Advanced Toxicology Testing in Urine using Ultra High Pressure Liquid Chromatography Tandem Mass Spectrometry

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Abstract

Drug testing, specifically advanced toxicology and pain management testing, has become an integral part of treatment and diagnostic programs during the last decade. The number of medication injuries caused by inappropriate use is in the millions [a]. As such, the need for accurate and precise testing is also on the rise. Many laboratories have converted their Gas Chromatography Mass Spectrometry (GC/MS) methods to more efficient Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS) methods. It has been documented that LC/MS/MS methods are more robust, specific, and efficient than GC/MS methods [b, c] . Here we developed a method that is faster, more sensitive, and has better resolution than the LC/MS/MS platform [d] ; this method utilizes the Ultra High Pressure Liquid Chromatography Tandem Mass Spectrometry (UPLC/MS/MS) platform.

Introduction

Toxicology testing, specifically in the pain management arena, has increased several-fold in the last decade. Regulations that require physicians who prescribe analgesics to monitor their patients are partly responsible for the increase. In fact, a CDC study showed that pain reliever abuse has increased 111% between 2003 and 2005 [e]. Moreover, the prevalence of abusing illicit drugs in the pain management population is approximately 11% [f]. Additionally, the increase in testing is also due to physicians wanting to provide the best relief to their patients while still ensuring their safety. Physicians are responsible for the patients' health; consequently they find it necessary to monitor the concentrations of the drugs being prescribed. Most opt to do so by performing urinalysis. Due to the inherent complexities of urinalysis, effective testing requires the physicians to work closely with a reliable laboratory [f]. Furthermore, physicians need extremely accurate results in a short time frame, generally within four days. As such, developing methods that are highly sensitive, highly specific, and can be done in minimal time are of great importance.

Methods & Materials

Urine samples are hydrolyzed, spiked with internal standard, and run in a 49-compound UPLC/MS/MS method

Sample Collection & Preparation:

Urine samples are shipped to our Troy, MI facility via two-day air.

Methods & Materials, cont.

A one milliliter aliquot is placed in a microcentrifuge tube and spun at 220 x *g* for 5 minutes. 60% of the supernatant is transferred to a 2mL glass vial and spiked with 2.5% *β-Glucuronidase Type HP-2* enzyme from *Helix pomatia* (activity ~330 units). Samples are incubated for 60 minutes at 40°C. Post incubation, the sample is split in half. One half diluted with 50%/50% Methanol/Water mixture spiked with six internal standards; the other is diluted with 50%/50% Methanol/Water mixture spiked with two internal standards.

Standards:

All standards were purchased from Cerilliant. Compounds tested includes four amphetamines, ten opiates/opioids, five benzodiazepines, eleven tricyclic antidepressants, twelve common prescription drugs, and two illicit compounds.

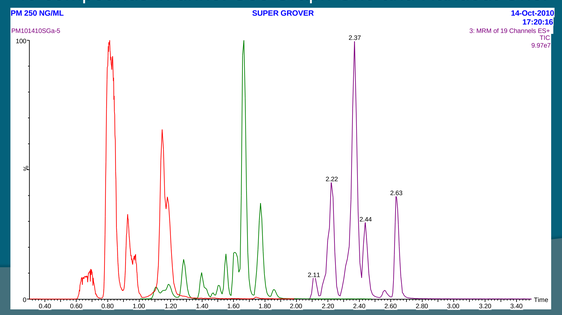
Instrumentation and Method:

Samples were run on a Acquity TDQ coupled to an Acquity UPLC Autosampler. The mass spectrometer was calibrated according to manufacturer's guidelines. Quality control checks were run after the standards and every ten injections thereafter. Mobile phase contained 2.5mM Ammonium Formate and 2.5mM Ammonium Acetate in Water and Methanol for A and B, respectively.

All samples were run on a Pinnacle® 1.9µm 140Å 50 X 2.1mm Column (Restek) with an Ultra Shield UHPLC Pre-column filter (Restek) and Acquity Inline Filter (Waters).

Results

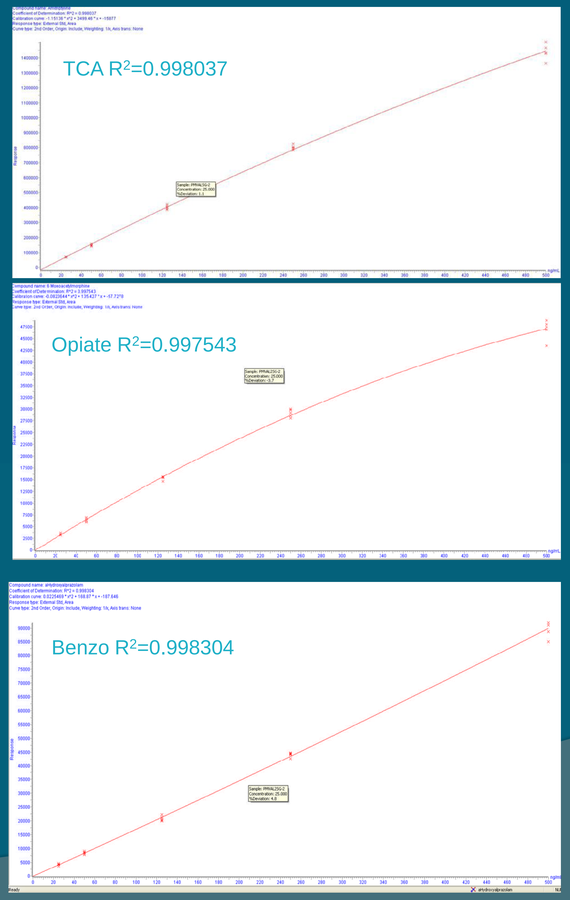
Great separation and resolution was achieved with the advanced toxicology panel using the described method with 49 compounds in urine. See Graph One and Handout One.



Graph 1: Total Ion Count for UPLC Advanced Toxicology Panel

Results, cont.

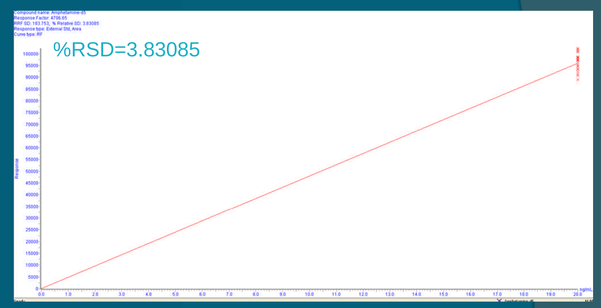
In addition to high separation efficiency, excellent sensitivity, good resolution, and increased analysis speed, linearity of at least 0.995 (R^2) is achieved for all compounds. Please see graphs below. Each demonstrates five sets of standards overlaid.



Graph 2: Calibration Curves Amytriptyline, 6-Monoacetylmorphine, and α-Hydroxyalprazolam, Respectively

Results, cont.

With repeated injections, the relative standard deviation (%RSD) for all compounds was below ten. See graph number three, which demonstrates 25 injections. Moreover, intrarun and interrun variability for each compound was low. Again, the %RSD was below ten for area. (Results not shown).



Graph 3: Repeated Injection Graph

Summary & Conclusion

In summary, we were able to develop an advanced toxicology panel that tests for 49 compounds in 3.5 minutes using the UPLC/MS/MS platform. This method has proven to be robust, sensitive, specific, and applicable in urine.

References

a. U.S. Food and Drug Administration Press Release November 25, 2009 www.fda.gov/NewsEvents/Newsroom
b. Giton *et. al.* Clinica Chimica Acta 2010 41(17-18): 1208-13
c. Krone *et. al.* The Journal of Steroid Biochemistry and Molecular Biology 2010 121(3-5):496-504
d. Nováková and Vickôvá Analytica Chimica Acta 2009 656(1-2):8-35
e. U.S. Centers for Disease Control and Prevention Press Release June 17, 2010 www.newsroom.cdc.gov
f. Cone and Caplan 2009 Postgraduate Medicine 121(4):91-102

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