K62 Rapid Detection and Separation of Isomeric Fentanyl Analogs Using Gas Chromatography-Atmospheric Pressure Chemical Ionization-Trapped Ion Mobility-Time of Flight/Mass Spectrometry (GC-APCI-TIMS-TOF/MS)

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Learning Overview: After attending this presentation, attendees will better understand a novel instrumentation technique for the separation and identification of isomeric fentanyl analogs.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by introducing an alternative high-resolution instrumentation technique for toxicological analysis that is complementary to traditional chromatography/mass spectrometry workflows.

The emergence of Novel Psychoactive Substances (NPS) in the United States, especially fentanyl analogs, has led to the need for alternative screening and quantitative techniques capable of achieving both high sensitivity and specificity. Due to the clandestine nature of these compounds, the potential for the development of isomeric sets is high, thus increasing the need for method development of a complementary separation technique for the detection and quantification of isomeric substances. With relatively unknown potencies among structural isomeric and isobaric sets, being able to distinguish between these compounds is paramount when properly certifying the cause and manner of death. In the present work, the use of complementary gas-phase separation was evaluated for the analysis of fentanyl analogs, specifically GC-APCI coupled to TIMS-TOF/MS.

Sixteen fentanyl analogs, as shown in Table 1, including six sets of isomers, were prepared in methanol using certified reference standards provided by the Miami-Dade Medical Examiner Department (MDME). All standards were fortified into whole blood and extracted via protein precipitation with acetonitrile. In addition, previously analyzed postmortem case samples, provided by the Institute of Forensic Medicine, Medical Center at the University of Freiburg, Germany, were also extracted and sampled to confirm the application of the method developed. Chromatographic separation was achieved using a GC equipped with a DB-5 non-polar capillary column, coupled to an APCI source in positive ionization mode. A 15min thermal ramping method was used, with compound elution between 6-10 minutes of the total run time. Using the standards provided, the GC-TIMS-MS method was optimized, and detection of analogs was achieved using a commercial TIMS-TOF with an internal mobility and accurate mass calibration.

Table 1. Analytes

Despropionyl ortho-fluoro fentanyl (298 Da)	Cyclopropyl fentanyl (348 Da)	Benzyl fentanyl (322 Da)	3-methyl fentanyl (350 Da)	Para-fluorobutyryl fentanyl (368 Da)	Meta-fluoro fentanyl (354 Da)	Fentanyl (336 Da)
Despropionyl para-fluoro fentanyl (298 Da)	Crotonyl fentanyl (348 Da)	Acetyl fentanyl (322 Da)	Butyryl fentanyl (350 Da)	Para-fluoroisobutyryl fentanyl (368 Da)	Ortho-fluoro fentanyl (354 Da)	Furanyl fentanyl (374 Da)
					Para-fluoro fentanyl (354 Da)	Methoxyacetyl fentanyl (352 Da)

All fentanyl analogs were positively identified using high resolution mass accuracy, with experimental *m/z* values within 1ppm of calculated theoretical *m/z* value. All analytes were linear from 1ng/mL to 500ng/mL post-extraction and no endogenous or exogenous interferences were observed. An increase in signal to noise was noted when signals were filtered in the GC, IM, and MS domains, allowing for increased sensitivity. A Collision Cross Section (CCS) value, which is a term that represents the interaction between the target ion and the drift gas (N_2) in the TIMS cell, is used to characterize the measured IM of a compound. CCS was measured for all fentanyl analogs (<1% error) and mobility separation was classified as a difference in CCS value larger than 0.6 $Å^2$; this separation was achieved for four out of the six sets of structural isomers. All analytes were also observed as having a characteristically high mobility resolving power (R>100). In addition, five out of the six sets of isomers were chromatographically resolved (r>1). Successful separation was achieved for each set of isomers either by IM, chromatography, or both. The analytes detected in the previously analyzed postmortem case samples, including furanyl fentanyl, and methoxyacetyl fentanyl, were confirmed positive on the present method, with quantitative values within 20% of the previously measured concentrations. The use of GC-IM spectrometry for the detection of fentanyl analogs in the field of toxicology demonstrates the advantage of orthogonal separation techniques and multi-dimensional identification (retention time, CCS value, and accurate mass). Ongoing experiments will assess the use of liquid chromatography, as well as the addition of more analytes to the method.

High Resolution Mass Spectrometry, Novel Psychoactive Substances, Ion Mobility

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