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Note d'application

The Important Contribution of Glucuronide Metabolite Hydrolysis to Detection and Quantitation in Urine Drug Testing

TG Rosano, JP Danaceau, M Wood, RM Konetchy Jr, SMT Islam, J M Rumberger

[a]National Toxicology Center at Life Sciences Innovation Building, Albany, NY

[b]Department of Pathology and Laboratory Medicine at Albany Medical Center, Albany, NY

[c]Waters Corporation, Milford, United States

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Abstract

Phase 2 drug metabolism, catalyzed by UDP-glucuronosyltransferase (UGT) isoforms, may contribute significantly to urinary elimination of many drugs and their Phase 1 metabolites. While direct testing for the glucuronidated metabolites is not routinely performed in definitive testing for large panels of drugs and metabolites (analytes), glucuronidase hydrolysis is widely used to test for combined free and glucuronidated forms. Drug-prevalence findings from definitive urine drug testing in 400 emergency department patients suspected of drug misuse was recently reported. A large panel of drugs and Phase 1 metabolites (analytes) were tested with use of a rapid glucuronidase hydrolysis and with identification of over 1500 drugs and metabolites.

This current work, featuring the Xevo™ TQD Mass Spectrometer and UPLC I-Class Plus, expands upon those data and determines the contribution of hydrolysis to positive drug findings.

Benefits

- Applies multi-analyte definitive UPLC-MS/MS method in urine drug and metabolite testing
- Demonstrates importance of glucuronidase hydrolysis in drug detection and quantification

Introduction

Drug testing by liquid chromatography–tandem mass spectrometry (LC–MS/MS) is widely used in clinical and forensic toxicology because of the technique's analytical specificity, broad analyte coverage, and ability to detect both parent drugs and metabolites.[1] Interpretation of urine drug results is complicated by extensive Phase II metabolism, particularly glucuronidation, which can account for the majority of urinary drug-related material for opioids, benzodiazepines, cannabinoids, antidepressants, and several other therapeutic or psychoactive drug classes.[2-4] Analytical approaches that measure only the unconjugated ("free") analyte may therefore underestimate total drug burden and risk clinically significant false-negative results.[5,6]

To mitigate this issue, laboratories often incorporate β-glucuronidase hydrolysis prior to LC–MS/MS analysis to cleave glucuronide conjugates and release the aglycone.[7] However, hydrolysis efficiency varies greatly depending on the enzyme preparation, reaction conditions, and the structural characteristics of the glucuronide being analyzed. Traditional β-glucuronidase enzymes derived from *Helix pomatia*, *Patella vulgata*, or bacterial sources show substantial variability in substrate specificity and reaction kinetics.[8,9] These limitations are especially pronounced for N-glucuronides, which are more resistant to enzymatic cleavage and may remain partially conjugated even under prolonged incubation.[10] Inadequate hydrolysis of benzodiazepines, opioids, and psychoactive compounds has been shown to reduce detection sensitivity and contribute to false-negative results in clinical and forensic settings.[11–13]

The development of recombinant β-glucuronidase enzymes has addressed several limitations of traditional preparations. Glucuronidase variants produced by site-directed mutagenesis show increased hydrolytic activity, and formulations that incorporates multiple recombinant β-glucuronidase isoenzymes improve substrate coverage and hydrolysis efficiency across structurally diverse conjugates.[14,15] Comparative studies show that

recombinant enzyme blends provide more complete and consistent hydrolysis of both O- and N-glucuronides than many traditional enzyme sources.[11] Despite these advances, relatively few investigations have assessed the clinical impact of hydrolysis on large, unselected patient populations. Most studies have evaluated hydrolysis performance using spiked matrices and analyte-focused method development. Consequently, the extent to which omission of hydrolysis affects detection rates, measured concentrations, and clinical interpretation in real-world patient care settings—such as emergency medicine—remains insufficiently characterized.

Findings from a definitive LC–MS/MS urine drug testing study involving 400 emergency department patients, illustrating the complexity of drug exposure patterns in acute care settings has been recently reported.[16] The definitive drug testing method, as well as an isomer specific cannabinoid method were developed and validated using a Waters Xevo TQD Mass Spectrometer coupled to a Waters ACQUITY™ I-Class PLUS UHPLC System. In the present study, all specimens with and without recombinant enzymatic hydrolysis, were reanalyzed allowing direct comparison of total (post-hydrolysis).

The objective of this study was to determine the extent to which recombinant glucuronidase enzyme hydrolysis improves detection of drugs and metabolites in an emergency department patient setting, along with a characterization of analyte-specific and drug-class-specific patterns in glucuronide-dependent detection.

Experimental

Sample Preparation

Definitive drug testing with and without β-glucuronidase hydrolysis was performed on urine samples obtained from 400 patients presenting to the Albany Medical Center Emergency Department. A study of drug prevalence, using only hydrolyzed urine samples from the patient cohort, has been previously reported.[16] The study was reviewed and authorized by the Human Study Committee of the Albany Medical College.

Analytical Methods

The additional testing with and without hydrolysis was performed for a panel of 110 drugs and Phase 1 metabolites (analytes) use two quantitative UPLC-MS/MS methods. A Threshold Accurate Calibration (TAC) method tested for 104 of the panel analytes and used a novel matrix normalization technique. A complete description of the concept and validation of TAC, along with a step-by-step protocol for the analysis, has been previously published.[17–19] All urine samples were tested by the TAC method with and without a 10-fold dilution

with analyte-negative urine to achieve the analytical ranges as shown in Table 1. A cannabinoid isomer-specific method was used for detection and quantitation of the additional analytes in the panel including Δ9 THC, Δ8 THC, Δ9 cTHC, Δ8 cTHC, 11-hydroxy THC and CBD (20). UHPLC conditions for both methods are shown in Tables 2-5. Both TAC and cannabinoid testing was performed with and without the glucuronidase hydrolysis using a recombinant-sourced reagent (IMCSzyme RT, Irmo SC) employing two β-glucuronidase variants selected for optimized hydrolysis.[16] Waters Application Notes for both analytical methods, including examples of validation workflow for addition of new panel analytes, are also available.[21,22] A list of all panel analytes along with their detection and quantitation limits is shown in Table 1.

Drug Class	Drug/Metabolite	Detection Limit (ng/mL)	Upper Quantitative Limit (ng/mL)
Amphetamines	Amphetamine	40	10000
	Methamphetamine	20	10000
	MDA	7.5	1000
	MDEA	4	1000
	MDMA	4	1000
	Ephedrine	20	10000
	Pseudoephedrine	20	10000
	Phentermine	20	10000
Benzodiazepines	Alprazolam	20	10000
	Alprazolam, α-hydroxy	20	10000
	Bromazolam	2	1000
	Clonazepam, 7-amino	20	10000
	Diazepam	20	10000
	Nordiazepam (Diazepam, nor)	20	10000
	Etizolam	4	1000
	Flualprazolam	2	1000
	Flunitrazepam, 7-amino	10	5000
	Lorazepam	20	10000
	Midazolam	20	10000
	Midazolam, α-hydroxy	2	1000
	Oxazepam	20	10000
	Temazepam	20	1000
	Triazolam, α-hydroxy	20	10000
Cannabinoids	Δ9 THC	10	1000
	Δ8 THC	10	1000
	Δ9 THC, carboxy	10	1000
	Δ8 THC, carboxy	10	1000
	11-OH THC	10	1000
	Cannabidiol (CBD)	10	1000
Other Illicits	Cocaine	10	5000
	Cocaethylene	10	5000
	Benzoylecgonine	10	5000
	6-Acetylmorphine	2	1000
	Phencyclidine (PCP)	2	1000
	Xylazine	10	5000
	Xylazine, 4-hydroxy	10	5000
Gabapentinoids	Gabapentin	400	100000
	Pregabalin	200	50000
Opioid Analgesics	Codeine	20	10000
	Dihydrocodeine	20	10000
	Hydrocodone	20	10000
	Hydromorphone	20	10000
	Hydrocodone, nor	20	10000
	Morphine	20	10000
	Oxycodone	20	10000
	Oxycodone, nor	20	10000
	Oxymorphone	20	10000
	Oxymorphone, nor	20	10000
	Buprenorphine	2	1000
	Buprenorphine, nor	4	1000
	Fentanyl	1	500
	Fentanyl, nor	1	500
	Fentanyl, beta-hydroxy	1	500
	Fentanyl precursor (4-ANPP)	1	500
	Meperedine	20	10000
	Normeperedine	20	10000
	Methadone	20	10000
	EDDP (Methadone metab.)	20	10000
	Nalbuphine	20	10000
	Tramadol	20	10000
	Tramadol, N-desmethyl	20	10000
	Tramadol, O-desmethyl	20	10000
	Tapentadol	20	10000

Drug Class	Tapentadol	20	10000
Opioid Antagonists	Naloxone	20	5000
	Naloxone, nor	20	5000
	Naltrexone	20	5000
Nicotine Agonist	Nicotine metab. (Cotinine)	20	10000
Ketamine	Ketamine	2	1000
	Ketamine, nor	2	1000
Dextromethorphan	Dextromethorphan	20	10000
	Dextrorphan	20	10000
Methylphenidate	Methylphenidate	20	10000
	Ritalinic acid	20	10000
Hallucinogen	Psilocin metab. (Psilocin)	2	1000
Mitragynine	Mitragynine	2	1000
	Mitragynine, 7-hydroxyl	2	1000
Antipsychotics	Aripiprazole	40	10000
	Clozapine	20	10000
	Haloperidol	20	10000
	Lurasidone	40	10000
	Olanzapine	20	10000
	Quetiapine	20	10000
	Risperidone	20	10000
	Ziprasidone	40	10000
Antidepressants	Amitriptyline	20	10000
	Nortriptyline	20	10000
	Bupropion	20	10000
	Bupropion, hydroxy	20	10000
	Citalopram	20	10000
	Clomipramine	20	10000
	Imipramine	20	10000
	Desipramine	20	10000
	Duloxetine	20	10000
	Doxepin	20	10000
	Doxepin, nor	20	10000
	Fluoxetine	20	10000
	Fluoxetine, nor	20	10000
	Mirtazapine	20	10000
	Paroxetine	20	10000
	Sertraline	40	10000
	Trazodone	20	10000
	Trazodone metab. (mCPP)	20	10000
	Venlafaxine	20	10000
Synthetic Stimulants	Alpha PVP	2	1000
	Eutylone	4	1000
Muscle Relaxants	Carisoprodol	20	10000
	Cyclobenzaprine	20	10000
	Meprobamate	20	10000
Sedatives	Zaleplon	10	5000
	Zolpidem	10	5000

Table 1. A test panel of 110 drugs and metabolites performed by definitive methods. Panel analytes

are listed by drug class and include detection limit (LOD) and upper limit of quantitation (ULOQ) in ng/mL.

Definitive Drug Testing LC Conditions

LC system:	ACQUITY UPLC I-Class PLUS Flow-Through Needle (FTN)
Column:	ACQUITY UPLC BEH™ Phenyl Column 1.7 µm, 2.1 x 50 mm; p/n: 186002884
Column temperature:	45 °C
Sample temperature:	10 °C
Injection volume:	5 µL
Flow rate:	0.6 mL/min
Mobile phase A:	2 mM ammonium formate + 0.1% formic acid in MilliQ® (MilliporeSigma) water
Mobile phase B:	2 mM ammonium formate + 0.1% formic acid in methanol
Wash solvent:	25:25:25:25 methanol:isopropanol:acetonitrile:water

Gradient Table

Time (min)	Flow (mL/min)	%A	%B	Curve
0.0	0.6	98	2	Initial
0.5	0.6	98	2	6
2.2	0.6	30	70	6
2.7	0.6	10	90	6
3.0	0.6	98	2	6
3.3	0.6	98	2	6

Table 2. General UHPLC and gradient conditions for the TAC method used in this application.

MS system:	Xevo TQD Mass Spectrometer
Ionization mode:	ESI+
Capillary voltage:	0.55 kV
Desolvation temperature:	550 °C
Source temperature:	150 °C
Desolvation gas:	800 L/Hr
Cone gas:	100 L/Hr
MS1 resolution:	Unit

MS2 resolution:	Unit

Table 3. Mass spectrometry parameters used for the TAC method.

Cannabinoid Isomer Testing LC Conditions

LC system:	ACQUITY UPLC I-Class PLUS (FTN)
Column:	Cortecs UPLC C_{18}+ Column 1.6 µm, 2.1 x 50 mm; p/n: 186007114
Column temperature:	30 °C
Sample temperature:	10 °C
Injection volume:	10 µL
Flow rate:	0.6 mL/min
Mobile phase A:	0.1% formic acid in MilliQ water
Mobile phase B:	LC/MS grade Acetonitrile
Wash solvent:	95:5 Acetonitrile:water

Gradient Table

Time (min)	Flow (mL/min)	%A	%B	Curve
0.0	0.4	49	51	Initial
3.3	0.4	49	51	6
3.31	0.4	31	69	1
6.3	0.4	13	87	6
6.4	0.4	49	51	1

Table 4. General UHPLC and gradient conditions for the cannabinoid isomer method used in this application.

MS system:	Xevo TQD Mass Spectrometer
Ionization mode:	ESI+/ESI-
Capillary voltage:	2.5kV
Desolvation temperature:	500 °C
Source temperature:	150 °C
Desolvation gas:	800 L/Hr
Cone gas:	100 L/Hr
MS1 resolution:	Unit
MS2 resolution:	Unit

Table 5. Mass spectrometry parameters used for the cannabinoid isomer method.

Results and Discussion

In the 360 patient samples with positive test results, a total of 1,866 specific drug and metabolite were positive with glucuronidase hydrolysis, representing positive findings involving 76 of the 110 analytes in the panel. Analyses were performed on each sample with and without hydrolysis to assess the relative free and conjugated analyte contribution to the routine practice of testing with hydrolysis. Data from an average of 25 matched test results (range 3-249) per analyte were employed in the study. Figure 1 displays the average percent contribution to hydrolysis testing for free versus glucuronidated analyte. The range of free and glucuronidated forms is represented by the minimum and maximum bars for each analyte. The co-analysis showed that hydrolysis contributed to greater than 50% of the test response for 39 out of the 76 analytes tested in the panel. Furthermore, the glucuronide metabolite form was the predominate (>80%) contributor to test response in 24 of the 76 analytes, including analytes in the cannabinoid, benzodiazepine, opioid and psychiatric drug classes.

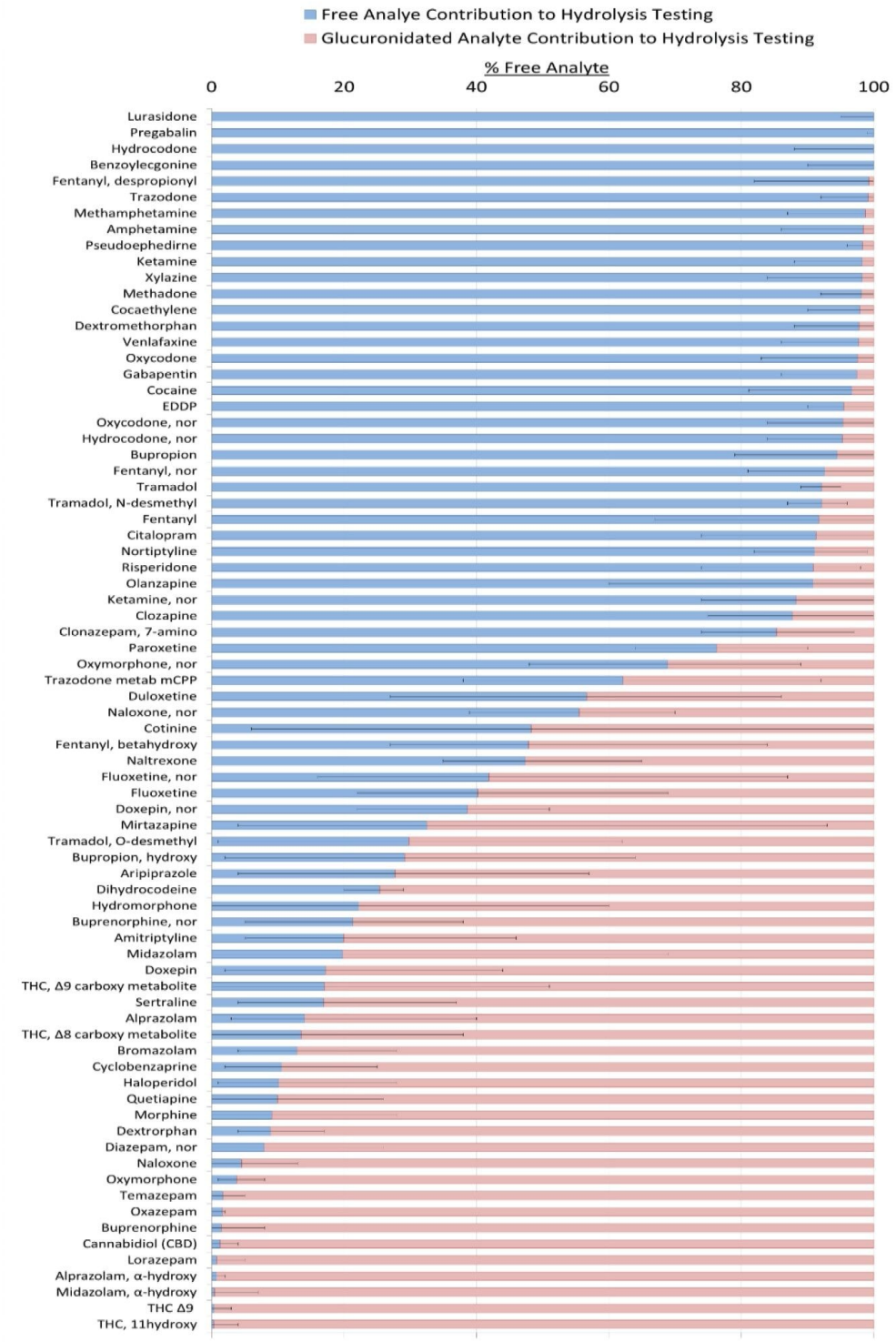

Figure 1. Relative percent free and glucuronidated analyte contribution to test response with

hydrolysis (Mean ± range represented).

Test performance without hydrolysis was initially evaluated qualitatively for detection limit sensitivity. The rate of detection without hydrolysis, which includes testing only for free analyte, was compared with hydrolysis testing in which 100% of the tests exceeded the detection limit by study design. Figure 2 shows the relationship between the average percent free form of analyte versus the rate of analyte detection without hydrolysis. All analytes with an average free-analyte contribution of greater than 90% had detection rates of greater than 99%. Detection rates without hydrolysis decreased precipitately for 32 of the analytes in which the average free-analyte contribution was below 40%. Detection rates in the lowest range of 0-20% were determined in 10% of the analytes tested. Therefore, hydrolysis is critical for detection of drug use for many of the analytes, based on the limits of detection used in this study.

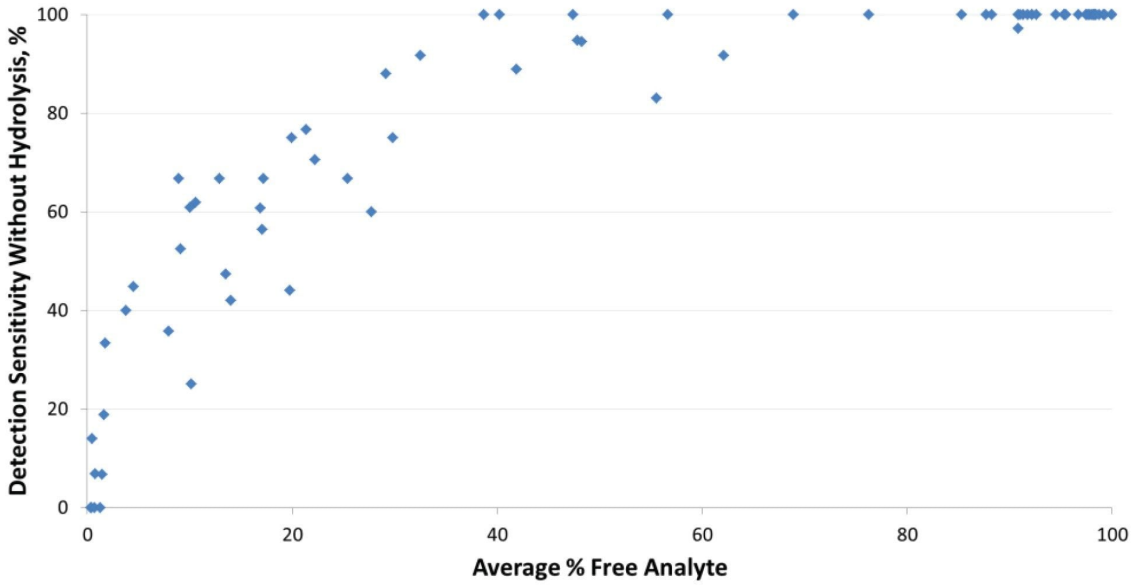

Figure 2. Relationship between the analyte detection sensitivity without hydrolysis and the average percentage of free analyte for the 76 analytes in the study.

False negative results are another risk of incomplete glucuronide hydrolysis. False negative test results were observed without hydrolysis for 39 of the analytes in the study. Figure 3 shows the false negative rate in ranked

order for these analytes. Over 40% (n=16) of the 39 analytes had false negative rates greater than 50% when hydrolysis was not performed, and four of these analytes (cannabidiol, α-hydroxy alprazolam, 11-hydroxy THC and buprenorphine) had false negative rates of 100%.

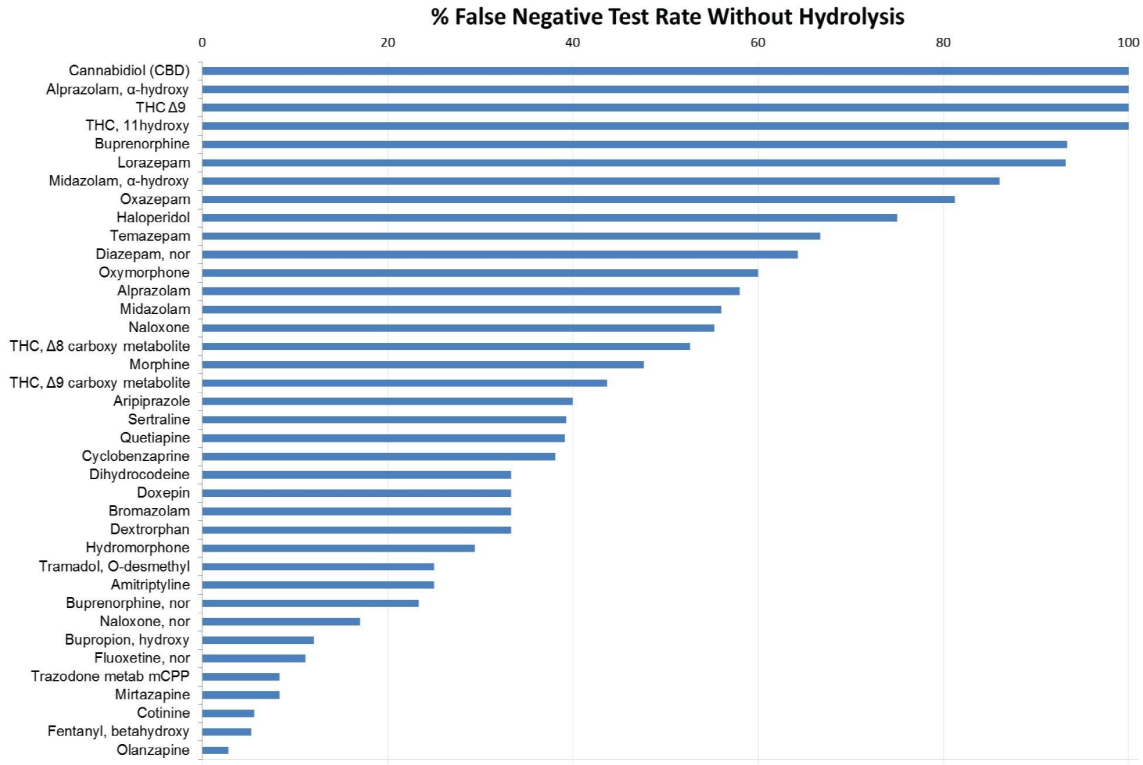

Figure 3. Percent false negative rate in ranked order for the 39 analytes with reduced detection sensitive without hydrolysis.

In addition to the false negative rates that were found in the identification of analytes, based on LOD, the quantitative performance of the testing was compromised for a majority of analytes when hydrolysis was not employed. Table 6 lists 39 analytes in the panel with a greater than >50% contribution of glucuronidated analytes to the total concentration as determined with hydrolysis. Both the median and range of analyte concentration for these analytes was significantly reduced without hydrolysis. A greater than 99% decrement in concentration was observed without hydrolysis for more than 20% (n=16) of the analytes in the panel, including testing across cannabinoid, benzodiazepine, opioid, muscle relaxant and psychiatric drug classes.

Analyte	Median Conc with Hydrolysis	Conc Range with Hydrolysis	Median Conc without Hydrolysis	Conc Range without Hydrolysis	Median Conc Decline
Fentanyl, betahydroxy	23	5.6–310	10.1	ND-210	56
Cotinine	1460	20 - >10,000	641	ND-3,490	56
Duloxetine	326	78–953	139	67–670	57
Hydromorphone	225	24–1,470	94	ND-522	58
Dihydrocodeine	52	76–750	20	ND-510	62
Doxepin, nor	222	146–462	83	48–223	63
Fluoxetine	993	92.2–2700	353	26–1,650	64
Naloxone, nor	65	31–127	20	ND-77	69
Sertraline	300	71–3540	90	ND-724	70
Tramadol, O-desmethyl	950	79 - >10,000	262	ND-2,590	72
Fluoxetine, nor	802	35–1690	215	ND-920	73
Bupropion, hydroxy	2790	73 - >10,000	630	ND-7,750	77
Quetiapine	375	22–2,900	72	ND-266	81
Mirtazapine	687	27–5700	121	ND-2,430	82
THC, Δ9 carboxy metabolite	88.5	10–1980	14	ND-791	84
Bromazolam	156	18–1,330	24	8–343	85
Buprenorphine, nor	387	15 - >1,000	55.5	ND-760	86
Amitriptyline	831	169 - >10,000	107	ND-377	87
Morphine	374	21–10,000	24	ND-4,750	94
Oxymorphone	350	58–2,790	22	ND-84	94
Doxepin	658	90–967	39	ND–39	94
Dextrorphan	5910	254 - >10,000	271	ND - >10,000	95
Cyclobenzaprine	966	45 - >10,000	42	ND-840	96
Alprazolam	192	38–672	0	ND-201	100
Alprazolam, α hydroxy	514	7.1–1050	0	ND	100
Aripiprazole	109	52–279	0	ND-86	100
Buprenorphine	98.5	9–930	0	ND-10	100
Cannabidiol (CBD)	86	18–937	0	ND-10	100
Diazepam, nor	180	46–2760	0	ND-78	100
Haloperidol	107	23–604	0	ND-99	100
Lorazepam	360	20–6910	0	ND-52	100
Midazolam	93	20–1210	0	ND-760	100
Midazolam, α hydroxy	290	23 - >1000	0	ND-15	100
Naloxone	600	14–4580	0	ND-259	100
Oxazepam	290	28–5010	0	ND-54	100
Temazepam	247	21–3610	0	ND-176	100
THC Δ9	23	10–112	0	ND	100
THC, 11 hydroxy	58	10–1,250	0	ND	100
THC, Δ8 carboxy metabolite	68	11–320	0	ND-126	100

Table 6. Analytes with greater than 50% reduction in concentration when tested without hydrolysis.

Median, range and percent decline in concentration are shown. (ND: none detected)

Glucuronidated analytes demonstrated a significant contribution to the quantitative performance of urine drug testing. As shown in Figure 4, the decrease in median analyte concentration without hydrolysis correlated directly, and linearly, with the percentage of glucuronidated analyte in the urine specimen. Regression analysis revealed a slope (1.09) and intercept (-4.4) that were statistically indistinguishable from 1 and 0, respectively. Excretion of an increasing proportion of the analyte in the glucuronidated form therefore decreases not only the detection sensitivity but also linearly decreases the quantitative performance of testing when hydrolysis is not employed.

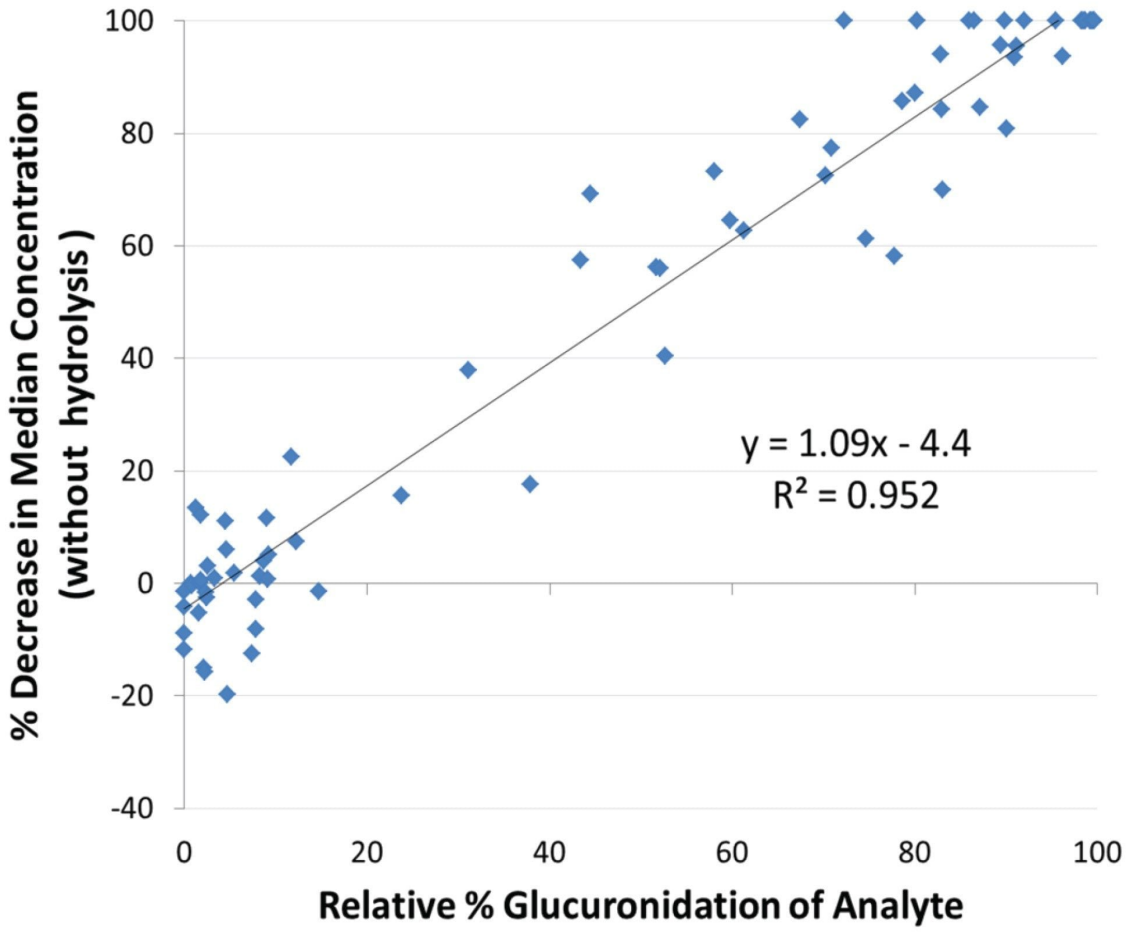

Figure 4. Regressed relationship of the decrease in quantitative test performance with the mean percent glucuronidation for each of the 76 analytes studied.

Conclusion

While beta glucuronidase hydrolysis is used widely in urine drug testing for combined analysis of glucuronidated and free forms of most analytes, knowledge is limited regarding the contribution of glucuronide metabolites to detection sensitivity and quantitative performance for many drugs and Phase 1 metabolites tested in routine forensic and clinical practice. The current study of 76 drugs and metabolite tests in urine, obtained from a cohort of emergency department patients who were suspected of drug misuse, shows a significant and wide range of

contribution of glucuronidated metabolites to urine drug testing performance. Glucuronidated metabolites are the major source (>50%) of analyte response for 39 of the 76 analytes and are a predominated source (>80%) of total analyte response in 32% of the analytes. The study shows that analyte detection often relies heavily on glucuronide metabolite hydrolysis, with analyte detection falling precipitately when the free-analyte component in the excreted urine falls below 40% and with detection rates of less than 20% for 10% of the analytes when tested without the hydrolysis step. False negative rates without hydrolysis show that over 40% of the analyte tests have false negative rates greater than 50% and that testing for cannabidiol, α-hydroxy alprazolam, 11-hydroxy THC and buprenorphine resulted in 100% false negative rates in the absence of a hydrolysis step. Quantitative performance of testing was also affected, with hydrolysis contributing to more than 50% of the concentration for 39 of the analytes and with the decrement in quantitative performance without hydrolysis correlating directly with the relative amount of glucuronidated analyte in urine. Effective glucuronide hydrolysis is a major contributor to the sensitivity and quantitative performance of urine drug testing for many of the drugs and Phase 1 metabolites tested in emergency medicine practice. These methods, developed and validated using Waters Xevo TQD Mass Spectrometer, ACQUITY I-Class PLUS and UPLC Columns, enabled a rapid, sensitive analysis with both methods used in this study.

References

1. D'Ovidio C, Locatelli M, Perrucci M *et al*. LC-MS/MS Application in Pharmacotoxicological Field: Current State and New Applications. *Molecules*. 2023;28:2127-39. doi: 10.3390.
2. Liston HL, Markowitz JS, DeVane CL. Drug Glucuronidation In Clinical Psychopharmacology. *J Clin Psychopharmacol.* 2001;21(5):500-515. PMID: 11593076.
3. Kamisha L Johnson-Davis. Opiate & Benzodiazepine Confirmations: To Hydrolyze or Not to Hydrolyze is the Question J *Appl Lab Med* 2018;2:564-572. doi: 10.1373/jalm.2016.022947.
4. Smith HS. Metabolism and Disposition of Prescription Opioids: a Review. *Pain Physician*. 2012;15(3 Suppl):ES133-ES150. PMID: 26227254.
5. French D, Wu AH, Lynch KL. Hydrophilic interaction LC–MS/MS Analysis of Opioids in Urine: Significance of Glucuronide Metabolites. *Bioanalysis*. 2011;3(23):2603-2612. doi:10.4155/bio.11.259. PMID: 22136049.

6. Matsuo T, Ogawa T, Iwai M, *et al*. Development of an LC–MS/MS Method for the Determination of Five Psychoactive Drugs in Postmortem Urine by Optimization of Enzymatic Hydrolysis of Glucuronide Conjugates. *Forensic Toxicol*. 2024;42(2):181-190. doi:10.1007/s11419-023-00690-7. PMID: 38557936.

7. Dinga Y, Penga M, Zhanga T. *et al*. Quantification of Conjugated Metabolites of Drugs in Biological Matrices After the Hydrolysis with β-glucuronidase and Sulfatase: A Review of Bio-Analytical Methods. *J Chromatogr B Analyt Technol Biomed Life Sci.* 2013;925:38-50. PMID: 23649485.

8. Wakabayashi M, Fishman WH. The Comparative Ability of β-glucuronidase Preparations (liver, Escherichia coli, Helix Pomatia, and Patella Vulgata) to Hydrolyze Certain Steroid Glucosiduronic Acids. *Biochim Biophys Acta*. 1961;48:198-199. PMID: 13782588.

9. Southworth MJ, *et al*. Morphine Glucuronide Hydrolysis: Superiority of β-glucuronidase from Patella Vulgata. *Pharmacology*. 1982;28:83-86. PMID: 6799227.

10. Namera A, Saito T, Nagao M. Investigation of Commercially Available Recombinant and Conventional β-glucuronidases to Evaluate the Hydrolysis Efficiencies Against O-glucuronides and N-glucuronides in Urinary Drug Screening. *Forensic Toxicol*. 2025;43:356-364. doi: 10.1007/s11419-025-00715-6. Epub 2025 Mar 5.

11. Lee LA, McGee AC, Sitasuwan P *et al*. Factors compromising glucuronidase performance in urine drug testing potentially resulting in false negatives. *J Anal Toxicol*. 2022;46(6):689-696. PMID: 34401904.

12. Cone EJ, Yousefnejad D, Darwin WD *et al*. Incomplete Recovery of Prescription Opioids in Urine Using Enzymatic Hydrolysis of Glucuronide Metabolites. *Drug Metab Dispos*. 2007;35(4):649-654. PMID: 17132254.

13. Klette KL, McMillin GA, Koons TL *et al*. Comparison of Purified β-glucuronidases in Patient Urine Samples Indicates a Lack of Correlation Between Enzyme Activity and Drugs of Abuse Metabolite Hydrolysis Efficiencies Leading to Potential False Negatives. *J Anal Toxicol*. 2018;43(3):221-227. PMID: 30517702.

14. Zhang X, Sitasuwan P, Horvath G *et al*. Increased activity of β-glucuronidase Variants Produced by Site-Directed Mutagenesis. *Enzyme Microb Technol*. 2018;109:20-24. doi: 10.1016/j.enzmictec.2017.09.005.

15. Schlachter CR, McGee, AC, Sitasuwan PN, *et al*. Variants of Glycosyl Hydrolase Family 2 β-glucuronidases have Increased Activity on Recalcitrant Substrates. *Enzyme Microb Technol*. 2021;145:109742. doi: 10.1016/j.enzmictec.2020.109742. Epub 2021 Jan 22.

16. Rosano TG, Islam SMT, Rumberger JM *et al*. Definitive urine drug testing in emergency medicine:

Recreational and psychiatric drug findings. *J Mass Spectrom Adv Clin Lab*. 2025;37:16-27. doi: 10.1016/j.jmsacl.2025.04.008.

17. Rosano TG, Rumberger JM, Scholz KL *et al*. Novel Matrix Normalization Technique for Accurate LC-ESI-MS/MS Detection and Quantification of Drugs and their Etabolites in Toxicology Research and Practice. *Current Protocols*, 3, e644. doi: 10.1002/cpz1.644.
18. Rosano TG, Rumberger JM, Wood M. Matrix Normalization Techniques for Definitive Urine Drug Testing. *J Anal Toxicol* 2021;45:901-912.
19. Rosano TG, Ohouo PY, Wood M. Screening and Quantification of 64 Drugs and Metabolites in Human Urine using UPLC-MS-MS Analysis with Threshold Accurate Calibration. *J Anal Toxicol* 2017;41:536-546.
20. Rosano TG, Cooper J, Scholz KL, Wood M. Confirmation of Cannabinoids in Forensic Toxicology Casework by Isomer-Selective UPLC-MS/MS analysis. *J Analyt Toxicol*, 47;709–718 (2023) https://doi.org/10.1093/jat/bkad048.
21. Rosano TG, Rumberge JM, Konetchy RM *et al*. Direct-to-definitive Urine Drug Testing Panel with Addition of xylazine and 4-hydroxy xylazine Identification and Quantitation. Waters Application Brief, 720008798 (2025).
22. Rosano TG, Cooper JA, Scholz KL *et al*. Cannabinoid Isomer Identification and Quantitation by UPLC-MS/MS Analysis in Forensic Urine. Waters Application Brief, 720008365 (2024).

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