



# Coated Blade Spray – Mass Spectrometry (CBS-MS) for clinical toxicology testing in urine

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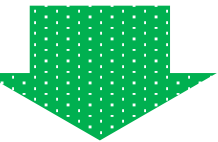
# Drug Testing – The two-step approach

Screen

- Screening for classes of abused or prescribed drugs (Immunoassay/POC cup) – rapid, minimal labor
- **Limitations:**
  1. Assays not available for all drugs of clinical interest
  2. Cross-reactivity
  3. Do not detect atypical/Designer Drugs



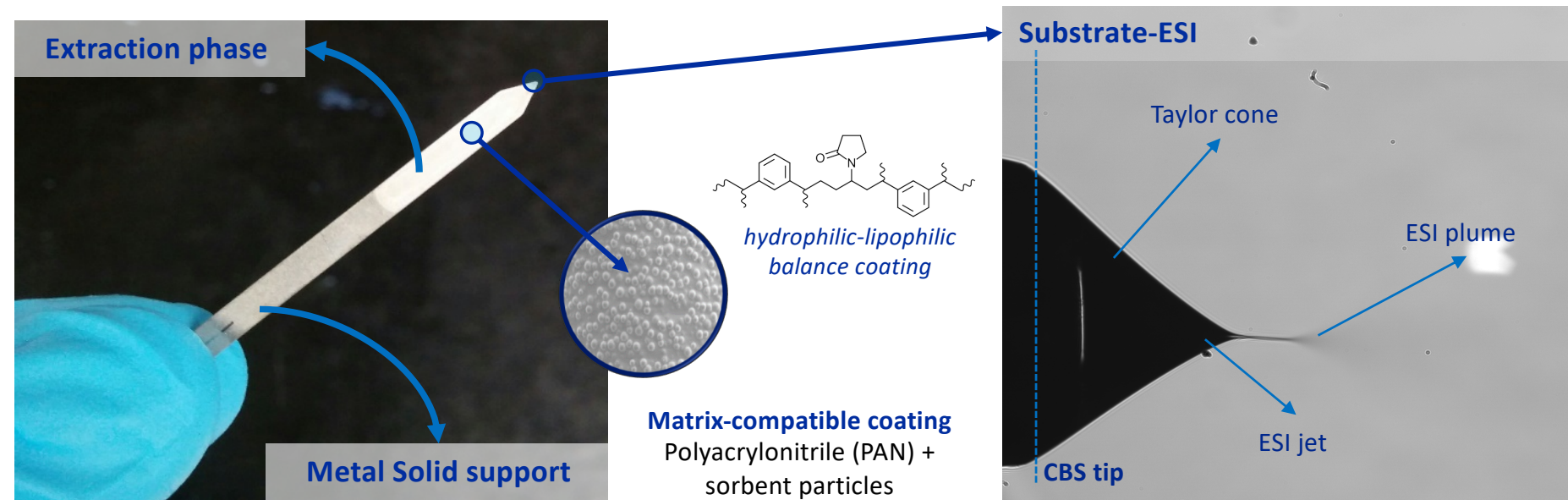
*False positives & False negatives*



Confirm

- Targeted confirmation testing (GC/MS or LC-MS/MS)
- **Limitations:**
  1. Testing not always available in-house
  2. Long turn around times

# CBS-MS



- Coated blade spray (CBS) is a solid-phase microextraction-based technology that can be directly coupled to MS to enable the rapid qualitative and quantitative analysis of complex matrices (*solid-phase microextraction (SPME) and substrate-spray ionization*)
- CBS is a 'sword-like' device made of stainless steel that is partially coated with a matrix compatible extraction phase. This extraction phase can either be a polymer or polymeric particles (e.g., hydrophilic–lipophilic balanced particles) that have been attached to the substrate surface using a chemical binder such as polyacrylonitrile.

Gómez-Ríos, G.A., et. al., Trends in Analytical Chemistry., 2019, 112, 201-211

Gómez-Ríos, G.A., et. al., Bioanalysis, 2018, 10(4), 257–271

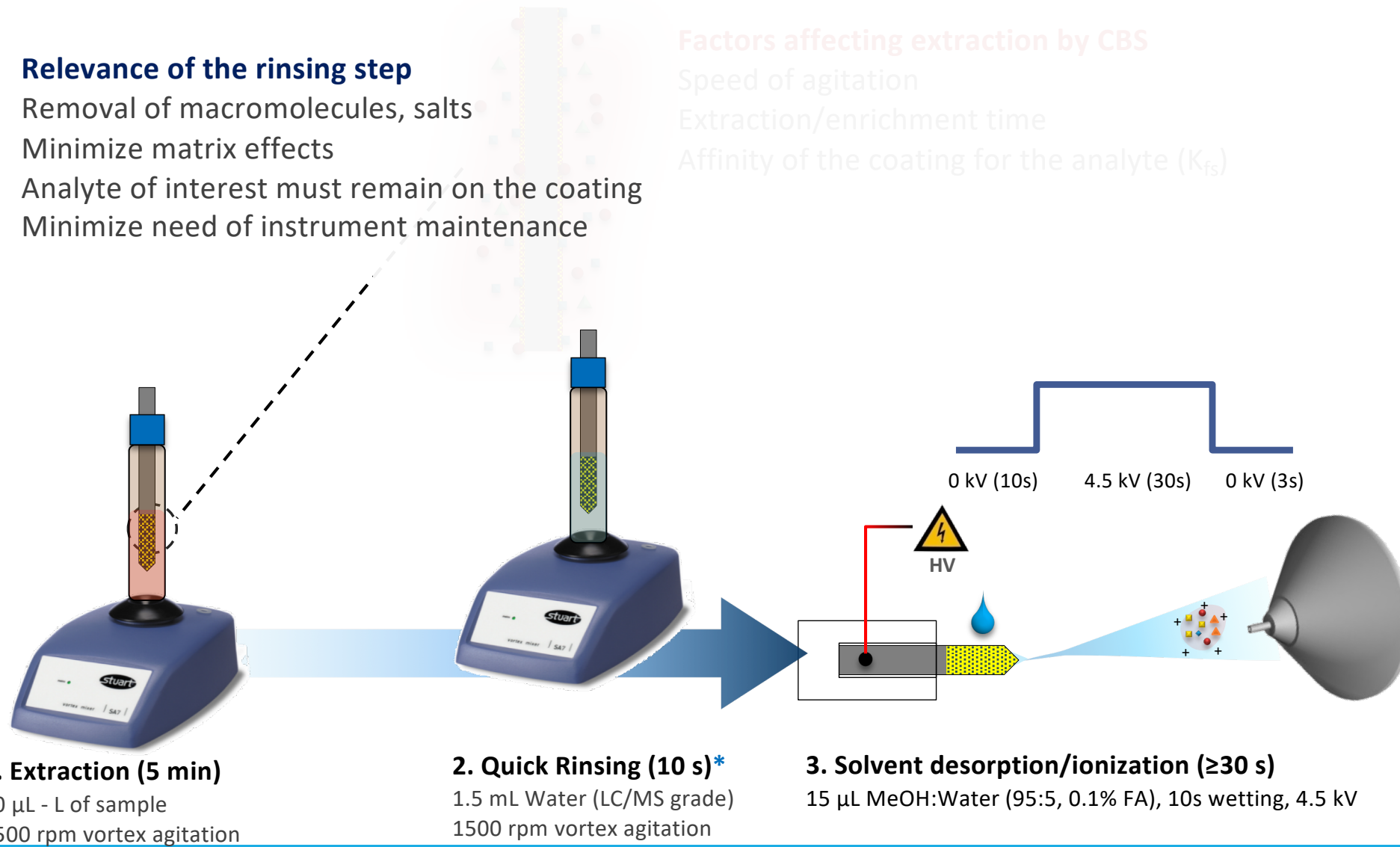
# Coated Blade Spray (CBS) mechanism of extraction

## Relevance of the rinsing step

- Removal of macromolecules, salts
- Minimize matrix effects
- Analyte of interest must remain on the coating
- Minimize need of instrument maintenance

## Factors affecting extraction by CBS

- Speed of agitation
- Extraction/enrichment time
- Affinity of the coating for the analyte ( $K_{fs}$ )



# Proof of concept experiment

(POC cup vs. CBS-MS/MS vs. LC-MS/MS)

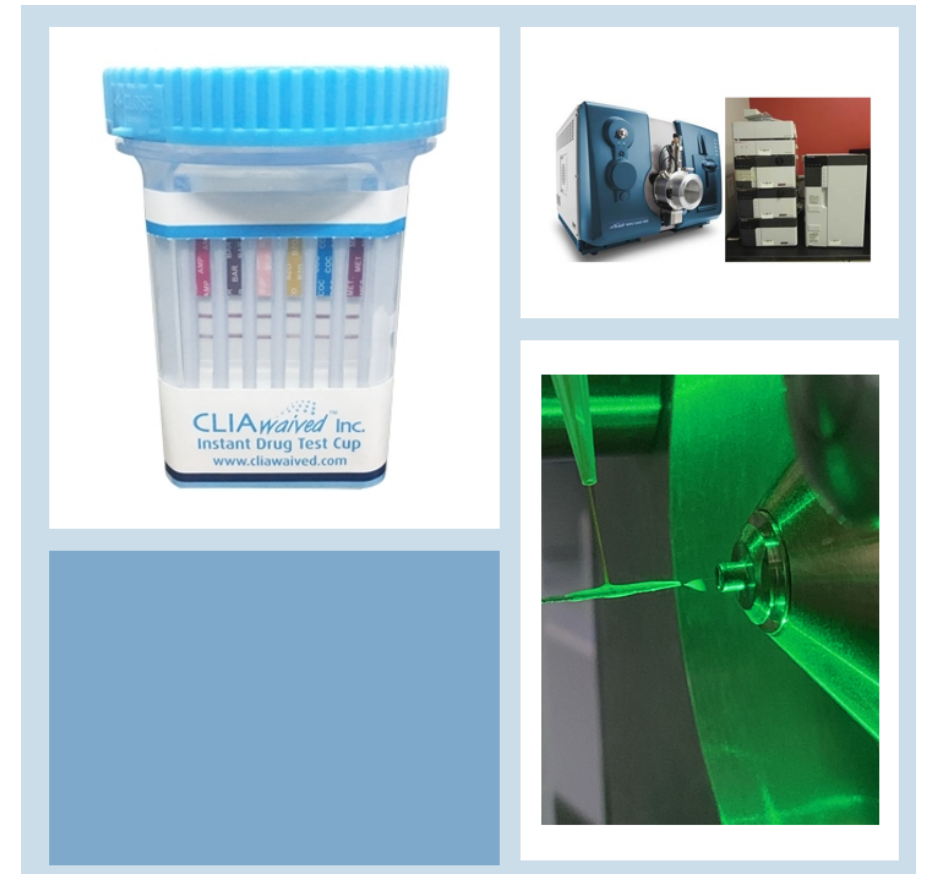
## 1. CBS-MS/MS pain panel: 54 drugs and metabolites

Proof of concept experiment / validation:

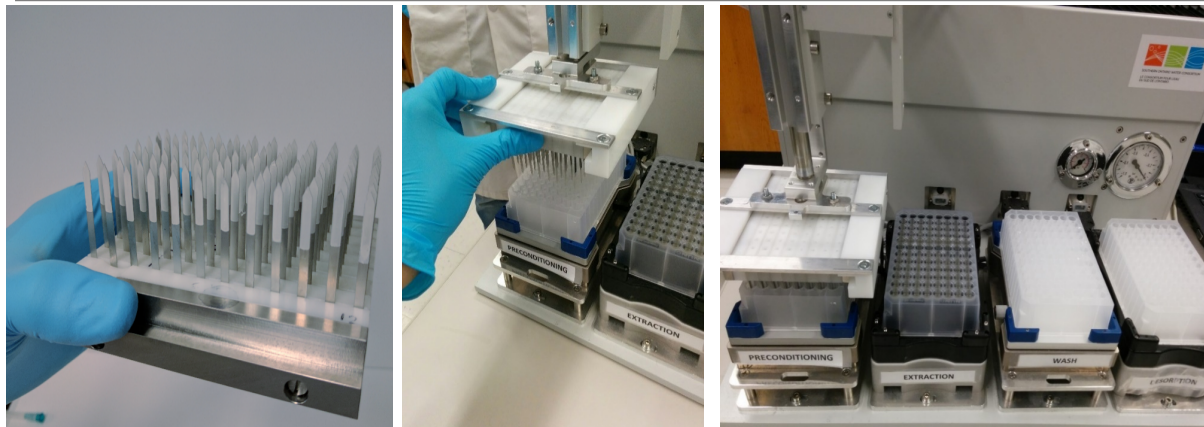
- Evaluation of **81** urine samples
- Method optimization
- Method validation
- **POC cup (pain management)**
- The immunoassay method was a point of care drug test cup (POC-CUP) for the following drug groups: amphetamines, benzodiazepines, buprenorphine, opiates, barbiturates, cocaine, methamphetamine, ecstasy, methadone, oxycodone and phencyclidine

## 2. LC-MS/MS pain panel: 54 drugs and metabolites

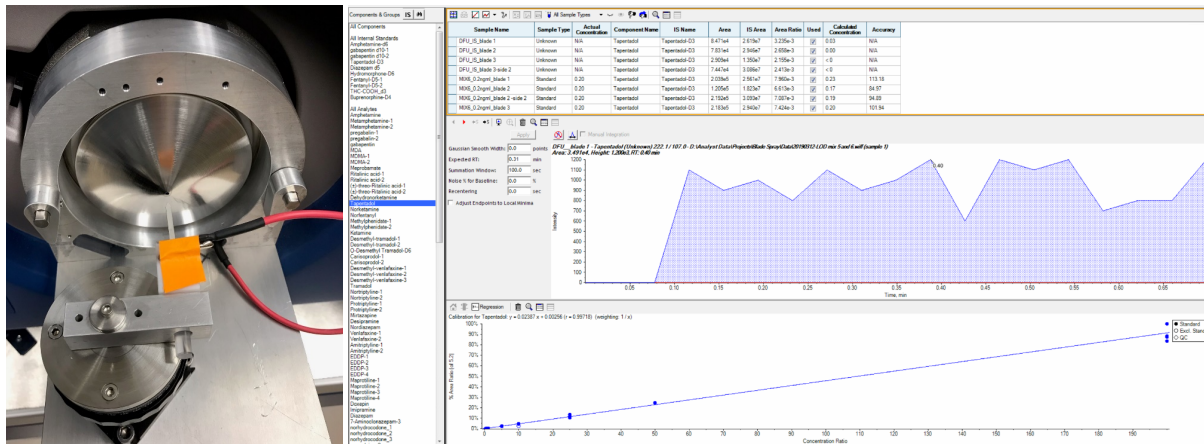
- All samples were analyzed with an LC-MS/MS method as a confirmation test.



# CBS analytical protocol

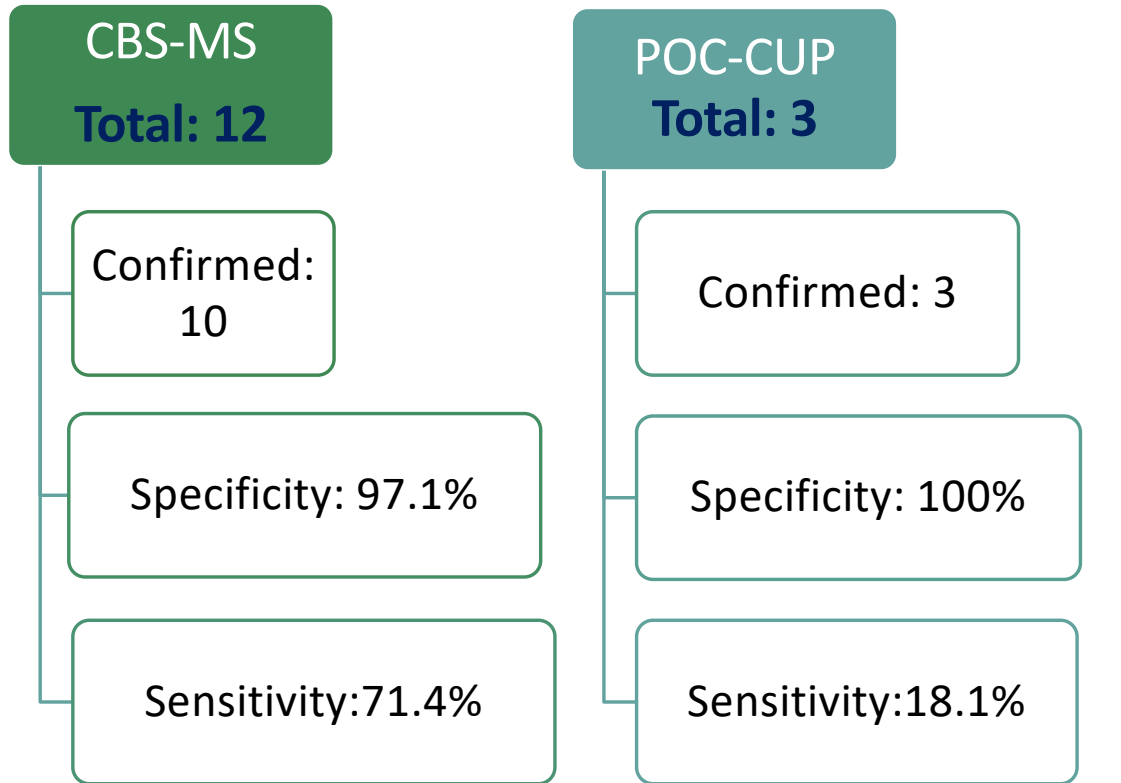


1. **Hydrolysis:** Incubate urine with IMCSzyme enzyme for 1 hr at 55°C.
2. **CBS pre-conditioning:** 300 µL LC/MS methanol:water (50:50), 10 min at 1500 rpm. 96 CBS devices
3. **Extraction:** Insert the CBS into 300 µL of hydrolyzed urine + ISs (1500 rpm, vortex agitation for 5 min)
4. **CBS coating rinsing:** removal of any remaining matrix on the surface (300 µL LC/MS water, 3 seconds)



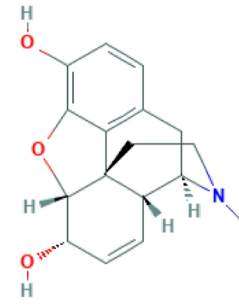
- A SCIEX 4500 QTrap mass spectrometer was used.
- Data was collected by use of a selected reaction monitoring (SRM) scan.
- The SRM window was 0.737 min (19 cycles) and the method contained 131 transitions.
- All analyses were carried out in positive ionization mode with ion spray voltage of 4500 V.
- Analyst® and MultiQuant software from ABSciex were utilized for MS system control, data acquisition and data analysis.

# Benzodiazepines

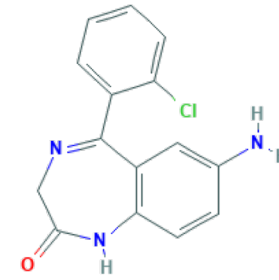


CBS False Negative, CBS-MS:N=4 (high matrix effects)  
 POC-CUP: N=11

*Sensitivity = True positives / (True positives + False negatives)*  
*Specificity = True negatives / (True negatives + False positives)*



Morphine  
 286.1 → 152.1



7-aminoclonazepam  
 286.1 → 222.2

	CBS-MS		POC-CUP
	LOD (ng/ml)	LOQ (ng/ml)	Cutoff (ng/ml)
Alprazolam	0.2	1	
Midazolam	0.2	0.5	
Diazepam	1	5	
7-Aminoclonazepam	5	10	
Nordiazepam	5	10	
Temazepam	1	10	300
alpha-hydroxyalprazolam	5	10	
alpha-hydroxymidazolam	1	5	
Oxazepam	10	50	
Lorazepam	10	25	

# Opiates

**CBS-MS**  
**Total: 35**

Confirmed: 31

Specificity: 92%

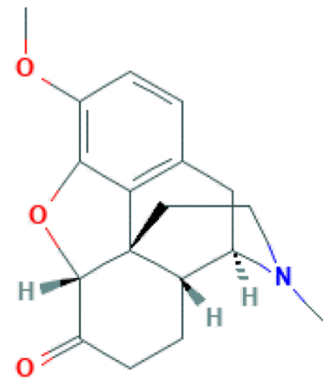
Sensitivity: 94%

**POC-CUP**  
**Total: 24**

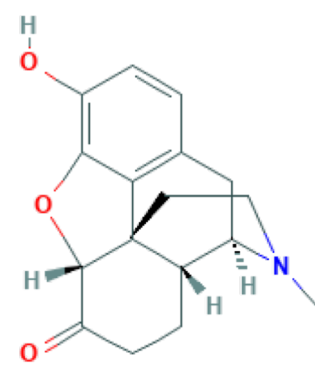
Confirmed: 24

Specificity: 100%

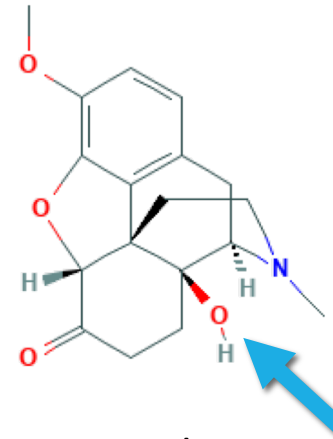
Sensitivity: 68%



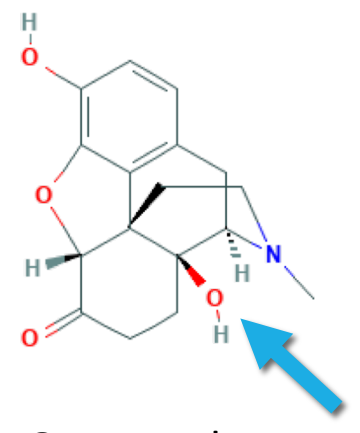
Hydrocodone



Hydromorphone



Oxycodone



Oxymorphone

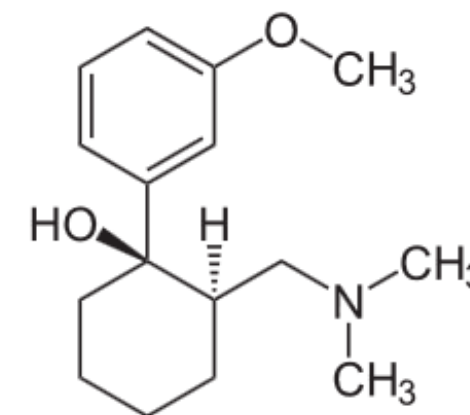
	CBS-MS		POC-CUP
	LOD (ng/ml)	LOQ (ng/ml)	Cutoff (ng/ml)
Codeine	0.5	5	2,000
Morphine	1	5	
Hydromorphone	1	5	
Hydrocodone	1	5	

- **False positives:** 4 false positive samples were true positive for OXY → In-source fragmentation of Oxycodone and Oxymorphone
- **False negatives:** 2 for CBS and 9 for CUP

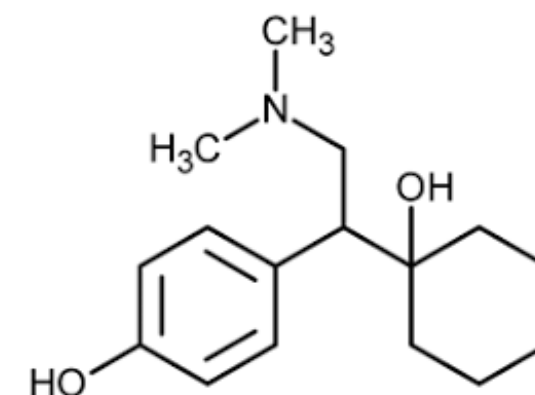


# Other Drugs/Metabolites Summary

Drug / Metabolite	CBS-MS/MS (FN = 2)	POC-CUP	POC- cutoff	CBS LOD (ng/mL)	CBS LOQ (ng/mL)
Oxycodone/ Oxymorphone	ID: 8 Confirmed: 7	ID: 4 Confirmed: 4	100	0.5 / 1	5 / 5
Buprenorphine/ Norbuprenorphine	ID: 3 Confirmed: 3	ID: 2 Confirmed: 2	10	0.5 / 5	10 / 10
Cocaine/ Benzoylecgonine	ID: 1 Confirmed: 1	ID: 1 Confirmed: 1	300	0.2 / 0.5	0.5 / 5
Methadone/ EDDP	ID: 2 Confirmed: 2	ID: 2 Confirmed: 2	300	0.5 / 0.2	5 / 0.5
Tramadol/ Desmethyltramadol	ID: 12 Confirmed: 10			0.2 / 0.2	1 / 0.5
Gabapentin	ID: 15 Confirmed: 15			50	500
Pregabalin	ID: 3 Confirmed: 3			500	1000
Zolpidem/ ZCA	ID: 1 Confirmed: 1			0.2 / 0.2	0.5 / 5
Fentanyl/ Norfentanyl	ID: 3 Confirmed: 3			0.05 / 1	0.1 / 5



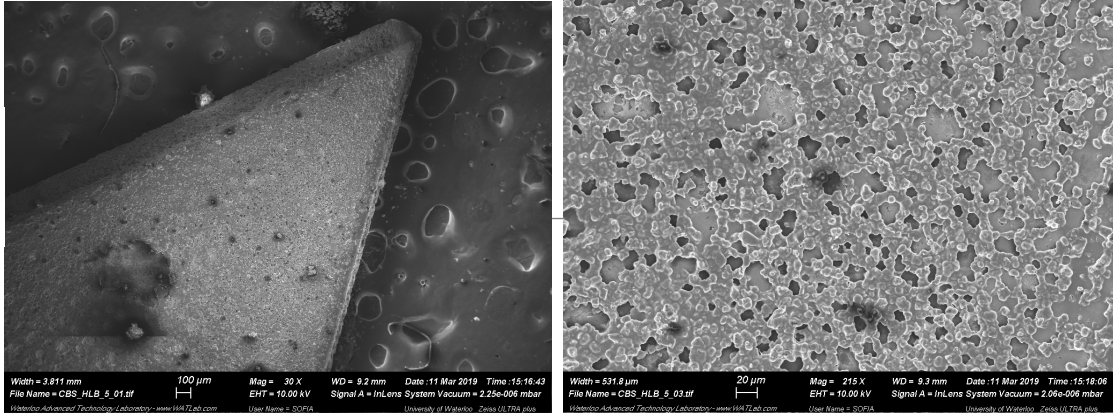
Tramadol  
264.2 → 58.0  
Desmethylvenlafaxine



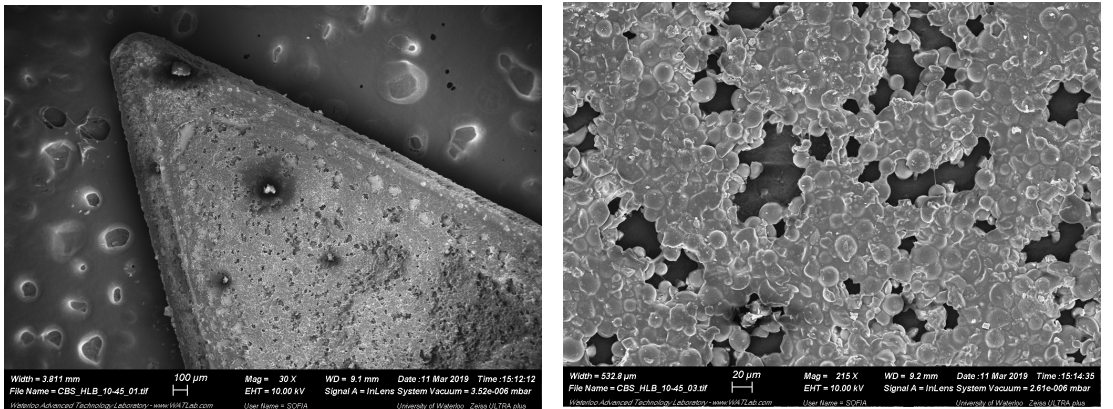
# Method Modification (Coating Particles)

Smaller particle size → More active sites & thinner coating → Higher & faster extraction → Higher sensitivity.

Component Name	Heat map is S/N <b>low</b> --> <b>high</b>			
	Commercial Waters HLB		In-house synthesized HLB	
	Blade-1	Blade2	Blade-1	Blade2
Amphetamine	27.4	35.7	59.0	52.3
MDA	17.5	23.5	20.8	24.3
Ketamine	357.9	536.4	804.9	638.7
Tramadol	154.9	224.7	464.4	396.4
Nortriptyline	1338.0	1029.7	1355.0	1277.5
EDDP	877.0	1081.8	10944.7	15073.2
Morphine	16.9	25.2	60.1	79.1
Temazepam	232.8	280.9	543.5	496.4
Alprazolam	451.9	562.1	1554.1	1468.3
Fentanyl	6.7	11.3	25.4	19.6
Ritalinic acid-1	190.3	227.2	191.1	204.3
Morphine-6-glucuronide-1	1.2	1.0	2.1	2.0
Morphine-6-glucuronide-2	0.6	0.6	1.4	1.3
Oxazepam Glucuronide-1	2.0	1.6	2.4	2.5
Oxazepam Glucuronide-2	39.3	25.2	35.8	34.3
Norbuprenorphine-3-glucuronide-1	30.2	25.6	40.1	46.9
Norbuprenorphine-3-glucuronide-2	8.4	7.2	9.7	8.0
Lorazepam Glucuronide-1	1.4	1.3	2.1	2.1
Lorazepam Glucuronide-2	28.9	20.0	17.8	19.9



In-house synthesized HLB particles – 5 µm particle diameter – total coating thickness ~10 µm



Commercial Waters HLB particles – 10-45 µm particle diameter – total coating thickness ~20 µm

Signal-to-noise ratio (Concentration: 50 ng/ml)

Heat map is S/N low --> high

# Method Modification

(Extraction time)

Component Name	1 min-1	1 min-2	5 min-1	5 min-2	10 min-1	10 min-2	15 min-1	15 min-2	20 min-1	20 min-2
Amphetamine	5.4	6.5	94.8	85.0	25.7	15.5	53.9	54.1	37.1	41.4
pregabalin	1.1	1.3	8.0	9.0	7.5	7.7	3.2	3.9	2.0	2.3
MDA	1.3	1.6	26.4	23.1	6.5	4.7	13.0	12.1	17.6	18.4
Ritalinic acid-1	11.2	7.9	11.7	18.4	133.4	75.6	24.5	59.2	150.8	29.3
Ritalinic acid-2	2.5	1.7	1.1	1.9	5.6	4.0	1.3	2.9	4.6	1.1
Norketamine	11.2	12.5	141.8	131.4	48.0	26.7	93.8	103.7	104.0	89.4
Desmethyl-tramadol-1	161.6	149.4	908.0	1412.8	655.1	639.1	357.5	525.8	499.7	447.2
Desmethyl-tramadol-2	1.0	1.0	5.3	6.0	2.9	3.2	2.0	2.6	2.3	2.1
Tramadol	6.2	6.7	60.9	56.4	14.7	11.4	34.0	36.0	31.4	28.4
Nortriptyline-1	1343.3	1887.3	10726.2	9887.6	4411.1	3823.5	11376.1	10285.9	5993.8	7983.6
Mirtazapine	3159.2	2617.7	21672.0	25795.3	16394.1	15887.9	4028.6	6275.9	3294.2	3118.2
Desipramine	1398.4	1183.0	8865.3	9028.1	8339.2	2929.5	7245.1	11006.4	12596.6	11515.3
EDDP-1	565.0	673.6	4853.3	4857.0	1109.7	998.5	1362.3	1458.9	1621.3	1559.7
EDDP-2	1704.1	2131.9	37040.7	35382.2	8446.4	7611.0	8068.7	8307.8	8482.8	7914.8
EDDP-3	429.7	553.7	1083.0	1028.9	609.5	556.7	984.8	1026.3	1237.8	1170.3
EDDP-4	2313000.0	2914000.0	15893.3	14771.1	27540.1	26208.4	8968.9	9679.3	10780.4	10180.5
Doxepin	102.1	84.2	1053.6	1040.1	478.8	472.9	287.6	491.8	495.2	467.0
Morphine-1	55.1	47.9	234.4	209.9	29.0	29.1	92.1	39.7	56.8	63.4
Morphine-2	17.6	20.2	72.5	62.1	13.0	13.3	41.3	23.2	15.9	17.7
Morphine-3	14.2	13.4	70.5	61.2	10.7	12.3	40.8	20.9	15.3	18.0
Benzoylcegonine	5.4	5.8	26.6	45.4	20.8	17.3	14.8	26.5	20.0	19.5
Temazepam	71.6	101.2	1018.3	953.3	316.2	377.3	655.5	542.2	819.7	946.9
Oxymorphone	44.7	37.1	202.1	209.7	119.7	114.7	69.9	115.1	63.2	55.5
Alprazolam	101.8	152.6	641.4	571.6	1566.0	1759.6	1526.2	1266.0	736.1	810.4
Clomipramine	4.0	3.1	13.9	12.4	8.1	6.8	6.5	4.3	4.9	4.6
Midazolam	955.5	704.5	3410.5	3196.5	4564.0	4651.0	4607.5	7050.0	7500.0	7090.0
Norbuprenorphine	48.6	36.8	81.1	87.2	51.4	44.5	26.0	42.1	33.4	33.2
Hydromorphone-3-β-D-glucuronide-1	1.7	1.2	4.2	3.7	1.7	1.4	1.2	1.7	1.4	1.2
Hydromorphone-3-β-D-glucuronide-2	1.9	1.9	4.1	2.4	1.9	2.6	1.9	2.5	1.5	1.2
Morphine-6-glucuronide-1	2.0	1.2	1.3	7.9	32.5	16.2	2.9	4.1	1.9	0.9
Morphine-6-glucuronide-2	3.3	3.5	15.2	16.1	8.5	3.3	1.9	2.8	2.3	0.7
Morphine-6-glucuronide-3	1.5	1.2	4.0	2.9	3.5	2.3	1.6	1.8	2.0	0.8
Oxazepam Glucuronide-1	1.5	1.0	2.0	3.7	18.4	10.8	1.9	2.2	11.1	5.6
Oxazepam Glucuronide-2	1.1	0.9	11.3	6.6	11.1	8.4	37.1	46.2	29.0	12.3
Lorazepam Glucuronide-1	1.5	1.0	1.3	1.6	3.7	2.2	1.6	2.0	1.6	0.7
Lorazepam Glucuronide-2	1.8	2.3	2.0	2.1	19.1	10.7	4.7	4.6	6.4	2.6
THCCOOH-gluc-1	1.4	1.1	2.1	1.5	3.7	2.1	1.6	2.0	1.4	0.7
THCCOOH-gluc-2	3.2	1.6	1.9	1.6	3.9	2.2	1.8	1.9	1.4	0.9
THCCOOH-gluc-3	4.3	1.8	1.1	1.4	5.8	2.4	1.8	2.4	1.7	1.3
Norbuprenorphine-3-glucuronide-1	2.8	2.2	3.9	3.4	7.3	3.6	2.5	3.4	3.6	1.2
Norbuprenorphine-3-glucuronide-2	2.0	3.1	2.0	2.6	5.0	3.7	3.2	3.4	4.2	1.9

# Method validation

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The method was verified (n=3) by evaluating the limit of detection (LOD), limit of quantitation (LOQ), linearity and matrix effects (ME).

- LOD: The lowest concentration that could be detected with a signal-to-noise ratio (S/N) of 3.
- LOQ: The lowest concentration that could be measured with a signal-to-noise ratio (S/N) of 10 within 20% precision and accuracy.
- Linearity: Calibration curves were made from peak area ratios between analytes and the IS using 1/x weighted linear regression.
- ME: Signal intensities ratio of post-preparation samples and neat standard samples at 50 ng/ml. A ratio over 100% demonstrates an enhancing ME, a ratio below 100% demonstrates a suppressing ME, and the percentage of enhancement or suppression is calculated as the absolute difference from 100%.

	LOD (ng/ml)	LOQ (ng/ml)	Linear range (ng/ml)	R <sup>2</sup>	ME (%)	Type of effect	
Oxycodone	0.5	5	5-200	0.996	77.9	-22	Suppression
Codeine	0.5	5	5-200	0.997	97.5	-2.4	Suppression
Morphine	1	5	5-200	0.992	90.3	-9.6	Suppression
Hydromorphone	1	5	5-200	0.998	91.3	-8.7	Suppression
Oxymorphone	1	5	5-200	0.995	80.4	19.6	Enhancement
Hydrocodone	1	5	5-200	0.996	103.2	3.2	Enhancement

	LOD (ng/ml)	LOQ (ng/ml)	Linear range (ng/ml)	R <sup>2</sup>	ME (%)	Type of effect	
Fentanyl	0.05	0.1	0.1-200	0.997	89.5	-10.4	Suppression
EDDP	0.2	0.5	0.5-200	0.999	82.9	-17.07	Suppression
O-desmethyl-cis-tramadol	0.2	0.5	0.5-200	0.998	98.4	1.6	Enhancement
Methadone	0.2	5	5-200	0.996	141.8	41.8	Enhancement
Tramadol	0.2	1	1-200	0.999	97.8	-1.3	Suppression
Buprenorphine	0.5	10	10-200	0.999	107.8	7.8	Enhancement
Norfentanyl	1	5	5-200	0.99	122.3	22.3	Enhancement
Norbuprenorphine	5	10	10-200	0.995	88.4	11.5	Enhancement
6MAM	5	25	25-200	0.995	92.6	-7.4	Suppression

	LOD (ng/ml)	LOQ (ng/ml)	Linear range (ng/ml)	R <sup>2</sup>	ME (%)	Type of effect	
Tapentadol	0.5	1	1-200	0.997	101.4	1.4	Enhancement
Gabapentin	50	500	500-100000	0.995	91.2	-8.8	Suppression
Pregabalin	500	1000	1000-100000	0.997	88.4	11.6	Enhancement
Carisoprodol	1	5	5-200	0.998	13.5	-86.4	Suppression

# Method validation

## Gabapentin and Pregabalin Concentrations Measured in 57,542 Pain Patients in Urine

Analyte	Number of Positive Specimens	Percent Positive Specimens	Mean ± SEM (µg/mL)	Median (µg/mL)	Range (µg/mL)
Gabapentin	7013	12.2	430.9 ± 8.3	259.8	2.5-35345
Pregabalin	4799	8.3	183.9 ± 3.6	108.0	2.5-6892

J Anal Toxicol. 2011 Jul;35(6):357-9.

	LOD (ng/ml)	LOQ (ng/ml)	Linear range (ng/ml)	R <sup>2</sup>	ME (%)	Type of effect	
Mirtazapine	0.2>	0.2	0.2-200	0.999	107.6	7.6	Enhancement
Venlafaxine	0.2	0.5	0.5-200	0.997	91.4	-8.5	Suppression
Desipramine	0.2	5	5-200	0.997	85	-26	Suppression
Imipramine	0.2	5	5-200	0.998	78.3	-21.4	Suppression
Maprotiline	0.2	5	5-200	0.991	147.6	47.6	Enhancement
Nortriptyline	0.2	5	5-200	0.997	87.5	-12.5	Suppression
Amoxapine	0.5	1	1-200	0.998	121.4	21.4	Enhancement
Doxepin	1	5	5-200	0.999	133	-33	Suppression
Amitriptyline	1	5	5-200	0.99	106.6	6.6	Enhancement
Desmethylvenlafaxine	5	10	10-200	0.985	93.5	-6.5	Suppression
Protriptyline	5	10	10-200	0.999	132.7	32.75	Enhancement

	LOD (ng/ml)	LOQ (ng/ml)	Linear range (ng/ml)	R <sup>2</sup>	ME (%)	Type of effect	
Cocaine	0.2	0.5	0.5-200	0.998	123.7	23.7	Enhancement
Benzoylcegonine	0.5	5	5-200	0.999	58.8	41.2	Enhancement
Methamphetamine	1	5	5-200	0.994	129.3	29.3	Enhancement
MDA	5	25	25-200	0.995	155.3	55.3	Enhancement
MDMA	5	10	10-200	0.991	124	24	Enhancement
Amphetamine	10	25	25-200	0.998	106.6	6.6	Enhancement

	LOD (ng/ml)	LOQ (ng/ml)	Linear range (ng/ml)	R <sup>2</sup>	ME (%)	Type of effect	
Dehydronorketamine	0.1	0.5	0.5-200	0.995	126.7	26.7	Enhancement
Ketamine	0.2	0.5	0.5-200	0.991	120.7	20.7	Enhancement
ZCA	0.2	5	5-200	0.984	130.3	30.3	Enhancement
Zolpidem	0.2	0.5	0.5-200	0.997	114.9	14.9	Enhancement
Norketamine	0.2	5	5-200	0.986	100.46	0.46	Enhancement
Ritalinic acid	5	10	10-200	0.996	105.6	5.6	Enhancement

# Method validation

# Method validation

	LOD (ng/ml)	LOQ (ng/ml)	Linear range (ng/ml)	R <sup>2</sup>	ME (%)	Type of effect	
Alprazolam	0.2	1	1-200	0.987	42.34	-57.65	Suppression
Midazolam	0.2	0.5	0.5-200	0.997	119.8	-19.8	Suppression
Diazepam	1	5	5-200	0.995	101.6	1.6	Enhancement
7-Aminoclonazepam	5	10	10-200	0.998	140.8	40.8	Enhancement
Nordiazepam	5	10	10-200	0.995	89.7	-10.2	Suppression
Temazepam	1	10	10-200	0.986	11.99	-88	Suppression
alpha-hydroxyalprazolam	5	10	10-200	0.996	22.4	-77.6	Suppression
alpha-hydroxymidazolam	1	5	5-200	0.998	102.9	2.9	Enhancement
Oxazepam	10	50	50-200	0.985	15.5	-84.4	Suppression
Lorazepam	10	25	25-200	0.982	7.1	-92.9	Suppression

## False negatives:

CBS-MS: 4 samples (5%),

➤ Lorazepam, Temazepam, Oxazepam, 7-Aminoclonazepam → High ME were observed → Change/Add the IS (Diazepam-D5)

# Summary & Future directions

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- CBS-MS shows great promise-“proof of concept data”
- CBS-MS identified 96 drugs in the urine samples, of which 86 (89%) were confirmed by an LC-MS/MS confirmation test.
- The CBS-MS method identified 46% more drugs than the immunoassay. 9 and 26 false negatives were reported by CBS and POC-CUP methods, respectively.
- CBS-MS allowed for direct identification of drugs and metabolites, not just drug class.
- CBS-MS has demonstrated that this technology is an attractive alternative to the immunoassay due to its simplicity, sensitivity and speed of analysis
- **Method modification for polar compounds such as glucuronide metabolites by using different coating materials.**
- **Evaluate more positive samples for each drug classes.**





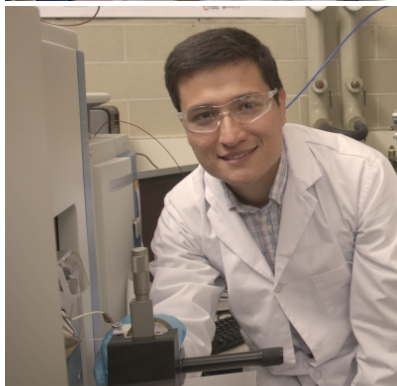
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SFGH



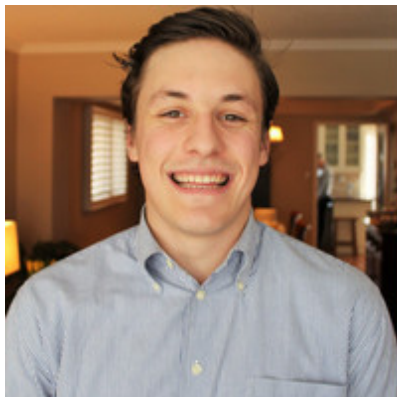
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Thank you!  
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