## K1 Identification of Fentanyl and Fentanyl Analogs by Using High-Resolution Mass Spectrometry and Machine Learning

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**Learning Overview:** After attending this presentation, attendees will be familiar with the fragmentation pattern of fentanyl as well as its derivatives under Collision-Induced Dissociation (CID). Attendees will also be introduced to a new methodology where the structure of major fragmentation ion could be predicted by Konstanz Information Miner (KNIME) workflow.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a novel KNIME workflow that is capable of predicting the major fragmentation ion as well its structural similarity against the reference based on the input tandem mass spectrum.

Fentanyl is a type of synthetic opioid that was originally developed as a pain management agent for cancer patients. Because of its stronger addictive reaction over morphine or heroin, it was diverted into a type of abused drug, which largely contributed to the current ongoing opioid crisis. One special emerging issue regarding the detection of fentanyl is the rapid evolution of fentanyl derivatives, as the analytical standards in the forensic laboratory may not be able to keep up with the development of newer fentanyl derivatives.

KNIME is a free and open-source data analytics and mining platform developed by a team of software engineers at the University of Konstanz in 2004. Compared with other data mining software such as Statistical Package for the Social Sciences (SPSS) and Statistical Analysis System (SAS), KNIME was chosen in the study for its user-friendliness as well as its versatility. Traditional cheminformatics platforms rely on command line interfaces to operate, which considerably hinders their application as they require the operator to have certain training in command-line language. KNIME, on the other hand, has a Graphic User Interface (GUI) and modular functionality, which greatly facilitates the operation as well as the modification of calculation as the operator could change the functionality of the whole workflow by connecting and disconnecting nodes. Datasets obtained from different sources could be integrated into one workflow. Currently, KNIME is widely used in biological as well as pharmaceutical applications.[1]

Regarding the techniques used in this study, quadrupole Time-Of-Flight (qTOF) Mass Spectrometry (MS) was used to obtain Tandem Mass Spectrometry (MS/MS) spectra and the design of workflow was performed on the KNIME platform. MS has become one of the leading techniques in multiple fields, including forensic science, because of its sensitivity and versatility. Reinforced by the capability of performing structure elucidation via CID, hybrid mass spectrometer such as qTOF/MS has become a powerful tool in the study of controlled substances, including fentanyl derivatives. In CID, pure nitrogen gas is pumped into the collision cell where it collides with the targeted ion, causing fragmentation of this ion, which could provide useful information for structure elucidation as different molecules will fragment differently depending on their structure.

Thus, in this study, a novel KNIME-based workflow was created based on a series of CID MS/MS spectra from unmodified fentanyl as well as five additional fentanyl derivatives, including benzyl carfentanil, cyclopropyl fentanyl, cyclopentyl fentanyl, furanyl fentanyl, and acryl fentanyl.[2,3] Preliminary results suggested that two common fragmentation patterns could be observed on the fentanyl ion, including product N-phenylpropionamide (m/z 188) and a neutral loss of cyclohexylamine (149 Da). Across a total of five different fentanyl derivatives, either one of these patterns could be observed and the appearance of fragmentation pattern is related to the structure of fentanyl derivative. Fentanyl could be considered as the structural combination of an N-phenylpropionamide with a cyclohexylamine and functionality modifications could be on either part. If the main product ion of m/z 188 showed up, this fentanyl derivative should have modification on the side of N-phenylpropionamide. If the neutral loss is 149 Da, it should be a fentanyl derivative that had a modification on the cyclohexylamine. By utilizing this observation, a KNIME workflow could be established based on the fragmentation pattern of fentanyl derivatives obtained from MS/MS on an Agilent® G6520B qTOF mass spectrometer. This KNIME workflow was able to read MS/MS spectrum file (in form of a CSV file) from different software vendors and determine the structure of major fragment ion by m/z and mass difference based on a set of preset rules. It could also predict structural similarity as a numeric factor of Tanimoto coefficient by calculating the proportion of common functional groups between unmodified fentanyl and fentanyl derivative.

**Reference(s):**

1. Berthold, Michael, Nicolas Cebron, Fabian Dill, Thomas Gabriel, Tobias Kötter, Thorsten Meinl, Peter Ohl, Kilian Thiel, and Bernd Wiswedel. KNIME—the Konstanz Information Miner. *ACM SIGKDD Explorations Newsletter* 11, 11, no. 1 (November 16, 2009): 26–31. https://doi.org/10.1145/1656274.1656280.
2. Madras, Bertha K. The Growing Problem of New Psychoactive Substances (NPS). *Current Topics in Behavioral Neurosciences* 32, 32 (January 1, 2017): 1–18. https://doi.org/10.1007/7854_2016_34.
3. Weinstein, Aviv M, Paola Rosca, Liana Fattore, and Edythe D London. Synthetic Cathinone and Cannabinoid Designer Drugs Pose a Major Risk for Public Health. *Frontiers in Psychiatry* 8, 8 (2017): 156. https://doi.org/10.3389/fpsyt.2017.00156.

**Fentanyl, Tandem Mass Spectrometry, KNIME**



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