TECHNICAL NOTE

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A summary of "Fast, Simple Method for the Analysis of Benzodiazepines in Meconium and an Interlaboratory Method Comparison"

Overview:

The quantitation of ten commonly prescribed benzodiazepines and their metabolites in meconium was developed using rapid enzyme hydrolysis using IMCSzyme™ followed by WAX-S dispersive pipette extraction (DPX) tips and LC-MS/MS analysis. This new hydrolysis process was simplified to directly adding buffer and enzyme solution to the meconium samples whereas prior processes required protein removal with acetonitrile and solvent evaporation prior to addition of the enzyme. The new method was evaluated for linearity, precision, extraction efficiency, and limits of detection and quantitation. A blind study was performed to illustrate the viability of the new method.

Materials and Methods:

All drug standards were purchased from Cerilliant Corporation. DPX WAX-S tips were purchased from DPX Labs, LLC. IMCSzyme™ β-glucuronidase was obtained from IMCS. To minimize the analysis time, the initial sample preparation used vortex mixing of the sample meconium matrix with water, instead of rigorous homogenization. The samples were hydrolyzed by β-glucuronidase enzyme at 55 °C for 1 hour. Post-hydrolysis samples were cleaned by acetonitrile protein precipitation and resulting supernatant processed by solid phase extraction using WAX-S tip prior to analyzing by LC-MS/MS.

Results:

The method was evaluated for percent recovery and percent ion suppression for each benzodiazepine. Ten common benzodiazepines and metabolites were evaluated, 7-aminoclonazepam, clonazepam, α-hydroxyalprazolam, alprazolam, nordiazepam, diazepam, midazolam, oxazepam, lorazepam, and temazepam. Analyte recoveries were greater than 50% (55% to 81%) and percent ion suppression did not exceed 45% for any analyte (Table 1). A ten point calibration plot covering the range of 5 ng/g to 1000 ng/g with four replicates at each concentration. The plot was used to evaluate the linear regression for this method, and all compounds had correlation coefficients above 0.994 with slopes ranging from 0.9948 to 1.0414 and y-intercepts below the limit of quantitation (LOQ). The limits of detection (LODs) ranged from 0.5 to 2.1 ng/g and the LOQs ranged from 1.5 to 6.4 ng/g, though thresholds were set at 5 to 10 ng/g. The average between-run imprecision, represented by the percent coefficient of variation, was 10.2% or below for each benzodiazepine at each concentration tested (100 ng/g and 1000 ng/g), and the total imprecision was found to be less than 13% for each analyte.

Conclusions:

The method established herein is characterized by simple vortexing for sample homogenation, fast and reliable IMCSzyme™ for hydrolysis in situ minimizing hydrolysis time to one hour, and a single WAX-S DPX tip for sample preparation that produces a small amount of clean, analyte rich acetonitrile to minimize sample preparation and solvent evaporation times. This method is quick yet effective. Correlation coefficients for each benzodiazepine were above 0.99. All precision values were below 15%. LODs and LOQs were below 5 and 10 ng/g, respectively.

Table 1. Percent recovery and percent ion suppression for each benzodiazepine

Compound	% Recovery	% Ion Suppression
Nordiazepam	60.33	13.21
Diazepam	54.97	27.19
7-Aminoclonazepam	64.23	38.20
Oxazepam	54.51	2.00
Temazepam	63.57	19.73
Alprazolam	80.74	6.54
Clonazepam	68.46	12.98
Lorazepam	56.32	-1.69
a-hydroxyalprazolam	80.95	6.03
Midazolam	77.95	42.82

Figure 1. ARUP Method vs. DPX Method

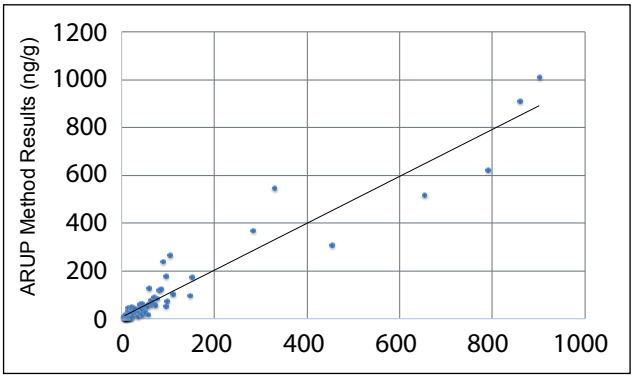

Figure 1. Correlation of positive patient sample results from the current method and the ARUP method. A blind study of 35 patient samples was also performed with a collaborating lab. The correlation of this method's results with the corresponding lab's results was greater than 0.92. The success of this blind study signifies the validity of this quick and easy method compared to a more intricate and lengthy method.

References:

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2. *Bar-Oz, B. "Comparison of Meconium and Neonatal Hair Analysis for Detection of Gestational Exposure to Drugs of Abuse" Archives of Disease in Childhood. Fetal and Neonatal Edition, 2003, 88, F98–F100*
3. *Williamson, S., "Determination of the Prevalence of Drug Misuse by Meconium Analysis" Archives of Disease in Childhood. Fetal and Neonatal Edition, 2006, 91, F291–F292*
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This information was summarized by IMCS from the technical poster "Fast, Simple Method for the Analysis of Benzodiazepines in Meconium and an Interlaboratory Method Comparison" presented by William Brewer –University of South Carolina at MSACL 2015.