A fast, sensitive, and high-throughput LC-MS/MS assay for Benzodiazepines/Z-Drugs/Barbiturates

Hui Qiao[1]; Sha Joshua Ye[1]; Ellie Majdi[1]; Sharon Lupo[2]; Frances Carroll[2]

[1]IONICS Mass Spectrometry Group, 32 Nixon Rd Bolton, ON, Canada L7E 1W2

[2]Restek Corporation, 110 Benner Circle Bellefonte, PA 16823

Background:

Benzodiazepines, Z-drugs, and barbiturates belong to a group of psychotropic drugs that are often prescribed for the treatment of anxiety, depression, and insomnia. However, as controlled substances, these drugs have the potential for over dosage or abuse. The development of fast and accurate methods for the screening and confirmation analysis of these drugs therefore becomes very critical in toxicological, clinical, and forensic laboratories. LC-MS/MS offers superior sensitivity, selectivity, and robustness for simultaneously detecting benzodiazepines and non-benzodiazepines in complex biological matrices. This work here presents a fast, reliable, and accurate LC-MS/MS method on an IONICS 3Q 120 triple quadrupole mass spectrometer with Restek Biphenyl column for the analysis of a total of 43 compounds with fast polarity switching.

Methods:

The mixed drug standard solutions and analytical LC column were obtained courtesy of Restek. The mixed drug standard solutions as received were further diluted with 50/50 mobile phase A (100% H2O, 0.1% formic acid) and mobile phase B (100% Methanol, 0.1% formic acid) to make a series of concentrations ranging from 0.024 to 50 ng/mL for Benzodiazepines, Z-drugs and other anxiolytic/sedatives/muscle relaxants, and 0.24 to 500 ng/mL for barbiturates. An IONICS 3Q 120 mass spectrometer, equipped with a heated coaxial flow ion source and Hot Source-Induce Desolvation (HSID^{TM}) interface was used for the best ionization and sampling efficiencies. Electrospray ionization was used for this analysis. The time-managed MRM in Molana^{TM} software was used to optimize the dwell time for each compound based on the retention times and the number of MRM transitions within given experiments. Fast polarity switching allowed for simultaneous analysis of positive and negative ions within a single run. A Shimadzu Prominence LC system was used. Restek Raptor Biphenyl column (50X2.1mm, 2.7um) gave good separation and nice peak shapes in the chromatogram. The injection volume

used was 10 µL. A gradient method was created with a flow rate of 0.6 mL/min and a total LC cycle time of 6.5 minutes.

Results:

All 43 compounds eluted within the 6.5 minutes run time showed good chromatographic separation with excellent peak shapes. No carryover was detected in the blank injection immediately following the upper level calibration sample. The calibration curves showed good linearity for all the analytes across the full concentration range with coefficients R^2>0.996. All calibration curves used a linear weighting regression of 1/x. The LLOQs for the 43 drugs were in the range of 0.024 to 4 ng/mL. At the LLOQs, the accuracy was between 83-116%, and CVs were <14% for all analytes.

Conclusion:

A rapid, accurate, and reproducible LC-MS/MS method for Benzodiazepines and non-Benzodiazepines was developed. The results in this study showed that in a 6.5-minute LC run, this LC-MS/MS method can effectively analyze 43 drugs with fast positive and negative polarity switching. The quantitation results also indicate that this method is accurate, precise, and reproducible. The LLOQs for all the 43 drugs is in the range of 0.024 to 4 ng/mL, which is much lower than the typical confirmation cutoff concentration (50 ng/mL) for most of the drugs. Therefore, this LC-MS/MS method with IONICS 3Q 120 mass spectrometer and Restek Raptor Biphenyl column can provide a fast, accurate and high throughput solution for clinical pain management medications, to monitor patient drug use and program adherence or for drugs of abuse or other workplace drug testing.