K20 Screening, Confirmation, and Quantitation of Synthetic Cathinones and Cannabinoids in Urine by High-Resolution Accurate-Mass Mass Spectrometry

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After attending this presentation, attendees will understand the capabilities of a high-resolution, accurate-mass mass spectrometer for the screening and confirmation of synthetic cathinones and cannabinoids. Attendees will see the impact the chromatographic method can have on the mass spectrometer method performance.

This presentation will impact the forensic science community by providing tools to detect and confirm current novel psychoactive compounds.

Introduction: Forensic laboratories need reliable, flexible methods for detecting novel psychoactive compounds such as synthetic cathinones and cannabinoids. The methods need to be easily modifiable to include new compounds. LC/MS is ideally suited for this type of application; it can easily detect different classes of compounds in a single analytical run.

Objective: To demonstrate the performance of high-resolution mass spectrometry for identification, confirmation, and quantitation of synthetic cathinones and cannabinoids in urine.

Methods: A single point calibrator at cutoff concentration (25ng/mL-500ng/mL, compound dependent) 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 two-minute elution intended for screening that provided limited chromatographic separation of isobaric compounds. The second was a nine-minute elution used for confirmation. The mass spectrometer collected 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* (5ppm mass window) 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. 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 a collaborating laboratory was injected three times with each method to determine identification accuracy.

Results: Data from the short method showed mass accuracies within 1 ppm for all but one compound which was within 2.2ppm. The long method, run several days after the short method and near the end of the recommended instrument calibration period, showed mass accuracies within 3ppm except for the same single compound, which was within 4.2ppm. Peak area precisions for all compounds and all concentrations were better than 13.9% and 8.1% for the short and long methods, respectively. Calculated concentration precisions across all compounds and all concentrations were better than 9.8 % and 8.5% for the short and long methods, respectively.

The short method was intended only to screen for compounds using retention time and accurate *m/z* from the FS data as identifiers. The longer method, which provided better chromatographic separation, included isotopic pattern

matching and fragment spectral matching for confirmation. Fragment spectral matching was included in the short screening method to determine if it could be of any utility. Three compounds (MDPV, mephedrone and methylone) were identified and confirmed in the unknown sample using both methods. The longer method gave higher library matching scores and isotopic patterns. A fourth compound was identified as Methedrone by *m/z*, retention time, and isotopic pattern matching. However, it failed confirmation by spectral matching. It was suspected that this unconfirmed compound might be a metabolite of mephedrone. A literature search revealed a possible match, which was confirmed with a theoretical fragmentation spectra match.

Conclusion: The developed methods accomplished their goals of identifying, confirming and quantifying 32 synthetic cathinones and cannabinoids in urine. The short method was intended as a screening-only method not requiring definitive confirmation. It surpassed that goal by also providing confirmation through library matching of fragmentation spectra. The longer method provided better confirmation and greater quantitative precision. Its higher quality fragmentation spectra resulted in higher library matching scores. The addition, isotopic pattern recognition also contributed to more confident confirmation.

Mass Spectrometry, Novel Psychoactive Substances, Confirmation