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

1. Scope

1.1. This practice covers the qualitative analysis of extracts using liquid chromatography-mass spectrometry (LC-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.

1.3. 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 standard 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 Standard Practice for Gunshot Residue Analysis by Scanning Electron Microscopy/Energy Dispersive X-ray Spectrometry.

E1732 Standard Terminology Relating to Forensic Science.

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

E2998 Standard Practice for Characterization and Classification of Smokeless Powders.

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

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

E3196 Standard 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-0002 Standard Practice for the Identification of Compounds Related to Organic Gunshot Residue (OGSR) by Gas Chromatography-Mass Spectrometry (GC-MS)

3. Terminology

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

3.2. Definitions of terms specific to this practice:

3.2.1. *Inorganic GSR (IGSR), n* – Gunshot residues from the primer, cartridge case, projectile (e.g., bullet or shot pellets), or the firearm that 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 the 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 s.

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. Liquid chromatograph (LC) - A liquid chromatograph (LC) that uses a reversed phase column and pump system providing a gradient of at least two solvents (refer to Appendix Table X1), coupled to a mass spectrometer with electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) ion source working in positive and negative ion mode.

5.2. The use of a guard column is not required but is recommended to protect the analytical column.

5.3. Mass Spectrometer (MS) - Mass analyzers with high resolution and mass accuracy are recommended. When unit resolution instruments such as triple quadrupoles are used, multiple reaction monitoring (MRM) techniques are recommended.).

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

5.5. 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, water, or other solvents compatible with LC-MS

6.1.1. Purity of Solvents – LC-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. Drying Gas – Nitrogen, air, or other inert gas of a purity 99.95% or higher.

6.5. Filters – Single use disposable filters of a hydrophobic membrane construction are recommended for us 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 lifts, swabs, and vacuum filters; refer to OSAC 2021-N-0009.

7.1.1. Alternative analyses can be performed on OGSR components that have been described elsewhere [OSAC 2021-N-0002] [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, acetonitrile, or a 20/80 ethanol:water mixture [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 [5,6].

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. Analyze the filtered or centrifuged extract directly or the extract can be concentrated, as required, for analysis.

7.2.4.1. Concentrate the extract by evaporating down to the required total injection volume using a gentle stream of nitrogen gas or another drying gas. If the extract is taken to complete dryness, some of the OGSR related compounds may be lost.

7.2.5. Store extracts at 0 ºC or colder to maximize preservation, when the sample is not being analyzed.

7.3. LC-MS analysis of OGSR extracts:

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

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

7.3.2.1. Validate the LC-MS parameters on the laboratory's instrument use in casework (Refer to E3255).

7.3.2.2. 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. Verify that the parameters are optimized to ensure that the mass spectral features of the reference standards are reproducible. When MRM techniques are used, validate the method for the precursor and product ions for each OGSR related compound in Table 1 [7, 8, 9] , Prior to analyzing samples, the LC-MS should be tuned and calibrated per validation protocols or the manufacturers 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 qualitative identification of the target compounds listed in Table 1.

7.3.4.2. Each specific retention time is measured by analyzing a suitable reference material (see section 6.2) using the LC-MS system and method under the same instrumental conditions [9].

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

Target Compound	**Ionization Source and Mode**	**Exact Mass (*m/z*)**	**Commonly Observed Ion**	**Ref.**
Akardite II (AK II)	ESI pos., APCI pos.	226.1106	$[M+H]^+$	[7,8]
2,4- and 2,6-dinitrotoluene (2,4/2,6-DNT)	ESI neg.	182.0327	$[M-H]^-$	[, 9]
Diphenylamine (DPA)	ESI pos., APCI pos.	169.0891	$[M+H]^+$	[7,8,9]
Ethyl centralite (EC)	ESI pos., APCI pos.	268.1575	$[M+H]^+$	[7, 8, 9]
Methyl centralite (MC)	ESI pos., APCI pos.	240.1262	$[M+H]^+$	[7, 9]

2 and 4-nitrodiphenylamine (2/4-NDPA)	ESI pos., APCI pos.	214.0742	[M+H]$^+$	[7, 9]
Nitroglycerin (NG)	ESI neg., APCI neg.	227.0024	[M+adduct]- dependent upon LC conditions	[9]
N-nitrosodiphenylamine (N-NODPA)	ESI pos., APCI pos.	198.0793	[M+H]$^+$	[8, 9]
Nitrotoluenes (NTs)	ESI neg.	137.0476	[M-H]-	[9]
2,4,6-trinitrotoluene (TNT)	ESI neg.	227.0177	[M-H]$^-$	[10]

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 samples 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 must meet identification criteria for liquid chromatography retention time and mass spectrometry. :

8.3.1. Liquid 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 compounds shall have the same base peak and the same molecular ion, if present.

8.3.2.2. The measured *m/z* value of the ions shall be within the instrument manufacturer's tolerance (mass accuracy) of the theoretical value of the target compound.

8.3.2.2.1. When MRM techniques are used, the precursor and product ions shall be the same for both the reference and unknown compounds.

8.3.2.2.2. When HRMS techniques are used, the exact mass shall be the same for both the reference and unknown compounds.

8.3.2.3. 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 chemicals 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 liner 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 7.2.4).

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 peak(s), the presence of new peaks. The laboratory can also determine an expiration date for the quality control sample with questioned extracts.

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. Documentation

10.1. Document the following, electronically or hard-copied:

10.1.1. LC-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; LC-MS.

12. **References**

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APPENDIX

(Non-mandatory Information)

X1. Instrumental Operation Parameters

X1.1 Suggested LC-MS Parameters

X1.1.1 Examples of LC-MS instrumental methods, from literature, to analyze OGSR compounds are provided below as a starting point for the laboratory validation process. These methods use a quadrupole-time of flight mass spectrometry (qTOFMS) [2,12] or triple-quadropole - linear iontrap hybrid mass detector (QTRAP) [3]; however, this method can be modified for any mass spectrometer system.

X1.1.2 It is the responsibility of the individual laboratory to validate the method parameters prior to analyzing case samples.

X1.1.3 Considering the individuality of each instrument, a gradient system and associated flow rate should be validated by the individual laboratory prior to analysis of case samples.

TABLE X1: Suggested LC-MS conditions from literature [2,3,7, 8, 9]

Parameter	Ref. [2]	Ref. [3]	Ref. [3]	Ref. [9]
LC column	C18 100 mm x 3 mm, 2.6 µm	C18 100 mm x 3 mm, 2.6 µm	C18 100 mm x 3 mm, 2.6 µm	Polar end-capped C18 150 mm x 1 mm; 3 µm
Column Temperature	40 ± 0.8 °C	40 °C	40 °C	35 °C
Elution Gradient				
Solvent A	Water + 1 mmol Ammonium acetate	Water + 0.1 % (v/v) formic acid	Water + 2.5 mmol ammonium acetate	Water
Solvent B	Methanol	Acetonitrile + 0.1 % (v/v) formic acid	Methanol + 2.5 mmol ammonium acetate	Methanol
Flow Rate (mL/min)	0.1 - 0.3	0.25	0.40	0.05 – 0.15
Injection Amount	5 µl	5 µl	2 µl	3 µl
MS Ionization	ESI	ESI	APCI	ESI

MS System	Agilent 6530 Accurate Mass QTOF with Jet Stream ionization source	AB Sciex QTRAP 6500 + Turbo V ESI ionization source	AB Sciex QTRAP 6500 + Turbo V APCI ionization source	Bruker compact QqTOF
MS Conditions according to reference	Dry Gas: 10 L/min, 300 °C Nebulizer pressure: 30 psi Sheath Gas: Nitrogen, 11 L/min, 300°C	Voltage: 5500 V Desolvation temperature: 500°C Curtain gas: 25 psi and a turbo gas of 50 psi	Source temperature: 137.5°C (NG), 425°C (DNT) Curtain gas: 30 psi (NG), 27.5 psi (DNT) Ion source gas: 36 psi (NG), 40 PSI (DNT)	End plate offset: 500 V Capillary voltage: 4000 V Nebulizer pressure: 2.5 bar Dry gas flow: 4 L/min Dry temperature: 200°C
Ionization Mode	positive and negative	positive	negative	positive and negative
MS range	50 - 1000	Not provided	Not provided	Not provided
Target compounds	AK II, 2,4-DNT, DPA, N-NODPA, 2-NDPA, 4-NDPA, EC, MC, TNT	AK II, DPA, N-NODPA, 2-NDPA, 4-NDPA, EC	NG, 2,4-DNT	AK II, 2,4-DNT, 2,6 DNT, DPA, N-NODPA, 2-NDPA, 4-NDPA, EC, NG