K5 Identification of Fentanyl in Urine From Drug Abuse Cases Using a Direct Multistage Mass Spectrometry Method

Diaa M. Shakleya, PhD, West Virginia University, PO Box 6045, Morgantown, WV 26506; James C. Kraner, PhD, Office of the Chief Medical Examiner, 619 Virginia Street, W, Charleston, WV 25302; and Patrick Callery, PhD, West Virginia University, PO Box 9530, Morgantown, WV 26505*

After attending this presentation, attendees will add to their knowledge the use of ion trap and similar mass spectrometers for the identification of drugs of abuse in urine. The direct-injection, multistage mass spectrometric methods are faster and often more specific than traditional mass spectrometric identification methods.

Because the method involves direct injection into a mass spectrometer and does not require a chromatographic step, this presentation will impact the forensic community and/or humanity by providing considerable savings of time and costs compared to the application of mass spectrometric methods for the identification of fentanyl currently in the literature.

Multistage mass spectrometric analysis has become a powerful tool for quantitative confirmatory analysis of chemicals and drugs of abuse and has begun to spread in the field of forensic toxicology. In this presentation, the identification of fentanyl from six fentanyl positive cases provided by the Office of the Chief Medical Examiner of West Virginia is discussed.

The application of multistage MS to the identification of fentanyl in drug abuse cases was evaluated by developing a simpler and more rapid mass spectrometric method for identification of fentanyl in urine. Urine from six fentanyl-positive cases under review by the Office of the Chief Medical Examiner of West Virginia was included in the studies. Each of the six cases described in the presentation was investigated as an apparent drug overdose. A complete autopsy was performed on each of the decedents including comprehensive toxicology testing. Alcohol analysis was by direct injection gas chromatography with *t*-butanol as an internal standard. Drugs of abuse were screened by enzyme-multiplied immunoassay technique. Fentanyl was identified in each case by either enzyme-linked immunosorbent assay or by GC/MS. Blood fentanyl concentrations were determined on an Agilent 1100 Series LC/MSD. Chromatography was performed on a Zorbax 5SB-C_{18}, 4 x 150mm column using an isocratic solvent system (20% ammonium formate, 80% acetonitrile). The APCI interface parameters were drying gas 10 L/min, drying gas temperature 350^{O}C, nebulizer pressure 25 psi, vaporizer temperature 300^{O}C, and capillary voltage 4000 V. The ions monitored under SIM mode were m/z 337, 338 for fentanyl and 251, 252 for methaqualone (internal standard). A negative control consisted of pooled urine from normal healthy volunteers. To quantify fentanyl concentrations, $^{2}H_{5}$-fentanyl was used as an internal standard. Urine (1mL) samples from overdose cases were spiked with 10 µL of deuterium labeled internal standard (10 µg/mL), then filtered through a 0.2 µm PTFE membrane. A 50 µL aliquot was diluted to 200 µL total volume with 0.1% formic acid in acetonitrile. Samples were centrifuged for 5 min at 13,000 rpm. The solution was injected into the electrospray ionization (ESI) source of an ion trap mass spectrometer operating in the positive ion mode. A standard curve from control urine was constructed from spiked fentanyl HCl concentrations. Blank methanol/water mixture (50:50 v/v) was injected between two samples for cleaning purposes. Multistage mass spectra recorded in MS, MS/MS and MS/MS/MS (MS^{3}) modes were used to quantify and confirm the presence of fentanyl in the samples. Although present, ion suppression was not a problem at the concentrations measured above 100 ng/mL of urine.

Because the method involves direct injection into a mass spectrometer and does not require a chromatography step, considerable savings of time (3 to 4 min per sample) and costs are possible compared to the application of literature mass spectrometric methods for the identification of fentanyl. Multistage mass spectrometry methods were also developed from blood and liver for methamphetamine and MDMA.

Multistage Mass Spectrometry, Fentanyl, Forensic

 ** Presenting Author*