Toxicological Drug Screening using Paper Spray High-Resolution Tandem Mass Spectrometry (HR-MS/MS)

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Abstract

Immunoassays and high-performance liquid chromatography (HPLC) coupled with mass spectrometry (MS) are both widely used methods for drug screening in toxicology. We investigated an alternative approach for rapid drug screening: paper spray MS (PS-MS). In paper spray, the biofluid sample is spotted onto a paper substrate. Upon application of a spray solvent and an electric potential, extraction and ionization occur directly from the paper without any need for additional sample preparation. We developed two paper spray high resolution MS/MS targeted drug screening assays using a quadrupole-orbitrap mass spectrometer, one the positive ion mode and one in the negative ion mode. In the positive ion mode, over 130 drugs and drug metabolites were semi-quantitatively screened at sub-toxic concentrations in a single 2.5 minute analysis. Limits of detection and calibration performances for each target compound are reported. The PS-MS/MS assay was tested on authentic postmortem specimens, and its screening ability and semi-quantitative performance were evaluated against independent LC-MS-MS screening and confirmation assays with good agreement. The paper spray MS/MS showed good qualitative agreement with LC-MS-MS; the true positive rate of paper spray MS/MS was 92%, and the true negative rate was over 98%. The quantitative results between the two methods were also acceptable for a screening application; Passing-Bablok regression yielded a slope of 1.17 and a Pearson's correlation coefficient of 0.996. A separate PS-MS/MS negative ion screening method was also developed for a small panel of barbiturates and structural analogs, demonstrating its potential for acidic drug detection and screening.

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Introduction

Forensic toxicology laboratories typically use a two-step process to detect toxicants in biological samples. The first step is screening, which employs a variety of analytical procedures to detect a broad range of targets. The best screening tests require little sample manipulation, cover a wide array of analytes, and are fast, inexpensive, sensitive, and selective. Immunoassays (IAs), gas chromatography (GC), GC-mass spectrometry (MS), and high performance liquid chromatography (HPLC) with spectroscopic or MS detection[1-4] are all commonly used methods for drug screening. Positive identifications during the initial drug screening step are confirmed and quantitated by an independent analytical procedure. LC-MS-MS is the most widely used method for drug confirmation and quantitation due to its excellent sensitivity and selectivity. According to the guidelines published by the Society of Forensic Toxicologists in conjunction with the American Academy of Forensic Science, a confirmatory test should be more specific than the screening test and based upon a different chemical principle[5].

Immunoassays are widely used for drug screening of toxicological samples on whole blood, plasma/serum, and urine specimens because they are inexpensive, simple, fast, and automatable. Several types of IAs are utilized in casework, including fluorescence polarization immunoassays (FPIAs), radioimmunoassays (RIAs), enzyme-multiplied immunoassay technique (EMIT), kinetic interaction of microparticles in solution (KIMS), and enzyme-linked immunosorbent assays (ELISAs)[6]. While IAs are a suitable screening tool in some scenarios, their limitations for drug screening are well-known, including inadequate sensitivity and selectivity, which can result in false negatives and unacceptably high false positive rates[7]. In addition, the number of drugs that can be screened by IA is often insufficient even in multiplexed systems. As a result, IA screening alone is not acceptable for some applications including postmortem toxicology[8].

GC-MS is widely used as a confirmatory method, and it has also been used in drug screening[9]. GC gives good chromatographic resolution, and when coupled to mass spectrometry the specificity is excellent. The reproducible fragmentation arising from electron ionization (EI) facilitates the use of database searching for peak identification. A significant limitation of GC-MS is that most drugs and drug metabolites cannot be analyzed with adequate sensitivity by GC methods unless the targets are first derivatized[10]. Analysis of blood and plasma samples therefore involves extensive sample cleanup and may require multiple derivatization reagents in order to obtain adequate detection limits and analyte coverage[9].

HPLC-MS has good sensitivity and specificity, especially when high-resolution or tandem mass spectrometry is used. It is also able to screen for a broad spectrum of analytes and is not limited to volatile or thermally stable compounds like GC-MS. Because of this, HPLC-MS is rapidly gaining prominence in toxicology laboratories for confirmatory testing. As a screening method, however, HPLC-MS is still not ideal. While it is an improvement on GC-MS, HPLC-MS still requires sample preparation. HPLC systems also have a reputation for being less robust than GC systems. Leaks, fluctuating back-pressure, shifting retention times, column deterioration, dead volumes, and carry-over are all technical issues that have persisted despite HPLC being a fairly mature technology. The instrument costs, the expertise required to maintain and operate the system, and the required sample preparation/handling all point to a need for an alternative approach for mass spectrometry based drug screening.

To simplify MS analyses, ambient or direction ionization methods have been developed. Beginning with DESI (desorption electrospray ionization)[11] and DART (direct analysis in real time)[12], these methods seek to analyze complex samples directly without sample preparation. This

research area has been very active, and numerous techniques and applications have been reported in the literature. Paper spray mass spectrometry, first described in 2010, is one such method for performing rapid, direct analyses of complex samples spotted on paper[13-14]. Paper spray MS/MS analysis of drugs at low or sub-ng/mL levels with quantitative accuracy and precision has been reported in numerous studies[13, 15-35]. In addition to quantitative analysis, some drug screening work has also been reported[36-37]. PS-MS has been widely investigated since its inception. Aside from analysis of drugs in biofluids, other applications have included food and agrichemical analysis[38-42], forensic applications[43-46], analysis of acylcarnitines[47-48], chemical warfare agents[49], natural products[50-52], paper-based immunoassays[53-54], and identification of bacteria[55-56]. Alternative spray substrates[15, 57-61] and cartridges with built-in sample preparation[18, 21] have also been investigated.

Paper spray is performed by first spotting blood or other biofluid specimens are onto paper and allowing them to dry before analysis, although analysis of freshly deposited blood samples has also been demonstrated[30]. A small volume of solvent (20-100 µL depending on the size of the paper substrate) is applied to the paper where it wicks through the substrate and sample by capillary action. The paper is cut to a sharp tip, which is positioned a few millimeters away from the atmospheric pressure inlet of a mass spectrometer. A high voltage (3-5 kV) is then applied directly to the paper, inducing an electrospray at the paper tip. The solvent evaporates from the charged droplets generated by the electrospray process, leaving gas-phase ions of the analyte molecules which can then be detected by a mass spectrometer[13]. Chemicals which are both soluble in the extraction/spray solvent and ionizable – typically by protonation or cationization with sodium or ammonium – will be detected immediately by the mass spectrometer. The entire analysis takes about 60 seconds and does not require any offline sample preparation.

Because of its speed, simplicity, and low sample consumption, paper spray shows good promise for drug screening applications. Here, we report on the development of a semi-quantitative paper spray high-resolution MS/MS screening method for 137 drug and drug metabolites that are commonly encountered in routine toxicology casework. The entire drug panel can be screened in a single 2.5 minute analysis, with cut-offs ranging from 1 ng/mL to over 1 µg/mL depending on the target. The screening and quantitative performance of the paper spray MS/MS assay was compared against independent toxicology laboratory results for 30 postmortem blood specimens. Furthermore, a separate negative ion method was also developed to screen for acidic compounds such as barbiturates.

Materials and Methods

Chemical Materials. Glacial acetic acid, Optima-grade ammonium hydroxide (NH_4OH), and HPLC-grade methanol, acetonitrile, isopropanol, and acetone were purchased from Fisher Scientific (Pittsburgh, PA, USA). Anhydrous carbon tetrachloride (CCl_4) was purchased from Sigma Aldrich (St. Louis, MO, USA). Single donor human whole blood for calibrator preparation was obtained from Innovative Research (Novi, MI, USA) in K_2EDTA tubes. Postmortem (PM) blood samples were obtained from banked material at Axis Forensic Toxicology (Indianapolis, IN, USA). Most of the 144 drugs and 12 stable isotope labeled internal standards (SIL ISTDs) used in the drug screens were purchased as analytical standards from Cerilliant (Round Rock, TX, USA) at concentrations of either 1.0 mg/mL or 100 µg/mL in methanol. Secobarbital and thiopental were purchased from Sigma Aldrich as 1.0 mg/mL standards. Acetaminophen, amlodipine, aripiprazole, benztropine, bupivacaine, carbamazepine, donepezil, etomidate, fluvoxamine, hydroxyzine, labetalol, metaxalone, methocarbamol, metoclopramide, papaverine, and ropinirole were

purchased as powders from Sigma Aldrich and dissolved in 95:5 methanol:water to create standard solutions.

Calibration Standards. Five-point calibration curves for each analyte were prepared in drug-free human blood. The 137 analytes in the positive ion screen were divided up into separate groups of about 15 to maintain low organic content in the blood samples. Structural isomers were places in separate groups prevent structural isomers from interfering in calibration standards. Calibration curves extended to 50 times higher than the cut-off concentration for most analytes (see Table 2 for cut-off concentrations).

The negative ion drug screen calibration standards were also prepared in blood, dividing up the analytes into two groups to maintain low organic content and separate the structural isomers amobarbital and pentobarbital. The concentrations of the analytes in the lowest calibrator were: 500 ng/mL for butabarbital, butalbital, amobarbital, pentobarbital, and secobarbital; 1000 ng/mL for phenobarbital and phenytoin; and 2000 ng/mL for thiopental. A five-point calibration series was used for each, spanning up to 15 times these cut-off concentrations.

Sample Preparation. All positive ion calibration standards and PM samples were mixed 1:3 (v:v) with an aqueous ISTD solution before spotting onto the paper spray cartridge. The relatively large volume of ISTD solution was used to dilute the blood because acetonitrile-based spray solvents did not penetrate dried undiluted blood quickly enough. The concentrations of each compound in the ISTD solution were: 65 ng/mL of alprazolam-d5 (A); 650 ng/mL of benzoylecgonine-d8 (B), cocaine-d3 (C), and methamphetamine-d11 (I); 260 ng/mL of flunitrazepam-d7 (D), hydrocodone-d3 (F), and trimipramine-d3 (J); 1300 ng/mL of gabapentin-d10 (E); 2600 ng/mL of metaxalone-d6 (G); 325 ng/mL of methadone-d3 (H); and 130 ng/mL of zolpidem-d6 (K). No analyte from any of the stable isotope labeled ISTDs was observed. For ease of reporting, the ISTDs—in alphabetical order—are given labels A-K. The internal standards were assigned to the analytes by hierarchical clustering analysis on logP and pKa, which were obtained from drugbank.ca. Alternative ISTDs were manually tested for targets in which poor calibration performance was obtained with the initial ISTD.

For the negative ion screen, 5 μL of an 8 μg/mL ISTD solution of phenobarbital-d5 was spiked into 100 μL of each sample. Phenobarbital-d5 was used as the internal standard for each target.

Paper Spray Ionization. Paper spray was performed using Velox sample cartridges on the automated Velox 360 source from Prosolia, Inc. (Indianapolis, IN, USA). 12 μL of the blood-ISTD mixture were spotted onto the paper contained within the cartridges and allowed to dry at room temperature for at least 90 minutes before analysis. The drying process can be accelerated if desired by heating [62] or blowing air over the cartridges. The extraction/spray solvent used was 85:10:5:0.01 acetonitrile:acetone:water:acetic acid for the larger positive ion screen. Acetonitrile was selected as the primary organic phase because it showed lower matrix effects for the PM samples, in agreement with earlier work[63]. However, acetonitrile-based solvents were problematic because they did not quickly permeate and fully wet dried blood spots (DBSs), which completely stymies the spraying process. Acetone was added as a co-solvent in conjunction with dilution using the ISTD solution to ensure faster solvent penetration of the DBS. In total, 136 μL of solvent were gradually applied to each cartridge by the automated paper spray source; 6 μL were deposited directly onto the DBS followed by 130 μL into the rear solvent well of the cartridge.

The method for negative ion mode detection of acidic compounds was adapted from recent work with organophosphonic acids[49]. The spray solvent was 90:10:0.01 methanol:CCl_4:NH_4OH. Carbon tetrachloride was included to decrease corona discharge. In total, 142 μL of this solvent

were added to the paper spray cartridge—12 μL first onto the DBS followed by 130 μL into the rear solvent well.

Mass Spectrometry. All data were acquired on a Q-Exactive Focus orbitrap mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA) with the S-lens set to 75 and capillary temperature set to 320°C for both positive and negative ion mode. The instrument method for the positive ion drug screen was 2.43 minutes long, operating in positive ion mode at +5.0 kV for the first 1.6 min before turning the voltage off to 0 kV for the next 0.83 min. The voltage was turned off for 0.83 min at the end of the run to generate zero-intensity scans for each drug, which were required for automatic integration in Thermo's TraceFinder. In the final 0.2 min, the instrument was switched to negative ionization at -4.0 kV to eliminate any charge buildup.

The mass spectrometer was operated in MS/MS mode using an inclusion list with an isolation width of 1.0 m/z in the first quadrupole to filter precursor ions. A maximum injection time of 50 ms was used. In the cases where the precursor ions of two or more targets had similar or identical precursor m/z and the optimal collision energy were similar, a single scan event was used for both targets. The precursor ions, fragment ions, and optimized collision energies (CEs) for all 137 drugs and the 11 SIL ISTDs in the positive ion screen can be found in Supplementary Table 1. For nearly all of the analytes, the intact $[M+H]^+$ ion predominated and was used as the precursor ion. The $[M+Na]^+$ ion was used for carisoprodol and topiramate. Norpropoxyphene, norsertraline, and propoxyphene all showed intense fragment ions in the full scan MS. Since these in-source fragment ions showed higher signal than the intact molecular ion, they were used as the precursor ions for MS/MS.

The inclusion list for the smaller negative ion screen can be found in Supplementary Table 2; each of the analytes generated $[M-H]^-$ precursor ions. Since this screen was significantly smaller than the positive ion screen in terms of the number of targets, a larger maximum injection time of 500 ms was used. The spray voltage was set to -4.0 kV. The lower spray voltage was used to minimize the risk of corona discharge. The voltage was left on for the first 1.1 min of the 1.4-min instrument method and was set to 0 kV for the final 0.3 min to acquire the zero-scans necessary for automated integration of the data.

Data Processing. All data were automatically processed using TraceFinder v. 3.3 (Thermo Fisher Scientific). Peak detection was restricted to a 5 ppm m/z window around the target compound's fragment ion. The area under the curve (AUC) of the analyte fragment ion over the entire analysis time was determined by automatic integration. The AUC of the analyte fragment ion was then divided by the AUC of the ISTD fragment ion. Each positive ion calibration point was run in duplicate, and the negative ion calibration points were run in triplicate. The ratios of analyte signal to ISTD signal were plotted against their known concentrations to generate the calibration curve, which was linearly fit using 1/x weighted least squares.

The signal-to-blank ratio (S:B) for each target compound at the cut-off was determined to provide a rough estimate of the detection limit. For most drugs, no blank signal was detected from drug-free blood samples. To calculate S:B in those cases, the instrumental noise level was used. In the few cases in which there was detectable signal in the drug-free blood, the average signal obtained across all matrix blanks was used.

PM Sample Drug Screen. All 30 unknown PM samples were run in triplicate alongside the calibration samples. The results of this PS-MS/MS drug screen were then compared to an LC-MS-MS confirmatory assay, which was run off-site and independently by Axis Forensic Toxicology.

Results and Discussion

Paper Spray Screening on a Quadrupole-Orbitrap Mass Spectrometer. An example of the total ion chronogram acquired using the described instrument method is shown in Figure 1a. The term chronogram is used here rather than chromatogram because no separations are being performed. The chronograms are plotted in stick mode rather than smoothed so that individual scans can be seen. A full cycle of the inclusion list, encompassing all target compounds, was completed within ~0.3 minutes. Five or six scans were acquired for each MS/MS scan filter followed by a zero-intensity scan at the end, which was obtained by turning off the spray voltage 1.6 minutes into the acquisition. Such zero-intensity scans were required for each channel for automatic peak integration through the TraceFinder software. The TIC for the MS/MS of m/z 304.2 (corresponding to cocaine) is shown in Figure 1b, demonstrating the number and frequency of MS/MS scans as well as the zero-scan. The MS/MS spectra for a neat infusion of cocaine and a low concentration of cocaine spiked into drug-free blood are shown in Figure 1c and 1d, respectively. The cocaine MS/MS spectrum from blood shows a number of extraneous peaks owing to the complex chemical background. However, the diagnostic fragment ions for cocaine, such as m/z 182.117, were clearly detected with mass accuracy better than 5 ppm.

When screening for a relatively large number of targets using an MS/MS inclusion list, the settings of the mass spectrometer must be adjusted to ensure an adequate number of scans are obtained for each target within the analysis time. The maximum injection time and the resolution of the orbitrap were set such that around five scans were obtained per target during the 90-second analysis time. The analysis is limited to 90 seconds primarily due to depletion of the extraction/spray solvent from the paper spray cartridge after analysis begins. Five scans were sufficient to give accurate m/z measurements and ion ratios and was deemed acceptable for semi-quantitative performance.

The injection time of the orbitrap is analogous to dwell time on a triple quadrupole. The injection time is directly proportional to sensitivity unless the orbitrap is filled to capacity. In this case, the orbitrap rarely reached capacity. The injection time could therefore be increased to several seconds with a concomitant increase in sensitivity if desired. However, increasing the injection time would lead to an undesirably long acquisition time considering the number of target compounds.

Resolution is another factor affecting both assay performance and scan time. The m/z resolution used here was 35,000, which lies in the middle of the range offered by this instrument. This resolution was considered an acceptable trade-off between selectivity and scan time. If fewer targets were included in the screening panel, the resolution and/or the injection time could be increased to improve selectivity and sensitivity, respectively.

In this PS-MS/MS assay, target detection was based off the presence of a single fragment ion within a 5 ppm m/z window with adequate signal to blank ($\geq$3). We opted for detection of only a single fragment ion in order to minimize the risk of false negatives. Because full MS/MS spectra were collected, additional fragment ions, with or without use of fragment ion ratios, can be added to the data analysis step to increase selectivity and decrease the rate of false positives.

Relative Matrix Effects. We compared the signal of the 11 ISTDs obtained for each of the 30 postmortem blood samples to the signal obtained from the fresh whole blood calibrators. The results, summarized in Table 1, showed that there is no systematic difference between the postmortem blood and the fresh blood used for the calibration curves. The absolute signal of the ISTD varied somewhat more in PM samples than the calibrators, likely due to variations across

the 30 PM samples. While the variation of the absolute signal was around 30%, the variation in the analyte:ISTD ratio was considerably less (<10% on average).

Calibration Curves and Detection Limits. Calibration curves prepared in whole blood are summarized in Table 2. Nearly all of the calibration curves had coefficients of determination (R^2) above 0.9 and relative errors of the slope less than 15%. Buspirone stood out as having a relatively poor calibration curve with an R^2 of 0.53 and a relative error of the slope of 33%. Although concentration determinations for this drug are not expected to be accurate, buspirone was nevertheless reliably detected throughout its calibration range with strong signal. The cut-off concentrations are also indicated in Table 2. These values were set within or below the therapeutic or "safe" range for each target compound. In many cases, the drug could be detected at significantly lower concentrations if desired. The S:B of the primary fragment ion at the cut-off concentration is indicated in the table to provide a rough estimate of the feasible detection limits for each drug using this method. For example, the cut-off for carbamazepine was set at 1 μg/mL because the therapeutic levels of this drug are in the low μg/mL range, and toxic levels are above about 10 μg/mL[64]. However, the magnitude of the S:B indicates that carbamazepine can feasibly be detected at concentrations less than 10 ng/mL if desired.

Selectivity. Because of the lack of chromatographic separation prior to the ionization step in PS-MS/MS, co-elution of drugs, drug metabolites, and matrix components can result in interferences. Several intratarget interferences were identified and are summarized in Table 3. Only intratarget interferences in which the highest calibrator of the interferent resulted in a signal above the cut-off for the target compound were included. Several of the interferences arose from structurally similar isomers that yielded the same fragment ions. In each of these cases, the intensity ratios of the major fragment ions generated by the two isomers were different; fragment ion ratios might therefore be used to distinguish these isomers if desired. In two cases, interference was observed from apparent hydrolysis of the parent drug standard in the blood calibrators. This interference was not observed in neat standards, ruling out impurities or in-source CID. Interference from non-target compounds can also occur, potentially resulting in false positives. For example, the non-target compound norcodeine could give false positives for morphine and hydromorphone[65] since all three of these compounds are structurally similar isomers.

Evaluating the PS-MS/MS Drug Screen on Postmortem Samples. Thirty blinded postmortem blood samples were obtained from a toxicology laboratory and analyzed using PS-MS/MS. The toxicology lab performed their typical screen and confirm workflow, which consisted of screening by a combination of immunoassay and LC-MS-MS followed by a separate quantitative LC-MS-MS assay for confirmation. A complete list of the drugs detected by the two methods and the determined concentrations can be found in Supplementary Table 3. PS-MS/MS detected a total of 97 drugs across all samples, whereas the LC-MS-MS screen and confirm detected 89. Of the 89 drug detections from the LC-MS-MS screen/confirm, 7 were not detected by the PS-MS/MS method (false negatives – FNs). Of the seven false negatives, five were detected by the LC-MS-MS assay at concentrations below the PS-MS/MS detection limit. For the other two results, quantitative analysis was not performed by the toxicology lab, so the drug concentrations are unknown. In total, some 4,000 negative results were determined by both screening methods.

Of the 97 PS-MS/MS screening detections, 7 instances were not detected by the LC-MS-MS screen/confirm and were treated as false positives (FPs). Of these 7 FP, two were opiates detected in the presence of other opiates. For example, the PS-MS/MS assay screened positive for both hydromorphone and morphine, whereas only morphine was confirmed positive by LC-MS-MS. Hydromorphone and morphine are isomers that yield many of the same fragment ions, albeit

in different ratios. Of the remaining 5 FP, the source of interference is not known; 4 of them were at low levels near the cut-off (within a factor of 2). Finally, of the 97 drugs screened positive by PS-MS/MS, 7 were not screened for by the toxicology lab because they were not included in the panel ordered by the customer; these detection events were excluded from the data analysis.

Qualitatively, the rates of occurrence for positive and negative results can be used in measuring a method's true positive rate, false positive rate, positive predictive value (PPV), and negative predictive value (NPV). These values are defined as[66]:

$$\text{true positive rate} = \frac{\text{TP}}{\text{TP} + \text{FN}} \tag{1}$$

$$\text{true negative rate} = \frac{\text{TN}}{\text{TN} + \text{FP}} \tag{2}$$

$$\text{PPV} = \frac{\text{TP}}{\text{TP} + \text{FP}} \tag{3}$$

$$\text{NPV} = \frac{\text{TN}}{\text{TN} + \text{FN}} \tag{4}$$

As summarized in Table 4, the true positive rate of the PS-MS/MS screen was 92.1% while the true negative rate was 99.8%. The calculated true positive rate for the PS-MS/MS assay included drugs at concentrations below the PS-MS/MS detection limits; the true positive rate for drugs within the reporting range was 100%. No drug targets above the assay cut-off were detected by LC-MS-MS but not by paper spray. These results compare favorably with immunoassay drug screens; a recent study showed that the true positive rate was about 85% across all drug classes, with some drugs (MDMA and PCP) having a 0% true positive rate[67]. 92.1% of all positive PS hits were genuinely present in the samples (positive predictive value) and 99.8% of all undetected drugs were confirmed to be absent in the samples (negative predictive value). While a significantly larger number of samples are needed to confidently assign the true positive and negative rates of paper spray MS/MS, these results indicate that the method shows good promise as a drug screening method.

The quantitative results obtained by the PS-MS/MS screening method were compared to the LC-MS-MS quantitative confirmations using Passing-Bablok regression (Figure 2). The two methods were highly correlated, with a Pearson's correlation coefficient (r) of 0.996. The concentrations determined by PS-MS were, on average, slightly higher than the LC-MS-MS values as reflected by the slope value of 1.17. A complete comparison of the quantitative results can be found in Supplementary Table 3. It is important to note that the PS-MS/MS method was developed as a rapid screening method, whereas the HPLC method was developed for quantitative confirmation. The quantitative performance of PS-MS can be improved by decreasing the number of targets and by using isotopically labeled internal standards for each analyte.

Screening for Acidic Drugs. The screening method described thus far was carried out via a single paper spray analysis in the positive ion mode. Some acidic drugs, such as barbiturates, did not form abundant positive ions and were suited for the negative ion mode. Using a different solvent system optimized for negative ionization, we tested the PS-MS/MS screening method for barbiturates in the negative ion mode. Phenytoin, which like the barbiturates has an acidic cyclic imide moiety, was also included. The limits of detection and calibration performances in the whole

blood calibrators are shown in Table 5. The LODs for each target lie below or within their therapeutic ranges, which are all >1 μg/mL[64]. The calibration curves for each target showed relative slope errors of 3-7% with R^2 of 0.95 or higher.

Conclusion

Paper spray-MS/MS drug screening methods were developed for the rapid screening of over 140 drugs and drug metabolites commonly encountered in toxicological analyses. Separate methods were developed in the positive and negative ion modes, each of which could be completed in a few minutes without sample preparation. Semi-quantitative analysis was carried out by generating calibration curves for each of the analytes. The positive ion drug screen, which included >95% of the target drugs, was tested on 30 postmortem blood specimens. Comparison with independent LC-MS-MS screening and confirmation testing showed good agreement.

The sensitivity of PS-MS/MS coupled to a quadrupole-orbitrap mass spectrometer was adequate for postmortem drug screening of all of the drugs investigated here. Screening for a large panel of drugs in a single run made it impossible to optimize the experimental conditions for each drug class; better sensitivity could be expected by reducing the number of target compounds. Also, some potent emerging drugs of abuse such as carfentanil require screening cut-offs well below 1 ng/mL. Direct analysis approaches that employ analyte preconcentration are one way to achieve such low detection limits[21, 68-69]. It is also important to realize the selectivity limitations of PS-MS. Because there is no chromatographic separation, co-elution of compounds can result in interferences. Use of fragment ion ratios or gas-phase separations via differential mobility[65] or ion mobility are potential solutions to this problem. Additional study on a more exhaustive list of potential interferences is needed. Finally, the results presented here represent the results of a single run; investigation into method robustness and precision will be needed. Nevertheless, this study showed promising results that demonstrate the capability of PS-MS/MS to perform rapid, sensitive, and selective drug screening from blood samples.

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Figure Captions

Figure 1. (a) Total ion chronogram (all scans combined). (b) Extracted ion chronogram for cocaine's MS/MS scans. (c) Tandem mass spectrum for a neat standard of cocaine at 200 ng/mL, infused via a commercial ESI source. (d) Tandem mass spectrum for blood spiked with 16 ng/mL cocaine (0.33 times its cut-off), ionized via paper spray.

Figure 2. Comparison of concentrations for the drugs detected and quantitated from the PM samples by both HPLC- and PS-based screening methods using a log-log plot. The Passing-Bablok regression is shown as a solid line; perfect correlation is shown as a dashed line.

Table 1. The area under the curve (AUC) and relative standard deviation (RSD) for each of the internal standards across all calibrators and all PM samples during a single batch. The percent difference between the PM samples and calibrators is also shown.

ISTD	Parameter	PM	Cal	Percent difference
Alprazolam-d5	**AUC**	$3.5x10^8$	$3.1x10^8$	+11%
	RSD	46%	26%	
Benzoylecgonine-d8	**AUC**	$3.6x10^8$	$3.1x10^8$	+15%
	RSD	28%	28%	
Cocaine-d3	**AUC**	$6.8x10^8$	$5.7x10^8$	+18%
	RSD	28%	24%	
Flunitrazepam-d7	**AUC**	$4.0x10^7$	$2.3x10^7$	+54%
	RSD	32%	27%	
Gabapentin-d10	**AUC**	$3.5x10^7$	$3.6x10^7$	-2%
	RSD	41%	31%	
Hydrocodone-d3	**AUC**	$7.0x10^7$	$8.6x10^7$	-20%
	RSD	30%	29%	
Metaxalone-d6	**AUC**	$2.4x10^7$	$2.9x10^7$	-19%
	RSD	32%	24%	
Methadone-d3	**AUC**	$9.4x10^8$	$^8.6x10^8$	+9%
	RSD	50%	32%	
Methamphetamine-d11	**AUC**	$6.9x10^8$	$9.0x10^8$	-27%
	RSD	37%	35%	
Trimipramine-d3	**AUC**	$6.2x10^8$	$5.8x10^8$	+6%
	RSD	48%	27%	
Zolpidem-d6	**AUC**	$2.3x10^7$	$1.9x10^7$	+19%
	RSD	29%	26%	

Table 2. Quantitative measurements for each of the analyte calibration curves which ran concurrently with the PM samples in the PS-MS/MS drug screen.

Analyte	ISTD	Cut-off [ng/mL]	S:B at Cut-off	Rel. Error in Slope [%]	R^2
6-Monoacetylmorphine	**F**	20	5	3	0.993
7-Aminoclonazepam	**F**	25	34	3	0.994
7-Aminoflunitrazepam	**F**	20	52	3	0.992
9-Hydroxyrisperidone	**F**	10	155	7	0.963
10,11-Dihydro-10-hydroxycarbamazepine	**J**	500	19	6	0.976
Acetaminophen	**E**	5000	4	9	0.942
Alfentanil	**K**	50	257	7	0.961
Alpha-PVP	**K**	50	214	9	0.936
Alprazolam	**A**	5	3	2	0.996
Amitriptyline	**J**	20	72	3	0.994
Amlodipine	**F**	80	9	7	0.959
Amphetamine	**I**	80	3	5	0.984
Aripiprazole	**H**	50	111	8	0.951
Atenolol	**F**	100	48	3	0.993
Baclofen	**E**	250	11	7	0.961
Benzoylecgonine	**B**	50	7	1	0.998
Benztropine	**H**	10	303	6	0.972
Benzylpiperazine	**F**	50	2	2	0.997
Brompheniramine	**H**	25	83	13	0.877
Bupivacaine	**H**	250	4696	5	0.983
Buprenorphine	**H**	10	6	4	0.986
Bupropion	**K**	50	156	11	0.915
Buspirone	**H**	6	69	33	0.528
Carbamazepine	**J**	1000	5925	9	0.940
Carbamazepine-10,11-epoxide	**J**	500	194	2	0.995
Carisoprodol	**B**	2000	10	7	0.961
Chlordiazepoxide	**K**	50	120	5	0.981
Chlorpheniramine	**H**	15	145	5	0.977
Chlorpromazine	**J**	50	9	5	0.973
Citalopram	**H**	10	63	10	0.926
Clomipramine	**J**	20	35	3	0.993
Clonazepam	**A**	30	3	4	0.984
Clozapine	**K**	50	542	8	0.951
Cocaethylene	**C**	50	136	2	0.997
Cocaine	**C**	50	36	12	0.903
Codeine	**F**	20	7	3	0.992
Cyclobenzaprine	**J**	10	208	2	0.997
Demoxepam	**D**	50	12	5	0.993
Desalkylflurazepam	**A**	50	64	6	0.972
Desipramine	**J**	20	21	4	0.991
Dextromethorphan	**H**	10	78	8	0.952

Diazepam	**A**	50	97	3	0.995
Diltiazem	**K**	50	129	11	0.910
Diphenhydramine	**H**	25	7	4	0.986
Donepezil	**H**	45	47	4	0.989
Doxepin	**J**	20	128	5	0.977
Doxylamine	**K**	25	60	6	0.974
Duloxetine	**H**	800	4	10	0.963
EDDP	**H**	25	141	6	0.973
Ephedrine/Pseudoephedrine	**I**	50	17	2	0.996
Etomidate	**A**	100	9	5	0.982
Felbamate	**B**	2500	4	12	0.889
Fentanyl	**C**	1	10	4	0.988
Flecainide	**K**	250	549	5	0.981
Flunitrazepam	**D**	20	5	4	0.989
Flurazepam	**H**	25	182	5	0.979
Fluvoxamine	**K**	15	7	9	0.933
Gabapentin	**E**	250	39	7	0.966
Haloperidol	**H**	10	264	7	0.963
Hydrocodone	**F**	20	35	2	0.998
Hydromorphone	**F**	20	11	6	0.973
Hydroxychloroquine	**K**	2000	336	12	0.899
Hydroxyzine	**K**	10	82	4	0.985
Ketamine	**H**	100	370	7	0.963
Labetalol	**F**	45	50	5	0.983
Lamotrigine	**F**	500	252	5	0.983
Levetiracetam	**G**	2000	3	4	0.984
Lidocaine	**K**	250	4303	3	0.993
Lorazepam	**D**	25	8	6	0.972
MDA	**I**	100	8	4	0.984
MDMA	**I**	45	79	3	0.994
MDPV	**K**	45	360	4	0.987
Meperidine	**K**	25	137	5	0.980
Mephedrone	**I**	45	64	5	0.983
Meprobamate	**B**	1000	3	11	0.910
Mescaline	**F**	100	3	6	0.971
Metaxalone	**G**	1000	37	7	0.966
Methadone	**H**	15	165	5	0.980
Methamphetamine	**I**	45	70	2	0.995
Methocarbamol	**G**	500	4	6	0.974
Methylone	**I**	45	17	5	0.983
Methylphenidate	**K**	20	464	2	0.997
Metoclopramide	**F**	100	726	5	0.982
Metoprolol	**F**	45	131	3	0.993
Midazolam	**K**	45	65	4	0.987
Mirtazapine	**K**	45	572	4	0.985
Morphine	**F**	30	3	5	0.980

Naproxen	**B**	14994	83	14	0.878
Norbuprenorphine	**H**	10	3	8	0.9575
Norclomipramine	**J**	36	276	2	0.998
Norclozapine	**J**	45	140	3	0.993
Nordiazepam	**A**	50	133	5	0.978
Nordoxepin	**J**	20	65	4	0.985
Norfluoxetine	**H**	20	7	2	0.989
Norketamine	**F**	91	114	5	0.978
Normeperidine	**F**	25	297	6	0.972
Norpropoxyphene (fragment)	**H**	50	16	3	0.995
Norsertraline (fragment)	**H**	50	113	3	0.993
Nortramadol	**K**	2500	3	8	0.953
Nortriptyline	**J**	20	66	3	0.994
Norvenlafaxine	**K**	25	3	5	0.9879
o-/m-Chlorophenylpiperazine	**F**	20	89	4	0.989
Olanzapine	**K**	50	164	7	0.966
Oxazepam	**D**	50	41	4	0.987
Oxycodone	**F**	50	18	3	0.992
Oxymorphone	**F**	15	5	5	0.981
Papaverine	**K**	250	2064	3	0.994
Paroxetine	**K**	15	57	7	0.959
PCP	**H**	250	7	12	0.901
Pentazocine	**H**	50	425	4	0.987
Pregabalin	**E**	250	7	7	0.958
Primidone	**B**	750	3	11	0.910
Promethazine	**H**	25	61	5	0.978
Propoxyphene (fragment)	**H**	50	39	3	0.994
Propranolol	**K**	50	194	2	0.996
Quetiapine	**K**	50	579	5	0.983
Ranitidine	**F**	250	231	5	0.981
Risperidone	**K**	10	48	7	0.961
Ropinirole	**K**	10	144	5	0.982
Sertraline	**H**	100	18	6	0.971
Sildenafil	**F**	100	14	3	0.994
Temazepam	**D**	50	86	2	0.997
TFMPP	**K**	50	386	3	0.992
Topiramate	**G**	5000	4	14	0.889
Tramadol	**K**	100	8	3	0.991
Trazodone	**K**	100	539	3	0.995
Triazolam	**A**	20	19	5	0.982
Trimipramine	**J**	20	179	3	0.993
Vardenafil	**F**	100	39	4	0.989
Venlafaxine	**F**	50	3	4	0.984
Verapamil	**H**	50	267	4	0.987

Zaleplon	**D**	15	3	4	0.986
Ziprasidone	**H**	40	49	9	0.944
Zolpidem	**K**	10	169	1	0.999
Zonisamide	**A**	750	3	13	0.875

Table 3. Intratarget interferences.

Target Compound	**Interferent**	**Extent of Interference†**	**Possible cause of interference**
Tramadol	Norvenlafaxine	45%	Isomers that generate the same fragment ions
Hydromorphone	Morphine	20%	Isomers that generate the same fragment ions
Morphine	Hydromorphone	5%	Isomers that generate the same fragment ions
Morphine	6-Monoacetylmorphine	25%	Partial hydrolysis of 6-MAM to morphine in blood
Codeine	Hydrocodone	5%	Isomers that generate the same fragment ions
Hydrocodone	Codeine	20%	Isomers that generate the same fragment ions
Benzoylecgonine	Cocaine	35%	Partial hydrolysis of cocaine to benzoylecgonine in blood

†The extent of interference is expressed as (apparent concentration of target compound)/(actual concentration of interferent)*100%

Table 4. Qualitative results of the PS-MS/MS based drug screen relative to LC-MS-MS based confirmatory assays.

Parameter	Result
True Positive (TP)	82
False Positive (FP)	7
True Negative (TN)	~4000
False Negative (FN)	7
True positive rate (eqn 1)	92.1%
True negative rate (eqn 2)	99.8%
Positive Predictive Value (eqn 3)	92.1%
Negative Predictive Value (eqn 4)	99.8%

Table 5. Limits of detection and calibration summary for acidic drug targets.

Analyte	LOD [ng/mL]	Rel. Error in Slope [%]	R^2
Butabarbital	229	3	0.99
Butalbital	263	4	0.98
Amobarbital	321	5	0.97
Phenobarbital	502	4	0.98
Secobarbital	286	4	0.98
Thiopental	1100	4	0.98
Phenytoin	919	7	0.95

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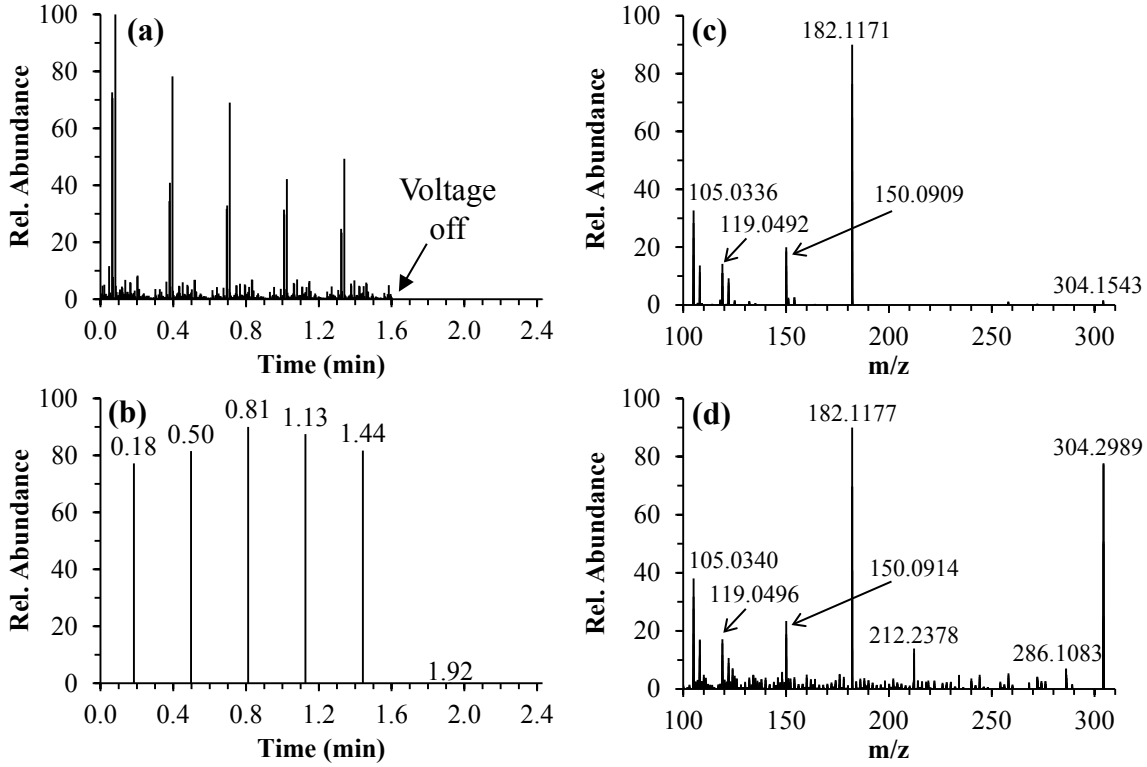

Figure 1. (a) Total ion chronogram (all scans combined). (b) Extracted ion chronogram for cocaine's MS/MS scans. (c) Tandem mass spectrum for a neat standard of cocaine at 200 ng/mL, infused via a commercial ESI source. (d) Tandem mass spectrum for blood spiked with 16 ng/mL cocaine (0.33 times its cut-off), ionized via paper spray.

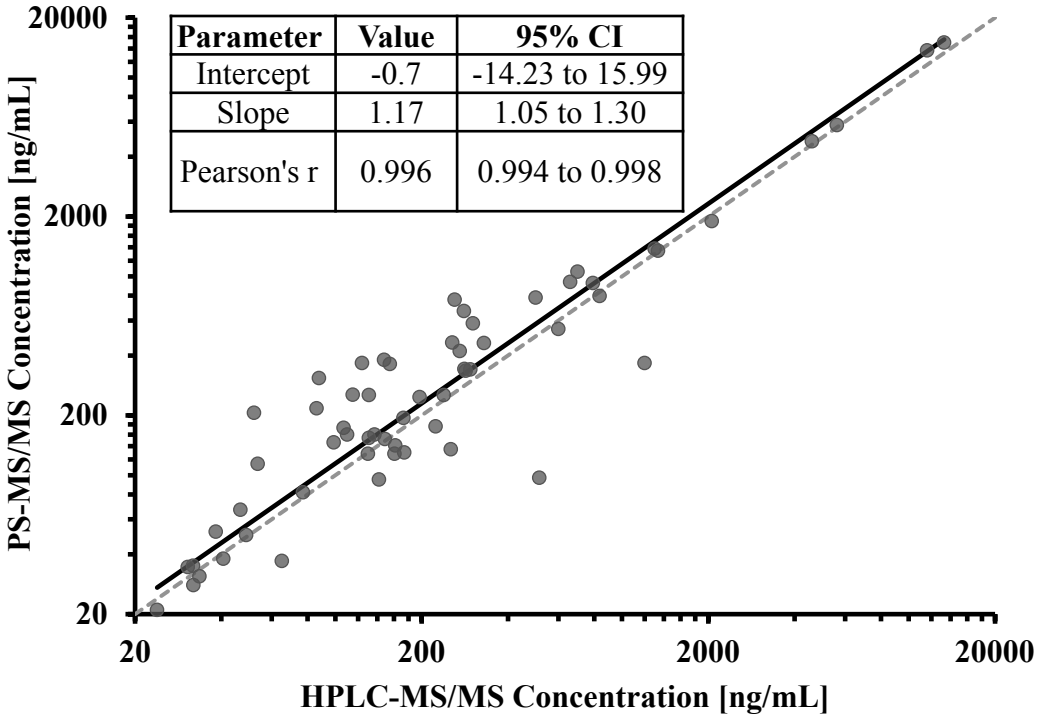

Figure 2. Comparison of concentrations for the drugs detected and quantitated from the PM samples by both HPLC- and PS-based screening methods using a log-log plot. The Passing-Bablok regression is shown as a solid line; perfect correlation is shown as a dashed line.

Toxicological Drug Screening using Paper Spray High-Resolution Tandem Mass Spectrometry (HR-MS/MS)

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Contents

Supplementary Table 1. Fragmentation of each analyte and SIL ISTD used in the PS-MS/MS positive ion drug screen. The fragment ion indicated was the primary fragment ion used for quantitation.

Compound	Precursor Ion [m/z]	Fragment Ion [m/z]	HCD CE
6-Monoacetylmorphine	328.1543	211.0753	25
7-Aminoclonazepam	286.1000	121.0760	30
7-Aminoflunitrazepam	284.1194	135.0915	30
9-Hydroxyrisperidone	427.2140	207.1127	33
10,11-Dihydro-10-hydroxycarbamazepine	255.1128	194.0965	30
Acetaminophen	152.0706	110.0602	20
Alfentanil	417.2609	268.1763	20
Alpha-PVP	232.1696	126.1276	22
Alprazolam	309.0902	281.0712	45
Amitriptyline	278.2000	191.0852	28
Amlodipine	409.1525	238.0625	15
Amphetamine	136.1121	119.0858	10
Aripiprazole	448.1553	285.0910	30
Atenolol	267.1700	145.0646	26
Baclofen	214.0629	116.0620	32
Benzoylecgonine	290.1387	168.1015	20
Benztropine	308.1850	167.0850	35
Benzylpiperazine	177.1386	91.0546	20
Brompheniramine	319.1400	274.0215	20
Bupivacaine	289.1000	140.1430	33
Buprenorphine	468.3108	414.2630	42
Bupropion	240.1150	131.0728	30
Buspirone	386.2551	122.0710	35
Carbamazepine	237.1022	194.0961	26
Carbamazepine-10,11-epoxide	253.0972	180.0806	24
Carisoprodol	283.1628	200.1644	16
Chlordiazepoxide	300.1000	227.0489	21
Chlorpheniramine	275.1000	230.0726	19
Chlorpromazine	319.1030	86.0967	30
Citalopram	325.1711	109.0449	24
Clomipramine	315.1623	86.0969	50
Clonazepam	316.0484	214.0416	45
Clozapine	327.1371	270.0786	25
Cocaethylene	318.1700	196.1328	25
Cocaine	304.1543	182.1170	30
Codeine	300.1594	215.1064	30
Cyclobenzaprine	276.1747	215.0853	60
Demoxepam	287.0582	179.9847	23
Desalkylflurazepam	289.1000	140.0260	33
Desipramine	267.1700	208.1117	26

Compound	Precursor Ion [m/z]	Fragment Ion [m/z]	HCD CE
Dextromethorphan	272.2009	147.0801	32
Diazepam	285.1000	154.0415	33
Diltiazem	415.1686	178.0315	23
Diphenhydramine	256.1000	167.0851	25
Donepezil	380.2220	243.1373	28
Doxepin	280.1696	107.0493	25
Doxylamine	271.1805	182.0962	18
Duloxetine	298.1260	267.0836	15
EDDP	278.1903	234.1271	38
Ephedrine/Pseudoephedrine	166.1226	115.0544	26
Etomidate	245.1285	113.0346	30
Felbamate	239.1026	117.0702	10
Fentanyl	337.2274	188.1431	25
Flecainide	415.1451	301.0284	46
Flunitrazepam	314.0936	239.0975	40
Fluoxetine	310.1413	148.1120	10
Flurazepam	388.1586	315.0685	28
Fluvoxamine	319.1400	71.0497	20
Gabapentin	172.1332	137.0959	17
Haloperidol	376.1474	123.0242	55
Hydrocodone	300.1594	199.0752	30
Hydromorphone	286.1000	185.0594	30
Hydroxychloroquine	336.1837	179.0370	20
Hydroxyzine	375.1834	201.0461	20
Ketamine	238.0993	125.0152	20
Labetalol	329.1860	162.0545	25
Lamotrigine	256.1000	210.9821	25
Levetiracetam	171.1128	126.0914	20
Lidocaine	235.1805	86.0967	20
Lorazepam	321.0192	275.0129	32
MDA	180.1019	133.0647	15
MDMA	194.1176	135.0438	25
MDPV	276.1594	126.1276	27
Meperidine	248.1645	174.1274	20
Mephedrone	178.1226	145.0883	25
Meprobamate	219.1100	162.0915	12
Mescaline	212.1281	180.0780	20
Metaxalone	222.1125	161.0960	10
Methadone	310.2165	265.1580	18
Methamphetamine	150.1277	91.0546	10
Methocarbamol	242.1023	118.0500	10
Methylone	208.0968	160.0754	20
Methylphenidate	234.1489	84.0811	20
Metoclopramide	300.1000	227.0575	21

Compound	Precursor Ion [m/z]	Fragment Ion [m/z]	HCD CE
Metoprolol	268.1907	116.1070	20
Midazolam	326.0855	244.0320	30
Mirtazapine	266.1500	195.0914	24
Morphine	286.1000	201.0905	30
Naproxen	231.1016	185.0963	20
Norbuprenorphine	414.2639	101.0965	60
Norclomipramine	301.1466	72.0813	20
Norclozapine	313.1300	270.0783	26
Nordiazepam	271.0633	140.0259	34
Nordoxepin	266.1500	107.0493	24
Norfluoxetine	296.1257	222.0491	20
Norketamine	224.0837	125.0151	15
Normeperidine	234.1489	160.1118	15
Norpropoxyphene (fragment)	308.2009	143.0856	25
Norsertraline (fragment)	275.1000	158.9759	19
Nortramadol	250.1802	189.1274	15
Nortriptyline	264.1800	191.0853	28
Norvenlafaxine	264.1800	133.0648	28
o-/m-Chlorophenylpiperazine	197.0840	154.0416	26
Olanzapine	313.1300	256.0893	26
Oxazepam	287.0582	241.0521	23
Oxycodone	316.1543	241.1089	33
Oxymorphone	302.1387	227.0934	35
Papaverine	340.1543	202.0858	28
Paroxetine	330.1500	192.1179	22
PCP	244.2060	159.1166	13
Pentazocine	286.2165	218.1535	20
Phenytoin	253.0972	182.0965	24
Pregabalin	160.1332	124.1118	12
Primidone	219.1100	162.0912	12
Promethazine	285.1000	198.0369	33
Propoxyphene (fragment)	266.1500	143.0854	24
Propranolol	260.1645	116.1070	20
Quetiapine	384.1740	253.0795	25
Ranitidine	315.1485	176.0484	18
Risperidone	411.2191	191.1172	35
Ropinirole	261.1961	114.1277	24
Sertraline	306.1000	158.9760	29
Sildenafil	475.2122	283.1183	45
Temazepam	301.0738	255.0677	36
TFMPP	231.1104	188.0679	32
Topiramate	362.0880	265.1047	18
Tramadol	264.1958	58.0659	10
Trazodone	372.1586	176.0814	25

Compound		Precursor Ion [m/z]	Fragment Ion [m/z]	HCD CE
Triazolam		343.0512	315.0318	35
Trimipramine		295.2169	100.1123	25
Vardenafil		489.2279	169.0968	50
Venlafaxine		278.2000	121.0648	28
Verapamil		455.2904	165.0906	30
Zaleplon		306.1000	236.0927	29
Ziprasidone		413.1197	194.0362	30
Zolpidem		308.1850	235.1224	35
Zonisamide		213.0328	150.0548	22
Zopiclone		389.1123	217.0270	40
Alprazolam-d5	**A**	314.1215	314.1215	45
Benzoylecgonine-d8	**B**	298.2000	171.1208	25
Cocaine-d3	**C**	307.1732	307.0732	30
Flunitrazepam-d7	**D**	321.1375	321.1375	40
Gabapentin-d10	**E**	182.1960	182.1960	17
Hydrocodone-d3	**F**	303.1783	303.1783	30
Metaxalone-d6	**G**	228.1501	228.1501	10
Methadone-d3	**H**	313.2354	313.2354	18
Methamphetamine-d11	**I**	161.1968	161.1968	10
Trimipramine-d3	**J**	298.2000	298.2357	25
Zolpidem-d6	**K**	314.2134	314.2134	35

Supplementary Table 2. Fragmentation of the analytes and ISTD used in the negative ion drug screen.

Compound	Precursor Ion [m/z]	Fragment Ion [m/z]	HCD CE
Butabarbital	211.1088	168.1029	11
Butalbital	223.1088	180.1030	10
Amobarbital	225.1245	182.1185	11
Phenobarbital	231.0775	188.0718	10
Secobarbital	237.1245	194.1187	10
Thiopental	241.1017	100.9812	14
Phenytoin	251.0824	102.0345	22
Phenobarbital-d5	236.1090	193.1032	10

Supplementary Table 3. Full comparison between the PS-MS/MS assay and the HPLC-MS/MS screen and confirm workflow. Paper spray concentrations are reported as the average value ± the standard deviation of three replicate measurements. If a PM sample is not listed, it screened negative by both PS-MS/MS and HPLC-MS/MS for all of the drugs in this panel. "FN" = false negative by PS-MS/MS. "FP" = false positive by PS-MS/MS. "<" = detected by PS-MS/MS, but no quantitative value is reported because the concentration was less than the lowest calibrator. "POSITIVE" = no quantitative result was obtained by HPLC-MS/MS. NA = not screened as part of the HPLC-MS/MS workflow because the relevant panel was not ordered by the original customer.

PM Sample	Drug	PS Conc. (ng/mL)	HPLC Conc. (ng/mL)	% Error
2	Clonazepam	FN	9	---
2	Metoprolol	<45	38	---
2	Bupropion	308 ± 18	88	251%
2	7-Aminoclonazepam	95 ± 15	142	-33%
2	Quetiapine	130 ± 21	174	-25%
2	Tramadol	1346 ± 80	1336	1%
2	Nortramadol	FN	1398	---
2	10,11-Dihydro-10-hydroxycarbamazepine	6965 ± 2248	4700	48%
2	Benztropine	20.0 ± 0.1	POSITIVE	---
2	Risperidone	15 ± 1	FP	---
2	Fluoxetine	31 ± 5	NA	---
3	Hydrocodone	82 ± 2	77	6%
3	Lidocaine	544 ± 85	600	-9%
3	Midazolam	<45	POSITIVE	---
3	Morphine	FN	POSITIVE	---
3	Promethazine	FN	POSITIVE	---
4	Gabapentin	781 ± 62	500	56%
4	Donepezil	84 ± 19	FP	---
5	Hydrocodone	38 ± 3	41	-7%
5	7-Aminoclonazepam	<25	49	---
6	Alprazolam	28 ± 3	32	---
6	Hydrocodone	253 ± 6	239	6%
6	Gabapentin	13679 ± 3536	11600	18%
6	Amlodipine	34 ± 7	FP	---
6	Nordiazepam	106 ± 13	FP	---
6	Norfluoxetine	49 ± 2	NA	---
8	Promethazine	37 ± 5	65	-43%
8	Dextromethorphan	146 ± 8	99	48%
8	Bupropion	366 ± 10	124	195%
8	Codeine	128 ± 7	161	-20%
8	Gabapentin	26882 ± 4278	19500	38%

PM Sample	Drug	PS Conc. (ng/mL)	HPLC Conc. (ng/mL)	% Error
8	Hydrocodone	29 ± 3	POSITIVE	---
8	Chlorpheniramine	41 ± 2	POSITIVE	---
8	Pseudoephedrine	<50	POSITIVE	---
9	Diphenhydramine	<25	58	---
9	Sertraline	128 ± 45	130	-2%
9	Mirtazapine	176 ± 17	224	-21%
9	Norsertraline	21 ± 5	254	-92%
9	Gabapentin	14981 ± 4293	13300	13%
11	Hydrocodone	<20	12	---
11	o-/m-Chlorophenylpiperazine	<20	22	---
11	Diphenhydramine	67 ± 8	47	---
11	Bupropion	253 ± 18	131	93%
11	Nortramadol	FN	835	---
11	Gabapentin	4781 ± 196	4600	4%
12	Diazepam	154 ± 32	131	18%
12	Metoclopramide	160 ± 39	137	17%
12	Nordiazepam	194 ± 34	173	12%
13	Hydrocodone	<20	10.	---
13	Nordiazepam	173 ± 14	107	62%
13	Oxycodone	152 ± 6	149	2%
13	Alprazolam	135 ± 12	253	---
13	Oxymorphone	57 ± 17	FP	---
14	Codeine	21.2 ± 0.4	24	-12%
14	6-Monoacetylmorphine	348 ± 14	296	---
14	Morphine	937 ± 88	660	42%
14	Hydromorphone	88 ± 10	FP	---
15	Diphenhydramine	160 ± 17	110	45%
16	7-Aminoclonazepam	<25	27	---
16	EDDP	114 ± 12	54	113%
16	Methadone	669 ± 61	281	138%
16	Cyclobenzaprine	59 ± 3	NA	---
19	Citalopram	580 ± 117	302	92%
19	Gabapentin	1056 ± 274	700	51%
20	Morphine	217 ± 22	86	152%
20	Metoclopramide	254 ± 51	115	121%
20	Amitriptyline	926 ± 35	792	17%
20	Paroxetine	796 ± 84	836	-5%
20	Pregabalin	1378 ± 626	1300	6%
20	Nortriptyline	1894 ± 26	2058	-8%

PM Sample	Drug	PS Conc. (ng/mL)	HPLC Conc. (ng/mL)	% Error
20	Naproxen	135781 ± 93196	26800	407%
21	Oxycodone	<50	37	---
21	9-Hydroxyrisperidone	46 ± 8	FP	---
22	Buprenorphine	FN	1	---
22	Norbuprenorphine	FN	1	---
22	Alprazolam	<5	5	
22	Zolpidem	35 ± 2	32	10%
22	Mirtazapine	141 ± 30	162	-13%
22	Diazepam	247 ± 59	197	25%
22	Buspirone	464 ± 74	256	81%
22	Nordiazepam	335 ± 90	285	18%
22	Bupropion	97 ± 17	515	-81%
22	Gabapentin	33397 ± 9022	15900	110%
23	Methylphenidate	<20	12	---
24	Hydrocodone	31 ± 2	34	-8%
24	Morphine	<30	53	---
24	Citalopram	462 ± 4	330	40%
25	Amphetamine	254 ± 35	148	157%
26	Doxepin	421 ± 29	272	55%
26	Nordoxepin	342 ± 9	282	21%
26	Alprazolam	33 ± 2	NA	
26	Methadone	362 ± 15	NA	---
26	Gabapentin	8358 ± 1255	NA	---
27	Alprazolam	35 ± 1	31	---
27	Mirtazapine	<45	52	---
27	Benzoylecgonine	206 ± 7	52	295%
27	Hydroxyzine	362 ± 44	155	134%
27	Citalopram	763 ± 160	261	192%
28	Hydrocodone	50 ± 2	49	2%
28	Doxylamine	73 ± 6	NA	---
28	Diphenhydramine	97 ± 2	NA	---
30	Morphine	52 ± 16	38	36%
30	Amphetamine	199 ± 58	1200	-70%
30	Methamphetamine	5761 ± 127	5621	2%