Forensic Technology
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Information provided herein is intended to be objective and is based on data collected during the technology evaluation. The information presented is intended to provide an overview and guide; it is not intended as an exhaustive summary.
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TECHNICAL NOTE

Evaluation of Existing Technologies for Novel Analysis and Probabilistic Interpretation of Organic Gunshot Residue

Introduction

Firearms exposure has traditionally been monitored by screening for the presence of inorganic particles prevalent in gunshot residue (GSR). These inorganic compounds are associated with the primer in ammunition. Recent research efforts have explored alternative approaches for monitoring firearms exposure that screen for the presence of organic GSR (OGSR) components, which come from propellants and stabilizers.

Through the National Institute of Justice Forensic Technology Center of Excellence (FTCoE), scientists at West Virginia University (WVU) evaluated novel adaptations to two existing technologies for their suitability as screening methods for OGSR. This report is a continuation of the OGSR evaluation, "Organic Gunshot Residue Analysis for Potential Shooter Determination," by WVU, first reported by the FTCoE in May 2015. The methods evaluated herein include ion mobility spectrometry (IMS) and inlet thermal desorption gas chromatography mass spectrometry (GC-MS).

Ion Mobility Spectrometry

A novel data analysis approach involving neural network predictive modeling was evaluated for its ability to improve the performance of IMS as a screening tool for OGSR. Results from positive and negative controls, as well as outcomes from testing a general population, were used to model probability distribution functions and generate likelihood ratios useful in defining cut-off thresholds and interpreting results.

This approach involved using the mobility spectrum generated from a swab of an unknown subject as input to a neural network predictive model that was trained to distinguish three spectral patterns: laboratory blanks, skin swabs from non-shooters, and skin swabs from known-shooters. This approach significantly reduced the false-positive rates compared to an arbitrary decision threshold technique and using some neural network models, reduced the false-positive rate to zero. A full description of the methodology and results were published in the journal *Forensic Science International* (Bell, 2016).

Inlet Thermal Desorption Gas Chromatography Mass Spectrometry

A thermal separation probe (TSP) was evaluated as an inlet to a GC-MS system for the analysis of OGSR components. The TSP is a commercially available probe that fits directly into an injection port. Samples were generated from swabbing shooters' hands with clean room wipes (CapSure®, Berkshire) designed for high absorbency and particulate trapping. As supplied, the TSP has a limited sample chamber capacity; therefore, a small portion of the swab (< 1cm2) was rubbed against the skin of both the left and right hands of the shooters to concentrate residues onto the smallest surface area possible. This smaller area was then cut from the main swab and inserted into the microvial sample chamber of the TSP. The WVU team used this technique to collect and analyze 16 samples from a known-shooter after firing 5 shots. In a subsequent shooting session, 10 additional samples were collected from a known-shooter after firing varied numbers of shots (1–4) using pre-cut swabs (~1.5 cm2) and an in-house fabricated swab holder. From these analyses, the team identified two potential markers for OGSR—ethyl centralite and diphenylamine—when using TSP-GC-MS.

The clean room wipes used to swab shooters' hands were very efficient at sample collection, but the target OGSR components proved difficult to desorb using the TSP. As shown in **Table 1**, estimated limits of detection are orders of magnitude higher for analytes spiked onto swabs and then placed into the TSP sample chamber (i.e., swab) than they are for analytes added directly to the TSP sample chamber (i.e., instrument). Thus, obtaining consistently detectable results associated with shooting events at relevant forensic concentrations (i.e., one to two shots fired, 9mm) proved difficult.

Table 1. Limits of Detection (LOD) for OGSR components. Bold denotes potential markers for OGSR when using TSP-GC-MS.

OGSR Compound	Instrument LOD (ng)	Swab LOD (ng)
2,4-Dinitrotoluene	1	500
Diphenylamine	**0.5**	**5**
Methyl centralite	0.05	5
Carbazole	0.05	5
Ethyl centralite	**0.05**	**0.05**
2-Nitrodiphenylamine	0.05	20
4-Nitrodiphenylamine	20	500

Similar to many GC-MS and liquid chromatography- MS-based analytical methods, OGSR components were detected based on their retention time and an ion ratio between a quantifier ion and a qualifier ion. Many method validation guidance documents agree that as a quality control check, the quantifier/qualifier ion ratio should be consistent between samples, and a suggested range of +/- 20% RSD is typically considered reasonable. However, there is very little empirical evidence for why this 20% RSD of ion ratio should be considered an acceptable quality control check. To establish a quality control metric with an experimentally determined level of reasonableness, the WVU team used a statistical approach based on analysis of an internal standard (nitrobenzene-d6) and two surrogate standards (1,3,5-trimethyl-2-nitrobenzene and 1,2,4-trimethyl-5-nitro-benzene) spiked onto swabs at known concentrations. This approach utilizes both retention time and ion ratio of the surrogate analytes analyzed throughout a sample set.

Using a method adapted from work done by Woldegebriel et al. (2016), bivariate probability density functions for each of the surrogate standards were created. **Fig 1** shows the bivariate plots for each of the surrogate standards.

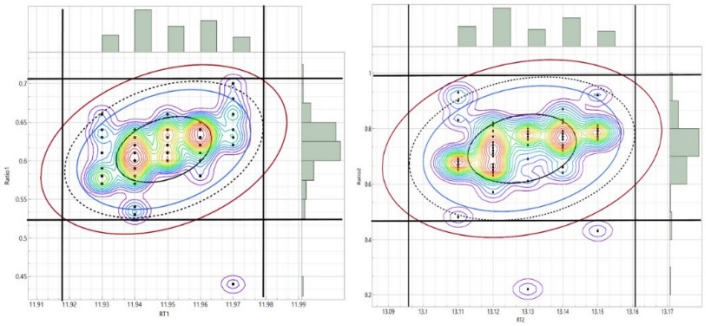

Fig 1. Bivariate probability density plots for two surrogate standards: Surrogate 1, Left: 1,3,5-trimethyl-2-nitrobenzene and Surrogate 2, Right: 1,2,4-trimethyl-5-nitrobenzene. Image reproduced with permission from Stevens, B., Bell, S., & Adams, K. (2016).

The x-axis is the retention time in minutes and the y-axis is the ion ratio between the quantifier ion and the qualifier ion (m/z 91 and 148). Each dot represents one GC-MS run, n = 70. The dotted black line corresponds to the 95% confidence level associated with the Mahalanobis distance from the center of the data mass. Histograms of the retention time and ion abundance are also shown. The ion abundance histograms suggest a normal distribution. In practice, this method could be used as a qualifier for data acceptance. If the retention time/ion ratio pair of the surrogate standards for any given sample falls outside of the 95% ellipse, it would suggest a procedural or recovery problem that would have to be considered in evaluating the results for that sample. Full details of this work were published in the journal Forensic Chemistry in 2016 (Stevens et al.).

Given the difficulties encountered with detecting forensically relevant levels of OGSR compounds with the commercially supplied TSP, the WVU team designed and evaluated an alternative sample collection and introduction setup, as well as a modified data analysis approach. Because retention times were determined to be stable (% RSD < 0.6%) density plots were created based solely on ion ratio, with the intensities of the quantifier and qualifier ions plotted on the x- and y-axes. Experimental modification consisted of stabilizing the collection swabs across the end of a glass stopper to concentrate recovered residues onto a minimal surface area. In addition, the microvial was removed from the TSP and the region of interest on the swab was mounted on a small hook at the end of the probe as shown in **Fig 2**.

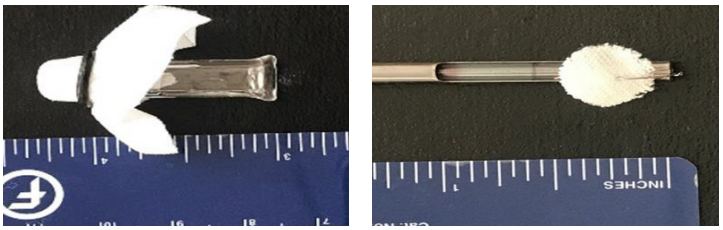

Fig 2. Left: Swab affixed to a glass stopper. Right: Cut portion of swab mounted on a wire hook for increased surface area exposure in the thermal separation probe. Image reproduced with permission from Stevens, B., Bell, S., & Adams, K. (2016).

This modification was thought to allow more gas flow and contact with the surface area of the swab to facilitate desorption and transport into the GC-MS system. However, analysis of both positive control samples and swabs from known shooters proved to be decidedly disappointing. Results were inconsistent and did not show a clear correlation between number of shots fired and detection of any of the target OGSR compounds. The adapted method did not show any overall improvement compared to the initial work.

Conclusions

The incorporation of a neural network predictive model into the interpretation of IMS screening data for OGSR showed marked improvement in the frequency of false-positives compared to traditional IMS data analysis techniques. Despite attempts to enhance the relatively high limits of detection seen with the TSP, the evaluation team determined that at this time, the TSP approach as implemented does not provide any improvement over IMS for OGSR screening.

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Disclaimer

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