Journal of High School Science

A comparative analysis of urine, blood, and hair testing in forensic toxicology

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Submitted: March 17, 2024, Revised: version 1, May 13, 2024
Accepted: May 13, 2024

Abstract
The field of forensic toxicology is a branch of forensic science and an amalgamation of chemistry, biology, and pharmacology. Through forensic toxicology, investigators gain crucial information on the influence of drugs and toxins in relevant crimes. The most recent development in the opioid epidemic is polysubstance abuse, in which opioids and non-opioid drugs are abused simultaneously, leading to dangerously high fatality rates. This paper is designed to inform forensic analysts about how to minimize error in the drug detection process, thus facilitating drug interventions amidst the current epidemic. To do so, this paper uncovers the circumstances in which certain methods of drug detection—urine testing, blood testing, or hair testing—are most efficacious. The accuracies for the three sample matrices are compared using data from both immunoassays and mass spectrometric techniques; while the former is used for quick, efficient detections, the latter assay method yields results that are more specific and less prone to error. It was discovered that hair testing yields less accurate results than testing urine and blood using both immunoassays and liquid chromatography-mass spectrometry (LC-MS). While urine and blood testing yield similar accuracies for immunoassays, accuracy for blood testing is significantly greater using LC-MS. However, hair is nonetheless the most effective at preserving drug evidence for long durations of time (up to years). Regarding short-term drug detection, blood testing is preferred if the test results are desired to be specific and comprehensive. Urine testing is preferred to blood testing for cheap, easy detections; even though urine and blood testing show similar performance, the detection windows in urine testing are longer than in blood testing, resulting in better preservation of evidence.

Keywords
Forensic toxicology, Drug detection, Immunoassay, Mass spectrometry, Urine testing, Blood testing, Hair testing, Drug metabolites, Detection window, Liquid chromatography

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Introduction

The opioid epidemic, a growing issue since the 1990s, has consumed America. While overdoses resulted mainly from prescription opioids from 1999-2010, the focus of the problem has recently shifted to illicit fentanyl and synthetic opioids. A particular issue is the non-transparency of illicit drug dealers, who sell opioids combined with stimulants to unknowing customers. The addition of fentanyl to stimulants is especially deadly, with 90% of the recent increase in cocaine-related deaths being accounted for by the simultaneous use of fentanyl and cocaine (1). Given the ever-evolving nature of the drug epidemic, it is imperative to develop sensitive techniques for drug detection. By implementing effective detection methods, more information can be obtained about the opioid epidemic, such as which opioid-stimulant combinations are popular in a particular region, and what ratios of opioid to stimulant are especially harmful.

The field of forensic toxicology utilizes various forms of evidence for drug detection. These evidence types have different applications depending on the relevant circumstances, thus distinguishing their respective contributions to forensic analysis. While toxicologists commonly prefer to work with blood and urine samples, new methods of evidence analysis, such as hair and saliva testing, have emerged in recent years, with each method introducing its own set of benefits and drawbacks (2). This paper compares the efficacies of urine, blood, and hair analysis relative to each other.

Regarding the technicalities of drug detection, two classes of drug testing exist: presumptive testing and confirmatory testing. Presumptive testing uses immunoassay techniques to detect the presence of a target substance given a certain threshold, meaning that one of four possible outcomes can arise during an immunoassay screening: 1) a true negative, 2) a true positive, 3) a false negative, and 4) a false positive. Confirmatory testing, on the other hand, uses chromatographic techniques—typically either gas chromatography (GC) or liquid chromatography (LC)—in conjunction with mass spectrometry (MS). These techniques are more specific than immunoassays, and the threshold values for detection in LC-MS and GC-MS are much lower (3,4) However, confirmatory testing requires a longer, more complicated preparation procedure, leading immunoassays to be commonly used as a precursor (4,5). Table 1 compares the parameters of immunoassays and LC-MS and shows that the latter exhibits superior performance.

Table 1. Basic parameters of immunoassays and LC-MS for urine, blood, and hair (6-19). LOQ is the limit of quantification, and LOD is the limit of detection. An immunoassay cut-off level is equivalent to an LC-MS LOD: the cut-off refers to the lowest value at which the method of detection can detect the presence of a drug. Since immunoassay screenings cannot quantify the concentration of a drug, they do not have LOQs. The LOQ is presented to compare it against the LOD. Finally, specificity refers to the total number of true negatives divided by the sum of true negatives and false positives. Ideally, the LC-MS has 100% specificity. Though the panels for LC-MS and immunoassays differ because of data limitations, clear patterns are visible from the table.

Detection Method	Medium	Drugs	LOQ	Cut-off Levels	Specificity (%)
Immunoassay	Urine	Methamphetamine	—	500 ng/ml	98.8
		Amphetamine	—	500 ng/ml	99.1
		Barbiturates	—	200 ng/ml	100.
		Benzodiazepines	—	60 ng/ml	85.6
		Cannabinoids	—	50 ng/ml	97.8
		Cocaine	—	150 ng/ml	100.
		Opiates	—	200 ng/ml	93.0
		Methadone	—	100 ng/ml	98.8
	Blood	Methamphetamine	—	100 ng/ml	89.0
		Amphetamine	—	100 ng/ml	89.0
		Barbiturates	—	20 ng/ml	95.0
		Benzodiazepines	—	100 ng/ml	98.2
		Cannabinoids	—	10 ng/ml	96.6
		Cocaine	—	50 ng/ml	97.0
		Opiates	—	100 ng/ml	96.0
		Methadone	—	5 ng/ml	98.3
	Hair	Methamphetamine	—	0.5 ng/mg	99.4
		Amphetamine	—	0.5 ng/mg	92.0
		Barbiturates	—	1.0 ng/mg	99.0
		Benzodiazepines	—	0.1 ng/mg	94.6
		Cannabinoids	—	0.5 ng/mg	99.1
		Cocaine	—	0.5 ng/mg	73.0
		Opiates	—	0.2 ng/mg	85.7
		Methadone	—	0.2 ng/mg	88.4
				LOD	
LC-MS	Urine	Methamphetamine	10 ng/ml	5 ng/ml	100.
		Amphetamine	5 ng/ml	2 ng/ml	100.
		MDMA	10 ng/ml	2 ng/ml	100.
		Codeine	10 ng/ml	5 ng/ml	100.
		Methadone	10 ng/ml	1 ng/ml	100.
	Blood	Methamphetamine	0.5 ng/ml	0.1 ng/ml	100.
		Amphetamine	0.5 ng/ml	0.1 ng/ml	100.
		MDMA	0.5 ng/ml	0.1 ng/ml	100.
		Codeine	0.98 ng/ml	0.5 ng/ml	100.
		Methadone	0.98 ng/ml	0.5 ng/ml	100.
	Hair	Methamphetamine	0.1 ng/mg	0.01 ng/mg	100.
		Amphetamine	0.1 ng/mg	0.01 ng/mg	100.
		MDMA	0.1 ng/mg	0.01 ng/mg	100.
		Codeine	0.01 ng/mg	0.025 ng/mg	100.
		Methadone	0.1 ng/mg	0.03 ng/mg	100.

On the other hand, hair testing seems to contain the solution for both issues—not only does it prevent interference from other ingested substances, but its window of detection is so long that it can retain evidence for months at a time (2,20). Furthermore, existing research shows that hair testing could be especially sensitive to lipophilic drugs, which deposit themselves in lipids such as the cell membranes of cuticle cells—the outermost layer of hair cells (21,22). These observations then raise the question of whether hair analysis can completely replace urine and blood testing. Since this is currently not the case, the appeal of traditional methods needs to be further studied.

Considering novel approaches to drug detection as well as the persisting popularity of established ones, this paper aims to determine the circumstances in which each form of evidence analysis proves optimal. To examine this subject, the paper is divided into two parts: 1) quantitatively delineating the differences between blood and urine analysis and 2) assessing the efficacy of hair analysis in comparison to traditional methods.

Methods

All relevant data was sourced from published articles accessible through the Internet. The Google search engine was used for the procurement of data and existing literature. Search terms included "forensic toxicology database" and "drug detection data." Figures were generated using Microsoft Excel.

For the statistical validation of results, the t-test was used, a method of determining whether the means of two different groups are significantly different. Though not integral to analyzing the results presented hereafter, the t-test is a statistical technique which, in the context of this paper, can be used to determine how different the detection capabilities of two evidence analysis methods are. The equal variance independent t-test was used across all cases, as in each case, the sizes of both test groups were the same. The formula for this kind of t-test is as follows:

$$T - value = \frac{mean1 - mean2}{\frac{(n1-1) \times var1^2 + (n2-1) \times var2^2}{n1+n2-2} \times \sqrt{\frac{1}{n1} + \frac{1}{n2}}} \quad (1)$$

n1 and n2 are the sizes of the test groups, mean1 and mean 2 are the averages of the groups, and var1 and var2 refer to the groups' variances.

The metric used will not be the t-value, however, but rather the p-value—the probability of obtaining the t-value given the assumption that the two groups are not statistically different (the null hypothesis). When the p-value is smaller than an established level of significance, there is a statistical significance between the two groups.

Table 2. The detection windows, MDs, and detected analytes for all major drug categories discussed in this manuscript. DO refers to "parent drug only," MO refers to "metabolite only," and "DM" refers to "parent drug and metabolite." Some MDs are not presented because of data limitations (24-50). *Though hair can show drug use over longer periods of time, the standard detection window for hair is 90 days

Drug	Detection Medium	Detection Window* (days)	MD	Analyte Detected (Immunoassay)	Analyte Detected (LC-MS)
Methamphetamine	Urine	3 – 6	0.14	DO	DM
	Blood	1 – 3	0.10 – 1.0	DO	DM
	Hair	90	0.004 – 1.16	DO	DM
Amphetamine	Urine	1 – 3	0.67 – 0.83	DO	DM
	Blood	~ ½	Exact figure unclear	DO	DM
	Hair	90	Exact figure unclear	DO	DM
Barbiturates	Urine	2 – 4	2.0 – 4.0 (long-acting barbiturates) Higher in short-acting barbiturates	DO	DM
	Blood	1 – 2	Exact figure unclear	DO	DM
	Hair	90	Exact figure unclear	DM	DM
Benzodiazepines	Urine	1 – 42	High MD (exact figure unclear)	MO	DM
	Blood	2 – 3	Exact figure unclear	DO	DM
	Hair	90	0.0052 – 110	DM	DM
Cannabinoids	Urine	7 – 30	4.0 – 4.5	MO	DM
	Blood	1 – 14	0.08 – 0.54	DO	DM
	Hair	90	0.016	MO	DM
Cocaine	Urine	3 – 4	43 – 62	MO	DM
	Blood	1 – 2	6.5	DO	DM
	Hair	90	0.005 – 0.57	DM	DM
Opiates	Urine	Codeine: ≤ 3 Heroin: 1 – 3 Morphine: ≤ 3	Codeine: High MD (exact figure unclear) Heroin: 7.1 – 8.6 Morphine: 6.7 – 30.	MO	DM
	Blood	Codeine: ≤ 1 Heroin: ≤ ¼ Morphine: ≤ 3	7.5	DO	DM
	Hair	90	0.01 – 10	DM	DM
Methadone	Urine	3 – 4	0.5 – 5	DM	DM
	Blood	2 – 3	0.079 – 0.088	DO	DM
	Hair	90	0.013 – 0.71	DM	DM
MDMA	Urine	1 – 3	0.02 – 0.65	DM	DM
	Blood	1 – 2	0.06 – 22.9	DO	DM
	Hair	90	0.029 – 0.11	DO	DM

The metabolite-to-parent drug ratio (MD) is a metric used to determine the relative concentrations of an unchanged drug and its major metabolites. This ratio will be used to examine results in urine, blood, and hair testing. Along with MDs, the detection windows of various drugs are presented in Table 2 to show how quickly they pass through the system using various detection mediums. Recording the detection windows will be crucial to later analyses.

This paper uses another a key metric to compare the performances of different evidence analysis methods when analyzed by LC-MS specifically. This metric is termed the accuracy deviation and is calculated through the following in Equation 2:

$$AccuracyDeviation = ExperimentalAccuracy - 100\% \vee \qquad (2)$$

The manuscripts documenting experimentation with LC-MS obtain accuracy measurements by comparing known concentrations of standard solutions to the measured concentrations of drug samples (2, 7, 23). As such, this paper defines accuracy in the same way.

Results

Urine vs. blood testing in immunoassays

While blood testing is known to be more specific than urine testing, the general purpose of the immunoassay is to provide a quick and easy diagnosis as to whether a specific drug is present (5, 20). Thus, the desired efficiency of immunoassays could undermine the more detail-oriented nature of blood testing, which requires specific techniques to overcome its shortcomings, such as limited sample volumes and short detection windows (20). Comparing urine and blood immunoassays helps reveal which evidence type is more suited for basic presumptive testing (53).

Verplaetse et al., (13) compared the accuracies of immunoassay and LC-MS of the two detection methods for various drugs in urine and blood. Since data was available for both blood and urine, one can alternatively examine how accuracy compares between the two evidence types. In analyzing the article, the first step is to verify that its primary claim is true (LC-MS produces more accurate results than immunoassays), meaning that its conclusions agree with existing literature. To support this claim, the article presented the number of false positives, false negatives, true positives, and true negatives that each method detected across a panel of seven drugs, juxtaposing the LC-MS and the immunoassay data (13). These seven drugs are amphetamine/methamphetamine (AMPH/METH), barbiturates, benzodiazepines (BZD), cannabinoids, cocaine, opiates, and methadone.

To establish a consistent metric for comparing LC-MS and immunoassays, the false-to-true-detection ratios (the total number of false detections divided by the total number of true detections) for all drugs were found. As seen in Figures 1 and 2, the LC-MS approach exhibits a significantly decreased error than the immunoassay approach; the LC-MS false-to-true ratio is at most 39.6% the immunoassay ratio (when testing for cannabinoids in blood) and as low as 0% (when testing for barbiturates in urine). The only exceptions to this trend occur when testing for either barbiturates or methadone in blood, in which cases both LC-MS and immunoassays exhibit no error.

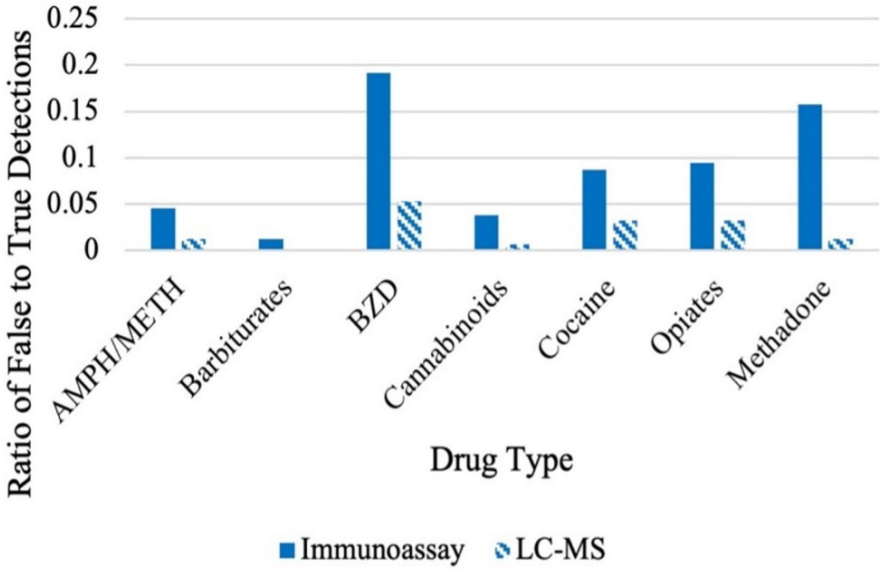

Figure 1. Ratio of false to true urine test detections using immunoassays vs. LC-MS (13). LC-MS performs better than immunoassays for all drugs, most notably benzodiazepines and methadone. This improved performance can be explained by Table 1: given the lower specificity of immunoassays, they are expected to result in more fallacious detections than LC-MS.

The significance of these discrepancies was then examined using a one-tailed t-test. With the level of significance set to 5%, it was found that the performances of LC-MS and immunoassays have a p-value of 1.02% in urine and 8.03% in blood. Although only the discrepancies for urine testing are statistically significant, it is nonetheless true that LC-MS performs as well as, if not better than, immunoassays for both urine and blood.

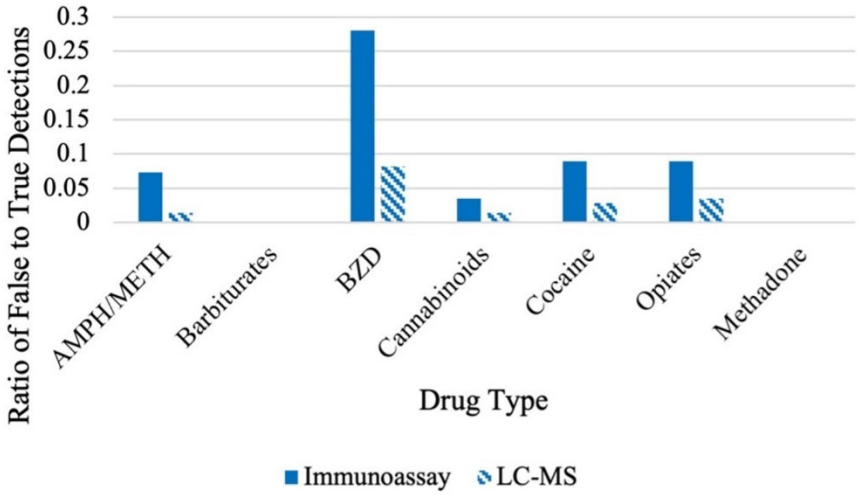

Figure 2. Ratio of false to true blood test detections using immunoassays vs. LC-MS (13). LC-MS again performs better than immunoassays for most drugs, with exceptions being barbiturates and methadone, for which both methods exhibit zero error. Table 1 explains this discrepancy, as the specificity of immunoassays are higher, meaning they are expected to cause more fallacious detections than LC-MS.

With the results of the article having been verified, it is now possible to explore the question of whether blood or urine testing yields greater accuracy using immunoassays. The data

from this article will not be used to examine the same question for LC-MS—because the article compares the accuracies of immunoassays and LC-MS, the data compensates for the limited specificity of immunoassays by simply summing the quantities of true or false detections. While valid, this approach does not do justice to the high specificity of LC-MS.

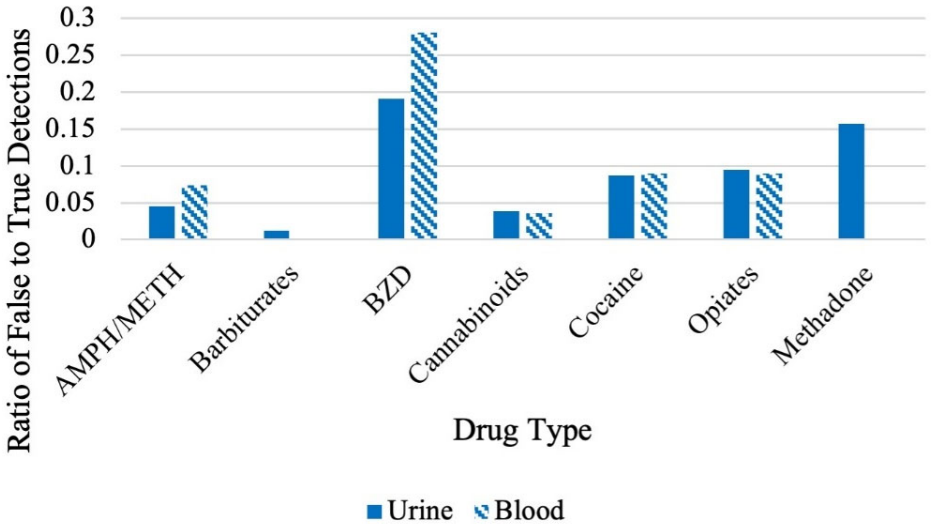

Figure 3. Ratio of false to true immunoassay detections in urine vs. blood (13) Urine and blood testing are comparable overall, with urine performing noticeably better when detecting benzodiazepines and amphetamines/methamphetamines. Blood performs noticeably better when detecting methadone.

To determine whether blood or urine immunoassays lead to better performance, the same metric as before was used as well as the same panel of drugs. The performances of blood and urine tests, as shown in Figure 3, are comparable for cannabinoids, cocaine, and opiates. However, they reveal interesting discrepancies for amphetamine/ methamphetamine, barbiturates, benzodiazepines, and methadone. For amphetamine/methamphetamine, the urine ratio is 61.4% that of blood, and in benzodiazepines, this percentage increases slightly to 68.1%. For barbiturates and methadone, this percentage is undefined because the blood screenings have no error. For the remaining cases, these percentages are 108.5% (cannabinoids), 97.4% (cocaine), and 105.6% (opiates).

The p-value for blood and urine immunoassays is 44.4%, showing that, while discrepancies exist, both analysis types exhibit similar detection abilities overall. Some of these discrepancies likely result from different detection windows for urine and blood, as seen in Table 2.

Noting that amphetamine/methamphetamine and benzodiazepines show the greatest quantifiable discrepancies between urine and blood testing, these two drug types should be the primary focus when analyzing detection windows. Benzodiazepines show a more noticeable correlation, as the much longer detection window in urine—compared to blood—clearly corresponds to a much lower frequency of false results. A longer detection window implies a longer half-life (the amount of time that needs to pass for the drug concentration to decrease by 50%), meaning that the drug concentration is decreasing at a much slower rate (54). In essence, a higher benzodiazepine concentration is available in

urine for a longer duration than in blood, allowing for more accurate detection.

However, only comparing the detection windows fails to account for the disparity in amphetamine/methamphetamine detections; although the detection window for urine is still longer, the discrepancy between the two windows has grown much smaller. Thus, the value of the detection window itself also needs to be considered. One value to note is the 12-hour window for amphetamine in blood. Applying the same rationale as above, it becomes clear that such a short window implies a short half-life, which makes capturing drug samples at peak concentration difficult because of how quickly the drug leaves the bloodstream. This complication leads to a greater possibility of false negatives and explains why blood performs worse than urine for amphetamine/methamphetamine—most likely, this phenomenon results more from the narrow detection window for amphetamine than the more generous detection window for methamphetamine.

One fact worth mentioning is that even though heroin, an opiate, has a 6-hour detection window in blood, blood testing still performs slightly better than urine testing for opiates as a collective. This could result from the fact that urine immunoassays only detect opiate metabolites, while blood immunoassays detect the parent opiate—ingested materials like poppyseed could cause false positives when screening for metabolites in urine. Similarly, blood is more effective than urine at detecting methadone, a purely synthetic opioid, since the substance can be mistaken for medications like verapamil (a blood pressure medication) in urine (55, 56). This can again stem from the fact that urine screenings for methadone detect the unchanged drug as well as metabolites, which can be confused with similar compounds.

As for barbiturates, the results are inconclusive: even though barbiturate detections in blood show zero error, the false-to-true-ratio in urine is the lowest among all the urine ratios; should more samples be studied, it is probable that the urine and blood tests would exhibit similar behavior.

However, one issue remains unresolved: the theory that a longer detection window leads to better accuracy is no longer supported when comparing urine tests across different drugs. For instance, although benzodiazepines have the longest detection window (3 – 6 weeks), they exhibit a much higher false-to-true-ratio than barbiturates (2 – 4 days).

To explain this issue, additional information needs to be considered, namely the analytes detected for each drug. Table 2 reveals why benzodiazepines have the highest false-to-true ratio and why barbiturates have the lowest: benzodiazepines are only excreted as metabolites, a possible source of error—even though various forms of benzodiazepines abound, the immunoassay itself has a limit as to which metabolites it tests for. The immunoassay typically only tests for nordiazepam, oxazepam, and temazepam, leaving other metabolites like lorazepam and clonazepam often undetected (4). The danger of undetected benzodiazepines lies in the fact that they are some of the most widely prescribed drugs for anxiety disorders, seizure disorders, and insomnia. Because of their prevalence as well as the ease of procuring them, many opportunities exist for abusing benzodiazepines. Potentially lethal practices include taking benzodiazepines with other drugs, like alcohol and other depressants (57).

On the other hand, barbiturate immunoassays test for the original compound and thus do not face many of the issues that benzodiazepine immunoassays do. Barbiturate excretions exhibit a moderate MD of 2.0 – 4.0, while benzodiazepines are rarely detected unchanged and hence exhibit a much higher MD. This can be seen from the fact that the MD of benzodiazepines in hair reaches a maximum of 110.

As for the remaining drugs, their MDs and relative detection windows largely align with their false-to-true-detection ratios in urine. It should be noted that, compared to benzodiazepines, the other drugs have relatively few metabolites. Thus, even when urine only detects metabolites of a certain drug, a high MD combined with metabolite-only detection could result in improved detection compared to the case of benzodiazepines.

Urine vs. blood testing in LC-MS

Attaining a level of specificity unparalleled in immunoassays, LC-MS can quantify the exact amount of a drug and/or its metabolites present in a sample. This precision counters the quick, yet generic, detection process of immunoassays, which can only reveal the drug type of the abused substance (58). Confirmatory testing, commonly using GC-MS or LC-MS, is only requested after an immunoassay produces positive results and more detailed analysis is required to ascertain whether the detection is a false or true positive (59). This section examines the LC-MS method instead of the GC-MS because the former involves a second separation and/or derivatization step during sample preparation and further reduces the probability of other substances interfering with detection results (4). Comparing how urine and blood perform with LC-MS reveals which evidence type is more suitable for precise, in-depth analysis.

Two research publications—one published by the Shimadzu Corporation and the other by Agilent Technologies—will be used to provide relevant data, with the former presenting the results from urine LC-MS and the latter describing blood LC-MS (7,23). The data used will mainly comprise of the observed accuracies of urine and blood testing; as opposed to simple tallies of true and false detections, as used with immunoassays, accuracies reveal much more about the specific detection capabilities of each evidence type.

The metric of comparison will be deviation in experimental accuracy (Equation 2). Even though it would be ideal to use the same panel of drugs to analyze both immunoassays and LC-MS, data limitations exclude barbiturates, cannabinoids, and cocaine from the LC-MS panel; 3,4-Methylenedioxymethamphetamine (MDMA), commonly known as ecstasy, will be analyzed as a replacement. Since LC-MS can detect not just the class but also the exact identity of the drug present, the category of opiates, as used to examine immunoassays, has been replaced by codeine, a specific opiate (58).

Another challenge for analyzing accuracies between the datasets is that the data for urine examines spiking concentrations of 20 ng/mL, 50 ng/mL, and 200 ng/mL, whereas the data for blood examines concentrations of 0.1 ng/mL or 0.5 ng/mL (depending on the drug), 1 ng/ mL, and 20 ng/mL. Thus, fair comparisons can only be made at 20 ng/mL. One final challenge is that the publication on blood LC-MS describes using two methods of sample elution: centrifugation and positive pressure. The accuracies for the two methods are dissimilar (7). Since this paper is not concerned with

distinguishing between the two, weighted averages of the accuracy deviations will be taken to represent the accuracy deviation of blood LC-MS as a whole; the reason for taking weighted averages is that more samples were eluted by positive pressure than by centrifugation.

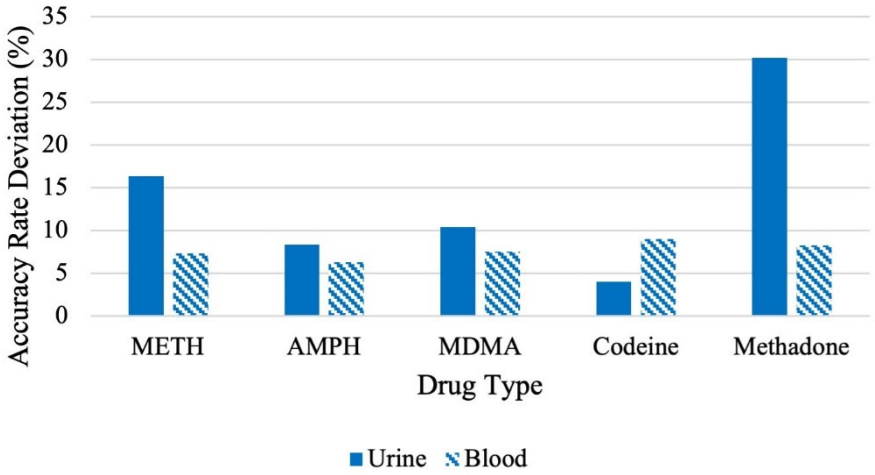

Figure 4. Accuracy deviations of urine vs. blood LC-MS at 20 ng/mL (7,23). Blood performs better than urine for all drugs (especially methadone) except codeine.

While comparable for immunoassays, the performances of urine and blood tests show drastic discrepancies for LC-MS. As opposed to the p-value of 44.4% in immunoassays, the p-value for LC-MS is 10.6%. Although neither p-value is statistically significant, the decrease from 44.4% to 10.6% suggests that the difference between the two analysis types has become more apparent.

Figure 4 shows the accuracy deviations of urine and blood across all five drugs. Blood tests perform noticeably better than urine tests at 20 ng/mL for all drugs except codeine. The accuracy deviation in blood is 2.05% (AMPH) to 21.95% (methadone) lower than in urine for the remaining drugs. For codeine, the accuracy deviation in urine is 5% lower than in blood. One reason that urine LC-MS performs better than blood LC-MS for codeine specifically is the high MD of codeine in urine, as presented in Table 2. Given that the LC-MS detects both metabolites and parent drugs, it would be beneficial to have a high MD to allow for enhanced detection: a high metabolite concentration can mean that a variety of metabolites are abundant in urine, increasing the number of analytes that can be reasonably detected. Since the LC-MS is able to detect specific analytes, the risk of confusing the target analyte with a similar substance is lowered. The MD of codeine in blood is likely to be lower than that the MD of codeine in urine, which means that urine LC-MS has superior detection capabilities for codeine.

The LC-MS results seemingly contradict the detection window argument made in the immunoassay section—since the detection windows of blood are often much lower than in urine (Table 2), it would be expected that the drug concentration in blood also decreases at a faster rate, making urine the better candidate for drug detection. However, blood testing performs significantly better than urine testing, a testament to the specificity of the LC-MS. Despite how rapidly drug concentrations may decrease in blood, the LC-MS can detect drugs

at significantly lower doses, negating the argument that short detection windows necessarily lead to worse performance. As presented in Table 1, the LOQs and LODs of blood LC-MS are much lower than those of urine LC-MS, explaining the superior detection ability of the former.

Another way to compare urine and blood LC-MS is to observe how changes in drug concentration affect accuracy rates. Figure 5 shows that drug concentration is inversely proportional to accuracy deviation in urine LC-MS. However, Figure 6 shows a more interesting relationship between drug concentration and the accuracy of blood LC-MS; going from 0.1 – 0.5 ng/mL to 1 ng/mL, accuracy deviation increases in methamphetamine, slightly increases in methadone, slightly decreases in MDMA and codeine, and steadily decreases in amphetamine. Going from 1 ng/mL to 20 ng/mL, accuracy deviation across all drugs increases.

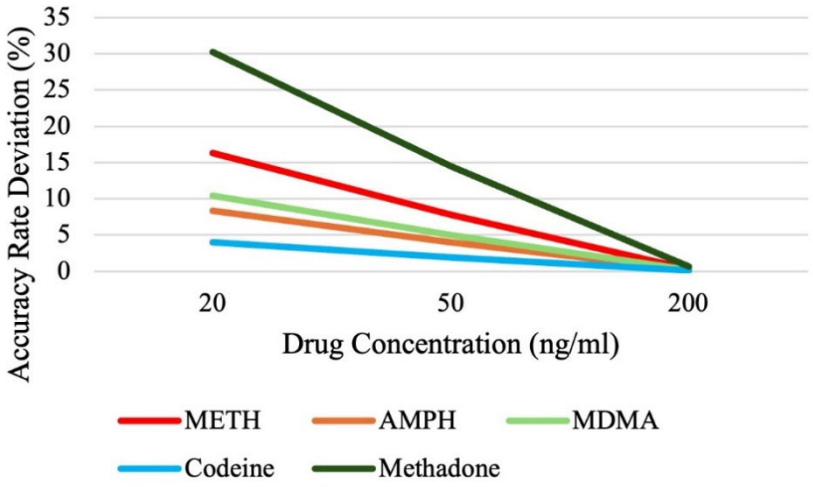

Figure 5. Change in accuracy deviations of urine LC-MS across three concentrations (7). As drug concentrations increase, the detection ability of urine LC-MS increases.

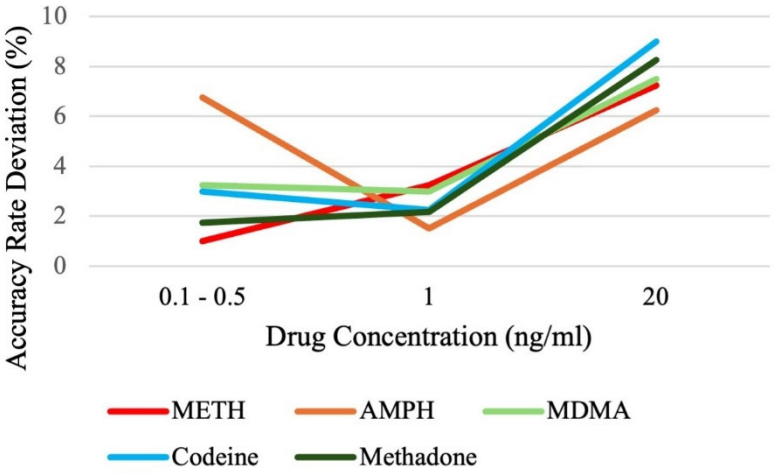

Figure 6. Change in accuracy deviation of blood LC-MS across three concentrations (7). Ambiguous trends exist for this dataset concerning blood LC-MS. As drug concentration changes from 1 ng/mL to 20 ng/mL, the detection ability of blood LC-MS decreases for all drugs.

While the trends in urine LC-MS accuracy are largely expected—higher spiking concentrations should ideally make the target drug easier to detect—those in blood LC-MS accuracy are counterintuitive. These results were initially attributed to matrix interference, the process in which unwanted substances in a sample interfere with the detection of the target substance. However, the publication on blood LC-MS shows that the experiment accounted for potentially problematic matrix interference by specifically increasing the LOQs of amphetamine, heroin, and lorazepam from 0.1 ng/mL to 0.5 ng/mL; this change indicates that matrix interference posed more of an issue for detecting these drugs at low concentrations, directly countering the prediction that matrix interference causes inconsistencies at high concentrations (7).

Based on conversations with Limian Zhao, the author of the publication, the trends in blood LC-MS likely stem from experimental error. The author related the fact that she used different spiking solutions for the different concentrations during matrix spiking, a process that tests for the accuracy of a detection method. The test works by applying the detection method to a solution with a known amount of analyte (the spiking solution) and finding if the amount added has been detected (60). Limian Zhao mentioned that the use of different spiking solutions across the different concentrations could have resulted in result variation. She further stated that, given the high possibility of human error, three data points are not enough to establish a trend. Thus, even if Figures 5 and 6 suggest that urine testing is more reliable at high concentrations, the data is insufficient to completely prove this claim.

Comparing hair testing to traditional methods

While urine and blood testing are commonly used to detect recent drug use, hair testing is known to retain evidence for up to 90 days (20). Thus, hair is the ideal medium through which toxicologists glean information about historical drug use, as the distribution of substances in hair reveals the quantities and chronology in which the substances were ingested. In 1994, a Chinese college student was poisoned by thallium, a case that initially left many investigators puzzled because her symptoms did not point to any common illness (61). Furthermore, the paucity of evidence meant that the circumstances surrounding her poisoning were unknown. However, a 2018 publication detailed extensive analysis of her hair samples, revealing a period of intermittent exposure to thallium in 1994 as well as a period of continuous exposure in 1995. These results provided the student's parents some lasting closure and prove how useful hair analysis can be for examining past ingestion of toxins (62). By examining how hair immunoassays and LC-MS compare with urine and blood testing, this section aims to uncover the extent to which hair testing proves effective.

Palamar et al. compared the performances of urine and hair immunoassays. The sensitivity (the number of true positives divided by the sum of true positives and false negatives) and specificity (the number of true negatives divided by the sum of true negatives and false positives) of both evidence types for all drugs was examined (19,63). The same metric as the one used in this paper—the ratio of false to true detections—was calculated using the sensitivities and specificities provided.

One important disclaimer to note is that the results of urine and hair testing were judged against self-reported drug use, hence while the reported results may largely be accurate, there

remains a certain level of under-reporting (19). This would mean that the calculated specificities are lower than the actual values.

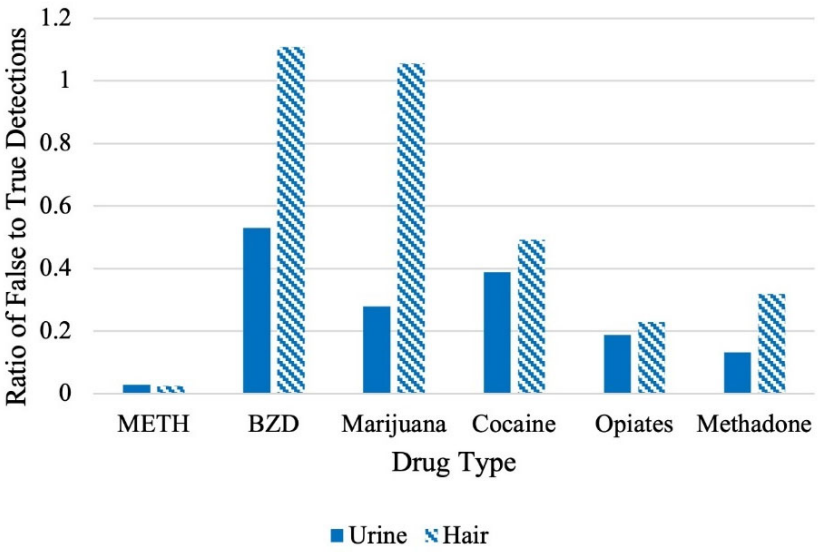

Figure 7. Ratio of false to true immunoassay detections in urine vs. hair (19). Hair immunoassays perform worse than urine immunoassays for all drugs, most notably benzodiazepines and marijuana, except methamphetamine.

Figure 7 shows that hair immunoassays largely perform worse than urine immunoassays; only for methamphetamine does the former exhibit better performance, with the hair false-to-true ratio being 80.6% that of the urine ratio. For the remaining drugs, the hair ratios are 210% (benzodiazepines), 377% (marijuana), 127% (cocaine), 122% (opiates), and 244% (methadone) those of their corresponding urine ratios. It should be noted that confirmatory testing via LC-MS was used to validate hair immunoassay results for methamphetamine and opiates, which could explain why these two drugs showed more success with hair analysis. Another reason why methamphetamine resulted in fewer false detections with both urine and hair is the low sample size, as only 2.3% (12 people) of the total sample population reported methamphetamine use (25).

After combining the results of Figures 3 and 7, it becomes clear that hair immunoassays are more susceptible to error than either urine or blood immunoassays. However, it should be noted that the p-value is 9.28%. While this value is not statistically significant, there nevertheless exists a noticeable pattern of hair showing greater error margins than urine.

One compelling reason why hair analysis performs worse is that hair can take 7 – 10 days to grow the segment that contains the target substances (25). However, because the experiment referenced in Figures 7 needed to ensure that both urine and blood samples could be captured at acceptable concentrations, the samples were likely taken within narrow detection windows (shown in Table 2). Such time constraints would have prevented the drugs present in hair from reaching detectable concentrations, and the fact that immunoassays lack high specificity by default exacerbates this issue.

For marijuana specifically, the reason why such a great discrepancy exists between urine and

hair analysis could be that hair testing naturally exhibits low sensitivity for cannabinoids. Intermittent or infrequent use of the drug could also result in challenges with hair testing (63). A study conducted by Huestis et al. revealed that hair immunoassays can detect 65% of cases involving daily cannabis use and 30% of cases involving non-daily cannabis use. Across all samples, the immunoassays detected 43% of cannabis use (19). These results reveal not only how frequency of use affects cannabinoid detections in hair, but also how hair testing often leave cases of cannabis use undetected.

To see how hair testing compares with urine and blood testing through LC-MS, an article published by Hegstad et al., which contains data on the accuracy of hair LC-MS, was used alongside the publications referenced in Section 4 (2). The same panel of drugs used to examine LC-MS performance in Section 4 was used here, although some computational changes will be implemented going forward. Since cutoff values for urine and blood are measured in ng/mL whereas those for hair are measured in ng/mg, comparing accuracy values at the same spiking concentration no longer proves feasible. While the same metric was used as before (deviation in accuracy), the accuracy deviations compared referred to the average deviations across all concentration levels.

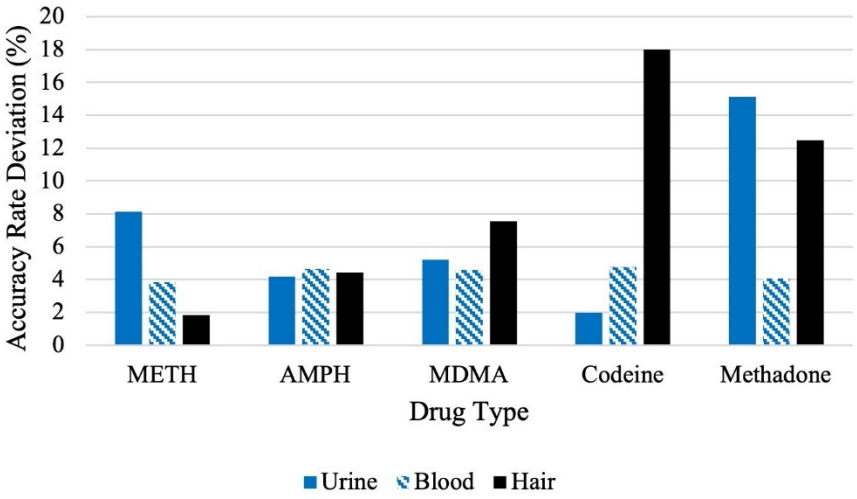

Figure 8. Average Accuracy Deviations of Urine vs. Blood vs. Hair LC-MS (2,7,23). Hair LC-MS is still slightly more error-prone than blood LC-MS and urine LC-MS. Hair performs better than both blood and urine when detecting methamphetamines but performs much worse when detecting codeine.

Figure 8 shows that hair performs worse than both urine and blood for MDMA and codeine, although it performs better than both for methamphetamine. For amphetamine, hair performs better than blood but worse than urine, and for methadone, hair performs better than urine but worse than blood. The reason why hair notably shows better performance for methamphetamine with reference to the other drugs can be that the MD of meth exhibits a wider range, as seen in Table 2. It can be that certain ratios of metabolite concentration to parent drug concentration allow for optimal detection, and with the narrower ranges of the other drugs, the optimal detection ratio is more difficult to discover.

To further put into perspective how hair testing compares with preferred methods of analysis, two separate t-tests were taken for the same

panel of drugs in Figure 8, one for hair and urine, and one for hair and blood. The p-value for hair and urine was 30.5%, and that for hair and blood is 7.97%. While hair testing is more comparable to urine testing through LC-MS (as opposed to immunoassays), the former is still lees accurate when compared to blood testing. In fact, errors in hair testing most commonly occur during opioid detections, with codeine and methadone being the opioids in question.

Even though it is unclear why hair testing shows much higher accuracy deviations for opioids, one factor could be the fact that some opioid specimens came from test subjects in a study on naltrexone implants (2). Naltrexone, a medication designed to mitigate opioid addiction, works by preventing opioids from reaching their targeted brain receptors, thus reducing their potency (65). While the specifics of the naltrexone study are unclear, it is possible that the test subjects experienced intermittent opioid exposure during their treatment; as was the case for marijuana detections with hair immunoassays, non-continuous drug use could have resulted in missed detections. This rationale would explain why the accuracy deviations in codeine and methadone indicate a high proportion of undetected specimens; the average accuracy deviations, without absolute values, are -18% for codeine and -12.5% for methadone (2).

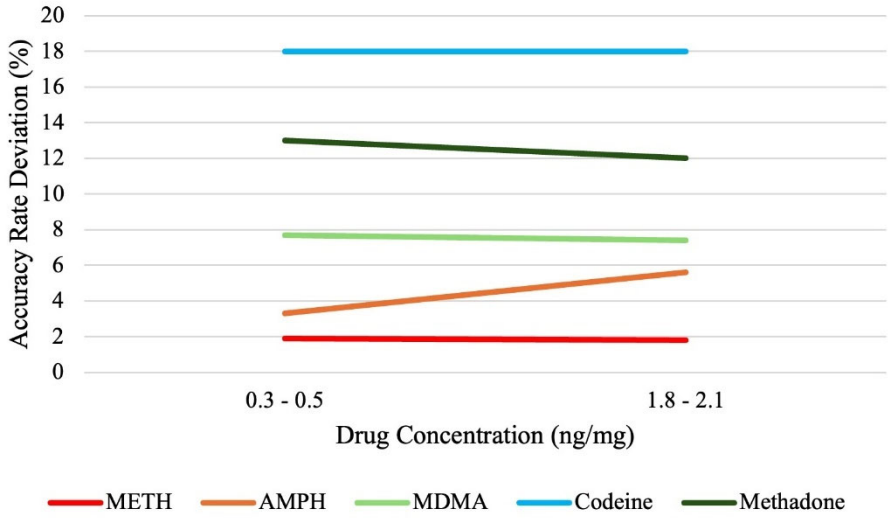

Figure 9. Change in hair LC-MS accuracy deviation across two concentrations (2). No clear trend exists when associating accuracy deviation to drug concentrations in hair LC-MS. Different drugs show different behavior.

As seen in Figure 9, changes in the accuracy of hair LC-MS across different concentrations are not significant, the reason for which could be that the gap between the observed concentrations is not as much as the ones observed for hair and blood LC-MS. Furthermore, two concentrations are insufficient to determine overall trends, so while there may appear to be unexpected results in the graphs, the difference in accuracy deviation for any of the five drugs never exceeds 2%.

Discussion

One of the objectives of this paper was to determine whether hair testing, a relatively niche form of toxicological analysis, has the potential to replace urine and blood testing. A thorough analysis into the efficacy of traditional methods was also performed, not only to

compare them with one another, but also to establish standards against which the performance of hair testing could be judged.

In general, the performances of urine and blood immunoassays were comparable. The only notable points of discrepancy occurred when testing for amphetamine/methamphetamine, benzodiazepines, and methadone; urine testing performed much better for the first two, whereas blood testing performed much better for the third. When LC-MS was involved, blood testing performed better than urine testing for most drugs. However, urine testing showed a consistent increase in accuracy as drug concentrations increased, whereas blood testing did not; while this detail could be taken to imply that urine testing was more reliable at higher concentrations, the unexpected trends in blood testing could simply be attributed to experimental error. Finally, hair immunoassays largely performed worse than both urine and blood immunoassays, exhibiting much higher error margins. With LC-MS, hair testing showed improvements, although there were significant error margins when detecting codeine and methadone. Thus, hair testing underperforms relative to urine and blood testing in terms of accuracy.

It should be noted that experimental limitations affected the outcome of this analysis. First, because obtaining data form the open Internet is much more difficult than it is from a comprehensive toxicology database, the lack of data was the most notable source of error. Another limitation would be that results from different experiments were used, and so the external factors present in each experiment were different from one another, thereby generating artifacts and reducing the comparability of the data sets. Thus, while systemic, quantitative methods of comparison were used throughout this paper, it was inevitable that undesirable circumstances impacted the outcomes. It was not known if all of the assays were validated for linearity and range, precision, accuracy, robustness and system suitability. It was also not known if the assay or metabolites were subjected to mass-balance. The time elapsed between drug ingestion and assay was not presented in any of the papers.

Overall, the results of this paper disprove the idea that hair analysis can act as a replacement for blood and urine testing. Nevertheless, hair testing remains a powerful tool for determining past drug ingestion. This property is unknown to both urine and blood testing, meaning that further research into the limits of hair testing can greatly advance the field of forensic toxicology.

For instance, one possible experiment could investigate how the lipophilicity of a drug correlates with accuracy in hair testing. Since lipophilic drugs are much more likely to be deposited in the cell membranes of hair cells, this observation posits that a higher degree of lipophilicity in drugs leads to more accurate detections (21). To calculate lipophilicity, a chemical parameter called log P was used, with a high log P value indicating high lipophilicity. However, even though methadone has a log P value of 5 (a very high log P value for a drug—amphetamine only has a log P value of 1.76, and morphine, another opioid, has values ranging from 0.8 to 2), hair LC-MS shows surprisingly high error margins for methadone detections (66). Thus, the relationship between lipophilicity and ease of detection seems more complicated. Further research into this topic would not only elucidate the exact relationship but also identify any other factors at play—for instance, it could be the case that different drugs permeate into different layers of hair, and those that are more easily detected have higher

permeability and reach deeper levels of hair. As evidence of drug use can be washed away, it would be expected that drugs with higher permeability face a lower chance of being removed beyond the limit of detection.

Conclusion

The accuracy of immunoassays and liquid chromatography-mass spectrometry (LC-MS) to analyze opioid and non-opioid substances in the blood, urine and hair is a function of the assay method itself; i.e., its sensitivity (LOD, LOQ) and its specificity; i.e., its ability to detect parent drug and/or metabolites. Therefore, judicious selection of one of these two methods must take into account, the recency of the analysis after drug(s) ingestion; i.e. the detection window, the drug's Absorption, Distribution, Metabolism and Excretion (ADME) profile and the in vivo metabolic pathway of the ingested drug(s).

Hair testing was less accurate than urine and blood testing for both immunoassays and LC-MS. While urine and blood testing yielded similar accuracy for immunoassays, the accuracy of LC-MS was significantly greater than immunoassays when testing for analytes in the blood. However, hair was nonetheless the most effective at preserving drug evidence for long durations of time (up to years). Regarding short-term drug detection, blood testing is preferred if the test results are desired to be specific and comprehensive. Urine testing is preferred to blood testing for cheap, easy detections; even though urine and blood testing show similar performance, the detection windows in urine testing are longer than in blood testing, resulting in better preservation of evidence.

Acknowledgements

I would like to thank my research mentor, Alexander Pomberger, for his continuous support and helpful insights, which generated numerous thoughtful discussions. I would also like to thank my writing coach, Rose Wang, for her willingness to help as well as her prompt, specific feedback. Finally, I would like to thank the Lumiere Research Scholar Program for giving me the opportunity to conduct and showcase my research.

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