6.89	8.39	6.95	12.02
25I-NBOMe	10.42	6.31	10.54	9.75	17.48	8.65	17.75	9.35	16.78
Amobarbital	9.11	6.37	4.92	3.09	5.02	10.69	8.21	2.62	TBD
Butalbital	8.40	6.21	4.78	2.94	4.61	12.59	6.93	2.07	TBD
Phenobarbital	8.20	8.88	4.41	2.92	5.68	9.65	7.44	3.44	TBD

Table A16: LOD values for drugs and metabolites extracted in blood

LOD (ng/mL)									
Compound	Blood								
	SPE A		SPE B		SLE		LLE		dSPE
	Lab A	Lab B	Lab A	Lab B	Lab A	Lab B	Lab A	Lab B	Lab A
Amphetamine	1	5	1	1	1	5	0.5	1	1
Methamphetamine	1	5	0.5	5	1	0.5	1	1	1
MDA	5	0.1	NR	5	5	0.5	5	1	NR
MDEA	0.1	0.5	0.5	0.1	0.1	0.5	0.1	0.5	0.1
MDMA	0.05	0.1	0.5	1	0.1	1	0.05	1	0.5
Benzoyllecgonine	0.5	0.5	0.5	5	1	1	NR	NR	0.5
Cocaine	0.05	0.5	0.05	5	0.05	0.1	0.05	1	0.05
Lidocaine	0.05	0.5	0.1	0.5	0.1	0.1	0.05	5	0.1
Codeine	0.5	5	0.5	5	1	1	0.5	5	1
Methadone	0.1	0.5	0.5	1	0.1	0.5	0.1	1	0.5
Morphine	1	5	1	1	0.5	1	NR	NR	0.5
Fentanyl	0.1	0.5	0.1	0.5	0.5	0.5	0.05	0.1	0.1
Oxycodone	0.1	0.5	0.5	1	0.5	0.5	0.05	1	0.5
6-AM	5	5	0.5	5	1	0.5	0.1	NR	0.5
LSD	0.5	0.5	0.5	0.5	0.5	0.1	0.05	0.5	0.1
PCP	0.5	0.5	1	1	1	0.5	0.5	1	1
Amitriptyline	0.5	1	0.5	5	0.5	0.5	0.5	0.5	0.5
Citalopram	1	1	1	5	5	0.5	0.05	5	0.5
Fluoxetine	1	5	0.5	5	1	0.5	0.5	5	0.5
Trazodone	0.5	0.5	1	5	0.1	0.1	0.05	0.5	0.1
Ethylone	0.1	0.5	0.1	0.5	0.1	0.5	0.05	0.5	0.5
Alpha-PVP	0.1	0.5	0.5	0.5	0.5	0.1	0.05	0.1	0.1
25I-NBOMe	0.05	0.5	0.5	0.5	0.05	0.5	0.05	0.5	0.5
Amobarbital	2	20	2	15	2	15	2	5	TBD
Butalbital	2	20	5	15	2	10	2	10	TBD
Phenobarbital	2	20	5	15	2	10	2	10	TBD

Table A17: Ion suppression/enhancement of drugs and metabolites in blood

Compound	Ion Suppression/Enhancement (%)																	
	Blood																	
	SPE A				SPE B				SLE				LLE				dSPE	
	Low		High		Low		High		Low		High		Low		High		Low	High
	Lab A	Lab B	Lab A	Lab B	Lab A	Lab B	Lab A	Lab B	Lab A	Lab B	Lab A	Lab B	Lab A	Lab B	Lab A	Lab B	Lab A	Lab A
Amphetamine	1.64	-31.09	-1.05	-5.76	-4.05	-20.06	-8.48	-22.46	16.94	-0.11	-0.19	-2.58	-0.11	-21.31	6.17	-15.58	5.77	4.35
Methamphetamine	-0.38	-27.91	-4.40	-4.02	-2.74	-16.00	-8.49	-26.51	13.03	-7.61	-2.83	-7.34	-0.18	-28.52	2.41	-24.87	1.61	-3.19
MDA	2.32	-38.80	-7.23	-27.74	NR	-26.76	NR	-31.08	20.22	-5.71	0.24	-5.60	0.07	-24.02	10.05	-19.92	NR	NR
MDEA	-3.92	-28.29	-6.48	-2.24	-2.44	-24.62	-2.56	-34.46	-2.44	-3.85	1.73	-1.44	6.11	-27.47	8.20	-25.15	-1.35	-0.12
MDMA	-0.59	-28.26	-1.88	-3.28	-1.84	-22.7	6.77	-30.77	18.48	-3.90	1.40	-4.58	-1.57	-28.46	6.19	-25.68	1.76	0.04
Benzoylecgonine	3.73	-25.57	0.39	-18.02	-1.45	-20.53	-2.10	27.97	13.59	4.55	0.59	-1.33	-2.58	-24.21	8.04	-20.97	6.26	2.29
Cocaine	-3.47	-27.41	-5.47	0.62	1.26	-17.18	-6.39	-26.63	15.85	-9.18	2.42	-6.87	-1.73	-23.96	9.83	-21.47	-5.62	-3.39
Lidocaine	-2.29	-24.93	-5.50	-0.57	-0.20	-17.79	-5.09	-27.73	21.65	-1.19	1.22	-2.13	2.94	-24.64	2.45	-21.22	-0.34	0.78
Codeine	0.09	-26.64	-8.14	-24.25	-6.75	-23.3	-15.58	-31.1	16.78	-9.36	4.53	-5.69	7.80	-27.71	7.12	-21.14	7.64	3.90
Methadone	-15.62	-38.15	-5.66	-13.93	-0.95	-19.8	-5.39	-29.5	21.77	-5.28	-1.00	-6.21	1.46	-31.54	0.49	-30.70	-9.74	-4.79
Morphine	-4.71	-28.89	-5.83	-25.46	-7.68	-31.1	-20.41	-39.1	14.91	-20.65	-0.60	-13.03	2.36	-27.06	6.19	-24.54	9.16	3.23
Fentanyl	-5.03	-33.70	-2.48	-20.06	-3.19	-27.8	4.40	-34.2	-5.11	1.86	-3.44	0.51	-7.04	-23.64	0.72	-18.93	-3.94	-10.31
Oxycodone	3.70	-39.27	-2.21	-36.04	-1.91	-8.8	-10.08	-15.4	15.85	1.37	2.50	-0.17	3.21	-26.18	4.97	-23.16	0.79	-0.34
6-AM	-2.01	-37.10	-2.60	-29.08	0.50	-30.1	-9.99	-40.5	15.08	-3.39	1.45	2.11	-2.48	-25.01	4.54	-23.93	-0.20	1.30
LSD	-4.96	-34.30	-2.51	-24.36	-5.17	-15.26	-1.60	-26.29	-8.30	-8.85	-3.70	-7.03	0.17	-21.75	2.62	-18.93	-5.08	-7.05
PCP	-5.14	-31.38	-6.88	-6.93	-2.99	-41.14	-5.11	-30.25	26.15	-5.51	-1.52	-5.08	-7.07	-22.55	1.18	-23.68	-5.96	-25.64
Amitriptyline	-26.75	-47.52	-14.41	-30.35	-1.64	-18.1	5.40	-27.06	15.31	-6.72	-2.72	-3.15	2.27	-29.33	-5.64	-27.31	-13.32	-13.30
Citalopram	-14.29	-41.59	-6.90	-26.01	3.31	-18.32	-7.24	-28.71	16.87	-1.27	2.65	-1.33	-10.38	-27.83	1.16	-23.45	-7.46	-12.72
Fluoxetine	-22.68	-46.55	-9.10	-28.00	25.71	-21.15	-0.24	-29.41	12.85	-10.34	3.83	-8.57	1.82	-28.79	-0.90	-27.35	-9.11	-3.78
Trazodone	-8.67	-32.22	-4.86	-24.32	-9.73	-10.72	-19.30	-26.9	19.90	-9.27	-0.57	-8.28	-1.45	-27.75	1.32	-25.29	-11.77	-15.39
Ethylone	-3.62	-34.32	-3.10	-25.99	-6.42	-19.89	-8.35	-27.23	4.03	-1.27	-1.32	-2.41	5.17	-23.08	10.94	-20.88	-0.46	-1.02
Alpha-PVP	-5.93	-30.78	1.07	-17.92	3.16	-17.75	-6.64	-21.05	-0.15	-2.76	1.40	-3.50	-3.86	-20.14	12.08	-15.18	1.69	1.10
25I-NBOMe	-16.64	-37.64	-1.90	-17.66	-1.39	-19.75	-13.97	-32.17	-6.37	-6.01	-2.61	-5.85	-6.87	-30.58	-2.52	-32.01	-14.67	-5.07
Amobarbital	-9.10	-4.52	-30.26	-12.12	-13.64	-23.28	-7.32	-14.28	-3.20	1.25	2.35	3.07	1.83	11.53	4.44	2.39	TBD	TBD
Butalbital	-17.15	17.50	-38.10	1.84	-15.88	-24.68	-6.37	-14.59	0.53	3.40	1.26	4.05	-3.50	12.52	0.63	3.06	TBD	TBD
Phenobarbital	-10.67	7.68	-30.84	8.26	-16.05	-27.87	-2.58	-24.37	3.41	7.68	7.12	4.79	-2.18	15.60	1.76	1.07	TBD	TBD

Table A18: Overall recovery of drugs and metabolites in blood

Compound	Overall Recovery (%)																	
	Blood																	
	SPE A				SPE B				SLE				LLE				dSPE	
	Low		High		Low		High		Low		High		Low		High		Low	High
	Lab A	Lab B	Lab A	Lab B	Lab A	Lab B	Lab A	Lab B	Lab A	Lab B	Lab A	Lab B	Lab A	Lab B	Lab A	Lab B	Lab A	Lab A
Amphetamine	89.07	79.83	88.28	86.30	63.13	94.67	73.64	95.16	75.50	88.29	70.13	89.91	54.38	71.01	57.57	78.01	46.45	50.26
Methamphetamine	79.12	84.74	83.64	92.08	55.75	106.55	71.69	102.67	65.16	89.30	66.75	90.51	80.22	83.40	83.16	82.85	45.13	52.28
MDA	87.86	78.42	98.36	83.46	NR	96.45	NR	100.94	68.92	89.25	67.44	89.93	51.01	72.38	54.79	77.56	NR	NR
MDEA	84.86	83.40	85.80	92.14	61.21	98.88	72.15	102.02	68.61	89.45	68.21	90.05	84.97	87.01	85.99	87.69	52.97	61.99
MDMA	84.23	83.27	86.75	91.78	59.15	98.27	75.67	101.18	70.12	89.04	70.12	91.74	75.68	80.95	75.18	81.90	53.14	61.93
Benzoylecgonine	69.87	68.67	78.84	79.07	62.48	89.5	77.86	93.54	63.81	93.52	67.16	93.57	0.24	0.30	0.22	0.38	56.91	64.24
Cocaine	83.98	85.73	89.78	90.68	58.78	96.46	72.96	96.43	62.71	86.03	66.26	85.92	89.81	85.51	84.67	85.52	58.35	62.97
Lidocaine	81.27	87.28	90.95	93.82	60.96	93.74	78.01	97.47	64.28	89.98	71.94	90.65	97.97	93.41	96.85	93.27	56.36	65.06
Codeine	77.39	76.95	84.72	80.82	56.84	99.80	67.48	101.50	66.72	89.43	67.00	87.77	75.99	61.17	71.98	63.98	57.85	62.39
Methadone	73.90	71.87	97.76	82.32	69.30	82.30	87.88	83.50	60.89	69.71	79.44	71.94	84.83	55.10	92.92	56.26	65.91	81.83
Morphine	60.68	58.97	62.56	65.99	30.21	17.89	49.51	19.52	57.87	71.66	62.70	73.63	4.67	2.23	6.34	2.27	47.90	56.84
Fentanyl	65.70	74.06	78.46	82.71	60.38	98.71	78.70	95.10	47.56	71.26	45.14	77.35	75.48	81.34	73.44	82.80	51.61	65.26
Oxycodone	80.36	83.45	85.43	85.53	59.03	104.44	69.31	107.11	65.11	89.54	62.15	89.46	78.60	69.19	79.83	72.63	53.49	58.59
6-AM	57.93	73.07	58.12	75.12	35.94	20.42	51.37	20.09	58.55	79.07	61.85	78.72	65.04	29.67	64.46	35.80	54.53	60.95
LSD	65.67	75.52	81.98	78.50	49.77	53.68	71.50	58.72	50.98	79.19	54.57	80.65	71.20	84.29	87.14	87.02	44.82	66.04
PCP	77.05	75.49	87.04	86.14	56.52	79.54	77.64	85.59	56.86	79.02	69.14	79.16	90.45	62.67	67.30	64.13	32.52	50.13
Amitriptyline	45.61	60.18	69.74	66.82	58.68	76.94	71.50	79.53	39.14	64.46	50.87	68.01	53.58	51.00	70.13	53.88	56.13	65.83
Citalopram	76.52	76.99	91.95	82.03	55.73	93.57	78.47	91.65	56.83	76.48	69.03	78.77	74.51	81.83	79.06	79.88	53.02	71.22
Fluoxetine	42.53	51.76	79.37	59.47	61.52	63.83	81.97	69.68	70.16	72.47	90.94	74.21	57.80	52.38	81.08	58.70	74.06	84.64
Trazodone	62.49	72.34	83.51	79.25	59.14	94.59	74.73	93.94	59.45	85.71	70.06	85.07	72.90	91.84	73.61	90.42	42.99	64.01
Ethylone	86.40	85.05	77.64	88.75	64.64	98.44	71.68	102.36	64.12	92.53	51.93	92.17	92.27	85.65	83.22	87.88	63.43	58.98
Alpha-PVP	75.77	80.80	73.67	87.04	56.61	82.85	66.64	86.81	58.60	79.06	44.41	84.27	97.71	82.43	80.07	84.91	40.22	36.65
25I-NBOMe	65.54	58.69	71.03	70.57	55.01	71.54	74.16	97.32	58.20	63.99	59.78	69.54	62.73	52.07	69.32	56.04	69.11	70.69
Amobarbital	60.66	34.97	63.08	40.67	73.28	46.31	77.64	45.42	69.06	52.83	78.04	46.32	49.75	61.34	57.88	59.72	TBD	TBD
Butalbital	54.59	34.47	59.00	43.75	73.01	47.73	75.67	48.95	70.66	55.59	81.54	48.56	51.43	61.63	59.43	55.23	TBD	TBD
Phenobarbital	43.55	12.20	48.39	26.30	74.12	51.42	76.42	48.22	70.14	53.34	79.37	47.38	57.39	64.13	62.04	57.11	TBD	TBD

Table A19: Time analysis of sample preparation for extraction of drug class mix (Table A1) in blood

LLE (blood)	SPE A (blood)	SPE B (blood)	SLE (blood)	dSPE (blood)
Add solvent (4 min)	Condition (3x) (8 min)	Condition (2x) (5 min)	Load samples (13 min)	Add acetonitrile (1 min)
Mix (10 min)	Load samples* (21 min)	Load samples* (6 min)	Absorb (5 min)	Centrifuge (5 min)
Centrifuge (5 min)	Wash (3x) (13 min)	Wash (2x) (5 min)	Elute (23 min)	Load samples* (8 min)
Transfer organic layer (40 min)	Dry (5 min)	Dry (1 min)	Evaporate (12 min)	Aspirate (3x) and perform airgap (3 min)
Evaporate (14 min)	Elute (10 min)	Elute (20 min)	Total Time: 53 min	Transfer to test tubes (7 min)
Total Time: 73 min	Evaporate (10 min)	Evaporate (10 min)		Evaporate (12 min)
	Total Time: 49 min	Total Time: 47 min		Total Time: 36 min

Figure A1: Solvent/reagent usage for 5 sample preparation techniques

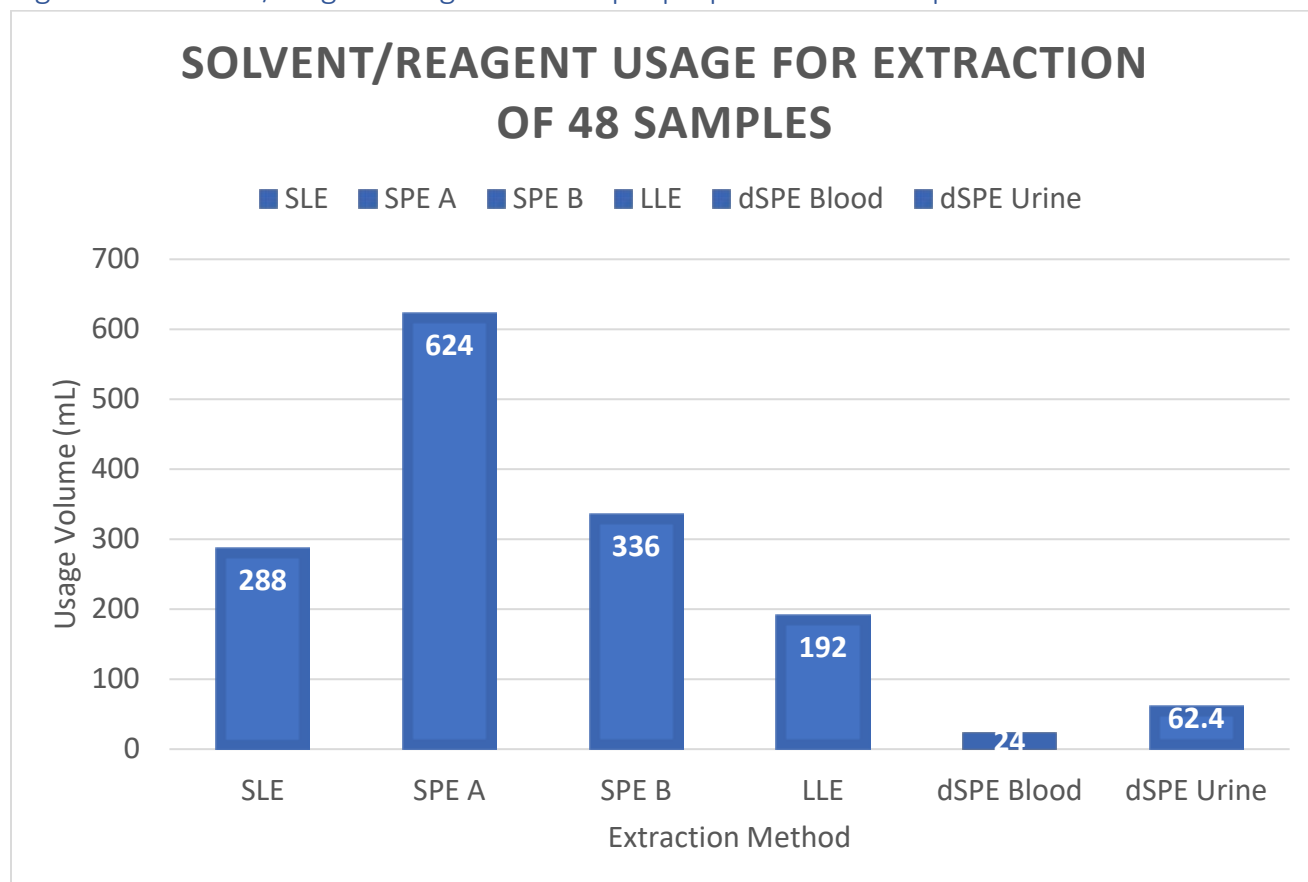


Figure A2: Sample extraction cost

