Screening and Confirmation of Fentanyl Analogues by High Resolution Mass Spectrometry using an Agilent 6545 QTOF Mass Spectrometer

Jason S. Hudson*, Rebekah Boswell, Curt Harper, Alabama Department of Forensic Sciences, Toxicology Section, Hoover, AL

Background/Introduction: Fentanyl analogues and other emerging synthetic opioids have become more prevalent in recent years leading to an increase in overdose related deaths in the State of Alabama. The chemical structure of fentanyl is being modified by the addition, removal, or replacement of functional groups to circumvent legal restrictions for the exportation and sale of fentanyl. These compounds are often more potent than fentanyl and information related to their toxicity is limited. These compounds are making their way into the illicit market through the sale of the powder form of the compound, in combination with heroin, or pressed into pill form to appear as other pharmaceutical medications.

Objective: Develop and utilize a detection and confirmation methodology for fentanyl analogues in whole blood utilizing high resolution mass spectrometry (HRMS) screening with fragment confirmation using an Agilent 6545 QTOF Mass Spectrometer.

Methods: The methodology employed for this work included initial screening of suspected fentanyl analogue cases in a low energy TOF MS only scan mode and the data processed by an accurate mass library search with an in-house constructed library. The library was created by the addition of empirical formulas for currently available fentanyl analogues and other synthetic opioids for accurate mass information. Standards were acquired and their retention times and fragmentation spectra were added to the library. Confirmation of initial screening results was conducted by a second injection in high energy mode for MS/MS fragmentation data. The MS/MS data was processed by both Mass Hunter Qualitative and Quantitive Analysis software packages for the evaluation of acceptance criteria parameters for structural confirmation of the TOF screen accurate mass screening results.

Results: Screening and confirmation by the QTOF mass spectrometer was accomplished by conducting two experiments with extracts from a liquid-liquid extraction. The first injection was for MS only full scan data collection in TOF mode. The second injection was for MS/MS mode with varying collision energies for fragmentation of the accurate mass precursor ions. The screening algorithm within the software was optimized for appropriate sensitivity and specificity for the target analytes. General acceptance criteria for MS mode were based on a scoring algorithm of extracted ion chromatograms that included mass accuracy, retention time, isotopic spacing, and isotopic abundance. Acceptance criteria for MS/MS data were based on the type processing software utilized. Mass Hunter Qualitative Analysis software fragmentation data criteria were based on mass accuracy, fragment coelution scoring, and number of qualified fragments. Mass Hunter Quantitative Analysis software fragmentation data criteria were based on more traditional acceptance parameters of mass spectral data such as ion ratios of fragments ± 20%, retention time, and S/N. Utilizing this approach we were able to detect and confirm the presence of fentanyl analogues or other emerging synthetic opioid compounds present in authentic whole blood samples (see table).

Target	# of Positives
4-ANPP	9
U-47700	6
Methoxyacetyl Fentanyl	5
Acetyl Fentanyl	3
FIBF	2
Butyryl Fentanyl	2
Acryl Fentanyl	1

Conclusion/Discussions: Utilization of HRMS screening instrumentation has become more common for forensic applications in recent years. There is limited information available regarding methodology and acceptance criteria for screening and confirmation using TOF/QTOF instrumentation. This work demonstrates the application of an Agilent 6545 QTOF for both accurate mass screening and fragment confirmation of fentanyl analogues and emerging synthetic opioids. MS data demonstrated high sensitivity and screening capability for the target analytes. MS/MS experiments allowed for confirmation of the accurate mass MS results by traditional fragmentation data confirmation criteria. This methodology has specific advantages over traditional immunoassay and GC-MS screening procedures that are unique to HRMS instrumentation such as increased sensitivity, higher throughput, reduced data processing time, and retroactive screening capability. An additional component of this work is the presentation of acceptance criteria being utilized in our laboratory for HRMS data.

Keywords: TOF, QTOF, Fentanyl Analogues