Evaluation of an Ultrafast Online SPE/TOF System to Screen for Drugs in Forensic Toxicology

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Introduction

Forensic drug screening has traditionally relied on immunoassays and then analytical confirmation with a quantitative method such as GC/MS or LC/MS. The steady increase in the number of samples requiring analysis has created a bottleneck in screening for these drugs across different classes and longer turnaround times for confirmatory tests. While traditional methods are convenient and well established, they are not as sensitive or specific as LC/MS. In the present study, we evaluated the ability of an ultrafast SPE/TOF system to screen across different classes of drugs in urine in a single injection while approaching the sensitivity and accuracy of LC/MS and maintaining the speed and efficiency of a screen (sample cycle times <15 seconds per sample



Experimental

Generic mass spectrometry and SPE methods were created for a panel of drugs that span multiple drug classes on a Highthroughput RapidFire Mass Spectrometry System interfaced to a 6550 QTOF. Drug-free urine was spiked with a panel of drugs for simultaneous analysis, treated with glucuronidase (if needed), diluted, and injected for analysis. Sample cycle times were under 15 seconds per sample. Data was acquired in MS mode using 2Ghz EDR, high sensitivity, 100-1000mz, and 3 spectra/s. Data analysis was performed using MassHunter Quantitative Analysis B.06.00 software.

Generic RapidFire conditions:

Buffer A = Water with 0.09% FA, 0.01% TFA Buffer B = 5% Acetonitrile in Water Buffer C = 50% Methanol, 50% Isopropanol, with 0.05% FA, 5mM ammonium formate

Injection volume: 10 µL SPE Cartridge: Agilent RapidFire Cartridge C

RF State 1: sip sensor RF State 2: 3000 ms. RF State 3: 1500 ms RF State 4: 7000 ms RF State 5: 1000 ms

Generic 6550 QTOF conditions:

Gas Temp: 200° C Gas Flow: 8 L/min Nebulizer: 45 psi Sheath Gas Temp: 250° C Sheath Gas Flow: 11 I/min Nozzle Voltage: 500 V Capillary Voltage: 3500 V

Benzodiazepine RapidFire conditions:

Buffer A = Water with 0.09% FA, 0.01% TFA Buffer B = 10% Acetonitrile in Water Buffer C = 50% Methanol, 50% Isopropanol, with 0.09% FA, 0.01% TFA

Injection volume: 10 µL SPE Cartridge: Agilent RapidFire Cartridge A2

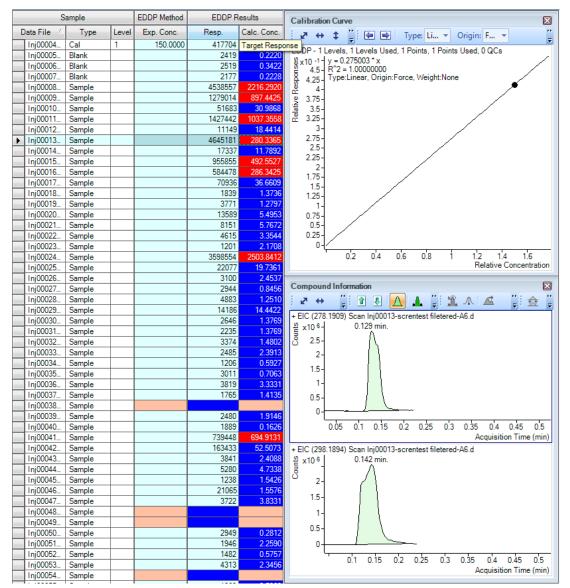
RF State 1: sip sensor RF State 2: 3000 ms. RF State 3: 3000 ms RF State 4: 6000 ms RF State 5: 500 ms

Benzodiazepine 6550 QTOF conditions:

Gas Temp: 350° C Gas Flow: 8 L/min Nebulizer: 50 psi Sheath Gas Temp: 400° C Sheath Gas Flow: 11 I/min Nozzle Voltage: 500 V Capillary Voltage: 3500 V

Results and Discussion

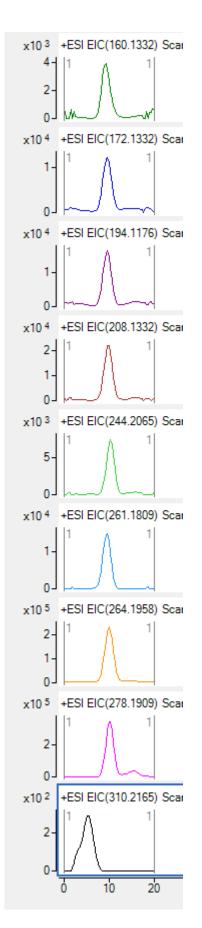
A panel of drugs including: gabapentin, pregabalin, tramadol, carisoprodol, methadone, EDDP, PCP, 6-MAM, benzoylecgonine, and amphetamines in urine had excellent linearity within their measured ranges with R² values greater than 0.995. A single cutoff value was determined for each analyte (5-5000 ng/ml) and combined into a single sample injection. The combined cutoff sample was injected multiple times and had an accuracy within 15% and a coefficient of variation within 10%. Human samples containing different analytes at varying concentrations both above and below the cutoff were tested. This screening method accurately identified positive samples as above the established cutoff concentration.



A forced single point calibration curve was used to determine if 48 human samples were above or below the cutoff threshold. Samples above are indicated in red and below in blue, making identification easy.

Sample ID	EDDP	Sample ID	EDDP	Sample ID	EDDP
1	X X	17	X X	33	
2	X X	18		34	X X
3		19		35	
4	X X	20		36	
5		21		37	
6	X X	22		38	
7		23		39	
8	X X	24		40	
9	Х	25		41	
10		26		42	
11		27		43	
12		28		44	
13		29		45	
14		30		46	
15		31		47	
16		32		48	

X indicate the expected EDDP positives (as determined by LC-MS/MS) for the human samples and X indicates the positives as determined by RapidFire/MS screen.



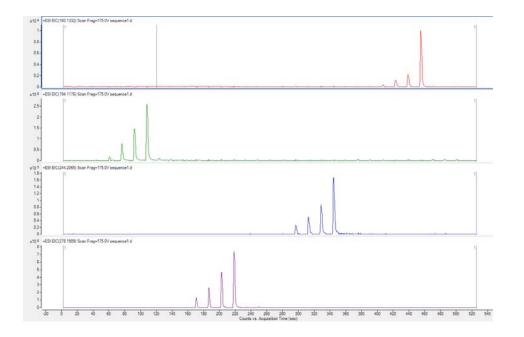
A panel of drugs including pregabalin (160.1332), gabapentin (172.1332), MDMA (194.1176), MDEA (208.1332), PCP (244.2065), Carisprodol (261.1809), Tramadol (264.1958), EDDP (278.1909), and Methadone (310.2165) were combined into a single injection at different concentrations.





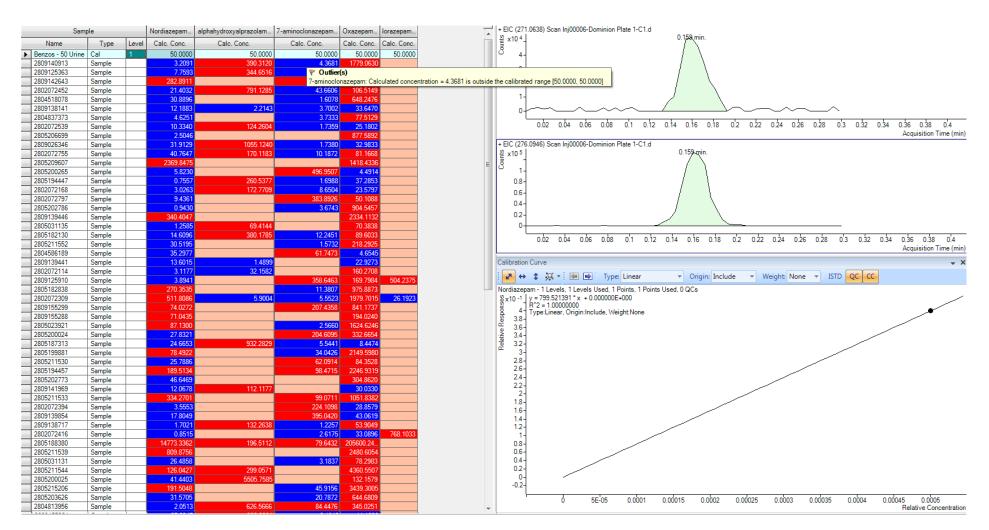


Results and Discussion



Representative standard curves: MDMA (50-1000ng/ml), EDDP (50-400 ng/ml), PCP (5-100 ng/ml), Pregabalin (100-5000 ng/ml) spiked into urine. All of the analytes were linear in the measured ranges and had R² values greater than 0.995. They all had good peak shape and no significant carryover was observed.

A panel of benzodiazepines including: nordiazepam, alpha-hydroxyalprazolam, 7-aminoclonezepam, oxazepam, lorazepam and temazepam in urine had excellent linearity within their measured ranges with R² values greater than 0.995. A single cutoff value was determined for each analyte (50 ng/ml) and combined into a single sample injection. Human samples containing different benzodiazepines at varying concentrations both above and below the cutoff were tested. This screening method accurately identified positive samples as above the established cutoff concentration – 100% concordance to LC/MS/MS results



A forced single point calibration curve was used to determine if 96 human samples were above or below the cutoff threshold. Samples above are indicated in red and below in blue, making identification easy.

Conclusions

Qualitative analysis of a panel of drug analytes was quickly, accurately and precisely measured in urine using an ultrafast SPE/TOF method (<15s/sample) with the potential to add more analytes to this panel. Using this SPE/TOF methodology, sensitivity and specificity comparable to LC/MS/MS were achieved without compromising the throughput and speed of traditional methods.