WESTERN SLOPE LABORATORY

Detection of Ethanol Consumption Biomarkers (Ethyl Glucuronide and Ethyl Sulfate) in Saliva by LC/MS/MS

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Abstract

Advanced toxicology testing, which is defined as comprehensive, flexible therapeutic drug monitoring at trace levels without screening, has been growing exponentially over the last decade. Three major segments of advanced toxicology testing are pain management, behavior management, and addiction cessation monitoring. In each, the need to ensure compliance with the treatment regimen is essential. Alcohol cessation is imperative for compliance in these segments due to drug-drug interactions and mediation of drug effects by alcohol. Most physicians rely upon blood or urine testing to demonstrate compliance in the aforementioned segments. Although both are appropriate matrices for drug testing, there are difficulties that limit the efficacy of these programs. Oral fluid testing has increased in popularity due to its ease of collection, relationship to plasma levels, and lack of adulteration potential. Here, we discuss the development of an advanced toxicology oral fluid method by high pressure liquid chromatography tandem mass spectrometry (LC/MS/MS) for ethyl glucuronide (EtG) and ethyl sulfate (EtS), alcohol consumption biomarkers.

Introduction

Ethanol consumption monitoring is essential in various aspects of drug cessation and management programs. The mediation of drug effects by ethanol can markedly increase the damage or harm done by the compound. In fact, alcoholism and alcohol misuse and abuse cost nearly $185 million to public health due to injury and illness in 1998[1]. Furthermore, these costs increased almost 4% in six years[1]. As physicians are responsible for the health of their patients in pain, behavior, and addiction management programs, it is imperative that testing for ethanol consumption is an integral part of the testing scheme of the aforementioned program. Currently, there are several methods, markers, and matrices that can be used for ethanol consumption testing. Each method has its benefits and downfalls, but critical to all would be the reduction of cost and adulteration potential while providing the most comprehensive result possible. As such, developing a highly specific, sensitive, and robust method that is applicable to oral fluid and utilizes advanced technology, with a large detection window, would be of great benefit to the physician and patients alike.

[1]Harwood (2000) Updating estimates of economic costs of alcohol abuse in United States: estimates, update methods, and data. NIAAA NIH Publication No 98-4327 pubs.niaaa.nih.gov/publications/economic-2000/

Methods and Materials

Saliva samples were collected using a modified Salivette® (Sarstedt) device. A 300 µL aliquot of saliva was mixed with three volumes of filtered (0.45 µm, Restek) acetonitrile containing internal standards of deuterated EtG and EtS. All standards were purchased from Cerilliant. The samples were centrifuged at 220 x *g* for 10 min and were filtered into sample vials. Samples were run on a MicroMass™Ultima mass spectrometer coupled to an Alliance 2795 HPLC. Mobile phase contained 0.2% formic acid in water/acetonitrile mixture. All samples were run on a Hypercarb 5µm, 100 x 2.1 mm column (Thermo Scientific). The method was validated for precision, accuracy, linearity, lower limits of detection and quantification in both matrix and solvent, where applicable. The method was further tested using a Synergi Hydro-RP 4µm, 100 x 4.6 mm column (Phenomenex) with 10mM ammonium formate mobile phase.

Results

We were able to achieve adequate resolution and separate the analytes from the matrix. In addition, all compounds were linear from 50- 5000 ng/mL with a coefficient of determination (r^2) of at least 0.999 for all compounds. Imprecision has a specification limit of ±20%, however repeated injections were under ±10% for all 6 transitions. Similarly, inaccuracy has a specification limit of ±20% and repeated injections were under ±10% at low levels (< 100 ng/mL). The accuracy was tighter at higher concentrations. Finally, the lower limit of detection and quantification was tested as low as 25 ng/mL for both EtG and EtS. These results were seen with both columns. Separation from the matrix was more pronounced with the Synergi Hydro-RP (Phenomenex) column.

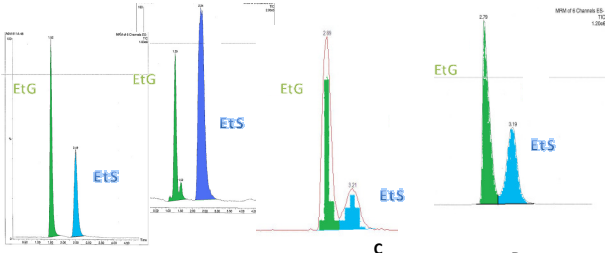

Figure One: (a) EtG and EtS in solvent on Hypercarb column (b) EtG and EtS in oral fluid matrix on Hypercarb column, (c) EtG and EtS in solvent on Synergi column, and (d) EtG and EtS in oral fluid on Synergi column

Results, cont.

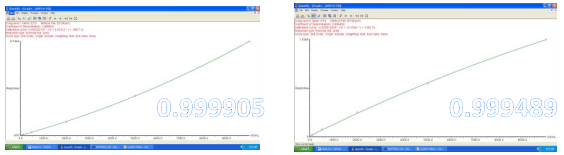

Figure Two: Calibration Curves for EtG and EtS, respectively

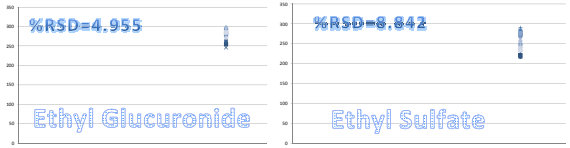

Figure Three: 30 repeat injections in matrix
EtG: RSD 4.955%, 112% recovery EtS RSD 8.842%, 103% recovery

As shown in figures 1-3, both methods were able to produce adequate chromatography even in the oral fluid matrix. Moreover, we were able to achieve great performance from the method in terms of its recovery, linearity, and repeatability. The lower limits of detection are tested in matrix for both analytes. At the present we have only tested as low as 25ng/mL. We were able to see both with a signal to noise ratio of greater than 15 (Figure Four).

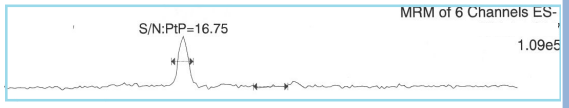

Figure Four: 25ng/mL EtG in Oral Fluid

Summary & Conclusions

In summary, we were able to develop an advanced toxicology method that tests for ethanol consumption in oral fluid in less than 5 minutes using LC/MS/MS. This method is robust, sensitive and specific.

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