Screening, Confirmation and Quantitation of Synthetic Cathinones and Cannabinoids in Urine by High-Resolution Accurate-Mass Mass Spectrometry

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BACKGROUND/INTRODUCTION: Forensic laboratories need reliable and flexible methods for detecting novel psychoactive compounds. The methods need to be easily modifiable to include new compounds. LC-MS is ideally suited for this type of application since it can easily detect different classes of compounds in a single analytical run.

METHODS: A single point calibrator at cutoff concentration and two quality controls (QC) one each at 50% and 150% of the calibrator concentration were prepared by fortifying blank urine with 32 synthetic cathinones and cannabinoids. The calibrator, QCs and an unknown sample were processed by protein precipitation followed by dilution. Processed samples were subject to HPLC separation followed by detection on a hybrid quadrupole-Orbitrap™ mass spectrometer. Two chromatographic gradients were used. The first was a “fast and dirty” two-minute screening method and the second was a nine-minute gradient used for confirmation. The mass spectrometer collected high-resolution full-scan (FS) spectra at a resolution of 70k (FWHM at 200 m/z) along with data-dependent fragmentation spectra (ddMS2) for masses on the target list. Compounds were identified using retention time and accurate m/z from the full-scan data. Semi-quantitation was performed on the FS extracted ion chromatographic peak using the single point calibrator and linear-through-zero calibration curves. Confirmation was accomplished by spectral library matching with the MS2 spectra in both methods. Isotopic pattern matching was added to the longer method. To assess method performance, the calibrator and each QC sample were injected ten times with each method to determine mass accuracy, peak area precision and quantitative performance. The unknown sample previously analyzed by collaborating laboratory was injected three times with each method to determine identification accuracy.

RESULTS: Data from the short screening method showed mass accuracies within 1 ppm for all, except one compound, which was within 2.2 ppm. The long method, which was run several days after the short method and near the end of the recommended instrument calibration stability, showed mass accuracies within 3 ppm except for the same single compound, which was within 4.2 ppm. Calculated concentration precision was better than 9.8 % and 8.5% across all compounds and all concentrations for the short and long methods, respectively. Three compounds were identified and confirmed in the unknown sample. A fourth compound was identified by m/z, retention time, and isotopic pattern matching. However, it failed the spectral matching. It was suspected that this compound might be a metabolite of one of the confirmed compounds.

CONCLUSION: The developed methods accomplished their goals of identifying, confirming and quantifying 32 synthetic cathinones and cannabinoids in urine.