SOFT 2022
Abstract Submission Form

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*****Do not exceed 600 words including tables and charts.*****

TITLE: Analysis of Benzodiazepines using Liquid-Chromatography Mass Spectrometry-Mass Spectrometry.

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Background/Introduction:

Novel benzodiazepines are prevalent within the community and are used to circumvent prosecution under the Controlled Substances Act. However, it has been demonstrated that benzodiazepines can cause impairment and thus affect the ability to safely operate any vehicle on roadways. At DFS, only one novel benzodiazepine is within the current scope (etizolam). Currently, DFS's procedure includes hydrolysis and solid phase extraction on day one which takes up to six hours to complete. These underivatized samples are analyzed by GC-MS for five benzodiazepines (diazepam, 7-amino-flunitrazepam, alprazolam, etizolam and midazolam). On day two, samples are derivatized for one hour and analyzed for the remaining eight benzodiazepines (7 amino-clonazepam, hydroxy alprazolam, hydroxy triazolam, nordiazepam, oxazepam, temazepam, hydroxy ethyl-flurazepam, and lorazepam). At the minimum, two and half days are required to complete analysis before the data can be reviewed.

Objectives:

The objective of this research was to shorten the analysis time, reduce consumption of case samples, expand the current scope of analysis, and move the assay from gas chromatography mass spectrometry (GC-MS) to liquid chromatography mass spectrometry-mass spectrometry (LC/MS/MS). Drug cases routinely screen positive using enzyme-linked immunoassay (ELISA) for the benzodiazepine drug class, but none detected is reported from the confirmation method. This is because the existing method lacks the ability to confirm many of these drugs.

Methods:

Blood samples are extracted by solid-phase extraction using United Chemical Technologies Clean Screen DAU SPE columns. After 0.5 mL of blood is extracted, the samples are analyzed by electrospray ionization tandem mass spectrometry in positive ion mode (Waters ACQUITY UPLC H-class/Xevo TQD). An ACQUITY Ultra-Performance Liquid Chromatography BEH C18 column (2.1 mm*100 mm, 1.7 µm) is used for separation with the following gradient:

Time (mins)	%Water	%Methanol	2% formic acid
Initial	65	30	5
2.50	30	65	5
3.25	25	70	5
4.50	18	77	5
4.51	5	90	5
5.80	5	90	5
5.91	65	30	5
9.00	65	30	5

Results:

This method was validated to ANSI/ASB standard 036, Standard Practices for Method Validation in Forensic Toxicology (1st edition, 2019). No interferences were observed from the matrix, internal standards, high drug concentrations and other commonly encountered drugs which include fentanyl, nor-fentanyl, codeine, hydrocodone, oxycodone, oxymorphone, morphine, hydromorphone, 6-monoacetyl morphine, amphetamine, methamphetamine, ephedrine, pseudoephedrine, MDA, MDMA, phentermine, tetrahydrocannabinol and metabolites, cocaine and metabolites. (List not all inclusive)

Flunitrazepam, 7-amino-flunitrazepam, midazolam, hydroxy etizolam, triazolam, hydroxy-triazolam, bromazepam, chlordiazepoxide, nor-chlordiazepoxide, clobazam, clonazolam, delorazepam, demoxepam, diclazepam, estazolam, flualprazolam, flubromazepam, flubromazolam, flurazepam, hydroxy ethyl-flurazepam, lormetazepam, nimetazepam, nitrazepam, phenazepam, pyrazolam, zaleplon, and zopiclone are qualitative only.

Drug	Quantitative range (ng/mL)	LOQ/LOD (ng/mL)	Low (% Bias)	Mid (% Bias)	High (% Bias)
7-amino clonazepam	5-240	5	6.11	4.90	1.01
Alprazolam			2.14	1.30	0.56
Clonazepam			3.34	3.47	1.60
Etizolam			2.30	1.52	7.11
Lorazepam			-0.93	-1.60	-3.62
Zolpidem			4.95	5.95	0.66
Hydroxy alprazolam			2.75	5.70	1.39
Diazepam	10-1000	10	5.41	7.48	3.89
Nordiazepam			13.60	13.37	5.18
Oxazepam			8.27	7.39	4.97
Temazepam			3.94	3.42	3.27

Conclusion/Discussion:

The SPE extraction for LCMSMS analysis and processing of 38 benzodiazepines was reduced to approximately 12 hours. This will allow for quicker results and more time for other processes. Currently, this new method is not approved for casework because dilution integrity has not been assessed and it will be qualitatively evaluated with urine samples. It is expected that benzodiazepine ELISA positive results will have more reportable drug confirmations once this method is approved because of an increased selectivity of this new method. In turn, this helps the community identify any drugged driving trends and district attorneys prosecute those that endanger others on Wisconsin roadways.

Keywords: benzodiazepines, solid-phase extraction, Liquid Chromatography Mass Spectrometry-Mass Spectrometry

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