Rapid Hydrolysis of Benzodiazepines in Urine

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**Objective**

Determine a more time effective method for the analysis of benzodiazepines in human urine.

- Use the recombinant β-glucuronidase enzyme, IMCSzyme™, to reduce enzyme hydrolysis by 2.5 hours.
- Use Thomson eXtreme|FV to reduce sample preparation time.

**Methods**

**Quality Control**

The patient samples and control urines were run with a 5 point calibration curve. The curves were spiked with concentrations of 75, 300, 1,000, 5,000, and 10,000 ng/mL of all drugs except Zolpidem. The Zolpidem curve consisted of concentrations of 75, 300, 500, 2,500, and 5,000 ng/mL. The calibrators were obtained from 1 mg/mL standards or 100 µg/mL standards (Zolpidem) purchased from Cerilliant Corporation.

**Sample Preparation**

Urine specimens were hydrolyzed using a combination of 50µL of IMCSzyme™ rapid hydrolysis buffer and 40µL of IMCSzyme™ enzyme per sample at 55°C for 30 minutes. Thomson eXtreme|FV 0.2µm PVDF containing 40% methanol in HPLC water and 50µL of hydrolyzed urine were pressed to filter out any particulate in the samples. Internal standards were incorporated into every sample. Patient samples were prepared, in addition to controls and calibration curve samples.

**LC/MS/MS**

An ABSciex 3200® MS/MS coupled with a Shimadzu Prominence LC system was used for analysis of the samples. Electrospray ionization (ESI) was used in conjunction with Multiple Reaction Monitoring (MRM) for mass spectrometry analysis.

**Results**

Validation studies were run on three different days. Method validation was accomplished using a calibration curve, LOD and LOQ samples run in triplicate, low and high benzodiazepine concentration controls, and patient samples. Within run and between run accuracy and precision were evaluated. An ion suppression study was also done to compare analyte recovery with the extraction method versus adding the analyte post extraction. The percent recovery ranged from 91%-111%, and the ion suppression ranged from -11% to -11% for the analytes.

Figure 1 shows chromatograms for a negative result for the drugs lorazepam, nordiazepam, and oxazepam.

Figure 2 shows chromatograms for a positive result for the drugs lorazepam, nordiazepam, and oxazepam.

**Conclusion**

This method shows the potential for the use of the recombinant β-glucuronidase enzyme, IMCSzyme™, in conjunction with Thomson eXtreme|FV 0.2µm PVDF, in the analysis of benzodiazepines. This method will dramatically decrease the amount of time needed to run benzodiazepine analysis in clinical and forensic toxicology settings.

**Acknowledgements**

I would like to thank Crystal Xander B.S., who provided direction and guidance throughout this project. I would also like to thank Dean Fritch, Ph.D. for his guidance, and assistance in data analysis.

**References**