OSAC 2022-S-0002 Standard Practice for the Identification of Compounds Related to Organic Gunshot Residue (OGSR) by Gas Chromatography-Mass Spectrometry (GC-MS)

Ignitable Liquids, Explosive, and Gunshot Residue Subcommittee
Chemistry: Trace Evidence Scientific Area Committee (SAC)
Organization of Scientific Area Committees (OSAC) for Forensic Science

OSAC Proposed Standard

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Prepared by
Ignitable Liquids, Explosive, and Gunshot Residue Subcommittee
Version: 2.0
November 2022

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Standard Practice for the Identification of Compounds related to Organic Gunshot Residue (OGSR) by Gas Chromatography– Mass Spectrometry (GC-MS)

1. Scope

1.1. This practice covers the qualitative analysis of extracts using electron impact ionization-gas chromatography-mass spectrometry (GC-(EI)MS) to identify compounds related to organic gunshot residue (OGSR).

1.2. This practice is intended for use by forensic science practitioners with the requisite formal education, discipline-specific training (see Practice E2917), and demonstrated competency to perform forensic casework.

1.1. This practice does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this practice to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.

2. Referenced Documents

2.1. ASTM Standards:

E1588 Practice for Gunshot Residue analysis by Scanning Electron Microscopy/Energy Dispersive X-ray Spectrometry.

E1732 Terminology Relating to Forensic Science.

E2917 Practice for Forensic Science Practitioner Training, Continuing Education, and Professional Development Programs.

E2998 Practice for Characterization and Classification of Smokeless Powders.

E2999 Test Method for Analysis of Organic Compounds in Smokeless Powder by Gas Chromatography-Mass Spectrometry and Fourier Transform Infrared Spectroscopy.

E3255 Practice for Quality Assurance of Forensic Science Service Providers Performing Forensic Chemistry Analyses.

WK56998 Terminology Relating to the Examination of Explosives.

OSAC 2021-N-0009 Standard Practice for the Collection and Preservation of Organic Gunshot Residue

OSAC 2022-S-0003 Standard Practice for the Identification of Compounds related to Organic Gunshot Residue (OGSR) by Liquid Chromatography-Mass Spectrometry (LC-MS).

3. Terminology

3.1. For definitions of terms that can assist in interpreting this standard, refer to Terminology E1732 and WK 56998.

3.2. Definitions of terms specific to this standard:

3.2.1. *Inorganic GSR (IGSR), n* – Gunshot residues from the primer, cartridge case, projectile (e.g., bullet or shot pellets), or the firearm which are typically identified using scanning electron microscope (E1588).

3.2.2. *Organic GSR (OGSR), n* – Gunshot residues from the propellant and the priming mixture that are organic (carbon-based).

3.2.3. *Primer GSR (pGSR), n* – Gunshot residues generating from the priming mixture that could be inorganic or organic.

3.2.4. *Reference Sample, n* – A solution containing known target OGSR compounds.

4. Significance and Use

4.1. Gunshot residue (GSR) examination is typically performed to determine if an individual or object was exposed to firearm discharge. GSR analysis has historically relied upon the detection of IGSR, as described in Practice E1588, which originates primarily from the ammunition primer (pGSR). OGSR analysis provides information which complements pGSR analysis [1].

4.2. OGSR originates from the combustion of the priming mixture and the smokeless powder following their ignition during the firearm discharge process. After a firearm has been discharged, the combined residue can be found on exposed surfaces in the vicinity of the fired weapon (e.g., hands, other skin surfaces, hair, clothing, and other surfaces). OGSR can also be found in the cartridge case after firing and can be recovered to provide information about the constituents of the propellant or the priming mixture, or both.

4.3. This practice is intended to be used in conjunction with a laboratory's validated standard operating procedures. Laboratory specific criteria should be established and supported by data obtained during method development and validation (E3255).

4.4. This practice does not cover how to interpret analytical data for reporting the absence or presence of OGSR.

4.4.1. This practice can be used in tandem with other practices (E1588) or approaches to simultaneously or independently identify components related to OGSR or IGSR/pGSR.

4.5. The analysis of smokeless powder grains is beyond the scope of this practice (refer to Practice E2998 and Test Method E2999).

4.6. This practice does not address the analysis of inorganic gunshot residue (IGSR) or primer gunshot residue (pGSR).

5. Apparatus

5.1. Gas chromatograph (GC) – A gas chromatograph (GC) capable of using capillary columns and being interfaced to a mass spectrometer (MS) (refer to Table X1).

5.2. Mass Spectrometer (MS) – A mass selective detector operating in electron ionization (EI) mode and capable of acquiring mass spectra from mass-to-charge (m/z) 10 - m/z 400 with unit resolution or better.

5.3. Sonicator – For use when extracting OGSR components from sample items collected.

5.4. Centrifuge – Recommended for use after sonication of extract and capable of achieving a minimum of 4000 RPM. Disposable centrifuge tubes that safely fit the centrifuge device are required.

6. Materials

6.1. Analytical Solvents – Acetone, acetonitrile, ethanol, isopropanol, methanol, methylene chloride, or other solvents compatible with GC-MS.

6.1.1. Purity of Solvents – GC-MS grade or higher.

6.2. OGSR Standard(s) or Reference Materials – Certified reference materials are to be used. Individual reference materials or standards, or mixture thereof, may be used in place of a certified reference standard provided that they are verified according to laboratory protocol before use.

6.3. Internal Standard – Use of an internal standard is not required for qualitative identification of OGSR but can be used to evaluate system sensitivity and reproducibility.

6.4. GC Carrier Gas – Helium or hydrogen of purity 99.995% or higher.

6.5. Drying Gas – Nitrogen, air, or other inert gas of purity 99.95% or higher.

6.6. Filters – Single use disposable filters of a hydrophobic membrane construction are recommended for use to filter the extract prior to analysis. A filter membrane porosity of 0.4 µm or smaller is required.

7. Procedure

7.1. Samples are submitted for OGSR analysis in one or more of the following forms: adhesive tape lifts, swabs, or vacuum filters; refer to OSAC 2021-N-0009.

7.1.1. Alternative analysis of OGSR components by LC-MS has been described elsewhere [OSAC 2021-S-0003] [2, 3].

7.2. Preparation of OGSR samples:

7.2.1. Extract collection items such as swabs, vacuum filters, or adhesive lifts using a suitable organic solvent, such as methanol, methylene chloride, or acetonitrile [4].

7.2.2. Place the entire collection item inside a new disposable vial suitable for the size of the collection item. Swabs can be folded to fit into the vial. Add a minimum volume of solvent required to submerge the item.

7.2.2.1. If an aluminum stub with an adhesive carbon tab was used to collect the sample, place the entire stub into a new disposable vial for subsequent extraction (using the process in Section 7.2.2.)

7.2.2.2. Alternatively, wash the surface of the stub with multiple aliquots of the solvent using repeated cycles of suction and addition [5].

7.2.3. Sonicate the vial with the item submerged in the solvent for a minimum of 5 minutes [6]. Remove the extract and either filter or centrifuge the item to remove any solid particulates from the solution.

7.2.4. The extract can be analyzed directly but concentration is often required for GC-MS analysis of OGSR components.

7.2.5. Carefully dry the extract down to an appropriate volume required for injection using a gentle stream of drying gas. If the extract is taken to complete dryness, some of the OGSR components may be lost.

7.2.6. Store extracts at 0°C or colder to maximize preservation when the sample is not being analyzed.

7.3. GC-MS analysis of OGSR extracts:

7.3.1. Common organic components of OGSR can be identified by GC-MS analysis.

7.3.2. Suggested GC-MS parameters are listed in Appendix Table X1.

7.3.2.1. Verify that these parameters are optimized to ensure that each peak in the reference test mixture has baseline resolution (less than 1% overlap between two peaks) on the laboratory's instrument before using the method in casework.

7.3.3. Prior to analyzing samples, the GC-MS should be tuned and calibrated per validation protocols or the manufacturer recommendations.

7.3.4. Table 1 lists the common compounds related to OGSR and their target ions .

7.3.4.1. Data analysis is limited to the identification of the target compounds listed in Table 1.

7.3.4.2. Each specific retention time is measured by analyzing a reference material (see Sec. 6.3), using the GC-MS system and method under the same instrumental conditions [7,8].

7.3.4.3. Nitroglycerin and trinitrotoluene are subject to thermal degradation in the injection port of the GC which may make them difficult to detect at trace levels.

TABLE 1: Typical OGSR related compounds and m/z of target ions in EI MS found by GC-MS analysis. Refer to the citations for further details on identification.

Target Compound	**Significance/ Application**	**Target Ions (*m/z*)***	**Ref.**
Akardite II (AK II)	Stabilizer	169, 226	[9, 10 E2999]
2-amine-4,6-dinitrotoluene (2-ADNT)	Degradation products	197, 180	[9]
Dibutyl phthalate (DBP)	Plasticizer	149, 223	[9, 10 E2999]
Diisoamyl phthalate (DIP)	Plasticizer	149	[E2999]
2,4- and 2,6-dinitrotoluene (2,4-DNT and 2,6-DNT)	Flash suppressor	165, 182	[9, 10 E2999]
Dioctyl phthalate (DOP)	Plasticizer	149, 279	[9, 10 E2999]
Diphenylamine (DPA)	Stabilizer	169	[9, 10 E2999]
Ethyl centralite (EC)	Stabilizer	120, 268	[9, 10 E2999]
Ethyleneglycol dinitrate (EGDN)	Explosive	46, 76	[9, 10 E2999]
Methyl centralite (MC)	Stabilizer	134, 240	[9, 10 E2999]
2 and 4-nitrodiphenylamine (2-NDPA, 4-NDPA)	Degradation products; stabilizer	167, 214	[9, 10 E2999]
Nitroglycerin (NG)	Explosive	46, 76	[9, 10 E2999]
4-nitrosodiphenylamine (4-NODPA)	Degradation products	167, 198	[9, 10 E2999]

N-nitrosodiphenlyamine (N-NODPA)	Degradation product	168, Detected as DPA	[9, 10]
Nitrotoluenes (NTs)	Sensitizer	65, 91, 120 or 137	[9, 10 E2999]
2,4,6-trinitrotoluene (TNT)	Flash suppressor/sensitizer	89, 210	[10]

* NOTE: Full scan or selected ion monitoring (SIM) analysis using all target ions

8. Identification of Compounds related to OGSR

8.1. The identification of a compound in an unknown sample shall be based upon direct comparison with a known reference standard of the compound.

8.1.1. The retention time or retention index and the mass spectral pattern (ions detected and their m/z values), and the mass spectral pattern as compared to the certified reference standard must be used for compound identification.

8.2. The unknown sample shall be run on the same instrument using the same instrument parameters and the same method as the known reference standard.

8.3. In order to identify any compound in an unknown sample, the compound should meet identification criteria for gas chromatography retention time and mass spectrometry.

8.3.1. Gas Chromatography

8.3.1.1. The retention time of the compound in the unknown sample shall be within ± 0.1 minute of the reference compound.

8.3.2. Mass Spectrometry

8.3.2.1. The unknown and reference samples shall have the same base peak and the same molecular ion, if present.

8.3.2.2. The measured m/z value of the molecular ion shall be within the instrument manufacturer's tolerance (mass accuracy) of the molecular ion peak observed in the corresponding mass spectrum of the certified reference standard.

8.3.2.3. The fragmentation patterns and relative abundances of target ions in the mass spectra of the unknown sample and the reference OGSR sample must not have unexplainable differences across the majority of the ions.

8.3.2.3.1. Analysts must document reasons for considering or not considering differences between the spectra.

8.3.2.4. When blank subtraction is performed, use the blanks described in 9.2.2.

8.3.2.5. There shall be no ions (above 5% of total spectral abundance) that cannot be attributed to the instrument's background.

9. Quality Control

9.1. For minimum quality assurance protocols refer to Practice E3255.

9.2. Quality assurance protocols specific to this standard practice:

9.2.1. Analyze a quality control sample with questioned extracts.

9.2.1.1. The quality control sample shall be analyzed, at minimum, at the beginning and the end of the analytical sequence on the instrument.

9.2.1.2. Quality control sample shall contain at least five compounds, as chosen by the individual laboratory, from the compounds s listed in Table 1.

NOTE: An example of a quality control sample is as follows: NG, EC, 2-NDPA, DPA, and 2,4-DNT prepared at a concentration within the method's linear dynamic range.

9.2.1.3. Establish protocols to ensure stability of the quality control sample.

9.2.1.4. Store quality control sample under appropriate conditions (see 7.2.5).

9.2.1.5. Replace the quality control sample when degradation is observed from the previous analysis of the sample. Examples of degradation include: the absence of peaks, the presence of new peaks. The laboratory can also determine an expiration date for the quality control sample in accordance with laboratory policy.

9.2.1.5.1. If degradation of the quality control was observed during analysis, any affected questioned samples must be reanalyzed with a new quality control.

9.2.2. Analyze the following blank samples with each batch of questioned extracts.

9.2.2.1. Prepare a method blank by extracting a clean swab or filter using the same procedure(s), reagents, and conditions used to extract the unknown sample(s).

9.2.2.2. A solvent blank, consisting of solvent alone, shall be analyzed between each sample to ensure no carryover occurs.

10. **Records**

10.1. Document the following, electronically or hard-copied, refer to Practice E3255:

10.1.1. GC-MS instrument settings and method parameters used for analysis.

10.1.2. Calibration and tuning of the instrument used for analysis.

10.1.3. Chromatograms of method blank(s), reference material(s), and questioned samples. Annotate all peaks relevant to the analysis with retention times.

10.1.4. Mass spectra of OGSR compounds identified and the associated material(s) used for comparison.

10.1.5. All analytical notes from the analysis including the details of sample preparation and instrument performance checks.

10.1.6. Maintain reports in accordance with laboratory policy and Practice E3255.

11. **Keywords**

11.1. OGSR; GC-MS

12. **References**

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APPENDIX

(Non-Mandatory Information)

X1. Instrumental Operation Parameters

X1.1 Suggested GC-MS parameters

X1.1.1 An example GC-MS instrumental method, from literature, to analyze OGSR compounds is provided below as a starting point for the method validation by the laboratory.

TABLE X1: Suggested GC-MS conditions derived from ASTM E2999.

Parameter	**Condition**
GC column:	30 meters, DB-5,
Injector Temp:	180° C
Initial Temp (T_0):	40-50° C
Hold time (T_0):	3 minutes
Ramp 1:	15° C/minute to 150° C (T_1)
Hold time (T_1):	2 minutes
Ramp 2:	20-40° C/minute to 265° C (T_2)
Hold time (T_2):	8 minutes
Mode:	EI
Scan Mode	Full Scan or Selected Ion Monitoring
Split Ratio:	Splitless (n/a)
MS range:	40 to 400 *m/z*
Source Temp:	230° C
Solvent delay:	1-2 minutes