Introduction

Law Enforcement officials, employers and pathologists use forensic drug screening extensively today. The steady increase in sample volume has led to the need for greater analytical capacity within forensic toxicology laboratories, placing a strain on traditional technologies like GC/MS or LC/MS. In the present study, we evaluated the ability of an ultrafast SPE/MS/MS system to quantitatively measure a panel of benzodiazepines or buprenorphine & norbuprenorphine (bup/nor) in urine at low ng/mL concentrations, with sample cycle times of <15 seconds per sample. Accuracy, precision, and linearity results achieved with this ultrafast SPE/MS/MS were comparable to LC/MS/MS at rates faster than 20-30 fold.

In the present study, blank matrix containing internal standard was spiked with the analytes of interest in a range of concentrations to prepare a calibration curve. The benzodiazepine panel consisted of: oxazepam, temazepam, lorazepam, nordiazepam, 7-aminoclonazepam and alpha-hydroxylorazepam. Benzodiazepine samples were subjected to enzymatic hydrolysis followed by centrifugation and dilution (1:50) with water, then injected on the SPE/MS/MS system. In case of the bup/nor, after the enzymatic hydrolysis, samples were extracted by an offline solid phase extraction procedure using 96 well SPE plates, dried down and reconstituted in 5% methanol in water. Analysis of all samples was performed at a rate of <15 seconds per sample using a RapidFire High-throughput Mass Spectrometry system coupled to Agilent 6490 and 6460 triple quadrupole mass spectrometers respectively. Online SPE methods were optimized for each analyte.

Experimental

Sample Preparation

A mixed analyte calibration curve was prepared in drug free urine from individual stock solutions for both panels. Then internal standards were added and followed by enzymatic hydrolysis and centrifugation. For the benzodiazepine panel after incubation, samples were centrifuged at 10,000 rpm for 5 minutes. Samples were then diluted 1:50 with water, transferred to a 96 well plate and centrifuged prior to injection on the Agilent RapidFire/MS/MS system. For the bup/nor panel, after enzymatic hydrolysis, samples were centrifuged at 3,000 rpm for 5 minutes. Then a solid phase extraction protocol was applied on the supernatant using Plexa PCX plates. The eluate from the SPE was dried down under nitrogen, reconstituted with 5% methanol and injected on the RapidFire/MS/MS system. The following MRM transitions were monitored for both panels using Agilent 6490 and 6460 triple quadrupole mass spectrometers.

Results and Discussion

Linearity: The analytes in both panels had excellent linearity within the measured ranges with R² values greater than 0.995

Table: Benzodiazepines were quantified between 50-12,500 ng/mL and were determined to have LOQs of less than 25 ng/mL.

Table: Buprenorphine was quantified between 200-400 ng/mL and determined to have an LOQ of 2.5 ng/mL for both analytes.

Method Comparison:

Temazepam (n=16)

Nordiazepam-D5 (IS)

Nordiazepam-D2 (IS)

Compound Q1 Q3 Fragmentor CE

Alpha-Hydroxylorazepam Quant 325.1 297.1 380 28

Alpha-Hydroxylorazepam Qual 325.1 215.9 380 44

Lorazepam Quant 321 275 380 21

Lorazepam Qual 321 229 380 35

Temazepam Quant 301.1 255.1 380 29

Temazepam Qual 301.1 177 380 45

Oxazepam Quant 287.1 241.2 380 21

Oxazepam Qual 287.1 104 380 36

7-Aminoclonazepam Quant 286.1 222.1 380 25

7-Aminoclonazepam Qual 286.1 121.1 380 33

Nordiazepam Quant 271 140 380 29

Nordiazepam Qual 271 165.1 380 25

Nordiazepam-D2 (IS) 276.1 140.1 380 11

Nordiazepam-D2 (IS) 276.1 165.1 380 31

Compound Q1 Q3 Fragmentor CE

Buprenorphine-d6 472.3 59.1 200 62

Buprenorphine Quant 468.3 55.1 200 62

Buprenorphine Qual 468.3 396.2 200 45

Buprenorphine-d3 417.3 83.1 188 60

Norbuprenorphine Qual 414.3 83.1 188 60

Norbuprenorphine Qual 414.3 101.1 188 50

Conclusions

• A panel of 6 benzodiazepine drugs were accurately and precisely quantified in urine within a linear range of 50-12500 ng/mL. All 6 benzodiazepines were simultaneously analyzed at 14s/sample using SPE/MS/MS.

• Buprenorphine and its metabolite norbuprenorphine were rapidly, accurately and precisely measured in hydrolyzed urine within a linear range of 2.5 to 400 ng/mL. Both analytes were analyzed at 15s/sample using SPE/MS/MS.

• SPE/MS/MS results from both methods were comparable to LC/MS/MS, but at >20x faster analysis times providing a high-throughput capacity of >240 samples per hour.

• The Agilent RapidFire/MS/MS system may be useful for fast and efficient detection of similar small molecule analytes in urine.

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