K19 A Comparison of Two High-Resolution Mass Spectrometry Data Acquisition Methods for the Screening, Quantitation, and Confirmation of Compounds in Postmortem Blood

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After attending this presentation, attendees will understand two high-resolution accurate-mass mass spectrometric methods for detecting drugs of abuse in postmortem blood and will be able to compare them for suitability in their laboratories.

This presentation will impact the forensic science community by providing forensic toxicologists with the tools to correctly identify, quantify, and confirm a large panel of compounds, including benzodiazepines, opiates/opioids, and more in a single analytical run with minimal sample preparation, thereby saving time and other resources.

Background/Introduction: Forensic toxicologists need to quantitate target compounds and screen for many more in as little time as possible. In the past, samples were screened either by GC/MS or immunoassay, both of which have significant limitations. GC/MS requires labor-intensive sample preparation, including derivatization. Multiple immunoassays must be used to cover different compound classes, and immunoassays are not specific to a particular compound. LC/MS techniques allow for simpler sample preparation and identify individual compounds, not just classes.

Methods: A single point calibrator (1ng/mL-1000ng/mL, compound dependent), two QCs (one at half and one at double the calibrator concentration), and five unknown post mortem blood samples were processed by a collaborating laboratory. Protein precipitation with a solution containing six internal standards was followed by evaporation of the supernatant and reconstitution with phosphate buffer. The calibrator and QCs contained 21 compounds including benzodiazepines, opiates/opioids, cocaine metabolite, gabapentin and pregabalin to evaluate method performance. Processed samples were subject to reversed-phase chromatographic separation followed by detection on a hybrid quadrupole-Orbitrap™ mass spectrometer. Data was collected using two methods. In the first, the mass spectrometer collected high-resolution full-scan spectra at a resolution of 70k (FWHM at 200*m/z*) along with data-dependent fragmentation spectra (FS-ddMS2) for any masses detected from a target list of over 400 compounds. In the second, full-scan spectra were again collected, followed by all-ion fragmentation (FS-AIF). Targeted compounds were identified using retention times and accurate mass *m/z* within 5ppm mass accuracy from the full-scan data. Confirmation was accomplished either by matching the MS2 spectra to a spectral library or by presence of known fragments in the AIF data. Detection limits were evaluated using the 21 representative compounds in the calibrator and QCs. Quantitation was performed on the full-scan extracted ion chromatographic peak using the single point calibrator and linear-through-zero calibration curves. Method performance was evaluated by analyzing the calibrator, QCs, and unknown blood samples previously analyzed by the collaborating laboratory and comparing the two sets of results.

Results: Desired limits of detection (0.75ng/mL-500ng/mL) were achieved for all 21 evaluation compounds in the calibrator and QC sample. All QC compounds that had deuterated analogs as internal standards were within 20% of nominal concentration. Accuracies for some of the compounds that did not have deuterated analogs were outside of the 20% range, suggesting that analogs are needed if rigorous quantitation is required. These data agreed with the results obtained by the collaborating laboratory (data not shown).

For screening of the five unknown samples, quantitative results were obtained for many of the 21 evaluated compounds. Qualitative results were obtained for compounds in the screening database of over 400 compounds. Results were reported for any peak that was both detected and confirmed. These values and results again agreed with those obtained by the collaborating laboratory.

FS-ddMS2 and FS-AIF performed equally well for confirmation of compounds within the concentration range of the QCs.

Conclusion/Discussion: The developed methods were able to both quantitate a target set of compounds and detect unknown compounds in post-mortem blood samples. Both methods performed similarly and met common industry requirements for sensitivity. The FS-ddMS2 data still offers the strongest identification since the fragmentation spectra ”fingerprint” is collected for a specific precursor. AIF data is less specific since the fragments are generated by all ions eluting at the same time. The advantage of collecting AIF data is the ability to conduct confident retrospective data analysis using fragmentation data. Compounds from many classes of drugs were successfully and specifically screened in a single analytical run.

Mass Spectrometry, Screening, Drugs of Abuse