A NOVEL METHOD FOR URINE DRUG SCREENING BASED ON DESORPTION ELECTROSPRAY IONIZATION (DESI) MS ANALYSIS

Julia Balog[1], Erika Stark[1], Eltahir I Elbakri[2], Rav Sheth[1], Jon Danaceau[1], Praveen Kumar[1], John Vukovic[1], Steven Pringle[3], Patrice Ohouo[2]

[1]Waters Corporation, Milford, MA, [2]CleanSlate Centers, Inc. and Total Wellness Centers, Holyoke, MA,[3]Waters Corporation, Wilmslow, UK.

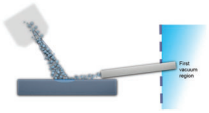
The goal of this study was to develop an ultra-rapid mass spectrometry-based screening method capable of routine detection of drug metabolites in urine samples.

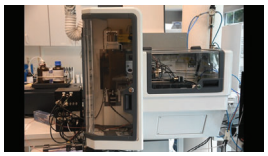
Drug of abuse screening – Can we use a fast mass spectrometry-based screening method instead of immunoassays?

- **All samples undergo screening**
- **Automated, cheap, rapid response time for the requesting clinicians**
- **Selective detection of illicit and therapeutic agents across many drug classes**
- **Higher specificity than conventional immunoassay-based screening**
- **Decreased false positive/negative results**
- **Flexibility and improved capacity to test for Novel Psychoactive Substances (NPS)**

DESI TECHNOLOGY

- A charged solvent is directed towards the sample mobilizing molecules from the sample surface
- Directly analyzed by MS – ambient ionization technology
- 1-2 seconds per sample





- Prototype DESI XS with automated slide sorting capability
- 20 plates (80 slides)
- Using 384 well plates with 3s per sample = 6.5 hours analysis time ~7500 patient samples

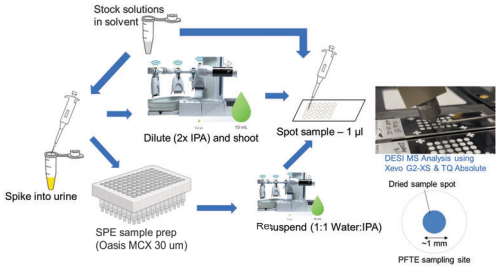
DRUG PANEL

Is DESI Sensitive enough: Cutoff concentrations between 2-100ng/mL, need to quantify 40% of cutoff (0.8—40 ng/mL)

General Class	Compound	MS ion Mode	Transition 1	Cutoffs (ng/mL)	40% LoQ (ng/mL)
Fentanyl	Fentanyl	ES+	337.2> 105.1	2	0.8
Fentanyl	Fentanyl, nor	ES+	233.1> 84.1	2	0.8
Buprenorphine	Buprenorphine	ES+	468.2> 84.1	5	2
Buprenorphine	Buprenorphine, nor	ES+	414.2> 83.1	5	2
Opiates/ Opiods	Morphine, 6-Acetyl	ES+	328.1> 165.1	5	2
Cannabinoids	(-)-Δ9-THC	ES (-)	213.3> 193.2	5	2
Creatinine	Creatinine (mg/dL)	ES+	114.0> 86.0	20	8
Gabapentanoid	Gabapentin	ES+	173.1> 155.2	500	200
Cannabinoids	(-)-11-nor-9-Carboxy-Δ9-THC	ES (-)	343.2> 299.2	5	2
Benzodiazepines	Alprazolam	ES+	309.1> 281.1	100	40
Benzodiazepines	alprazolam, a-Hydroxy	ES+	325.1> 297.1	100	40
Antidepressants	Amitriptyline	ES+	278.2> 91	100	40
Amphetamines	Amphetamine	ES+	136.1> 119.1	100	40
Carisoprodol	Carisoprodol	ES+	261.2> 176.1	100	40
Benzodiazepines	Clonazepam, 7-Amino	ES+	286.1> 121.1	100	40
Opiates/ Opiods	Codeine	ES+	300. > 215.1	100	40
Antidepressants	Cyclobenzaprine	ES+	276.2> 215.2	100	40
Dextrophan/ Levorphanol	Dextrorphan	ES+	258.2> 133.1	100	40
Benzodiazepines	Diazepam, nor	ES+	271.1> 140.1	100	40
Methadone	EDDP	ES+	278.2> 234.2	100	40
Benzodiazepines	Flurazepam, 2-Hydroxyethyl	ES+	333.1> 109.	100	40
Opiates/ Opiods	Hydrocodone	ES+	300.1> 199.1	100	40
Opiates/ Opiods	Hydrocodone, nor	ES+	286.1> 199.1	100	40
Opiates/ Opiods	Hydromorphone	ES+	286.1> 185.1	100	40
General Class	Compound	MS ion Mode	Transition 1	Cutoffs (ng/mL)	40% LoQ (ng/mL)
Benzodiazepines	Lorazepam	ES+	321> 275.1	100	40
Amphetamines	MDA	ES+	180.1> 163.1	100	40
Amphetamines	MDMA	ES+	194.1> 163.1	100	40
Carisoprodol	Meprobamate	ES+	219.1> 158.1	100	40
Methadone	Methadone	ES+	310.2> 105.1	100	40
Amphetamines	Methamphetamine	ES+	150.1> 119.1	100	40
Opiates/ Opiods	Morphine	ES+	286.1> 201.1	100	40
Ketamine	Norketamine	ES+	224.1> 125	100	40
Antidepressants	Nortriptyline	ES+	264.2> 105	100	40
Benzodiazepines	Oxazepam	ES+	287.1> 241.1	100	40
Opiates/ Opiods	Oxycodone	ES+	316.1> 241.1	100	40
Opiates/ Opiods	Oxycodone, nor	ES+	302.1> 227.1	100	40
Opiates/ Opiods	Oxymorphone	ES+	302.1> 227.1	100	40
Gabapentanoid	Pregabalin	ES+	160.1> 97.1	100	40
Opiates/ Opiods	Tapentadol	ES+	222.2> 107.1	100	40
Benzodiazepines	Temazepam	ES+	301.0> 255.1	100	40
Tramadol	Tramadol	ES+	264.1> 58.1	100	40
Tramadol	Tramadol, n-desmethyl	ES+	250.2> 44.1	100	40
Benzodiazepines	Triazolam, a-Hydroxy	ES+	359.1> 176.1	100	40
Cocaine	Benzoylecgonine	ES+	290.2> 168.1	100	40
Cocaine/ alcohol	Cocaethylene	ES+	318.2> 196.1	100	40
Sedative/ Z drugs	Zolpidem phenyl-4-carboxylic acid	ES+	338.2> 92.1	100	40
Kratom	Mitragynine	ES+	399.2> 174.1	20	8
Alcohol	Ethyl sulfate (EtS)	ES (-)	124.9> 97	250	100

SAMPLE PREPARATION

In addition to dissolving in solvent two preparation methods were used (dilute and shoot and SPE) before spotting 1uL onto the sample plate

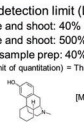


XEVO™ G2-XS TOF MS DATA

Dextrophan

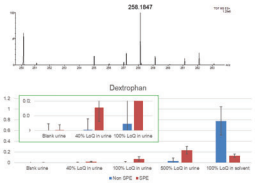
- DESI detection limit (LoD)
  - Dilute and shoot: 40% LoQ in solvent
  - Dilute and shoot: 500% LoQ in urine
  - SPE sample prep: 40% LoQ in urine

*LoQ (Limit of quantitation) = Threshold or cutoff



[M+H]+ = 258.185

Concentration for 100% LoQ is 100 ng/mL

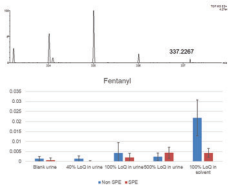


Fentanyl

- DESI detection limit:
  - Dilute and shoot: 40% LoQ in solvent
  - Dilute and shoot: >500% LoQ in urine
  - SPE: 100% LoQ in urine

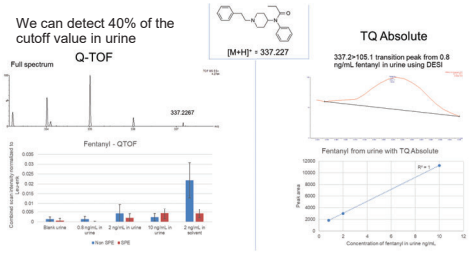
[M+H]+ = 337.227

Concentration for 100% LoQ is 2 ng/mL



XEVO TQ ABSOLUTE MS DATA

We can detect 40% of the cutoff value in urine



OVERALL PERFORMANCE

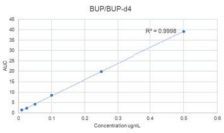
Number of drug molecules detected from urine with different methods (n=45)

	Xevo G2-XS no SPE	Xevo G2-XS SPE	Xevo TQ Abs SPE
40% LoQ	6	25	35
100%LoQ	16	35	39
500%LoQ	30	40	41

- Molecules not detected at 100% LoQ:
  - Fentanyl, nor (2 ng/mL)
  - Buprenorphine; Buprenorphine, nor; 6-MAM (5 ng/mL)
  - Pregabalin, MDA (100 ng/mL)

TOWARDS QUANTITATION

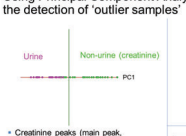
- Measuring Buprenorphine concentration vs Buprenorphine-d4 standard
  - BUP-d4 concentration 100ng/mL
  - BUP concentration: 10, 25, 50, 100, 250, 500 ng/mL
  - Area under the curve

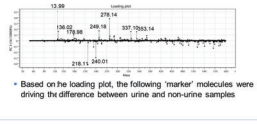


Quantitation with R^2 = 0.9998 correlation

ANOMALY DETECTION

- Using Principal Component Analysis (PCA) and Linear Discriminant Analysis (LDA) for the detection of 'outlier samples'





- Creatinine peaks (main peak, isotopes, dimer and adduct) excluded from analysis
- Based on he loading plot, the following 'marker' molecules were driving the difference between urine and non-urine samples

	Urine	Non-urine	Total
Urine	19	1	24
Non-urine	2	2	24
Total	21	7	48

- A total of 85.42% correct classification rate with only 8.3% of the non-urine samples falsely classified as urine (false negative)

CONCLUSIONS

- **Drug of abuse molecules ionize well with DESI – that is mandatory if we want to push the sensitivity limits**
- **We have created an automated, flexible, rapid plate reading system using DESI technology with options to run 80 slides or 20 plates without supervision and export data on the fly**
- **Using some specific sample preparation, we can reach the desired sensitivity for most of the molecules with DESI (2 ng/ml ->100 ng/ml) depending on the molecule**
- **Using parallel TOF and MRM methods allow us to:**
  - **Spot anomalies regarding urine or non-expected molecules**
  - **Immediately include novel psychoactive substances in the detection method once identified**

For Research Use Only

TO DOWNLOAD A COPY OF THIS POSTER, VISIT WWW.WATERS.COM/POSTERS

720009085EN