K14 The Development of a Simultaneous Separation and Identification Ultra Performance Liquid Chromatography/High-Performance Liquid Chromatography (UPLC/HPLC) Tandem Mass Spectrometry (MS/MS) Screening Method for Sulfur-Containing Fentanyl Analogs (SFA)

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Learning Overview: After attending this presentation, attendees will understand a screening method to separate and identify SFA and isomers.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by presenting a method capable of separating and identifying newly emerging SFA utilizing UPLC-MS/MS.

Background/Introduction: In February 2018, the Drug Enforcement Administration (DEA) released a statement of the emergency scheduling (Schedule 1) of all illicit fentanyl analogs not already regulated by the Controlled Substances Act due to an alarming increase in overdose deaths linked to synthetic opioids. Fentanyl analogs are pharmacologically similar to fentanyl, but often more potent. This increased potency can create problems with proper dosing of fentanyl analogs and can lead to untoward effects, including an increase in overdoses and deaths. These newly emerging fentanyl analogs often appear on the street as diverted research chemicals; therefore, their identification can be difficult. Given the increase in overdose deaths, an analytical method for identification and separation of these analogs is needed to help the clinical and forensic communities overcome this epidemic.

Objective: To develop a method for the simultaneous identification and separation of 12 SFA: cis-3-methyl thiofentanyl, trans-3-methyl thiofentanyl, α-methyl Thiofentanyl, β-hydroxythioacetylfentanyl, β-hydroxythiofentanyl, sufentanil (metabolite: norsufentanil), tetrahydrothiophene fentanyl, thienyl fentanyl, thiofentanyl, thiophene fentanyl, and 13C6 β-hydroxythiofentanyl.

Methods: To improve laboratory method transfer, the fentanyl analogs were evaluated on two LC/MS/MS systems: UPLC-MS/MS (Waters® ACQUITY® UPLC-TQs-micro) and HPLC-MS/MS (Shimadzu® LC20AD XR coupled to AB SCIEX™ triple quadrupole 5500). In order to optimize these systems and identify ion transitions, the 24 transitions with their respective declustering potential and collision energy were determined using Electrospray Ionization (ESI) via manual infusion. Table 1 contains the ion transitions identified for identification. UPLC-MS/MS and HPLC-MS/MS conditions such as initial aqueous phase, organic phase, and gradient, as well as column chemistry were adjusted to achieve optimal separation and identification of the SFA. Organic phases evaluated were formic acid in acetonitrile and methanol; the aqueous phase for all analyses was formic acid in LC/MS water. The mobile gradients evaluated were curved, linear, and step type. Chromatography conditions were evaluated using LC and UPLC columns: Pentafluorophenylpropyl (PFPP), biphenyl, and several C18 columns, including CSH C18 and BEH C18, of variable lengths and end caps. Selectra PFPP 100 x 2.1mm 3µm; Restek® Ultra Biphenyl 50 x 2.1mm 3µm; Phenomenex® Kinetex® 100 x 3.0mm 2.6µm; UCT Selectra® C18 100 x 4.6mm 3µm; Waters® X-Select® CSH C18 50 x 2.1mm 2.5µm; Waters® ACQUITY® UPLC BEH C18 50 x 2.1mm 1.7µm.

Results: The Waters® X-Select® CSH C18 column resulted in the most chromatic separation of the 12 SFA of the columns evaluated on the Shimadzu® LC coupled AB SCIEX™-5500 triple quad. The Phenomenex® Kinetex® biphenyl column resulted in comparable resolution to the CSH C18, the advantage of the CSH C18 is the partial spectral separation of the methylthiofentanyl isobars using one of the common transitions. The Water's® ACQUITY® UPLC BEH C18 resulted in the best chromatic separation of the 12 SFA on the Water's® UPLC-MS/MS. A linear organic phase gradient 5% to 95% on the BEH C18 and 15% to 95% on the CSH C18 led to all compounds eluting after the solvent front with narrow, tall peaks and spectral separation of isomeric sulfur containing fentanyl analogs.

Conclusion: The BEH C18 had better overall chromatographic resolution: the BEH C18 uses a sub-2-micron particle size, which requires a UPLC system (>10,000psi). The CSH C18 had sufficient chromatographic resolution to be used on an HPLC system. One aspect of this study was to chromatographically separate and qualitatively identify the SFA; however, the cis and trans isomers of 3-methylthiofentanyl were not sufficiently resolved chromatographically to be able to identify them as separate analogs.

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Table 1 identifies the transitions utilized for the identification of the 12 analogs on both the UPLC-MS/MS and AB SCIEX™-5500 triple quad MS.

SFA	Parent *m/z*	Transition 1 *(m/z)*	Transition 2 *(m/z)*
α-Methyl Thiofentanyl	357	125	259
β-Hydroxythioacetylfentanyl	345	192	327
β-Hydroxythiofentanyl	359	146	192
cis-3-Methyl Thiofentanyl	357	111	208
Norsufentanil	277	184	245
Sufentanil	387	238	355
Tetrahydrothiophene Fentanyl	395	105	188
Thienyl Fentanyl	329	97	180
Thiofentanyl	343	111	194
Thiophene Fentanyl	391	188	105
trans-3-methyl Thiofentanyl	357	209	259
13C6 βOH Thiofentanyl	365	152	192

Thiofentanyl, UPLC/MS/MS, Screening Method

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