WESTERN SLOPE LABORATORY

Detection and Analysis of 31 Compounds in Oral Fluid by Ultra High Pressure LC/MS/MS Without Solid Phase Extraction

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Abstract

In the fields of pain management and addiction control, there is a need for an efficient and reliable oral fluid testing method for a multitude of commonly prescribed and illicit drugs. Oral fluid testing has become more desirable in the last decade because it provides a less invasive, accurate alternative to urinalysis should a urine specimen be unobtainable or questioned. Use of saliva as a testing matrix is also advantageous because there is decreased potential for sample adulteration. In recent years, methods for analysis of oral fluid have been published utilizing the Ultra High Pressure LC (UHPLC) technology and solid phase extraction (SPE) as a sample preparation technique. Avoiding SPE would save hours of preparation, increase efficiency and reduce cost. We present a validated method developed to test thirty-one commonly prescribed and abused compounds in oral fluid without utilizing SPE in sample preparation.

Introduction

Oral fluid has gained in popularity as an alternative matrix to urine for drug testing. Oral fluid is easy to collect, medically non-invasive, hard to adulterate, and correlates to plasma levels. Thereby making oral fluid a suitable matrix for advanced toxicology testing in the fields of pain management and addiction control monitoring. Western Slope Laboratory, LLC has always been on the cutting edge of innovative techniques, using a dilute and shoot method over SPE, and using a comprehensive panel over suites for each class of drugs. Western Slope Laboratory, LLC was founded on oral fluid drug testing at an affordable cost using novel instrumentation. This method utilizes an oral fluid matrix run on UHPLC/MS/MS instrumentation.

Materials and Methods

Saliva samples were prepared for analysis by removing existing protein with acetonitrile spiked with internal standards (Cerilliant). Samples were vortexed and then centrifuged at 220 x g for ten minutes. The supernatant was removed, filtered and injected onto the UHPLC/MS/MS. Saliva samples were run on a Pinnacle DB 1.9µm 100 x 2.1mm column (Restek) with an Ultra Shield UHPLC Pre-Column (Restek) and Acquity Inline Filter (Waters) in a thirty-one compound advanced toxicology method (Figure 2). The run time was seven minutes. The compounds include three amphetamines, seven opiates and opioids, three commonly prescribed medications, five benzodiazepines, nine illicit drugs, and four internal standards. Mobile phases of water and methanol were used, each with ammonium formate and ammonium acetate as modifiers.

Figure 1: Separation and Resolution of Isobaric Compounds

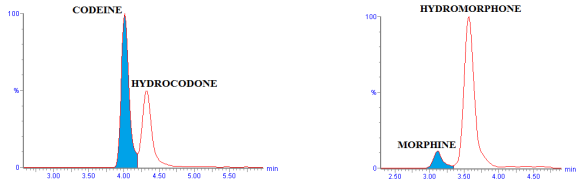


Figure 2: Total Ion Count for Pain Management Saliva Method

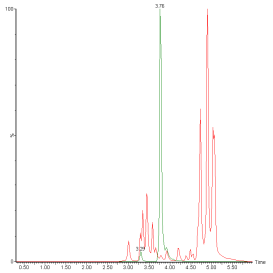
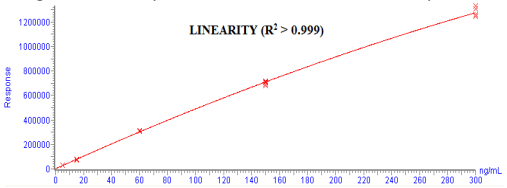


Table 1: LLOD/LLOQs and Signal-to-Noise Ratios of Drug Classes in the Method

Drug Class	LLOD*	LLOQ*	Signal–to–Noise (Concentration)
Amphetamines	1 ng/mL	1 ng/mL	194.28 (4.2 ng/mL)
Benzodiazepines	2 ng/mL	2 ng/mL	142.08 (3.6 ng/mL)
Commonly Prescribed Meds	1 ng/mL	1 ng/mL	191.49 (5.0 ng/mL)
Illicit Drugs	5 ng/mL	5 ng/mL	242.04 (16.6 ng/mL)
Opiates and Opioids	10 ng/mL	10 ng/mL	145.19 (5.4 ng/mL)

* LLOD and LLOQ are based on the highest compound in the drug class

Figure 3: Linearity of Five Sets of Standards Run In Replicate*



* Compound shown is of an opiate/opioid

Results

Using this advanced toxicology method we have been able to improve separation and resolution, especially among the isobaric compounds tested for in the saliva matrix (Figure 1). The linearity of all compounds was at least 0.990 (R^2) for a set of five standards that were run in replicate (Figure 3). A series of thirty injections, for intra and inter-run variability, gave %RSDs of under 20%, with most under 10%. The lower limits of detection (LLOD) and quantitation (LLOQ) are superior to our previous method (HPLC/MS/MS), with most detected at less than 5 ng/mL (Table 1). The advancements in LC/MS/MS technology have allowed for better resolution and sensitivity to be achieved. Resolution of isobaric compounds is needed because opiates are a common feature in pain management and addiction control monitoring, and many opiates have the same mass to charge ratio. Greater sensitivity is required for the detection low concentrations of analytes in oral fluid. With this greater sensitivity and better resolution, signal-to-noise is not compromised in a thirty-one compound method. Compounds such as methamphetamine, alprazolam, methadone, THC, and hydrocodone maintain a great signal-to-noise ratio even at concentrations less than 20 ng/mL in patient samples (Table 1).

Conclusion

An advanced toxicology method was developed to test thirty-one compounds in the saliva matrix using UHPLC/MS/MS technology that saves time and expenses in sample preparation, and is specific, robust and applicable to the pain management and addiction management treatment industries.

Acknowledgements

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