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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.



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## Project Design

Twenty-six drugs and metabolites (Table 1) were extracted in blood and urine, in small volumes (≤ 500 µL) 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.

**Table 1: 26 compounds analyzed in blood and urine**

| Drug Class | Compound |
|---|---|
| Barbiturate | Amobarbital |
| | Butalbital |
| | Phenobarbital |
| Amphetamine | Amphetamine |
| | Methamphetamine |
| | MDA |
| | MDEA |
| | MDEA |
| Miscellaneous | Benzoylecgonine |
| | 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 |

## 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



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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 Phenomonex 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 ±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 ±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



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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



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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 if 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 | • Add 200 µL of 1% ammonium hydroxide to each sample<br>• Mix/vortex<br>• **LOAD SAMPLES**<br>• Apply pressure for 3-5 seconds<br>• Allow to absorb for 5 min<br>• **ELUTE:**<br>• Apply 3 mL of dichloromethane (DCM)/isopropyl alcohol (IPA) 95/5 (v/v). Allow to flow by gravity for 5 min<br>• Apply a second 3 mL aliquot of DCM/IPA. Allow to flow by gravity for 5 min<br>• Apply pressure to pull through remaining solvent |
| SPE A | • Add 200 µL of 100 mM phosphate buffer (pH 6.0) to each sample<br>• Mix/vortex<br>• Centrifuge 4000 rpm for 5 min (blood)<br>• **CONDITION:**<br>o 2 mL methanol<br>o 2 mL D.I. $H_2O$<br>o 2 mL 100 mM phosphate buffer (pH 6.0)<br>• **LOAD SAMPLES**<br>• **WASH:**<br>o 2 mL D.I. $H_2O$<br>o 1 mL 100 mM acetic acid<br>o 1 mL methanol<br>o Dry column (5 minutes at full vacuum or pressure)<br>• **ELUTE:**<br>o 3 mL DCM/IPA/ammonium hydroxide ($NH_4OH$) 78/20/2 (v/v) |
| SPE B | • Add 200 µL of 1% formic acid to each sample<br>• Mix/vortex<br>• Centrifuge 4000 rpm for 5 min<br>• **CONDITION:**<br>o 1 mL methanol<br>o 1 mL 1% formic acid<br>• **LOAD SAMPLE**<br>• **WASH:**<br>o 1 mL 1% formic acid<br>o 1 mL 50:50 methanol:water<br>o Dry column (1 minute at full vacuum or pressure) |



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| | |
|---|---|
| | • **ELUTE:**<br>o 2 x 1.5 mL DCM/IPA/$NH_4OH$ 78/20/2 (v/v) |
| LLE | • Add 500 µL of 0.2 M sodium carbonate solution (blood samples)<br>• Add 4 mL of n-butyl chloride<br>• Mix for 10 minutes<br>• Centrifuge samples for 5 min at 3500 rpm<br>• Transfer organic layer to test tube |
| dSPE Urine | • Add 20 µL of 1.0 M HCl<br>• Vortex mix<br>• **CONDITION:**<br>• Condition with 500 µL 50% methanol in water by aspirating and then waiting 30 seconds before dispensing<br>• Perform an air gap<br>• **LOAD SAMPLE**<br>• Load the samples by aspirating and then waiting 30 seconds before dispensing. Do this three times.<br>• Perform an air gap<br>• **WASH:**<br>• Wash with 400 µL 10% methanol in water by aspirating and then waiting 30 seconds before dispensing<br>• Perform an air gap<br>• **ELUTE:**<br>• Elute analytes with 400 µL 4% ammonium hydroxide in methanol by aspirating and then waiting 30 seconds before dispensing |
| dSPE Blood | • Add 500 µL acetonitrile to blood<br>• Vortex mix<br>• Centrifuge for 5 min at 4000 rpm<br>• Remove organic layer into well plate<br>• 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



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

| Extraction | Steps |
|---|---|
| SLE | • Add 200 µL of 100 mM ammonium acetate (pH 5) to each sample<br>• Mix/vortex<br>• **LOAD SAMPLES**<br>• Apply pressure for 3-5 seconds<br>• Allow to absorb for 5 min<br>• **ELUTE:**<br>• Apply 1.5 mL of DCM. Allow to flow by gravity for 5 min<br>• Apply a second 1.5 mL aliquot of DCM. Allow to flow by gravity for 5 min<br>• Apply pressure to pull through remaining solvent |
| SPE A | • Add 200 µL of 100 mM phosphate buffer (pH 6.0) to each sample<br>• Mix/vortex<br>• Centrifuge 4000 rpm for 5 min (blood)<br>• **CONDITION:**<br>o 2 mL methanol<br>o 2 mL D.I. $H_2O$<br>o 2 mL 100 mM phosphate buffer (pH 6.0)<br>• **LOAD SAMPLES**<br>• **WASH:**<br>o 2 mL D.I. $H_2O$<br>o 1 mL 100 mM acetic acid<br>o Dry column (5 minutes at full vacuum or pressure)<br>o 1 mL hexane<br>• **ELUTE:**<br>o 3 mL ethyl acetate:hexane (50:50) at 1 to 2 mL/min |
| SPE B | • Add 1 mL acetonitrile to each sample (800 µL for urine at Lab A)<br>• Mix/vortex<br>• Centrifuge 4000 rpm for 5 min<br>• **CONDITION** (performed at Lab A for blood and urine and at Lab B only for blood)<br>o 2 mL methanol<br>o 2 mL D.I. $H_2O$<br>• **LOAD and ELUTE SAMPLE**<br>o Load the sample and allow to flow through the column into collection test tubes |
| LLE | • Add 500 µL of phosphate buffer (pH 5)<br>• Add 1.5 mL of 50:50 (hexane: ethyl acetate)<br>• Mix for 5 minutes<br>• Centrifuge samples for 5 min at 3500 rpm<br>• 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



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## 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 |
| Benzoylecgonine | 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

| Concentration (ng/mL) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 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 | 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 |
| Benzoylecgonine | 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 |



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## 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 |
| Benzoylecgonine | 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 |

## 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 |
| Benzoylecgonine | 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 |



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## 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 |
| Benzoylecgonine | 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 |



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## 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 |
| Benzoylecgonine | 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 |



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

| LOD (ng/mL) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 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 | 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 |



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## 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 |
| Benzoylecgonine | -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 |



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## 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 |
| Benzoylecgonine | 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 |



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## 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 |



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## 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 |



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## 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 |
| Benzoylecgonine | 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 |



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## 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 |
| Benzoylecgonine | 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 |



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## 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

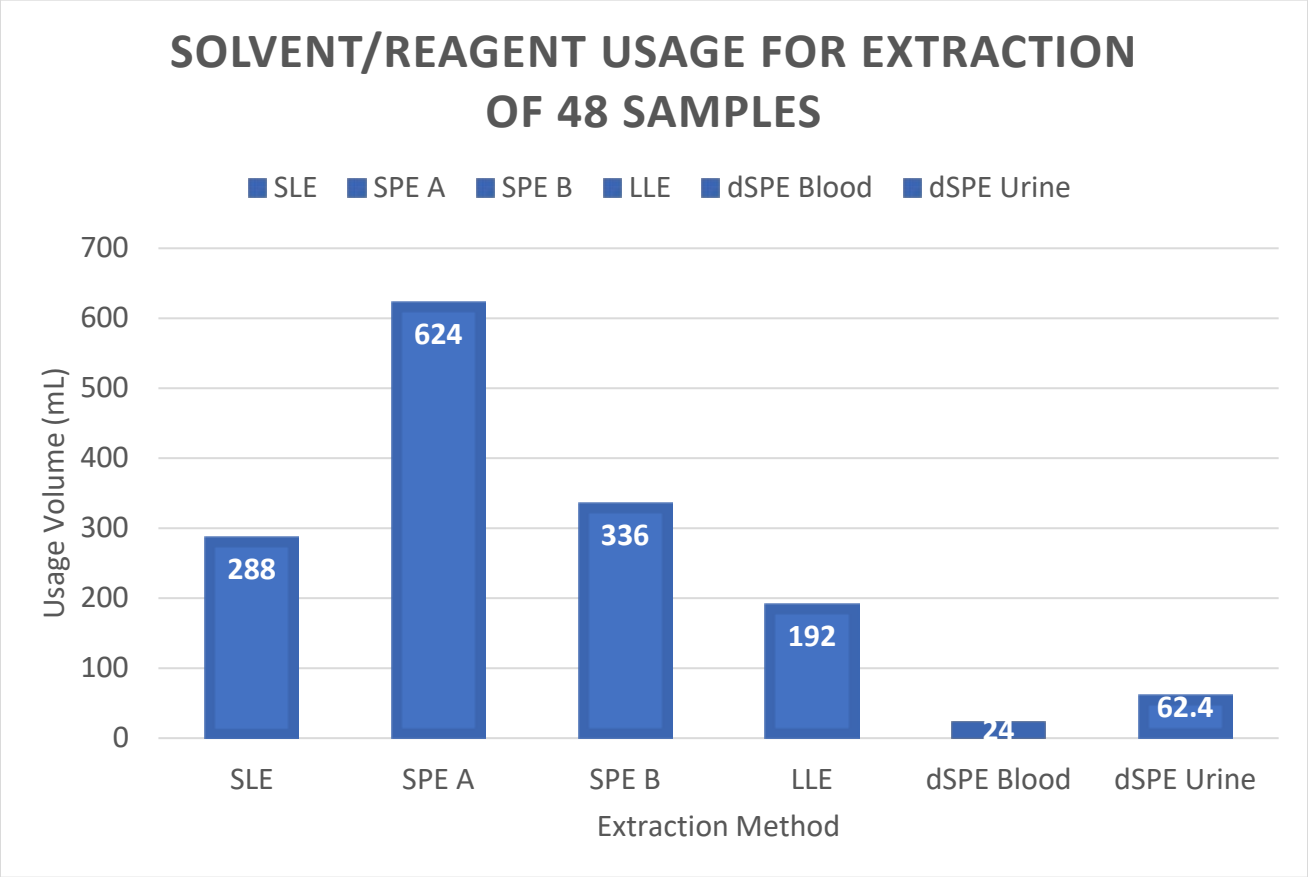

Figure A2: Sample extraction cost