K75 Quantitative Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) Analysis of 39 Fentanyl Analogs and Metabolites in Blood, Urine, and Oral Fluid

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Learning Overview: After attending this presentation, attendees will know that many fentanyl analogs and metabolites can be identified and quantified in blood, oral fluid, and urine in a single sensitive and specific LC/MS/MS procedure.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by showing how to identify and quantify fentanyl analogs in diverse biological matrices to document cause of death, the drugs involved with driving under the influence of drugs, and substance abuse.

Fentanyl and its novel psychoactive substance analogs are of strong interest due to increased potency, increased abuse liability, and the large number of intoxications and deaths occurring in the United States. Fentanyls are opioids with a rapid onset of effects that are injected or absorbed from a skin patch or oromucosally from an oral lollipop or buccal tablet. Fentanyls activate the μ-opioid receptor and may be as much as 10,000 times more potent than morphine. Food and Drug Administration-approved synthetic fentanyls include pain medications and anesthesia agents. Recently, dozens of fentanyl analogs were introduced by clandestine laboratories located in China and other countries, resulting in many overdoses and deaths. Clinical hospital laboratories and local, state, and federal forensic toxicology laboratories need to identify the novel psychoactive substances responsible for these adverse events to alert public health authorities, first responders, and drug users about the presence of a new toxic drug, as well as to provide psychiatric follow up for the drug user. This fentanyl analytical method was developed and optimized for accurate and robust drug analysis, while also demonstrating the challenges associated with investigating multiple compounds of similar structure and physicochemical properties.

A Thermo Scientific™ Vanquish™ HPLC system and a Thermo Scientific™ TSQ Quantis™ tandem mass spectrometer in positive electrospray mode were utilized for the analysis. Sample size was only 100uL urine, oral fluid, or blood. The selected column was a Thermo Scientific™ Accucore™ C18 100mm x 2.1mm, 1.5um column heated to 50‘C, with 5µL injected. Mobile phase A was a water: methanol mixture containing 0.01% formic acid and 2mM ammonium formate and mobile phase B was methanol. The gradient began at 90% A and 10% B ending at 8.5min with 2% A and 98% B. Analyte elution occurred from 0.52min (NPP) to 7.2min (Valeryl Fentanyl). Baseline chromatographic separation was achieved for all analytes within a total run time of 10min.

Mass spectrometer settings were: vaporizer temperature: 475°C, ion transfer tube temperature 300°C, sheath gas: 70, aux gas:1 0, sweep gas: 0, spray voltage: positive ion (V): 500, Q1/Q2 resolution: 0.7/0.7 (FWHM), cycle time (sec): 0.6, CID gas (mTorr) 2 and chromatographic peak width 6secs. Collision energies were optimized for each transition and ranged from 10V to 45V. Quantitative analysis was performed with Selective Reaction Monitoring (SRM) transition pairs for each analyte and internal standard. Reference materials for method validation were from UTAK.

Sample preparation techniques included protein precipitation for blood samples and simple dilution of urine and oral fluid samples, enabling high throughput analysis. Limits Of Quantification (LOQ) in blood ranged from 5pg/mL (acetyl norfentanyl) to 100pg/mL (remifentanil), in oral fluid 10pg/mL (norfentanyl) to 100pg/mL (ß-hydroxythiofentanyl), and in urine 25pg/mL (4-Anilino-4-piperidine) to 250pg/mL (N-methyl norcarfentanyl). Good linearity and reproducibility were obtained for all fentanyl analogs and metabolites with a coefficient of determination R^2>0.98 or better for all drugs in the different matrices. Excellent imprecision and accuracy were achieved (CV<15%) for all compounds in all matrices. The method achieved sensitive results for the analysis of 39 new fentanyl analogs with subtle structural differences and demonstrated the versatility of the mass spectrometer to consistently quantify multiple, closely related drugs at pg/mL concentrations.

Fentanyl Analogs, LC/MS/MS, Blood, Oral Fluid, and Urine