



K56 Ethylone: Development and Validation of a Quantitative Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) Method With Analytical Confirmation in Toxicology Casework

Stephanie Kumor, MA, NMS Labs, 3701 Welsh Road, Willow Grove, PA 19090; Joseph Homan, MS, 3701 Welsh Road, Willow Grove, PA 19090; Annette Ervin, BS, 3701 Welsh Road, Willow Grove, PA 19090; Donna M. Papsun, MS, Willow Grove, PA 19030; and Barry K. Logan, PhD, NMS Labs/CFSRE, 3701 Welsh Road, Willow Grove, PA 19090*

After attending this presentation, attendees will be able to discuss the emerging designer drug class of synthetic cathinones with a focus on ethylone, and will be able to undertake the development of an analytical assay using LC/MS/MS for their detection in biological fluids.

This presentation will impact the forensic science community by raising awareness of the abuse and toxicity of ethylone and other novel synthetic cathinones as well as improving their detection and quantitation in biological matrices.

Ethylone is considered a next generation synthetic cathinone since detection in laboratories began in late 2011 and has been steadily increasing. The effects of oral doses of ethylone are reported to be similar to those of cocaine and amphetamine, which include excitation, increased energy, and euphoria. The abuse of designer cathinones has increased in popularity since 2009 and they are often sold as “bath salts” or “plant food,” although they are labeled “not for human consumption.” Synthetic cathinone products are continually changing with slight structural alterations in order to circumvent drug control regulations of early-generation analogs such as methylone, mephedrone, and MDPV. As this drug is not part of many laboratories’ routine testing procedures, there is limited available information on its toxicity and pharmacological effects, which raises a public health concern due to the increase in its abuse. The development of a sensitive and reliable validated method of detection was undertaken to fill this gap.

This assay was developed to detect and quantitate ethylone in addition to four related synthetic cathinones: butylone, pentylone, flephedrone (4-fluoromethcathinone (4-FMC)), and 3-fluoromethcathinone (3-FMC). Whole blood and urine samples were extracted using Solid Phase Extraction (SPE). Analytes were quantified using positive mode LC/MS/MS. Separation was achieved on a BEH C18, 2.1mm x 100mm column with mobile phases consisting of ammonium acetate buffer, pH9, and acetonitrile. The two pairs of structural isomers, ethylone and butylone, and flephedrone and 3-FMC, were separated chromatographically using the high pH-buffered mobile phase.

The optimized method was fully validated according to the Scientific Working Group for Toxicology (SWGTOX) guidelines. Linearity was established from 10ng/mL to 1,000ng/mL using six calibration points. Replicates (n=5) at each concentration level were analyzed and the correlation coefficient was >0.99 for all analytes. All five synthetic cathinones were measured at three different concentrations to give precision $\leq 10\%$ Coefficient of Variation (CV) and accuracy $\pm 10\%$ for both within- and between-run experiments. For ethylone, the maximum average intra- and inter-run imprecision were 8.1% and 5.1%, respectively. The lower limit of detection for ethylone was 1.25ng/mL. Stability experiments (n=6) indicated that ethylone is stable in blood for up to two days at room temperature and for at least 28 days if kept refrigerated or frozen.

A set of 21 blood subject samples which had screened positive for ethylone were tested using this method. Of the cases with demographic information available, the median age was 26 years, and included 12 males and 5 females. The ethylone results of the 21 blood cases ranged from 7ng/mL to 24,500ng/mL with a mean and median concentration of 1,982ng/mL and 461ng/mL, respectively. Five were positive for ethylone in combination with other designer stimulants (methylone, butylone, flephedrone, and alpha-PVP). It was noted in the case history for the highest value of ethylone detected that the individual experienced excited delirium prior to collapsing. The detection of ethylone and other designer cathinones in these forensic toxicology and postmortem cases demonstrates the need for a reliable quantitative method over a wide concentration range.

The increased use of ethylone and other newer synthetic cathinone analogs required the development of a quantitative analytical assay. The observed concentrations in biological fluids provide insight to forensic toxicology in regard to the toxicity and expected levels of ethylone in casework.

Ethylone, Psychoactive Substances, Validation