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# Technical Note

# Detection of Organic Gunshot Residue Using Capillary Microextraction of Volatiles with Cryofocusing

## Executive Summary

Researchers at Florida International University's (FIU's) International Forensic Research Institute evaluated the capability of capillary microextraction of volatiles (CMV) to extract the volatile organic compounds (VOCs) that constitute organic gunshot residue (OGSR) on the hands of shooters. The team at FIU worked to optimize a field sampling method for the detection of OGSR and designed a custom-built Peltier cooling device for CMV to assess whether cryofocusing during the extraction process could improve the detection of OGSR in real-world samples. The benefits of cryofocusing, which were demonstrated in this work for the improved extraction of volatiles from OGSR, may be extended to other important areas of forensic chemistry, such as fire debris analysis and headspace signature analysis of drugs of abuse.

*Key findings of the study*:

- Cryofocusing of the CMV device with a thermoelectric cooler led to improved recoveries of nitroglycerin (NG) extracted from the headspace of double-base smokeless powder samples.
- Improving the extraction method

resulted in greater recoveries from mock swab samples spiked with VOCs typically present in OGSR.

- The equilibrium time, sample temperature, pump flow rate, extraction time, and sampling volume were all key factors requiring optimization.
- The volume of the container (15-mL vial vs. 1-L can) in which a sample is stored and extracted from significantly affects the amount recovered from the headspace. Vials provide better recoveries and may be more suitable for the storage of field samples.
- The CMV desorption temperature in the inlet of the gas chromatograph (GC) is important for the sensitive detection of NG.
- Hand blank samples collected in a similar fashion to the actual shooters' swabs did not show chromatographic interference.
- Sample extraction and analysis with GC-mass spectrometry can be completed in approximately 30 min.

## Introduction

Upon pulling the trigger of a firearm, a hammer strikes the back of an ammunition cartridge, detonating the sensitive primer and igniting the gunpowder. This leads to rapid increases in the temperature and pressure in the chamber, which propel the bullet outward and result in the vaporization of compounds from the primer and gunpowder. These compounds subsequently condense when they encounter ambient conditions to form gunshot residue (GSR), which is deposited onto the shooter's hands and clothing. GSR is composed of various organic and inorganic compounds. The inorganic components originate from the primer, cartridge case, and weapon barrel and consist of nitrates and metallic particles. The organic components generally originate from the propellant and consist of hydrocarbons (Dalby, Butler, & Birkett, 2010).

Traditional methods for GSR detection focus on inorganic components, such as lead, antimony, and barium. However, the foreseeable increase in the use of lead-free ammunition will complicate existing inorganic GSR analysis methods. This report summarizes a recent development in the sampling, collection, and analysis of organic GSR (OGSR) on the hands of shooters. This is an extension of work previously published by Tarifa and Almirall (2015). The methodology discussed herein features a novel extraction and pre-concentration device based on the capillary microextraction of volatiles (CMV) for the detection of OGSR. Beyond OGSR, CMV also holds potential for other forensic applications, including fire debris analysis and the identification of drugs based on their headspace signatures.

## Organic Gunshot Residue

OGSR contains organic compounds that stem from the smokeless powder used as a propellant in firearms ammunition. Many of these components can be classified as volatile organic compounds (VOCs). Characteristic VOCs present in OGSR include nitroglycerin (NG), 2,4-dinitrotoluene (2,4-DNT), diphenylamine (DPA), and ethyl centralite (EC) (**Figure 1**) (Goudsmits, Sharples, & Birkett, 2016).

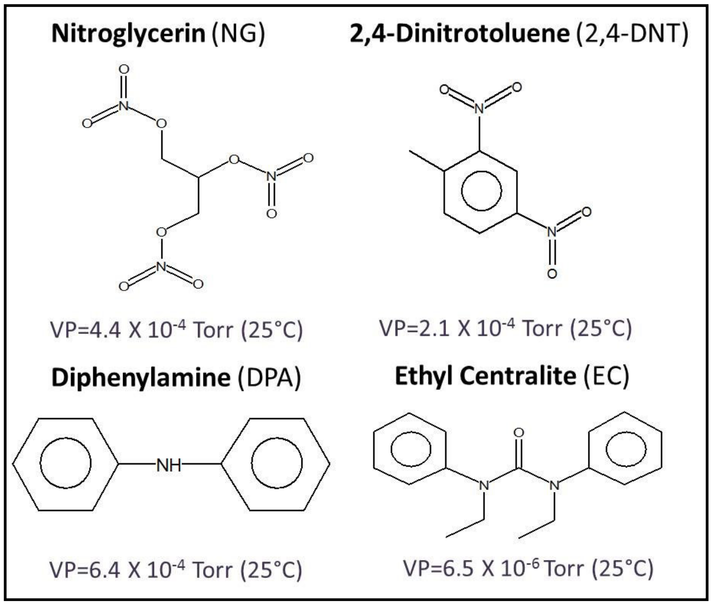

**Figure 1:** *Four characteristic VOCs commonly found in OGSR and their vapor pressures.*

Although various techniques have been developed, there is no universal method for the collection, extraction, and analysis of OGSR (Goudsmits, Sharples, & Birkett, 2015). VOCs tend to evaporate readily at room temperature because of their low boiling points and high vapor pressures. As a result, they can be efficiently sampled using headspace extraction. That is, when a sample containing VOCs is kept inside a sealed container, the volatile analytes partition into the headspace of the container and can then be transferred to a gas chromatograph (GC) for separation and detection.

## Capillary Microextraction of Volatiles

This report discusses the capabilities of an innovative CMV device developed by researchers in Dr. José Almirall's laboratory at Florida International University's (FIU's) International Forensic Research Institute. The CMV device is a versatile sorbent tube designed to collect and capture volatiles from the air for analysis via GC-mass spectrometry (GC-MS). CMV was previously employed for the detection of VOCs in smokeless powders and explosives (Fan & Almirall, 2014). Additionally, this device was demonstrated to be capable of detecting the VOCs present in GSR samples collected from the hands of shooters at a firing range (Tarifa & Almirall, 2015).

The CMV device consists of a glass capillary with a diameter of 2 mm and a length of 2 cm that is filled with rectangular strips of glass filters coated with polydimethylsiloxane (PDMS) (Fan & Almirall, 2014). The CMV device is open ended and can be connected to a vacuum pump to dynamically sample the air, which drastically reduces sampling times compared to static solid-phase microextraction (SPME) fibers. Volatiles absorb onto the PDMS as they are sampled, and the CMV device can be subsequently transferred to a thermal separation probe for GC-MS analysis. Following thermal desorption, the CMV device can be reconditioned for further sampling, making it cost effective.

## Instrumentation

The GC-MS system used for this study consisted of an Agilent Technologies 7890A GC connected to a 5975C Inert XL single quadrupole MS detector and a micro-electron capture detector. An Agilent thermal separation probe was installed for thermal desorption of the VOCs from the CMV device into the GC injection port at 180°C, and a Restek single taper inlet liner with an inner diameter of 4 mm was used. The GC method is described in **Table 1** and utilized a DB-5ms Ultra Inert column (5.8 m × 0.25 mm × 0.25 µm). Data analysis was conducted with Agilent's ChemStation software.

**Table 1:** *GC method for the analysis of GSR VOCs.*

| | | |
|---|---|---|
| Inlet Temp: 180°C | Flow: 1.2mL/min | Split (5:1) |
| Transfer Line to MS: 280°C | Ion Source: 230°C | Quadrupole: 150°C |
| | | |
| *Temp. Program (21.17 min total)* | **Temperature** | **Hold Time** |
| **Ramp Rate** | Initial: 40°C | 0.5 min |
| 15 °C/min | 240°C | 5 min |
| 30 °C/min | 280°C | 1 min |

Field samples were stored in either 1-L metal cans with lids (All-American Containers, Miami, FL, USA) or 15-mL glass vials with rubber/polytetrafluoroethylene (PTFE) septa (Supelco, Bellefonte, PA, USA). The cans were heated in an oven baked out for 3 days at 250°C prior to use. Standard solutions (1000 ppm) of NG, DPA (Sigma-Aldrich, St. Louis, MO, USA), and 2,4-DNT (Cerillant, Round Rock, TX, USA) were purchased; EC (97%) was purchased as a solid (Sigma-Aldrich). An Escort ELF portable air sampling pump (Zefon, Ocala, FL, USA) capable of flow rates of 0.5–3 L/min was used for the extractions.

## Calibration Curves

Calibration curves were created by spiking a known amount of a mixture of NG, 2,4-DNT, DPA, and EC directly onto the CMV device and placing it into the GC inlet for desorption. Solutions with concentrations ranging from 0 to 25 ppm were prepared in methanol. A 1-µL direct spike of each solution onto the CMV device was performed in triplicate. The CMV device is capable of quantification with linearity of 0.991 or better for all four compounds in single-ion-monitoring mode.

## Cryofocusing of the CMV Device

Previous work has shown that CMV is capable of detecting VOCs in smokeless powders and explosives (Fan & Almirall, 2014). In this evaluation, the team at FIU sought to determine whether cryofocusing of the CMV could improve the extraction efficiency of GSR VOCs by trapping the volatile compounds more effectively during sampling and, thereby, reducing breakthrough and increasing recovery. A custom-built Peltier cooler apparatus was designed to fit the CMV device and cool it to –10°C in under 5 min (**Figure 2**). The device includes a thermocouple and digital readout for precise temperature adjustment, monitoring, and control.

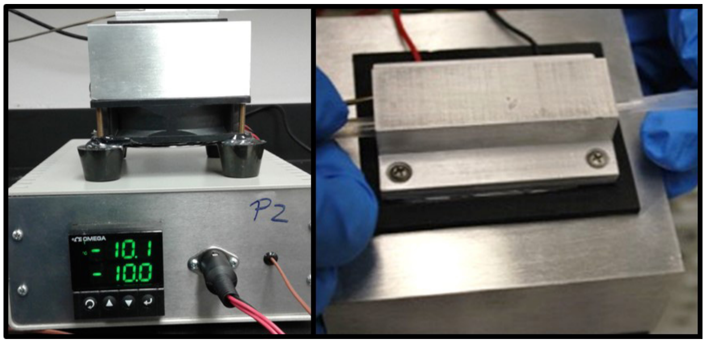

**Figure 2.** *Customized Peltier cooler for cryofocusing to facilitate sampling volatiles from air with the CMV device (Almirall and Tarifa, US Non-Provisional Patent Application [US 15/246,866]).*

Samples (100 mg) of Hodgdon BLC-2 double-base smokeless powder were placed into 1-L cans. The sealed lid of each can was pre-punctured with one hole and sealed with a rubber septum. After incubation overnight, the septum was removed, and the headspace of each can was extracted at a flow rate of 1 L/min for 2 min with the CMV device at room temperature (20°C) or at lower temperatures (2.5°C or –10°C). At –10°C, water condensation was observed in the CMV device during pumping, but at 2.5°C, this was not an issue. Compared to room-temperature extraction, at 2.5°C and –10°C, cryofocusing increased the amounts of NG detected in the headspace by 38% and 29%, respectively (**Figure 3**). The procedure was repeated with silica gel desiccant in the cans during the incubation, which resulted in a 62% improvement in the recovery of NG at –10°C and visibly less condensation in the CMV device.

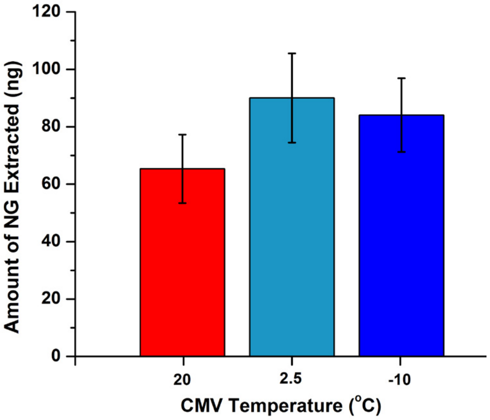

**Figure 3:** *Extraction of NG from the headspace of a 1-L can containing double-base smokeless powder using the CMV device at different temperatures.*

Higher flow rates (up to 1.3 L/min) significantly improved the recoveries of all compounds at both room temperature and –10°C. In the 1-L cans, increasing the extracted volume from 0.5 to 4 L resulted in corresponding increases in the recoveries at both temperatures for all compounds. One of the primary challenges in this technique is managing the water vapor produced from humid ambient air condensing on the cooled CMV device and tubing during the extraction. Cooling the CMV device alone did not result in moisture buildup, but pumping air through the cold CMV device did lead to condensation. This buildup of condensation exerted a greater effect when the extraction was performed using the 15-mL vials. Techniques for managing water condensation on the tubing and CMV device are being evaluated to determine their effects on the recoveries obtained. These techniques include passing a reverse flow of nitrogen through the CMV device before the extraction to prevent moisture buildup and after the extraction to prevent water entering the GC inlet during desorption.

## Preliminary Field Sampling, Extraction, and Analysis

Initial field sampling was conducted to determine if extraction using CMV and cryofocusing could detect OGSR on shooters' hands at forensically relevant concentrations (Figure 4). Institutional Review Board approval was granted to sample the hands of police officers conducting shooting practice at a firing range and volunteers at the university who had not fired or handled a gun. Two volunteers were asked to fire a series of consecutive shots with a handgun using Winchester 9 mm Full Metal Jacket ammunition. Hand blanks were collected prior to touching or firing the handgun and revealed no interferences or GSR compounds. After each shot, the shooters' hands were swabbed using cotton swabs moistened with deionized (DI) water, and the shooters did not wash their hands between shots. After sampling, the swabs were stored in either 15-mL glass vials with rubber/PTFE septa or 1-L metal cans with lids to determine the effect of the container volume. Six different volunteers at the university had their hands swabbed in a similar fashion. The samples were returned to the lab and analyzed within 72 h.

The containers containing the swabs were heated and kept stationary for an equilibration period (i.e., 20 min at 60–70°C) immediately prior to extraction to allow the buildup of volatiles in the headspace. Next, dynamic headspace extraction of the vials was performed with the CMV device connected to an adjustable vacuum pump via inert and airtight tubing. The CMV device was placed inside the cooler block of the Peltier cooler, and the temperature was kept at room temperature or set to −10°C. Following the extraction, the CMV device was placed in the inlet of the GC-MS via a commercially available thermal separation probe. The headspace extraction and GC-MS analysis could be completed within 30 min, and thus, this technique has the potential to speed up and simplify the process compared to other OGSR analysis methods.

In the preliminary field samples, NG and DPA were detectable after four shots were fired when the vials were extracted with the CMV device at room temperature. At −10°C, however, a buildup of condensation was observed in the tubing and CMV device as a result of the moisture from the wet swabs. In contrast, when the 1-L cans were used, the four target compounds were not detected, possibly because of the larger headspace volume. For the non-shooter hand blanks, no similar compounds or interferences were observed at the expected retention times for the selected GSR volatiles.

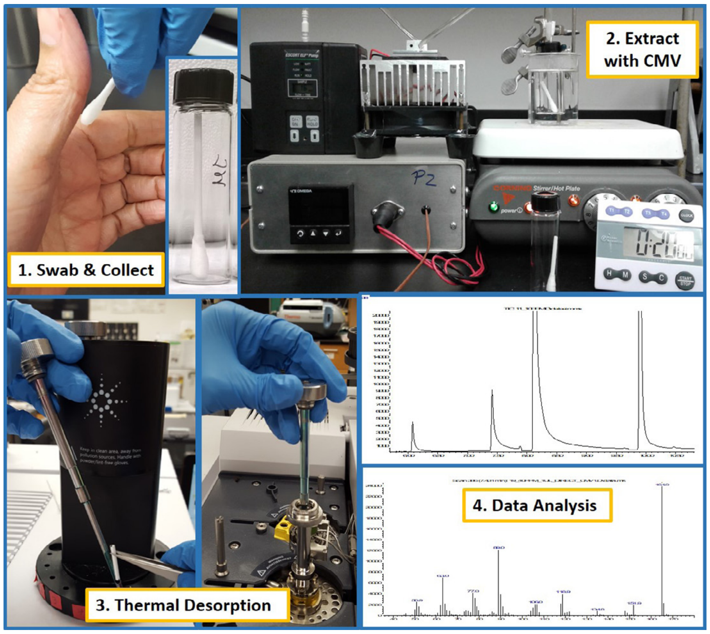

**Figure 4:** *Sampling protocol for OGSR using the CMV device. Extraction and GC-MS analysis can be completed in 30 min. Reprinted with permission from Tarifa, Kreitals, Mulloor, Gura, and Almirall (2017).*

## Optimization of Field Sampling

Experiments were conducted on mock field samples to optimize relevant parameters and improve the extraction and recovery of the four compounds of interest. Cotton swabs moistened with DI water were spiked with a mixture containing 100 ng each of NG, 2,4-DNT, DPA, and EC. The swabs were placed inside 15-mL glass vials and capped to prevent loss of volatiles.

A water bath was used to evenly heat and incubate the vials to increase the concentrations of the compounds in the headspace. Heating the samples is necessary because the compounds may otherwise remain trapped in the moist cotton and remain undetectable. A 20-min equilibration time in the 80°C water bath provided the highest recoveries for NG while maintaining adequate recoveries for the other three compounds. Longer equilibration times resulted in worse recoveries for DPA.

The Escort ELF sampling pump was utilized for dynamic sampling of the vials' headspaces (Figure 5). This pump was connected to the CMV device using Teflon tubing, which was selected because of its inertness and to avoid the interferences observed with other types of tubing. The septa of the vials were punctured with wide 16-gauge needles to create two holes: one for drawing air out towards the CMV device and the other to introduce air into the vial to prevent the development of a vacuum. The flow rate of the pump, sampling time, and extraction volume are important parameters that require fine tuning. Higher flow rates were found to increase the recoveries of the OGSR compounds. The maximum attainable stable flow rate through the CMV was 1.3 L/min. A sampling volume of 3 L led to the highest recovery from the vials.

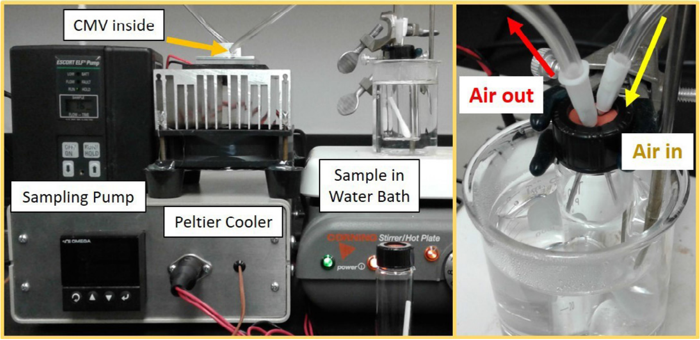

**Figure 5**: *Extraction setup for swab samples in vials.*

Comparing the optimized extraction method to the previous method revealed improvements in the recoveries of the four compounds. Samples (50 ng) of the four compounds were spiked on moistened cotton swabs, placed in 15-mL vials, and extracted using both methods. The initial setup consisted of Tygon tubing with 20 min of equilibration at 60°C and a 2-min extraction at 1 L/min. Using the new parameters—20 min of equilibration at 80°C, a 3-min extraction at 1 L/min, and Teflon tubing—led to significantly higher recoveries, as shown in Figure 6.

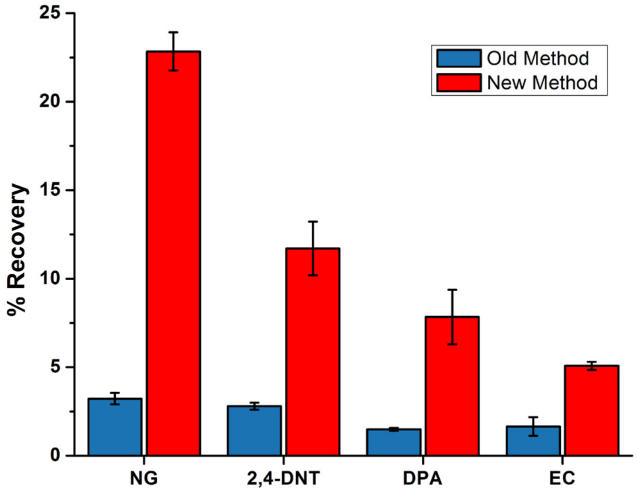

**Figure 6:** *Comparison of the old extraction setup to the new extraction setup using a 50-ng spike of NG, 2,4-DNT, DPA, and EC on a moistened cotton swab in a 15-mL vial.*

The volume of the container being sampled is also a crucial parameter. In this work, 15-mL vials provided better recoveries than 1-L cans. Using the 15-mL vials, 10-ng samples of the four target compounds could be detected, whereas when the extractions were performed from the 1-L cans, the minimum sample mass required for detection was 200 ng. Further optimization of the extraction process using the cans will be required before this technique can be applied to hand swab samples.

The inlet temperature used for desorption from the CMV also required optimization. Higher inlet temperatures were not suitable for NG because this compound decomposes at high temperatures. Therefore, although higher temperatures improved the method's sensitivity to the other three compounds, the inlet temperature was set at 180°C to optimize the detection of NG, which had the lowest integrated peak areas.

## Conclusions

A sampling, extraction, and analysis method for OGSR would complement inorganic GSR analyses and increase the evidentiary value of GSR. The ability of the innovative CMV device described here to extract VOCs from GSR has been demonstrated. Further optimization of the sample collection and extraction protocol and cryofocusing of the CMV device have the potential to enhance this qualitative and quantitative collection, extraction, and analysis method for OGSR from the hands of shooters.

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