Automated and High-throughput Urine Drug Screening using Paper Spray Mass Spectrometry

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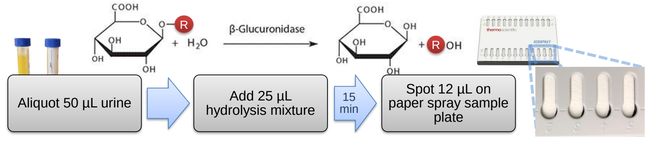
Background

Paper spray mass spectrometry (PS-MS/MS) has been shown to be a rapid, simple, and inexpensive alternative to traditional forensic drug screening methods. It can address the limitations of both immunoassays and chromatography-based techniques due to its non-reliance on sample preparation and its ability to rapidly screen for a wide array of compounds. In this study, an automated PS-MS/MS system was employed to screen for 40 commonly abused drugs and metabolites in urine after a 15-minute room temperature glucuronidase reaction.

Method

A 50 µL aliquot of urine was mixed with a solution containing internal standards, buffer, and IMCSzyme®RT recombinant β-glucuronidase. After 15 minutes of incubation at room temperature the sample was spotted directly onto a preassembled paper spray sample plate.



IMCSzyme®RT recombinant β-glucuronidase, buffer, and internal standard solution were combined in one spiking solution to minimize sample handling. The automated paper-spray sample cassette holds 24 samples. 240 samples could be processed automatically per run at 2 min/sample**.** Analysis was carried out using the VeriSpray™ paper spray system coupled to a TSQ Altis™ triple quadrupole mass spectrometer.

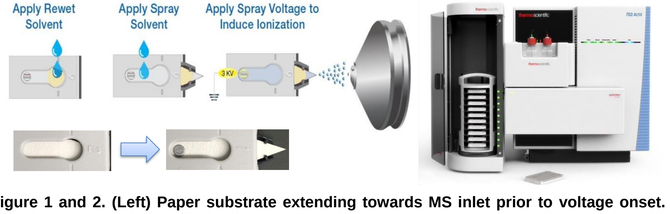


Figure 1 and 2. (Left) Paper substrate extending towards MS inlet prior to voltage onset. (Right) Thermo Scientific™ VeriSpray™ PaperSpray ion source. Thermo Scientific™ TSQ Altis triple quadrupole mass analyzer

VeriSpray™	
Solvent composition	57:57:6 EA:ACN:H_2O, 0.1% H_2CO_2
Solvent dispense delay (s)	Rewet: 1 : 1-1-1-1-3-3-3-3-5-5-5-5-7-7-7

TSQ Altis™			
Spray voltage	+4000 V	Ion transfer tube	300 °C
Q1, Q3 resolution	0.4, 0.4 FWHM	CID gas	1.5 mTorr

Analysis

- Single reaction monitoring (SRM)
- Two SRM transitions per target compound
- Nine isotopically-labeled internal standards

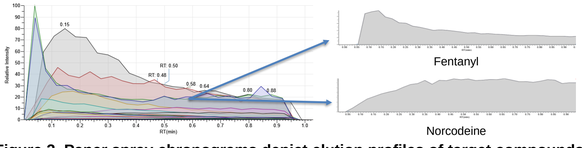


Figure 3. Paper spray chronograms depict elution profiles of target compounds

Data was analyzed using TraceFinder 3.3 (Thermo Fisher Scientific). Positive results were considered valid if primary and secondary transitions were present in a pre-established ratio. Isobaric interference yielded failed ion ratios and an inconclusive result.

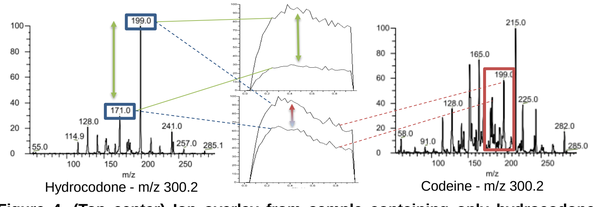
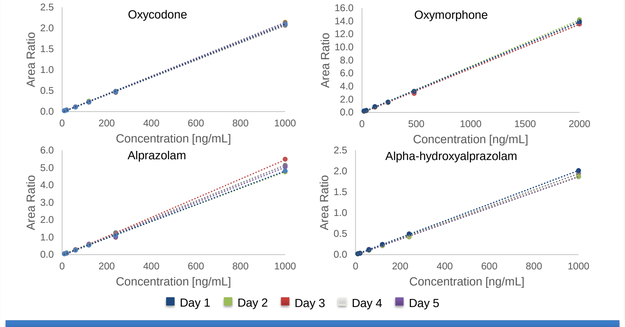


Figure 4. (Top center) Ion overlay from sample containing only hydrocodone. (Bottom center) Ion overlay from sample containing hydrocodone and codeine.

Results

The method was validated according to SWGTOX guidelines. Limits of quantification were defined as 10 times the standard error of the intercept divided by the slope of the calibration curve.



Compound	Internal Standard	Collision Energy(V)	Calibration Range	LOQ [ng/mL]	R^2	Cutoff [ng/mL]
Benzoylecgonine	A	19.8	10-1000	5.5	0.9984	10
Cocaethylene	B	18.8	10-1000	5.2	0.9974	10
Cocaine	B	19.6	10-1000	1.5	0.9997	5
Methylphenidate	B	19.4	10-1000	8.2	0.9919	15
Amphetamine	C	18.9	10-1000	10.2	0.9938	20
MDA	C	18.3	40-4000	40	0.9917	60
MDEA	C	13.6	10-1000	6.2	0.9965	15
MDMA	C	13.2	10-1000	5.5	0.9966	10
Methamphetamine	C	11	10-1000	10.5	0.9982	25
Ketamine	C	14	10-1000	7.2	0.9949	15
Phentermine	C	11	10-1000	11	0.9942	20
Alpha-OH-Alprazolam	D	25.1	10-1000	6.6	0.9964	10
7-Aminoclonazepam	D	30	40-4000	41	0.9805	60
Alprazolam	D	26.6	10-1000	6.2	0.9946	10
Clonazepam	D	24.9	20-2000	14.5	0.9954	25
Diazepam	D	33.4	10-1000	4.5	0.9946	10
Nordiazepam	D	27	10-1000	5.8	0.9982	10
Lorazepam	D	29	20-2000	22	0.9870	40
Oxazepam	D	35.1	20-2000	16	0.9927	30
Temazepam	D	19.5	20-2000	24	0.9856	40
Amitriptyline	E	18.4	10-1000	5	0.9980	10
Desipramine	E	16.7	10-1000	6	0.9957	10
Doxepin	E	18.4	10-1000	5.5	0.9958	10
Nortriptyline	E	22.1	10-1000	7	0.9954	10
Trimipramine	E	17.7	10-1000	2.4	0.9995	5
Morphine	F	38.7	20-2000	20	0.9917	40
Oxymorphone	F	29	20-2000	11	0.9983	20
Hydromorphone	G	42	20-2000	21.5	0.9873	40
Codeine	G	55	10-1000	10	0.9950	20
Norcodeine	F	43	20-2000	20	0.9913	40
6-MAM	G	38.7	10-1000	7.5	0.9968	10
Hydrocodone	G	29.9	10-1000	3.5	0.9993	5
Oxycodone	G	28.2	10-1000	6	0.9982	10
Buprenorphine	H	41	10-1000	4.8	0.9982	10
Methadone	I	15	10-1000	2.5	0.9995	5
EDDP	I	36.7	10-1000	7.5	0.9926	10
Tramadol	B	18.5	10-1000	7	0.9929	15
N-Desmethyltramadol	B	15	30-3000	22	0.9944	40
Fentanyl	B	24	1-100	0.7	0.9968	1
Norfentanyl	B	19	4-400	4.3	0.9838	6

A:Benzoylecgonin-d8 B:Cocaine-d3 C:Methamphetamine-d5 D:Nordiazepam-d5 E:Timipramine-d3 F:Oxymorphone-d3 G:Hydrocodone-d3 H:Buprenorphine-d4 I:Methadone-d3

Spike and recovery experiments for oxazepam, morphine, and codeine glucuronide were carried out in 200 remnant clinical urine specimens. Recoveries of >70% was achieved in nearly all cases.

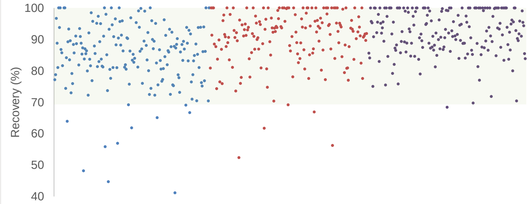


Figure 5. Recoveries of codeine (blue), morphine (red), and oxazepam (purple) in clinical urine specimens spiked with respective glucuronides

The effects of inhibitor compounds on enzyme activity sporadically yielded lower than expected recovery. Significant inhibition of IMCSzyme®RT was infrequent (n=9) and recoveries of <40% were not observed in any urine specimens. Further dilution of urine specimens to reduce inhibition was counterproductive in absence of subsequent sample reconcentration.

Conclusions

- Rapid glucuronide hydrolysis and automated paper-spray mass spectrometry facilitated a high rate of urine drug screening
- Cut-off levels were well below federally mandated requirements and commonly used forensic laboratory standards
- Throughput was comparable with immunoassays for large batches with the option of screening for a much wider array of drugs at significantly lower concentrations
- This method may prove very useful for forensic laboratories as an alternative to traditional MS techniques as it allows for up to 10 times the through-put of complex samples without extraction, separation, and sample cleanup.

References and Acknowledgements

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