AI-POWERED DIAGNOSTICS

Rapid Detection And Classification Of Fentanyl Analogs By 2D MS/MS Coupled With A Novel Library-less Machine Learning Algorithm

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The drug epidemic continues to ravage the United States, causing more than 100,000 deaths in annually in recent years. Due to increasing availability, one of the illicit culprits contributing to the epidemic is fentanyl, where as little as 2 mg can be lethal. Fentanyl continues to be illegally synthesized resulting in hundreds of molecular variants, making it difficult to detect, classify, and identify these threats using conventional library-driven algorithms. This is further compounded by newly emerging synthetic opioids such as nitazenes as well as novel combinations of new and existing drugs (e.g. fentanyl + xylazine). Due to this increasing trend, there is a critical need for identification and classification of near-neighbor variants of both fentanyl and nitazenes utilizing mass spectrometry; however, onboard mass spectral libraries can quickly become out-of-date. Therefore, the work described here aims to demonstrate library-less identification and/or classification of fentanyl and fentanyl variants by machine learning of novel two-dimensional mass spectrometry (2D MS/MS) data.

Various fentanyl analogs from the Cayman Fentanyl Analog Screening Kit were analyzed utilizing 2D MS/MS on a miniature Teledyne FLIR linear ion trap mass spectrometer. Each fentanyl variant was diluted in methanol to concentrations ranging from 1-100 ng/µL and were sampled/ionized by thermal desorption from a Nomex ticket followed by atmospheric pressure chemical ionization (APCI). In addition, mixture of fentanyl variants, other drugs, various solvents, and ‘background’ materials were analyzed. Several technical replicates of each sample were collected over the course of multiple days, and analytes were pseudo-randomly mixed with each other and with ‘background’ analytes (such as common solvents and other drugs) to generate a comprehensive dataset. Mass calibration and a smoothing 2D Gaussian filter were applied to each dataset prior to being ‘duplicated’ to generate copies of each file with randomized intensities and mass shifts to simulate real world samples. All of the 3708 files collected were classified as either ‘fentanyl containing’ or ‘other’. Four Python machine learning algorithms – a convolutional neural network (CNN) and random forest, multilayer perceptron, and k-nearest neighbors classifiers - were trained and validated using a 70/30 train/test split.

Of the four machine learning models, the CNN by far performed the best, resulting in 99.9% or higher accuracy on both training and validation datasets. Out of the 3708 files, only 1 was misclassified by CNN. In general, files that were misclassified by the four models had low signal-to-noise ratios or high background. In addition, the CNN could classify samples as fentanyl-containing when all files containing a particular variant were removed from the training set. For example, all data files corresponding to m-fluorofentanyl and p-fluoro furanyl fentanyl (as well as isobars and isomers) were removed from the training set and the model was retrained; after this, the model achieved 95% accuracy classifying the ‘unseen’ fentanyl variants.