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Method Consolidation to Improve Scope and Efficiency in Postmortem Toxicology

Jirair Gevorkyan [1]

Megan Wong [1]

Sue Pearring [1]

Luke N. Rodda [1, 2] *

[1] Office of the Chief Medical Examiner, San Francisco, California, USA

[2] Department of Laboratory Medicine, University of California, San Francisco, California, USA

* corresponding author: Luke.Rodda@sfgov.org; Office of the Chief Medical Examiner, City and County of San Francisco, 1 Newhall Street, San Francisco, California 94124, USA; Tel: +1 415-641-3688

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Abstract

Systematic toxicological approaches that employ both ideology changes and improvements in instrumentation and sample extraction allow for improved toxicology testing efficiency through lower sensitivities, higher specificity and minimized resource use. Historically, the San Francisco Office of the Chief Medical Examiner relied heavily on a GC-MS testing regime, comprised of individual drug-class confirmation and quantitation assays. Traditional methods utilizing GC-MS typically require iterations of testing, exhausting sample volume, and hindering productivity and turnaround times. Particularly for polypharmacy cases frequently seen in modern postmortem toxicology. The method described here consolidated the scope of seven legacy methods into a single LC-MS/MS method for better sensitivity, higher throughput, quantitation of drugs of abuse with minimal sample consumption, and incorporation of smart automated processing for improved quality assurance. One hundred microlitres of blood or urine were rapidly extracted using a simple acetonitrile protein crash and subsequent in-vial filtration and injected on to an LC-MS/MS system. The developed method was fully validated to SWGTOX and international guidelines and incorporated 55 analytes and a customized query that facilitates rapid and consistent application of acceptability criteria for data processing and review. Applicability was demonstrated with the analysis of 1389 samples (858 blood and 531 urine) where at least 41% of positive results may have been potentially missed due to their decreased sensitivity, and 11% of results were not within the scope, of the previous analytical methods estimated. On average, cases in this study would have previously required three distinct GC-MS assays, 3 mL of blood, and upwards of 30 hours of active staff time. The described LC-MS/MS analytical approach has mitigated the need to perform multiple assays, utilized only 0.1 mL of sample, significantly reduced analyst work time, incorporated 10 additional analytes, and allowed for a more comprehensive testing regime to better inform cause of death determinations.

Keywords

LC-MS/MS, Sensitivity, Postmortem, Efficiency, Polypharmacy,

Highlights

Seven GC-MS methods were consolidated into a single LC-MS/MS method on a 6500+ QTRAP to improve postmortem toxicology case management.

Introduction

The advent of the opioid crisis and rising number of cases involving drugs of abuse has placed increasing workloads on toxicology laboratories [1-5]. In addition, laboratory workloads have become more complicated due to polypharmacy and the emergence of novel psychoactive substances [6-10]. This presents difficulties for historical approaches of single drug category extraction and GC-MS analysis, where specimens that screen positive for more than one drug would be required to go through multiple iterations of confirmation and/or quantification for full toxicological analysis. The staff time and resource cost of the sample preparation, extraction, acquisition, processing, and reporting becomes burdensome for polypharmacy cases and significantly affects the efficiency of the toxicology laboratory and turnaround times for reporting. Typical extractions that require 1 mL of sample due to GC-MS sensitivity, rapidly deplete available sample in cases where multiple method analysis must be performed to target different drug classes. Limited sample volume may also force case managers to determine, subjectively, which tests make best use of available sample volume and what drug result is of most importance to the case. Additionally, derivatization strategies are often required during extraction to promote analyte volatilization and injection port parameters are optimized to prevent degradation of thermally labile analytes for GC-MS analysis [11]. This requirement becomes complicated by large drugs scopes with diverse chemical and physical properties due to challenges in finding a single injection configuration that produces optimal volatilization and stability for all analytes involved [12]. Efficient and high sensitivity confirmation and quantification of multiple drugs of abuse with minimal sample use is imperative for optimization of case management, resource use and turnaround times.

Alternatively, the issues of injection temperature, stability, and derivatization can be ameliorated by operation in the liquid phase with LC methodologies, which do not require volatilization or injection temperature optimization for well-defined peak shapes and resolution [11, 13]. Pairing of the LC with triple quadrupole mass analyzer systems (LC-MS/MS) further improves the method's selectivity and sensitivity. The characterization of unique precursor to product reactions (transitions) of an analyte and multiple reaction monitoring (MRM) with high scan rates are critical advantages of the tandem mass analyzer system that provides higher sensitivity and specificity. These advantages in LC-MS/MS often allow for a single method to confirm and quantitate multiple drug classes with improved detection and time efficiency.

Forensic toxicology laboratories must also make certain that methodologies follow quality assurance standards beyond data acquisition [14]. Analysts must ensure that each injection and peak integration perform correctly and consistently, calibration samples produce an acceptable fit to the validated model, quality control samples fall within their validated ranges, and all identified analytes exhibit appropriate MRM ion ratios. This quality assurance is often difficult to automate due to variability between methods and software, and becomes tedious and time consuming for both processing and review when multiple single drug class methods must be employed to complete individual cases.

The work presented here describes a full validation of an LC-MS/MS method that consolidated seven historical GC-MS methods and a colorimetric spot test previously employed by the San Francisco Office of the Chief Medical Examiner and incorporated other pivotal drugs not previously within the scope of the laboratory. The described method targets 55 drugs of abuse and therapeutics quantitatively in blood, and qualitatively in urine, with greater sensitivity and efficiency than previously achieved. In conjunction, a customized query was developed to rapidly calculate and consistently evaluate the analytical range, quality control and ion ratios based on validation guidelines for an improved quality assurance program [15-18]. Casework data gathered over a 12-month period utilizing this analytical method was cross referenced to the scope and sensitivity of the historical methodologies as an assessment of applicability and estimation of effectiveness.

Experimental

Chemicals, Reagents and Gases

Certified reference standards for analytes and isotopically labeled internal standards (IS) were obtained from Cerilliant (Round Rock, Texas, US), Lipomed (Cambridge, Massachusetts, US), Cayman Chemicals (Ann Arbor, Michigan, US), Wyeth (Madison, New Jersey, US), and MP Biomedical (Irvine, California, US), and Alltech (Deerfield, Illinois, US). All analyte stocks were prepared at 0.1, 1.0, or 10 mg/mL concentrations, as appropriate, if not already provided as such by the manufacturers. Optima LC/MS grade acetonitrile (ACN), methanol, isopropanol and ammonium formate were purchased from Fisher Scientific (Hampton, New Hampshire, US). All water utilized was purified to deionized water (dH_2O) using a Millipore Milli-Q Ultrapure Water System from Millipore Sigma

(Burlington, Massachusetts, US). All nitrogen gas utilized was produced in-house to 1-5 ppm purity (Peak, Inchinnan, Scotland, UK)

Specimens

Calibration and validation studies were performed using blank porcine blood (Del Monte, San Francisco, California, US) and certified blank urine samples containing 0.01 % sodium azide (UTAK, California US). Porcine blood was prepared in house with 10 g/L of sodium fluoride and 6 g/L potassium oxalate. Application studies were performed using blood and urine proficiency samples and authentic case samples. Proficiency samples were received from the California Association of Toxicologists (CAT) and College of American Pathologists (CAP) from 2016 to 2018. Authentic blood and urine specimens were obtained by the Forensic Laboratory Division of the San Francisco Office of the Chief Medical Examiner during routine postmortem casework obtained during autopsy over an approximate 12-month period during 2017-2018.

Apparatus

The LC-MS/MS system consisted of a Sciex Nexera X2 LC-30 (California, US) containing a degasser, two binary pumps, temperature-controlled autosampler, and temperature-controlled column chamber, coupled with a Sciex QTRAP 6500+ mass spectrometer utilizing an Ion Drive™ Turbo V electrospray ionization (ESI) source operating in positive MRM mode. Data acquisition and processing with custom built-in automation incorporating quality assurance was performed with Sciex Analyst and Multiquant software, respectively.

LC Parameters

Chromatography was performed using a gradient on a Kinetex Phenyl Hexyl 100 Å LC Column (100 x 4.6 mm, 2.6 µm) column coupled with a SecureGuard cartridges for Phenyl columns, both purchased from Phenomenex (California, US). Mobile phase A (MPA) consisted of 5 mM ammonium formate in dH_2O and pH adjusted to 4.5 using formic acid and mobile phase B (MPB) ACN containing 0.1% formic acid. The flow rate of the total mobile phases was consistent at 1.0 mL/min and degassed throughout use. The elution gradient was as follows: 0-1.0 min hold at 6% MPB; 1.0-2.5 min MPB increased to 9%; 2.5-4.0 min MPB increased to 12 %; 4.0-5.5 min MPB increased to 15 %, 5.5-7.0 min MPB increased to 21 %, 7.0-8.5 min MPB increased to 24 %, 8.5-10.0 min MPB

increased to 27 %, 10.0-11.5 min MPB increased to 30 %, 11.5-13.0 min MPB increased to 33 %, 13.0-14.0 min MPB increased to 36 %, 14.0-15.0 min MPB increased to 39 %, rapidly change MPB to 90 % at the 15 min, 15.01-15.5 min hold MPB at 90 %, rapidly change MPB to 97 % at 15.5 min, 15.51-18.5 hold MPB at 97 %, rapidly change MPB to 6 % at 18.5 min and hold until 22.25 for re-equilibration. The column oven was maintained at 40 °C, the autosampler was operated at 6 °C and the autosampler needle was rinsed before injection of each sample using a solvent mixture of 20 % methanol, 20 % acetonitrile and 60 % isopropanol.

MS/MS Parameters

The MS data were acquired with the following Ion Drive ™ Turbo V conditions: curtain gas, ionization gas 1 and gas 2 were all nitrogen gas set to flow with 40 (275.7), 60 (413.6) and 70 (482.6) PSI (kPa), respectively. The ion spray voltage was set to 2500 V and the temperature was set to 600 °C. Two MRM ions per analyte were monitored as per internationally-accepted guidelines [15-17, 19] with consideration for selectivity and the most abundant ion chosen as the quantifier ion and the ion selected to evaluate the validation results for each target analyte (**Table 1**). The mass spectrometer was set to operate in positive polarity using advanced scheduled MRM scan type. Target scan time per MRM experiment was set to 0.3 sec. MRM window detections were set to 60 sec with the exceptions of amphetamine, doxylamine, fentanyl, norsertraline, and olanzapine which were set to 30, 120, 45, 45, and 180 sec, respectively. Dwell times are automatically calculated by the software and range from 3 to 250 msec. A pause of 5.007 msec was set to between mass ranges to mitigate any crosstalk. A full cycle of MRM transition cycle lasted 0.3 sec and occurred 3496 times per injection. MRM transitions proceeded with nitrogen as the Collision Gas (CAD) set to the High setting.

Analyte Grouping

Target analytes were designated into four groups (A, B, C and D) according to concentrations typically encountered in forensic casework. Analyte grouping and respective internal standard are provided in **Table 1**. Benzodiazepines and cannabinoids were not incorporated into this method as these drug groups pre-existed within other LC-MS/MS methodologies.

Preparation of Stock Solutions, Calibrators and Controls

The calibration and quality control stock solutions were prepared separately by pooling individual analytes from groups A, B, C and D to stock concentrations of 15,000, 150,000, 1,500, and 300,000 ng/mL in methanol, respectively. Calibrant and controls were prepared from respective stocks by individually diluting to levels as shown in **Table 2**. A qualitative reporting limit stock was prepared independently to final analyte concentrations described in **Table 4** and was designated as "LOD" stock. IS stock was prepared to a final analyte concentration (and final in matrix concentration) of 1,000 (100) ng/mL with the exceptions of fentanyl-D5, carisoprodol-D7 and acetaminophen-D4 which were prepared at 100 (10), 10,000 (1,000) and 20,000 (2,000) ng/mL, respectively. All stocks, calibrators and controls were stored in multiple aliquots of 1 mL crimp top sealed vials at -20 °C.

Extraction Procedure

Blood or urine (100 µL) were aliquoted into 2 mL 0.2 µm PTFE with pre-silt caps Extreme FV Filter vials (Thomson Instrument Company, California, US), combined with premixed solution of -20 °C acetonitrile fortified with IS stock to deliver 390 µL of acetonitrile and 10 µL IS stock per sample, partially capped with the filter piston and vortexed for 5 minutes at 2,500 rpm. Samples were filtered by pressing down on the pistons over 30 seconds and uncapped where 350 µL of filtrate was aliquoted to glass autosampler vials with tapered bottoms. The filtrate was evaporated to dryness using a gentle flow of nitrogen gas over approximately 15 minutes at room temperature. Samples were then reconstituted with 50 µL of reconstitution mix consisting of 90:10 MPA:MPB . Finally, the autosampler vials were vortexed briefly before being placed into the LC-MS/MS system for 1 µL injection.

For urine qualitative casework, large analyte concentrations that saturate the detector could produce MRM ion ratios that are outside of the acceptable range. Therefore, these samples were subsequently diluted 1/100 with reconstitution mix and reinjected where appropriate confirmation of the qualifier ion ratio could occur.

Validation

Selectivity

Interference studies were carried out to assess the effect of the matrix, fortified internal standards and target drugs used in the analytical method, and the effects of other drugs possibly encountered during routine analysis. The studies also ensured that the method was selective and that there was no crosstalk within the quadrupole system.

Blank matrix interferences were assessed with the extraction of 20 authentic sources (5 postmortem blood, 5 postmortem urine, 5 antemortem blood, 5 antemortem urine) without the addition of internal standard. In addition, the evaluation of the blank matrix used for routine analysis was assessed by the extraction of porcine blood and authentic urine without internal standard. The lack of detectable drug responses in these samples indicated the blank matrix did not interfere with the identification or quantitation of analytes in this method.

All 40 available isotope-based internal standards were evaluated with the neat injection of each internal standard into the analytical system individually and confirmed no interference with the identification of target analytes. All 55 targeted drugs were evaluated in the same manner. Isobaric analytes (codeine and hydrocodone, morphine and hydromorphone, ephedrine and pseudoephedrine) were confirmed to be chromatographically resolved and not interfering.

The evaluation of interference from 51 other common therapeutic and illicit drugs and poisons was accomplished by analyzing fortified matrix samples, listed in **Supplementary Table 1**.

Matrix Effects, Extraction Recovery and Process Efficiencies

The matrix effects (ME), extraction recovery (ER), and process efficiencies (PE) were estimated with a set of three different samples at two concentrations (QC Low and High) with five different authentic samples [20]: the neat standard (set 1), blank matrix spiked with target analytes after extraction (set 2), and blank matrix spiked before extraction (set 3). Extraction efficiencies were estimated by comparison of the peak areas set 2 to those of set 3. Matrix effects were estimated by comparison of the peak areas of set 2 to those of set 1. For process efficiencies, the peak areas set 3 was compared to set 1. All comparisons are made as percentages. Values over 100 % for ME

indicate ion enhancement, while values below 100 % indicate ion suppression. The targeted range for each analyte ME was ≤ ±25% and the targeted ER and PE for validation studies was ≤ 50%, with a consideration of the consistency within the sample range.

Linearity

Linearity was evaluated through the analysis of all blood calibration points over five analyses on separate days. Standardized residual plots analysis were visually analyzed to evaluate linear and quadratic calibration models for suitability. The acceptability criteria for all quantitative blood calibration curves was set as a coefficient of determination (R^2) greater than 0.99 and ± 20 % accuracy on any individual calibration point, and no more than two of eight calibrators exclusions.

Urine casework was assessed qualitatively and consisted of a single calibrator point. Calibrator 1 (**Table 2)** was utilized as a qualitative reporting limit sample. Targeted analyte concentrations are described under Urine LOD in **Table 4**.

Processed Sample Stability

The stability of the extracted and processed samples during batch analysis under the conditions of the described method was estimated. Extracted matrices spiked with both QC Low and High and the targeted IS concentrations were suspended in the analytical method reconstitution matrix, pooled, redistributed into autosampler vials and subsequently analyzed for stability. Injections occurred approximately every 4 hours for a total of 96 hours. The autosampler tray was controlled at the same temperature as during the analytical method throughout the testing period to replicate authentic analytical protocols. Acceptable stability was ≥ 80% peak area throughout the said period.

Accuracy and Precision

Accuracy, or bias, and precision was evaluated in triplicate samples over five different days at the QC Low, Med, High, and UltraHigh concentrations fortified in blood matrix as specified in the validation plan. Accuracy was calculated as the relative difference of the grand mean from the nominal value per analyte per QC level. The

acceptability criterion for accuracy was ≤ ± 20 % for each target analyte and at each concentration. Precision was expressed as the coefficient of variation (CV). Two different types of precision studies were assessed during method validation: within-run precision (within-run CV) and between-run precision (between-run CV). Within-run and between-run CV were calculated using the one-way ANOVA approach [16, 18]. The acceptability criterion for within-run and between-run CV precision studies was ≤ ± 20 % for each target analyte and at each concentration. Dilution integrity was assessed at 1/2, 1/4, 1/9, and 1/18 ratios with the same accuracy and precision criteria applied to triplicate data repeated over five days, and using fortified matrix samples prepared at concentrations of 200, 2,000, 20,000, and 40,000 ng/mL above of the analytical range for group A, B, C, and D, respectively.

Lower Limit of Quantification and Limit of Detection

The lower limit of quantitation (LLOQ) for each analyte was administratively established as the lowest calibrator (i.e. Calibrator 1) for the assay whilst demonstrating at least a signal-to-noise ratio (S:N) of at least 10:1. The limit of detection (LOD) for each analyte was established through parallel dilutions to the lowest concentrations that would still retain a S:N of at least 3:1. The LLOQ and LOD were evaluated from spiked blank matrix samples from three different sources carried out concurrently with accuracy and precision in five trials over five days. Additionally, visual inspection was undertaken to ensure appropriate chromatography and acceptable integration.

Applicability

Applicability experiments were carried out by testing proficiency samples received within recent years. Thirty one (31) blood and 21 urine samples were analyzed using the method described and assessed for qualitative and quantitative acceptability with consideration for sample integrity, degradation over time and additional information from the proficiency sample providers.

In addition, a retrospective analysis was performed on all postmortem casework completed using the method within the last year and all reported positives for each analyte. Results were compared to the scope and sensitivity of the prior GC-MS methods. Specifically, the potential number of missed results due to lack of sensitivity and total absence from methodology were estimated. This was performed by comparing the concentration determined by the described method and with historical limits of detection.

Results and Discussion

Validation

Selectivity

Peak resolution was calculated using retention time of a given analyte, the retention time of the preceding or proceeding analyte, full width half max of the given analyte, and the full width half max of the preceding or proceeding analyte. Chromatographic resolution (peak resolution ≥ 1.0 widths) was achieved for most compounds, depicted by **Supplementary Figure 1**, and analytes were grouped together in a single set of standards or control samples. The chromatographic method yielded baseline resolution between hydromorphone and morphine, and hydrocodone and codeine. The peak resolution of ephedrine and pseudoephedrine was determined to be 1.17 widths and strict integration parameters were utilized to consistently identify the correct diastereomer, as show in **Supplementary Figure 2**. Methamphetamine and phentermine are resolved by retention time and clearly distinguished by ion ratio through the utilization of a qualifier transition unique to methamphetamine, shown in **Supplementary Figure 3**. Chiral resolution was not established for this method and, thus, all identified analytes are reported racemic. All peaks displayed a general Gaussian distribution, with the exception of olanzapine due to its physical and chemical properties.

Matrix Effects, Extraction Recovery and Process Efficiencies

Matrix effect, extraction recovery and process efficiency are listed in **Supplementary Table 2**. Bolded analytes were outside of the defined targeted ranges. Blood (doxylamine, fluoxetine, norbuprenorphine, norfentanyl, olanzapine, and sertraline) and urine (amitriptyline, chlorpheniramine, doxylamine, fluoxetine, gabapentin, hydroxyzine, morphine, norfluoxetine, norsertraline, nortriptyline, olanzapine, paroxetine, quetiapine, and sertraline) matrix effect outliers exhibited ion enhancement. Observed outliers were understandably due to the wide range of chemical and physical properties of the analytes within the method scope and the simple approach to extraction. The impact of observed matrix effects and signal attenuation was mitigated by pairing of targeted analytes to matching internal standards where possible, or to its best pairing available after consideration of retention time, structure and calibration model assessment.

Linearity

Residual plots generally showed an inverted "U" shape distribution for linear, non-forced through zero and equally weighted regression fitting, and showed improved random distribution with quadratic, non-forced through zero and weighted 1/x regression fitting. Thus, a quadratic regression model with 1/x weighting was deemed appropriate for quantitation of the analytes within the scope of the method in blood across the chosen analytical range. All quantitative data collected for pre- and post-validation applicability studies contained calibrations with R^2 values >0.990, calibrator accuracies with ±20 % the target, and no more than two of eight calibrators exclusions. Blood analysis also included an additional standard designated as the Blood LOD (targeted analyte concentrations listed under Blood LOD in **Table 4)** that was utilized as a qualitative reporting limit for those results with area ratios were between the Blood LOD and Blood LLOQ.

The qualitative urine casework simply utilized the single point Urine LOD.

Processed Sample Stability

All analytes exhibited >80% stability over 24-hour period. All data collected for post-validation applicability studies was acquired within 24 hours of extraction.

Accuracy and Precision

Accuracy and precision data for each analyte in blood is averaged across triplicate data repeated over five days, shown in Error! Reference source not found.. All analytes produced acceptable results for within-run CV, between-run CV. All analytes produced acceptable results for bias at the QC Low, Med and High levels. Hydroxyzine was not assessed at the QC UltraHigh or dilution levels. Six analytes (ketamine, meprobamate, mirtazapine, oxymorphone, venlafaxine, and zolpidem) produced bias results outside of the targeted criteria. These bias outliers were likely attributed to preparation due to the low within-run and between-run CV. Furthermore, concentration near QC UltraHigh for these six analytes were not encountered in authentic casework as described by the application study. Data for QC Med and UltraHigh was collected and analyzed to provide a more complete characterization of the analytical range.

Accuracy and precision data for each analyte with 1/2, 1/4, 1/9, and 1/18 dilutions of fortified matrix samples prepared at concentrations above of the analytical range (i.e. ULOQ) were analyzed in triplicate over five days. Dilution integrity showed generally acceptable results for most compounds. Nine analytes (buprenorphine, EDDP, ketamine, mirtazapine, norsertraline, olanzapine, oxymorphone, sertraline, and venlafaxine) produced results beyond the targeted criteria.

Lower Limit of Quantitation and Limit of Detection

The analytical limits of quantitation are summarized in **Table 2** and limits of detection are summarized in **Table 4**. The LOD and LLOQ for norbuprenorphine, norfluoxetine, norsertraline, olanzapine and acetaminophen were both set to calibrator 2 in blood. For all other analytes in blood, the area ratio of the Blood LOD sample was used for qualitative reporting below the Blood LLOQ concentration. To ensure suitable applicability to concentrations typically encountered in forensic casework, the LOD for analytes were administratively set higher and resulting in significantly greater S:N than the required 3:1, often well above 50:1. The LOD for urine was set at the same concentrations as blood Calibrator 1 in order to produce a qualitative threshold for positive identification.

Applicability to Proficiency and Authentic Samples

The pre-implementation application study of 31 blood and 21 urine previously analyzed proficiency samples included 127 positive analytes in blood and 67 in urine, of which 77 (blood) and 38 (urine) were within the scope of the described method. All 77 expected blood results were detected, 50 results were quantified within 20 % of expected concentration, 7 results above the method ULOQ, and 20 results quantified at concentrations beyond 20 % of the expected value. The outlier blood results were isolated to samples that did not contain preservative, ranged in age up to 2 years, and were observed to contain microbial growth. Outlier blood analytes (amphetamine, benzoylecgonine, cocaine, dextromethorphan, fentanyl, hydromorphone, MDA, morphine) were correctly identified and quantified in other blood sample results. Some outlier blood results may be attributed to degradation (6-monoacetylmorphine, amitriptyline, cocaine, diltiazem) or accumulation as a degradation product (benzoylecgonine) [21-25]. Of the 38 expected urine results, 36 were detected above the urine qualitative reporting limit and 2 results (benzoylecgonine and diltiazem) presented peaks with acceptable shape and S:N greater than 3:1 but produced area ratios below that of the qualitative reporting limit sample and as such, were deemed not detected. Similarly to blood,

the 2 urine results that were not detected were attributed to degradation over time. Additionally, the 50 blood and 29 urine analytes that were outside of the scope of this method, did not interfere with those within the scope. The results were generally accepted with the aforementioned considerations and the method was deemed satisfactory for implementation.

A second post-implementation application study was performed using authentic medical examiner casework data collected over an approximate 12-month period which included 1,389 samples (858 blood and 531 urine). A summarized total of the blood and urine analyte counts, and an assessment of the percentage of results that would have been potentially missed based on the scope and limits of historical methods are summarized in **Table 4** and depicted in **Figure 1**. A total of 2,551 blood and 1,940 urine positive results averaging 3 and 3.6 per sample, respectively, were quantified. The study indicated that 41% of blood results would likely have been missed due to the lack of sensitivity of historical methods. Additionally, 11% of all results would have been missed due to prior absence of buprenorphine, carisoprodol, ephedrine, gabapentin, hydromorphone, meprobamate, norbuprenorphine, norfentanyl, oxymorphone, and pseudoephedrine from the scope of historical methods. Potentially missed detection in urine due to lack of sensitivity could not be determined as all urine results were assessed qualitatively. Stimulant and opioid analytes constituted a majority of the total and potentially missed detections. These detections agreed with the observed trends in substance abuse, addiction and overdose in California and substantiate the need for more comprehensive methodology to feasibly approach the workloads generated by current drug epidemics [26, 27].

The consolidation of previous method scopes eliminated the need for iterative testing that would deplete sample volume. Furthermore, the greater sensitivity of the 6500+ QTRAP facilitated lower sample volume use in extraction while maintaining, or improving, low limit of detection and quantitation. The sample volume consumption, assuming a detection rate of 3 drugs on average per sample, decreased from approximately 3 mL on average to 0.1 mL (3,000 % increased efficiency) due to this consolidation and sensitivity. Active staff time to extract, process, and review data has been reduced from an approximate minimum of 45 hours to an approximate 8 hours (550 % increased productivity). A customized query that is incorporated into the instrument software during processing further streamlined the method by facilitating the acceptability criteria, performing calculations, color-coding results and outliers, and parsing results into appropriate confirmation or quantitation formats that are ready to report.

Conclusion

To relieve toxicological testing backlogs and lengthy turnaround times, the fully validated LC-MS/MS method described combined the scopes of seven previous GCMS methods and one colorimetric spot test employed by the San Francisco Office of the Chief Medical Examiner, decreased sample consumption, provided lower limits of detection and quantitation, and more efficient use of staff time. The successful application and assessment of toxicology casework indicated that 41 % of results would have been missed due to the detection limits of previous methodologies for the 12-month study. Additionally, the described method yielded an approximate 3,000 % more efficient use of sample and 550 % more productive use of staff time for the average analytes detected per case. The improved ability to detect key analytes (6-monoacetylmorphine, buprenorphine, fentanyl, gabapentin, morphine) has been significant for interpretation of the toxicology in the context of death investigations. This method and approach also incorporated more compound categories and serves as an example of the feasibility and effectiveness of well-implemented use of newer technology towards the advancement of toxicology laboratories.

Declarations

The authors declare no conflicts of interest.

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Tables and Figures

Table 1: Summary of MS/MS conditions for analysis of 55 drugs in postmortem samples

Group	Analyte	Q1 Mass (Da)	Q3 Mass (Da)	Retention Time (min)	MRM Window (sec)	DP[1] (volts)	EP[2] (volts)	CE[3] (volts)	CXP[4] (volts)	Assigned ISTD
A	Buprenorphine	468.26	396.1	12.48	60	11	10	53	42	Fentanyl-D5
	(Qualifier)		414.2	12.48	60	11	10	47	36	
A	Fentanyl	337.15	188.1	11.81	45	66	10	31	20	Fentanyl-D5
	(Qualifier)		105	11.81	45	66	10	39	14	
A	Norbuprenorphine	414.216	152	9.5	60	140	10	119	16	Fentanyl-D5
	(Qualifier)		165	9.5	60	140	10	95	18	
A	Norfentanyl	233.144	84.1	6.75	60	61	10	23	10	Fentanyl-D5
	(Qualifier)		55.1	6.75	60	61	10	53	26	
B	6-monoacetylmorphine	328.097	165	5.37	60	100	10	49	18	6-monoacetylmorphine-D6
	(Qualifier)		211	5.37	60	100	10	35	20	
B	Amitriptyline	279.29	234.1	14.72	60	61	10	23	26	Amitriptyline-D3
	(Qualifier)		192.1	14.72	60	61	10	31	22	
B	Amphetamine	136.044	91	4.01	30	60	10	21	10	Amphetamine-D5
	(Qualifier)		119.1	4.01	30	60	10	11	14	
B	Benzoylecgonine	289.764	168.2	6.66	60	80	10	35	18	Benzoylecgonine-D8
	(Qualifier)		105.1	6.66	60	80	10	35	12	
B	Bupropion	240.052	184	9.06	60	60	10	29	22	Bupropion-D9
	(Qualifier)		166	9.06	60	60	10	37	18	
B	Chlorpheniramine	274.884	167.1	10.65	60	100	10	61	18	Dextromethorphan-D3
	(Qualifier)		230	10.65	60	100	10	51	26	
B	Citalopram	326.012	109.1	12.54	60	100	10	41	12	Citalopram-D6
	(Qualifier)		263	12.54	60	100	10	37	30	
B	Cocaethylene	317.9	82	10.3	60	60	10	63	10	Cocaethylene-D8
	(Qualifier)		196.1	10.3	60	60	10	47	22	
B	Cocaine	303.772	182.1	8.92	60	100	10	32	20	Cocaine-D3
	(Qualifier)		77.1	8.92	60	100	10	84	34	
B	Codeine	300.259	152	4.57	60	80	10	83	14	Codeine-D6
	(Qualifier)		165.1	4.57	60	80	10	51	16	
B	Dextromethorphan	273.052	216.2	11.18	60	36	10	33	24	Dextromethorphan-D3
	(Qualifier)		214.1	11.18	60	36	10	37	22	
B	Diltiazem	415.091	109	13.02	60	60	10	119	12	6-monoacetylmorphine-D6
	(Qualifier)		178	13.02	60	60	10	71	18	
B	Diphenhydramine	255.897	167	11.83	60	90	10	20	18	Venlafaxine-D6
	(Qualifier)		165.1	11.83	60	90	10	53	16	
B	Doxepin	279.9	107	12.67	60	60	10	34	12	Doxepin-D3
	(Qualifier)		77	12.67	60	60	10	78	8	
B	Doxylamine	272.014	168	9	120	60	10	45	18	Doxylamine-D5
	(Qualifier)		182.7	9	120	60	10	21	20	
B	EDDP	277.789	234	13.36	60	60	10	61	26	Doxylamine-D5
	(Qualifier)		249	13.36	60	60	10	45	28	
B	Ephedrine	166.09	148	3.45	60	60	10	30	16	6-monoacetylmorphine-D6
	(Qualifier)		133	3.45	60	60	10	37	14	
B	Fluoxetine	309.888	44.1	15.21	60	60	10	51	20	Fluoxetine-D6
	(Qualifier)		148.1	15.21	60	60	10	11	16	
B	Hydrocodone	300.077	199	5.86	60	60	10	39	22	Hydrocodone-D6
	(Qualifier)		171	5.86	60	60	10	51	18	
B	Hydromorphone	285.8	185	3.12	60	60	10	39	20	Hydromorphone-D3
	(Qualifier)		157.1	3.12	60	60	10	57	16	
B	Hydroxyzine	375.269	201.1	14.64	60	100	10	33	10	Hydroxyzine-D8
	(Qualifier)		166.1	14.64	60	100	10	61	18	
B	Ketamine	237.777	125.1	6.77	60	90	10	33	14	Ketamine-D4
	(Qualifier)		220.1	6.77	60	90	10	21	24	
B	Lidocaine	235.142	86	6.08	60	60	10	63	10	Hydrocodone-D6
	(Qualifier)		58.1	6.08	60	60	10	51	26	
B	MDA	180.079	163.1	4.62	60	60	10	21	18	MDA-D5
	(Qualifier)		133	4.62	60	60	10	25	14	
B	MDMA	194.098	163.1	5.35	60	60	10	32	18	MDMA-D5
	(Qualifier)		105	5.35	60	60	10	68	12	
B	Methadone	310.127	265	14.8	60	60	10	36	14	Methadone-D9
	(Qualifier)		105	14.8	60	60	10	73	12	
B	Methamphetamine	150.058	91.1	4.82	60	45	10	63	10	Methamphetamine-D5
	(Qualifier)		119.1	4.82	60	45	10	23	12	
B	Methylphenidate	234.117	84	7.93	60	60	10	65	10	Methylphenidate-D9

Group	Analyte	Q1 Mass (Da)	Q3 Mass (Da)	Retention Time (min)	MRM Window (sec)	DP[1] (volts)	EP[2] (volts)	CE[3] (volts)	CXP[4] (volts)	Assigned ISTD
	(Qualifier)		56.1	7.93	60	60	10	87	26	
B	Mirtazapine	266.1	194.1	9.15	60	60	10	85	16	Mirtazapine-D3
	(Qualifier)		195	9.15	60	60	10	71	20	
B	Morphine	286.217	165.1	2.3	60	80	10	53	16	Morphine-D6
	(Qualifier)		153	2.3	60	80	10	53	16	
B	Norfluoxetine	296.074	134.1	14.74	60	60	10	9	12	Norfluoxetine-D6
	(Qualifier)		30.1	14.74	60	60	10	41	14	
B	Norketamine	223.765	207.1	6.26	60	90	10	17	24	Norketamine-D4
	(Qualifier)		125	6.26	60	90	10	31	14	
B	Norsertraline	274.952	158.9	15.13	45	60	10	23	18	Paroxetine-D6
	(Qualifier)		129	15.13	45	60	10	19	14	
B	Nortriptyline	265.403	234.1	14.26	60	100	10	19	26	Nortriptyline-D3
	(Qualifier)		192.1	14.26	60	100	10	29	22	
B	Olanzapine	313.102	256.1	7.5	180	60	10	36	28	Olanzapine-D8
	(Qualifier)		198	7.5	180	60	10	56	22	
B	Oxycodone	316.218	298	5.3	60	60	10	40	36	Oxycodone-D6
	(Qualifier)		241	5.3	60	60	10	52	26	
B	Oxymorphone	302.071	227.1	2.5	60	60	10	39	24	Oxymorphone-D3
	(Qualifier)		284	2.5	60	60	10	27	30	
B	Paroxetine	331.007	193.1	13.88	60	40	10	29	22	Paroxetine-D6
	(Qualifier)		192.1	13.88	60	40	10	29	22	
B	Phencyclidine	244.143	86	10.82	60	60	10	20	10	Phencyclidine-D5
	(Qualifier)		159	10.82	60	60	10	24	18	
B	Promethazine	285.097	86.2	13.19	60	50	10	59	10	Promethazine-D3
	(Qualifier)		198	13.19	60	50	10	67	22	
B	Pseudoephedrine	166.095	148.1	3.45	60	50	10	32	16	6-monoacetylmorphine-D6
	(Qualifier)		133	3.45	60	50	10	42	14	
B	Quetiapine	384.118	221.1	12.35	60	60	10	89	24	Quetiapine-D8
	(Qualifier)		253	12.35	60	60	10	66	28	
B	Sertraline	305.965	275	15.65	60	60	10	17	24	Fluoxetine-D6
	(Qualifier)		158.9	15.65	60	60	10	33	18	
B	Tramadol	264.131	58	7.8	60	60	10	89	26	Venlafaxine-D6
	(Qualifier)		42.1	7.8	60	60	10	115	18	
B	Trazodone	374.024	176.1	10.96	60	100	10	38	20	Trazadone-D6
	(Qualifier)		148.1	10.96	60	100	10	48	16	
B	Venlafaxine	277.862	58.1	9.41	60	100	10	56	26	Venlafaxine-D6
	(Qualifier)		260.3	9.41	60	100	10	22	14	
B	Zolpidem	308.083	235.1	9.66	60	60	10	82	26	Zolpidem-D7
	(Qualifier)		263	9.66	60	60	10	55	30	
C	Carisoprodol	261.111	158.1	13.7	60	16	10	30	18	Carisoprodol-D7
	(Qualifier)		176.1	13.7	60	16	10	28	20	
C	Gabapentin	172.115	95	2.84	60	40	10	46	10	Oxymorphone-D3
	(Qualifier)		137	2.84	60	40	10	36	16	
C	Meprobamate	219.13	158.1	8.87	60	40	10	23	10	Doxylamine-D5
	(Qualifier)		97.1	8.87	60	40	10	31	10	
D	Acetaminophen	152.059	110	2.69	60	56	10	39	18	Acetaminophen-D4
	(Qualifier)		93.1	2.69	60	56	10	46	8	
ISTD	6-monoacetylmorphine-D6	334.146	165	5.35	60	100	10	49	18	
ISTD	Acetaminophen-D4	157.034	115	2.66	60	71	10	23	18	
ISTD	Amitriptyline-D3	281.513	104.9	14.74	60	60	10	29	12	
ISTD	Amphetamine-D5	141.109	124	4.02	60	60	10	13	14	
ISTD	Benzoylecgonine-D8	298.136	171	6.58	60	86	10	37	18	
ISTD	Bupropion-D9	249.132	185.1	9.03	60	60	10	27	10	
ISTD	Carisoprodol-D7	268.109	183.1	13.6	60	26	10	23	10	
ISTD	Citalopram-D6	330.786	262.1	12.55	60	100	10	27	30	
ISTD	Cocaethylene-D8	326.148	204.1	10.28	60	60	10	47	20	
ISTD	Cocaine-D3	307.109	185.1	8.94	60	60	10	45	20	
ISTD	Codeine-D6	306.132	218.1	4.52	60	100	10	35	24	
ISTD	Dextromethorphan-D3	274.993	215.1	11.2	60	80	10	38	22	
ISTD	Doxepin-D3	282.981	107	12.69	60	60	10	31	12	
ISTD	Doxylamine-D5	276.136	187.1	8.95	120	60	10	41	20	
ISTD	Fentanyl-D5	342.208	188.1	11.78	60	86	10	31	20	
ISTD	Fluoxetine-D6	316.057	44.1	15.19	60	60	10	47	4	
ISTD	Hydrocodone-D6	306.144	202.1	5.82	60	60	10	41	22	
ISTD	Hydromorphone-D3	289.05	185.1	3.12	60	60	10	41	18	
ISTD	Hydroxyzine-D8	383.153	201	14.61	60	60	10	50	22	
ISTD	Ketamine-D4	242.053	129	6.75	60	60	10	35	14	
ISTD	MDA-D5	185.105	168.1	4.6	60	60	10	13	20	

Group	Analyte	Q1 Mass (Da)	Q3 Mass (Da)	Retention Time (min)	MRM Window (sec)	DP[1] (volts)	EP[2] (volts)	CE[3] (volts)	CXP[4] (volts)	Assigned ISTD
ISTD	MDMA-D5	199.2	165.1	5.35	60	60	10	15	4	
ISTD	Methadone-D9	319.217	268.2	14.78	60	60	10	36	14	
ISTD	Methamphetamine-D5	155.139	121	4.81	60	60	10	15	14	
ISTD	Methylphenidate-D9	243.167	92.7	7.89	60	60	10	27	10	
ISTD	Mirtazapine-D3	268.86	195.1	9.2	60	100	10	50	22	
ISTD	Morphine-D6	292.119	152.1	2.23	60	80	10	77	18	
ISTD	Norfluoxetine-D6	302.073	140.1	14.71	60	60	10	9	16	
ISTD	Norketamine-D4	228.041	211.1	6.23	60	90	10	17	24	
ISTD	Nortriptyline-D3	267.147	233.1	14.28	60	60	10	21	12	
ISTD	Olanzapine-D8	321.074	260.7	7.35	180	60	10	33	30	
ISTD	Oxycodone-D6	322.1	304.2	5.25	60	60	10	37	34	
ISTD	Oxymorphone-D3	305.12	287.1	2.45	60	41	10	27	32	
ISTD	Paroxetine-D6	336.093	198.1	13.88	60	40	10	29	22	
ISTD	Phencyclidine-D5	249.194	86	10.79	60	60	10	17	10	
ISTD	Promethazine-D3	288.08	89.1	13.21	60	50	10	46	10	
ISTD	Quetiapine-D8	392.15	258	12.31	60	60	10	53	28	
ISTD	Trazadone-D6	379.093	182.1	10.95	60	60	10	33	20	
ISTD	Venlafaxine-D6	283.879	266.3	9.42	60	100	10	17	14	
ISTD	Zolpidem-D7	315.17	242.1	9.62	60	60	10	72	22	

[1]Declustering potential, [2]Entrance potential, [3]Collision energy, [4]Collision cell exit potential.

Table 2: Blood Calibration and Quality Control Levels for Each Analyte Group (ng/mL)

Group	Calibrator 1*	Calibrator 2	QC Low	Calibrator 3	Calibrator 4	QC Med	Calibrator 5	QC High	Calibrator 6	Calibrator 7	QC UltraHigh	Calibrator 8 (Calibrant or Control Stock)
A	1	2	3.75	7.5	11.25	18.75	37.5	56.25	75	100	120	150
B	10	20	37.5	75	112.5	187.5	375	562.5	750	1,000	1,200	1,500
C	100	200	375	750	1,125	1,875	3,750	5,625	7,500	10,000	12,000	15,000
D	200	400	750	1,500	2,250	3,750	7500	11,250	15,000	20,000	24,000	30,000

*Blood Calibrator 1 additionally used as Urine LOD.

Table 3: Accuracy (Bias) and Precision (Within-run and Between-run CV) of Blood QC Low, Med, High and UltraHigh, and Dilutions

Compounds		Low	Med	High	Ultra High	Dil 1/2	Dil 1/4	Dil 1/9	Dil 1/18
6-Monoacetylmorphine	Within-run CV	2.4%	3.2%	3.5%	3.1%	5.5%	3.2%	6.6%	3.3%
(6-MAM)	Between-run CV	4.8%	7.3%	4.0%	5.0%	11.7%	12.2%	11.4%	7.8%
	Bias	-2.9%	0.0%	-2.2%	1.1%	0.0%	-11.2%	-6.0%	-9.2%
Acetaminophen	Within-run CV	1.3%	3.8%	3.8%	3.6%	3.8%	1.2%	5.0%	4.3%
	Between-run CV	6.0%	8.6%	4.4%	5.7%	12.7%	14.9%	12.3%	9.6%
	Bias	-1.4%	-1.4%	-0.5%	-2.3%	-4.9%	-16.2%	-12.5%	-16.1%
Amitriptyline	Within-run CV	3.2%	2.3%	3.6%	3.9%	3.7%	3.0%	6.7%	4.1%
	Between-run CV	5.1%	6.0%	5.1%	6.9%	10.8%	15.4%	11.2%	10.3%
	Bias	-0.7%	3.3%	7.9%	0.5%	-4.9%	-14.3%	-7.6%	-14.5%
Amphetamine	Within-run CV	1.6%	2.0%	3.5%	3.2%	3.0%	1.0%	5.0%	3.2%
	Between-run CV	4.6%	6.8%	4.2%	5.4%	8.9%	11.4%	10.6%	7.4%
	Bias	-4.5%	-2.8%	-1.9%	12.0%	9.0%	-2.1%	3.6%	-1.0%
Benzoylecgonine	Within-run CV	2.6%	3.2%	3.0%	4.7%	3.5%	1.7%	5.0%	5.4%
	Between-run CV	6.0%	7.0%	5.3%	7.0%	13.4%	13.3%	12.3%	12.3%
	Bias	-2.4%	2.0%	2.4%	-0.5%	-2.0%	-13.8%	-9.9%	-14.3%
Buprenorphine	Within-run CV	4.8%	8.3%	3.7%	3.6%	4.5%	3.8%	6.4%	6.0%
	Between-run CV	6.7%	15.8%	10.2%	8.1%	13.6%	17.9%	14.0%	11.9%
	Bias	-6.8%	1.7%	1.2%	-4.5%	-16.2%	**-23.1%**	-17.6%	**-21.9%**
Bupropion	Within-run CV	3.7%	1.6%	5.0%	4.2%	4.9%	2.2%	4.3%	3.6%
	Between-run CV	5.1%	7.9%	4.5%	6.1%	11.9%	12.5%	10.1%	8.8%
	Bias	-3.2%	-0.6%	2.8%	13.7%	6.3%	-5.7%	-0.9%	-8.6%
Carisoprodol	Within-run CV	3.9%	4.6%	4.2%	5.3%	4.8%	2.4%	5.1%	5.2%
	Between-run CV	7.7%	10.6%	6.6%	7.9%	10.6%	12.3%	11.6%	9.9%
	Bias	-14.0%	-11.5%	-11.1%	11.1%	5.2%	-7.4%	-2.0%	-6.6%
Chlorpheniramine	Within-run CV	1.5%	3.2%	3.9%	3.9%	4.1%	2.2%	5.3%	3.7%
	Between-run CV	6.0%	7.8%	5.1%	5.2%	9.7%	13.8%	11.2%	9.7%
	Bias	-2.4%	-1.6%	2.7%	15.1%	12.3%	1.5%	7.7%	4.5%
Citalopram	Within-run CV	1.1%	2.6%	3.4%	4.3%	3.5%	1.9%	5.8%	4.9%
	Between-run CV	5.5%	7.6%	5.1%	6.0%	16.1%	14.9%	13.9%	13.1%
	Bias	-5.0%	-3.3%	-2.1%	11.7%	8.2%	-3.7%	3.4%	-2.9%
Cocaethylene	Within-run CV	1.6%	5.4%	2.6%	3.7%	3.9%	1.1%	4.2%	4.6%
	Between-run CV	5.5%	9.1%	6.3%	5.2%	9.7%	12.5%	10.0%	9.0%
	Bias	4.5%	9.0%	7.0%	3.1%	0.9%	-11.9%	-8.5%	-13.2%
Cocaine	Within-run CV	2.4%	2.6%	4.1%	4.3%	3.2%	2.1%	5.4%	4.9%
	Between-run CV	6.0%	8.0%	4.9%	6.4%	16.8%	18.6%	14.4%	9.6%
	Bias	-10.2%	-9.5%	-5.3%	3.3%	0.5%	-12.3%	-8.8%	-13.2%
Codeine	Within-run CV	2.3%	3.5%	3.3%	3.3%	3.5%	1.5%	4.4%	3.7%
	Between-run CV	4.8%	8.2%	4.6%	6.5%	11.8%	12.7%	11.4%	9.6%
	Bias	-5.1%	-1.8%	-3.1%	0.6%	-2.2%	-13.2%	-8.0%	-11.2%
Dextromethorphan	Within-run CV	1.2%	2.0%	3.4%	3.3%	3.5%	1.3%	5.0%	4.3%
	Between-run CV	4.5%	6.7%	3.2%	4.8%	9.2%	13.5%	10.8%	8.5%
	Bias	-0.4%	0.6%	3.4%	14.9%	10.3%	-1.1%	6.4%	1.8%
Diltiazem	Within-run CV	3.1%	4.3%	4.4%	3.5%	3.4%	3.3%	6.3%	3.2%
	Between-run CV	6.4%	9.0%	7.9%	4.5%	9.6%	13.1%	11.7%	7.0%
	Bias	-4.0%	-0.9%	3.2%	2.2%	-0.8%	-11.5%	-6.9%	-11.0%
Diphenhydramine	Within-run CV	3.2%	2.7%	5.1%	2.8%	5.4%	6.8%	6.3%	7.1%
	Between-run CV	5.6%	8.0%	4.8%	12.4%	13.4%	19.2%	12.5%	15.9%
	Bias	-13.6%	-5.7%	-1.4%	6.2%	5.5%	2.1%	3.9%	2.0%
Doxepin	Within-run CV	2.1%	1.3%	3.4%	2.7%	3.7%	2.3%	5.8%	4.4%
	Between-run CV	5.6%	6.1%	3.2%	5.2%	10.1%	14.4%	12.4%	11.0%
	Bias	-4.7%	-2.3%	1.5%	15.6%	12.3%	1.1%	9.0%	2.3%
Doxylamine	Within-run CV	2.9%	1.9%	2.6%	3.3%	4.0%	1.7%	4.3%	4.8%
	Between-run CV	6.1%	6.3%	3.5%	5.7%	9.4%	12.1%	10.7%	8.7%
	Bias	-5.6%	-4.9%	-0.9%	16.6%	13.4%	1.0%	7.9%	3.4%
EDDP	Within-run CV	3.0%	3.3%	3.9%	5.9%	4.1%	2.7%	4.7%	5.7%
	Between-run CV	5.9%	7.8%	3.6%	8.8%	**30.7%**	**30.6%**	**25.2%**	15.0%
	Bias	-1.0%	-0.8%	3.1%	0.9%	-3.5%	-14.2%	-11.5%	-16.2%
Ephedrine	Within-run CV	3.9%	2.9%	3.9%	3.7%	4.6%	5.7%	5.6%	3.2%
	Between-run CV	4.8%	5.9%	3.6%	6.9%	12.3%	13.9%	12.4%	11.4%
	Bias	-2.5%	1.4%	4.8%	5.2%	5.7%	-4.9%	-2.9%	-6.2%
Fentanyl	Within-run CV	2.1%	2.8%	3.3%	3.3%	3.6%	1.7%	4.3%	4.1%
	Between-run CV	4.6%	7.6%	2.9%	5.6%	10.7%	12.9%	11.1%	8.6%
	Bias	-10.5%	-6.2%	-3.5%	-3.7%	-7.7%	-17.9%	-13.2%	-17.2%
Fluoxetine	Within-run CV	1.7%	3.4%	3.7%	3.5%	3.9%	3.8%	7.7%	5.2%
	Between-run CV	4.4%	7.0%	3.6%	4.9%	7.5%	13.4%	10.4%	10.3%
	Bias	-11.7%	-10.0%	-9.6%	13.3%	8.2%	-1.5%	8.8%	2.4%
Gabapentin	Within-run CV	4.3%	4.5%	4.7%	4.3%	4.6%	1.7%	5.2%	4.7%
	Between-run CV	7.4%	9.2%	8.7%	6.0%	13.0%	17.5%	15.3%	12.6%
	Bias	0.5%	1.0%	11.8%	7.9%	4.1%	-4.2%	-0.4%	-5.3%
Hydrocodone	Within-run CV	2.8%	2.7%	3.7%	4.2%	4.1%	1.9%	5.0%	3.9%
	Between-run CV	6.3%	8.1%	6.1%	6.2%	11.9%	14.7%	12.4%	9.3%
	Bias	4.7%	6.4%	11.6%	17.4%	14.8%	3.5%	10.5%	6.7%
Hydromorphone	Within-run CV	1.9%	1.9%	2.5%	3.6%	3.7%	2.1%	4.1%	3.7%

Compounds		Low	Med	High	Ultra High	Dil 1/2	Dil 1/4	Dil 1/9	Dil 1/18
	Between-run CV	6.9%	5.7%	3.6%	4.5%	10.3%	13.4%	11.8%	9.7%
	Bias	-1.9%	-0.6%	3.9%	15.3%	14.3%	1.6%	8.0%	4.7%
Hydroxyzine	Within-run CV	3.4%	5.9%	3.6%					
	Between-run CV	5.9%	10.5%	5.5%					
	Bias	-16.5%	-10.8%	-14.4%					
Ketamine	Within-run CV	2.2%	2.0%	2.5%	3.5%	3.6%	1.6%	4.7%	4.5%
	Between-run CV	5.4%	7.7%	3.4%	5.2%	10.3%	12.1%	9.7%	8.6%
	Bias	1.1%	5.9%	6.8%	**24.3%**	**21.5%**	6.8%	12.2%	6.6%
Lidocaine	Within-run CV	2.8%	2.8%	4.0%	4.6%	4.9%	4.8%	5.5%	4.3%
	Between-run CV	6.0%	9.6%	7.5%	7.2%	11.5%	14.6%	12.6%	9.9%
	Bias	-2.2%	2.5%	4.5%	17.6%	13.0%	4.5%	9.2%	4.4%
3,4-Methylenedioxyamphetamine (MDA)	Within-run CV	1.8%	1.9%	4.1%	3.5%	2.9%	1.5%	4.7%	3.7%
	Between-run CV	5.4%	6.6%	4.3%	5.4%	11.1%	13.4%	11.6%	9.0%
	Bias	0.5%	0.6%	4.4%	13.6%	10.2%	-1.8%	4.6%	-0.1%
3,4-Methylenedioxymethamphetamine (MDMA)	Within-run CV	1.3%	1.6%	3.8%	3.6%	3.7%	0.9%	5.2%	4.1%
	Between-run CV	4.9%	6.2%	3.8%	5.4%	13.8%	14.9%	13.3%	10.9%
	Bias	-7.6%	-6.5%	-4.8%	18.2%	13.6%	1.8%	8.2%	3.9%
Meprobamate	Within-run CV	3.8%	2.5%	4.2%	6.3%	9.3%	7.0%	5.8%	7.5%
	Between-run CV	11.0%	9.0%	5.8%	12.6%	17.9%	16.6%	11.8%	10.0%
	Bias	-2.2%	-1.4%	1.5%	**25.4%**	19.1%	1.0%	8.3%	3.8%
Methadone	Within-run CV	2.3%	2.5%	3.8%	3.6%	3.8%	2.4%	4.5%	4.9%
	Between-run CV	5.7%	7.6%	3.6%	5.1%	11.1%	14.6%	12.2%	9.3%
	Bias	0.2%	3.9%	6.3%	12.8%	10.1%	-1.3%	5.1%	-0.3%
Methamphetamine	Within-run CV	2.6%	3.6%	3.9%	3.5%	2.9%	3.1%	7.0%	3.2%
	Between-run CV	4.3%	6.8%	3.9%	6.8%	11.1%	15.2%	14.9%	11.1%
	Bias	1.8%	4.1%	7.2%	17.4%	13.5%	4.7%	10.2%	4.3%
Methylphenidate	Within-run CV	2.9%	2.9%	4.8%	3.7%	3.7%	1.6%	5.8%	5.1%
	Between-run CV	3.9%	9.0%	6.1%	5.9%	9.2%	11.5%	10.9%	9.8%
	Bias	-2.7%	-2.0%	-5.1%	15.6%	13.1%	-0.5%	3.9%	-1.0%
Mirtazapine	Within-run CV	3.0%	4.1%	3.0%	3.1%	3.5%	1.3%	5.0%	4.1%
	Between-run CV	4.2%	7.7%	3.5%	6.1%	9.7%	13.2%	11.2%	8.8%
	Bias	-8.4%	-7.5%	-5.3%	**38.4%**	**33.4%**	18.6%	**28.1%**	**22.3%**
Morphine	Within-run CV	1.4%	1.8%	3.3%	3.7%	3.7%	1.3%	4.9%	4.4%
	Between-run CV	5.9%	6.6%	4.4%	5.3%	10.4%	13.7%	12.3%	9.4%
	Bias	1.5%	2.9%	5.7%	12.1%	9.4%	-3.8%	2.1%	-1.4%
Norbuprenorphine	Within-run CV	5.6%	2.7%	4.9%	4.2%	4.0%	2.6%	5.8%	6.5%
	Between-run CV	13.7%	11.5%	9.4%	4.4%	13.5%	14.6%	12.8%	10.4%
	Bias	13.5%	13.6%	19.5%	13.3%	11.4%	-1.7%	4.3%	-2.1%
Norfentanyl	Within-run CV	2.3%	4.6%	4.7%	3.6%	5.0%	2.3%	5.2%	4.2%
	Between-run CV	7.6%	8.4%	4.1%	6.3%	13.1%	13.9%	10.7%	7.9%
	Bias	-6.0%	-7.9%	-2.7%	-7.9%	-8.9%	-18.8%	-15.4%	-16.5%
Norfluoxetine	Within-run CV	3.7%	6.6%	6.2%	5.9%	6.8%	7.0%	8.8%	10.8%
	Between-run CV	5.8%	7.0%	5.4%	8.7%	13.0%	18.0%	16.9%	11.5%
	Bias	9.3%	1.8%	7.4%	2.0%	-5.1%	-10.8%	-2.5%	-4.4%
Norketamine	Within-run CV	2.2%	3.5%	3.2%	4.2%	3.9%	1.2%	4.6%	4.7%
	Between-run CV	5.5%	6.7%	3.2%	6.1%	8.7%	11.0%	9.6%	9.3%
	Bias	3.6%	6.9%	8.5%	13.7%	11.5%	-2.1%	2.3%	-2.0%
Norsertraline	Within-run CV	11.6%	13.4%	12.8%	12.3%	15.6%	16.6%	**21.8%**	8.3%
	Between-run CV	18.8%	13.1%	13.3%	16.6%	18.1%	**27.8%**	**20.2%**	15.2%
	Bias	-11.8%	-9.7%	-5.1%	12.9%	1.2%	**21.1%**	**30.5%**	**31.6%**
Nortriptyline	Within-run CV	2.4%	3.0%	3.5%	3.5%	4.2%	3.6%	6.7%	5.4%
	Between-run CV	5.1%	7.3%	3.0%	5.9%	10.2%	14.0%	11.0%	11.0%
	Bias	-2.1%	-0.4%	1.5%	11.9%	6.6%	-3.8%	5.2%	-2.0%
Olanzapine	Within-run CV	4.8%	4.2%	7.2%	3.6%	6.4%	3.2%	5.9%	5.7%
	Between-run CV	5.4%	10.2%	7.7%	6.4%	**23.7%**	**20.4%**	15.4%	13.3%
	Bias	-2.7%	-8.8%	-9.1%	1.4%	3.1%	-9.6%	-5.3%	-8.5%
Oxycodone	Within-run CV	5.2%	5.4%	6.1%	3.2%	5.9%	7.8%	7.2%	8.8%
	Between-run CV	7.3%	8.4%	5.1%	4.7%	11.6%	15.3%	17.8%	12.1%
	Bias	-9.9%	-6.8%	-5.0%	9.6%	8.2%	-1.2%	3.2%	-2.4%
Oxymorphone	Within-run CV	3.4%	3.5%	2.7%	3.6%	3.8%	2.0%	4.7%	5.0%
	Between-run CV	6.6%	9.1%	4.6%	4.6%	12.3%	15.1%	14.7%	10.2%
	Bias	1.9%	4.5%	6.9%	**-20.1%**	**-21.7%**	**-30.9%**	**-27.7%**	**-31.9%**
Paroxetine	Within-run CV	2.3%	2.2%	3.2%	4.6%	4.1%	4.1%	6.5%	4.7%
	Between-run CV	5.1%	6.8%	4.4%	7.0%	9.2%	15.6%	10.7%	11.5%
	Bias	-1.2%	-1.3%	0.3%	13.2%	6.0%	-3.3%	5.9%	-0.5%
Phencyclidine (PCP)	Within-run CV	1.2%	2.2%	3.5%	4.2%	4.0%	1.3%	3.8%	4.7%
	Between-run CV	4.8%	6.9%	3.8%	6.8%	11.6%	14.6%	12.7%	10.4%
	Bias	-3.3%	-0.8%	2.2%	12.7%	8.3%	-4.2%	1.0%	-5.2%
Promethazine	Within-run CV	1.9%	1.9%	3.7%	4.1%	3.7%	3.3%	6.6%	4.7%
	Between-run CV	6.9%	7.6%	3.9%	6.8%	10.3%	14.9%	11.0%	10.0%
	Bias	-2.9%	-0.9%	2.1%	14.9%	9.8%	-1.3%	6.6%	-2.2%
Pseudoephedrine	Within-run CV	4.5%	2.9%	4.1%	4.5%	4.0%	5.9%	6.0%	2.8%
	Between-run CV	4.8%	5.9%	3.6%	8.1%	12.0%	14.2%	13.3%	11.5%
	Bias	-7.1%	-3.4%	0.5%	9.9%	11.6%	1.5%	4.8%	1.5%
Quetiapine	Within-run CV	2.2%	2.1%	3.6%	3.9%	4.4%	3.0%	5.7%	4.3%

Compounds		Low	Med	High	Ultra High	Dil 1/2	Dil 1/4	Dil 1/9	Dil 1/18
	Between-run CV	5.3%	7.4%	4.9%	5.9%	11.3%	14.7%	11.9%	10.7%
	Bias	-3.3%	-1.2%	1.3%	15.7%	11.3%	-0.1%	6.6%	0.9%
Sertraline	Within-run CV	10.9%	10.9%	12.4%	7.3%	11.6%	12.9%	17.6%	10.1%
	Between-run CV	14.5%	11.1%	14.1%	13.5%	14.4%	**24.9%**	18.7%	13.7%
	Bias	-2.2%	1.4%	3.1%	9.1%	5.3%	11.6%	**26.8%**	**24.0%**
Tramadol	Within-run CV	3.1%	2.6%	4.3%	2.8%	5.0%	4.3%	6.7%	4.1%
	Between-run CV	5.6%	9.8%	4.3%	6.8%	10.9%	13.1%	12.2%	11.8%
	Bias	-4.1%	-1.5%	1.8%	18.7%	15.9%	8.0%	12.8%	9.6%
Trazodone	Within-run CV	1.9%	4.6%	3.2%	3.6%	5.1%	2.2%	4.8%	5.0%
	Between-run CV	5.6%	8.4%	3.2%	6.0%	12.0%	14.4%	12.5%	10.0%
	Bias	4.0%	7.7%	9.4%	17.0%	12.3%	0.7%	6.3%	1.1%
Venlafaxine	Within-run CV	3.2%	2.7%	3.8%	3.1%	5.5%	1.9%	4.3%	5.6%
	Between-run CV	5.6%	8.1%	3.9%	6.1%	10.2%	11.6%	11.6%	11.5%
	Bias	10.4%	13.1%	17.1%	**24.9%**	**21.7%**	9.4%	16.0%	11.6%
Zolpidem	Within-run CV	3.8%	1.7%	4.2%	3.6%	4.5%	2.3%	4.5%	4.6%
	Between-run CV	6.3%	7.8%	5.4%	4.9%	11.4%	14.2%	12.4%	10.0%
	Bias	-0.8%	3.5%	4.3%	**23.5%**	18.6%	4.6%	10.2%	7.8%

Bold denotes values greater than ± 20 %. Hydroxyzine was not assessed at the QC UltraHigh or Dilution levels.

Table 4: Summary of Consolidated Scope, Analytical Limits, and 12-Month Applicability Study

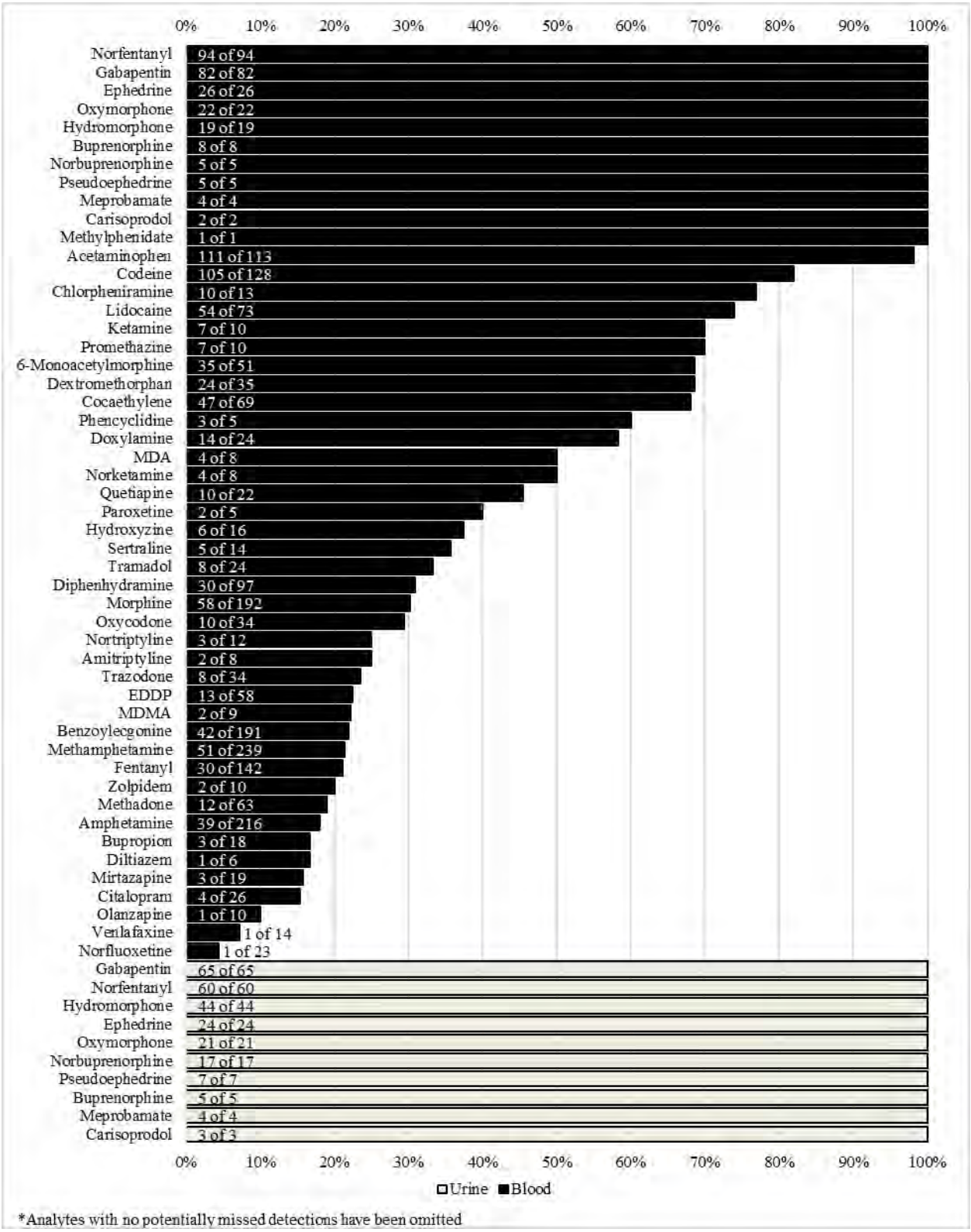
Analyte	Analytical Limits (ng/mL)					Blood			Urine		
	Historical LOD	Urine LOD	Blood LOD	Blood LLOQ	Blood ULOQ	Count	Potential Miss	Potential Miss (%)	Count	Potential Miss	Potential Miss (%)
6-Monoacetylmorphine	10	10	1	10	1,500	51	35	69%	57		-
Acetaminophen [a]	100,000	200	400	400	30,000	113	111	98%	134		-
Amitriptyline	25	10	2	10	1,500	8	2	25%	5		-
Amphetamine	10	10	2	10	1,500	216	39	18%	139		-
Benzoylecgonine	60	10	5	10	1,500	191	42	22%	176		-
Buprenorphine [b]	N/A	1	0.5	10	1,500	8	8	100%	5	5	100%
Bupropion	25	10	5	10	150	18	3	17%	16		-
Carisoprodol [b]	N/A	100	50	100	15,000	2	2	100%	3	3	100%
Chlorpheniramine	25	10	1	10	1,500	13	10	77%	7		-
Citalopram	10	10	1	10	1,500	26	4	15%	13		-
Cocaethylene	25	10	1	10	1,500	69	47	68%	52		-
Cocaine [c]	5	10	1	10	1,500	158		-	117		-
Codeine	25	10	1	10	1,500	128	105	82%	78		-
Dextromethorphan	25	10	1	10	1,500	35	24	69%	24		-
Diltiazem	25	10	1	10	1,500	6	1	17%	4		-
Diphenhydramine	25	10	2	10	1,500	97	30	31%	71		-
Doxepin	25	10	5	10	1,500	0		-	1		-
Doxylamine	25	10	5	10	1,500	24	14	58%	25		-
EDDP	10	10	2	10	1,500	58	13	22%	40		-
Ephedrine [b]	N/A	10	5	10	1,500	26	26	100%	24	24	100%
Fentanyl	1.25	1	0.1	1	150	142	30	21%	72		-
Fluoxetine	25	10	2	10	1,500	22		0%	11		-
Gabapentin [b]	N/A	100	50	100	15,000	82	82	100%	65	65	100%
Hydrocodone [c]	5	10	1	10	1,500	40		-	34		-
Hydromorphone [b]	N/A	10	2	10	1,500	19	19	100%	44	44	100%
Hydroxyzine	25	10	1	10	1,500	16	6	38%	10		-
Ketamine	25	10	1	10	1,500	10	7	70%	7		-
Lidocaine	25	10	1	10	1,500	73	54	74%	40		-
MDA	10	10	5	10	1,500	8	4	50%	11		-
MDMA	25	10	5	10	1,500	9	2	22%	8		-
Meprobamate [b]	N/A	100	20	100	15,000	4	4	100%	4	4	100%
Methadone	25	10	1	10	1,500	63	12	19%	41		-
Methamphetamine	25	10	2	10	1,500	239	51	21%	171		-
Methylphenidate	25	10	1	10	1,500	1	1	100%	0		-
Mirtazapine	25	10	5	10	1,500	19	3	16%	18		-
Morphine	25	10	1	10	1,500	192	58	30%	131		-
Norbuprenorphine [b]	N/A	1	2	2	150	5	5	100%	17	17	100%
Norfentanyl [b]	N/A	1	0.2	1	150	94	94	100%	60	60	100%
Norfluoxetine	25	10	20	20	1,500	23	1	4%	14		-
Norketamine	25	10	2	10	1,500	8	4	50%	7		-
Norsertraline [c]	5	10	20	20	1,500	14		0%	14		-
Nortriptyline	25	10	2	10	1,500	12	3	25%	10		-
Olanzapine	25	10	20	20	1,500	10	1	10%	8		-
Oxycodone	25	10	5	10	1,500	34	10	29%	35		-
Oxymorphone [b]	N/A	10	1	10	1,500	22	22	100%	21	21	100%
Paroxetine	25	10	5	10	1,500	5	2	40%	1		-
Phencyclidine	25	10	1	10	1,500	5	3	60%	1		-
Promethazine	25	10	2	10	1,500	10	7	70%	6		-
Pseudoephedrine [b]	N/A	10	5	10	1,500	5	5	100%	7	7	100%
Quetiapine	25	10	2	10	1,500	22	10	45%	11		-
Sertraline	25	10	1	10	1,500	14	5	36%	9		-
Tramadol	25	10	1	10	1,500	24	8	33%	16		-
Trazodone	25	10	5	10	1,500	34	8	24%	27		-
Venlafaxine	25	10	2	10	1,500	14	1	7%	14		-
Zolpidem	10	10	2	10	1,500	10	2	20%	4		-

[a] Estimated LOD as historical methodologies was a colorimetric spot test with not well-defined parameters.

[b] Not previously in scope of historical methodologies.

[c] Potentially missed blood detections could not be discerned due to qualitative reporting of values below LLOQ.

Figure 1: Authentic Casework Drug Detections of Described Method that would Potentially be Missed with Historical Scopes and Limits



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