B160 Analysis of Change in Nitrite-to-Nitrate Ratios in Gunshot Residue Over Time Using Ion Pairing High-Performance Liquid Chromatography (HPLC)

Anusha Rankoth, 100 College Drive, Allentown, PA 18104; Marianne E. Staretz, PhD, Cedar Crest College, Dept of Chemical & Physical Sc, 100 College Drive, Allentown, PA 18104; Peter J. Diaczuk, BS, 445 W 59th Street, New York, NY 10019-2925; Thomas H. Pritchett, MS, 100 College Drive, Allentown, PA 18104; and Elana Conant, MS, Cedar Crest College, 100 College Drive, Miller #10, Allentown, PA 18104*

After attending this presentation, attendees will be familiar with an ion-pairing HPLC method for measuring nitrate and nitrite that can be a useful analytical tool in Gunshot Residue (GSR) analysis.

This presentation will impact the forensic science community by introducing an HPLC method that can measure the levels of nitrate and nitrite in GSR in the barrel of a firearm. This method has the potential to determine the time frame in which a firearm was last discharged.

There is a high level of nitrites and nitrates present in GSR due to the high nitrogen content of the propellant used in smokeless powder cartridges. After discharge, these components are left behind within and on the firearm. Given that nitrite oxidizes to nitrate, it is feasible to assume that the nitrate-to-nitrite ratios within the gun barrel residue will change with time. Therefore, a gun that was fired several days prior might be expected to have a lower nitrite-to-nitrate ratio than a gun that was fired more recently; however, because different ammunition manufacturers use different ingredients when constructing their cartridges, these ratios may differ among various ammunition types.

This study has developed an HPLC method which can be utilized to measure the nitrite-to-nitrate ratios within the barrel of a firearm after it has been discharged and can determine if there is any change in this ratio over time that may prove useful for estimating the time frame in which this discharge occurred. This method consisted of an ion-pairing system using the ion-pairing agent tetrabutylammoniumhydrogen sulfate. Successful separation of the target analytes is possible in less than five minutes. The HPLC data was collected using an Agilent® 1100 HPLC system. The system utilized a diode array detector and absorbance was monitored at 205nm. Separation was accomplished using a 100mm x 2.1mm x 2.6μm Kinetex® C_{18} column. The mobile phase consisted of 95% 10mM tetrabutylammonium hydrogen sulfate in a 2.0mM sodium phosphate buffer adjusted to a pH of 8.4 and 5% HPLC-grade acetonitrile. Quantitation of nitrite-to-nitrate ratios was completed by analyzing the respective peak area via Agilent® Chemstation. Sample injections of 20μL were made using an autosampler at a flow rate of 0.20ml/min.

Gunpowder (smokeless powder) was burned in the open and samples were collected at various time intervals using Absorbond® polyester swabs dampened with HPLC-grade water. The swabs were then placed into 2ml of mobile phase. Extraction was carried out for 10-15 minutes followed by filtration using 0.2μm syringe filters. Filtered samples were placed into autosampler vials for analysis.

The retention time of nitrite present in GSR using this method is 2.4-2.5 minutes. The retention time for nitrate in GSR samples is 3.9-4.0 minutes. Starting ratios of nitrite to nitrate at time zero range from 1 to 0.92. After 24 hours, the ratios decreased to a range of 0.45 to 0.49. After an additional 24 hours, the ratios decreased again to approximately 0.27. These results indicate that nitrite-to-nitrate ratios decrease approximately 50% over a 24-hour period. With this type of data, it may be possible to construct a calibration curve which will be an important tool in determining the approximate time a firearm was last discharged.

Separation of nitrite and nitrate using this isocratic ion pairing system proved to be swift and reliable. The method was applied to the analysis of gunpowder residue. The oxidation of nitrite to nitrate in the sample was confirmed using this method. It was also confirmed that the nitrite/nitrate ratios decreased over time. This method will be utilized to analyze GSR in the barrel of a firearm at various time intervals after firing. These results will also be presented.

Gunshot Residue, HPLC, Ion Pairing Chromatography