Advanced Toxicology Testing for 80 Compounds using Core-Shell Technology on Ultra High Pressure Liquid Chromatography Tandem Mass Spectrometry in Saliva

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Introduction

Pain Management is one the fastest growing fields in medicine. As we develop more technology, diseases and disorders that could not be diagnosed and treated previously are now being documented. A large segment of the pain management arena is pediatric pain management. In addition to the common issues with opioid therapy, which are aberrant behavior, dependence, and aversion, physicians tasked with the job of alleviating pediatric pain must contend with the experimental behavior of adolescents and teenagers. With the advent of things like crunk parties, advanced toxicology testing is even more imperative. This testing is used to determine if patients are in compliance with the drug regimen by the presence or absence of particular drugs.

Methods and Materials

Saliva samples were prepared for analysis by removing existing protein using acetonitrile spiked with internal standards. Samples were vortexed and then centrifuged at 220 x g for 10 minutes. The supernatant was removed, filtered and injected from an ultra high pressure liquid chromatography system onto a core-shell column (Phenomenex e.g. *Kinetex 1.7µm C18 50 x 2.1 mm*) into a tandem mass spectrometer. The method uses mobile phases of water and methanol with ammonium formate and ammonium acetate buffers. This method was developed to quantitate eighty compounds in one run. Compounds includes opiates/opioids, amphetamines, cannabinoids, benzodiazines, commonly prescribed medications, drugs of abuse, and tricyclic antidepressants. All standards were purchased at Cerilliant or Cayman Chemical. Finally, it has a run time of 4.5 minutes and has great sensitivity while allowing for the resolution of isobaric compounds.

Results

Using this advanced toxicology method, we have been able to improve separation and resolution, especially amongst the isobaric compounds. The compounds were linear from 1-1500 ng/mL with a coefficient of determination (R^2) of at least 0.995 for all compounds. Imprecision has a specification limit of ±20%, however the method performed better than specification across all 96 transitions. Similarly, inaccuracy has a specification limit of ±20% but many compounds performed better than ±10% with several within ±5% (23 of the 96 transitions). The lower limits of detection and quantification was as low as 1ng/mL for most of the compounds; range 1-25ng/mL. Please note 1ng/mL is the lowest level tested, based on the absolute counts many compounds can be detected below the 1ng/mL level. In the saliva matrix, we saw little to no interference for the compounds of interest. Enhancement of the signal was also not experienced. The interference of the signal was such that internal standard correction was not required in order to obtain repeatable, accurate results across all compounds. Moreover, there was no decrease in resolution for the isobaric compounds. This was demonstrated repeatedly with real patient samples that had multiple positives.

The column was selected based on its retention of the compounds of interest and its life of use. Four batches of the column were tested over six months with an average of 3162 injections per column; 80% of the injections were patient samples or matrix injections.

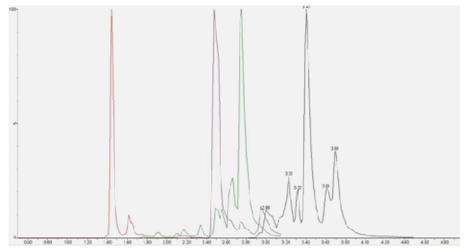

Figure 1: Total Ion Chromatogram of Method (96 transitions)

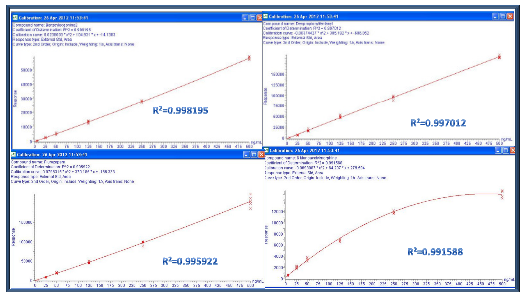

Figure 2: Linearity of 5 sets of standards for Illicit, Common Prescribed Medication, Opiate, and Benzodiazepine respectively

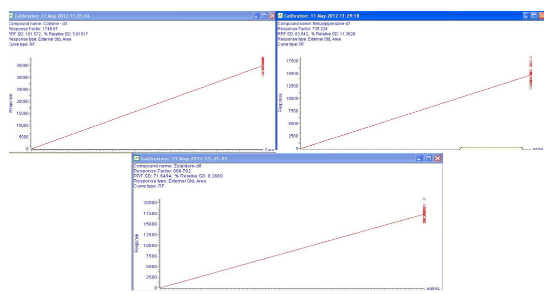

Figure 3: Precision of 25 injections (intrarun; over eight hours) Cotinine, BZP, and Zolpidem Internal Standards

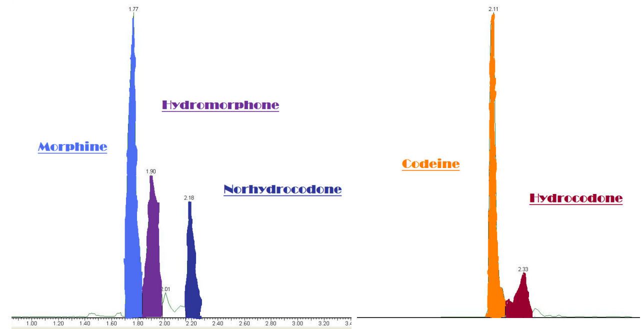

Figure 4: Chromatography of the Isobars (m/z 286 and 300, respectively)

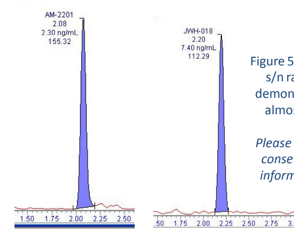

Figure 5: Chromatography of two spice compounds in oral fluid with s/n ratios above 100 with concentrations below 10ng/mL. This demonstrates the sensitivity of the method even though there are almost 100 transitions as well as the lack of interference of the matrix at low concentrations.

Please note that these are two different donor samples. All donors consented to drug testing. All testing, data collection, and donor information was utilized according to the WSL IRB requirements.

Discussion and Conclusions

With the ever changing landscape of drug testing due to designer compounds and user education, laboratories must adjust their approach frequently. As labs also adjust to the reimbursement changes, new testing paradigms must be developed. We were able to develop a method that was robust, efficient, and durable to changes as new compounds of interest emerge. At initial development, this method has 70 transitions for 52 compounds. In the following months we have added nearly 30 compounds without vast consequences to chromatography, limits of detection and quantification, precision, and accuracy. Lastly, we are able to support the industry as changes have occurred. Future plans are on developing a 173 compounds panel and increase isobar separation.

Acknowledgements

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