OSAC 2022-S-0003
Standard Practice for the Analysis of 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

Draft OSAC Proposed Standard

OSAC 2022-S-0003 Standard Practice for the Analysis of Organic Gunshot Residue (OGSR) by Liquid Chromatography-Mass Spectrometry (LC-MS)

Prepared by
Subcommittee for Ignitable Liquids, Explosive, and Gunshot Residue
Version: 1.0 - OSAC Open Comment
October 2021

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Standard Practice for the Analysis of Organic Gunshot Residue (OGSR) by Liquid Chromatography– Mass Spectrometry (GC-MS)

1. Scope

1.1. This practice covers the analysis of organic gunshot residue (OGSR) by liquid chromatography-mass spectrometry (LC-MS). This practice does not address the analysis of inorganic gunshot residue (IGSR) or primer gunshot residue (pGSR).

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

1.3. Units – The values stated in SI units are to be regarded as the standard, unless otherwise stated.

1.4. 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

WK56998 Standard Terminology Relating to the Examination of Explosives

WK72856 Standard Practice for the Collection and Preservation of Organic Gunshot Residue

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 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 smokeless powder and the priming mixture 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 exposed 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.

4.4. This practice does not cover the interpretation or significance of the OGSR results. Laboratory specific criteria should be established and supported by the data obtained during method development and validation (E3255).

4.5. Individual laboratory protocol will determine if IGSR/pGSR and OGSR will be analyzed from the same sample or separate samples [2,3].

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

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, with mass measurements to the 4^{th} decimal place, are recommended (e.g., time-of-flight, quadrupole-time-of-flight, orbitrap, etc.).

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 to achieve a minimum of 4000 RPM. Disposable centrifuge tubes that safely fit the centrifuge device are required.

6. Materials

6.1. Purity of Solvents – LC-MS grade or higher.

6.2. Analytical Solvents – Acetone, acetonitrile, ethanol, isopropanol, methanol, water, or other appropriate solvents.

6.3. 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 before use.

6.3.1. The concentrations of standards or reference materials used must be above the limit of detection for the instrument to be used in analyzing samples.

6.4. Concentrations of OGSR compounds recovered in forensic samples can be as low as 25 ppb [4].

6.5. 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.

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

6.7. 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 a 0.4 μm or smaller is recommended.

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 WK72856.

7.2. Additional analyses can be performed on OGSR components that have been described elsewhere [WK GC-MS] [5, 6].

7.3. Preparation of OGSR Samples:

7.3.1. Extract swabs, vacuum filters, or adhesive lifts using a suitable organic solvent, such as methanol, acetonitrile, or a 20/80 ethanol:water mixture [7].

7.3.1.1. The volume of the solvent used must be sufficient to extract the entire surface or area of the sample collection item used.

7.3.2. Extract the sample by placing the collection item inside a new disposable vial of a minimum volume required to hold the entire item. Swabs can be folded to fit into the vial. Add a minimum volume of solvent required to submerge the entire item.

7.3.3. Sonicate the vial with the item submerged in the solvent. Remove the extract and either filter or centrifuge the item to remove any solid particulates from the solution.

7.3.4. Analyze the filtered or centrifuged extract directly or the extract can be concentrated if required for analysis.

7.3.4.1. Concentrate the extract by evaporation down to the required volume using nitrogen gas or another dry gas.

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

7.4. LC-MS analysis of OGSR extracts:

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

7.4.2. The extract may be diluted, if required, to improve chromatography or mass spectral features.

7.4.3. The extract can be reconstituted using a suitable organic solvent to an appropriate volume if required for analysis (see section 6.2 [8]).

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

7.4.5. Validate this method on the laboratory's instrument before using the method in casework (E3255).

NOTE: Modify LC conditions to ensure that each peak of the reference test sample has baseline resolution. If a peak is not observed in the total ion chromatogram, then view the extraction ion chromatogram for the base peak ion of the targeted molecule.

7.4.6. Prior to analyzing samples, the LC-MS should be tuned and calibrated per validation protocols or the manufacturers recommendations.

7.4.7. Table 1 lists the common target compounds and their key ions used to identify the presence of OGSR.

7.4.7.1. Data analysis is limited to the qualitative identification of the target compounds listed in Table 1. Parameters used for identification can be observed retention time(s), ions detected, their ion masses, and associated fragmentation pattern of the molecule, if any.

7.4.7.2. Each specific retention time is measured by analyzing a suitable reference material using the LC-MS system and method under the same instrumental conditions [8].

7.4.7.3. At this time, there are no satisfactory studies completed to determine the common background presence of OGSR. Therefore, quantitative analysis of OGSR by LC-MS is not recommended.

7.4.7.3.1. It is the responsibility of each individual laboratory to verify any research study completed in their region concerning the background presence of OGSR [9, 10].

7.4.7.4. Interpretation of the presence or absence of target compounds in Table 1 is not covered in this standard practice (see Section 4.4).

TABLE 1: Typical OGSR related compounds and m/z of target ions found by LC-MS analysis

Target Compound	Ionization Source and Mode	Exact Mass (*m/z*)	Commonly Observed Ion	Ref.
Akardite II (AK II)	ESI pos., APCI pos.	226.1106	$[M+H]^+$	[11]
2,4- and 2,6-dinitrotoluene (2,4/2,6-DNT)	ESI neg.	182.0327	$[M-H]^-$	[5]
Diphenylamine (DPA)	ESI pos., APCI pos.	169.0891	$[M+H]^+$	[5]
Ethyl centralite (EC)	ESI pos., APCI pos.	268.1575	$[M+H]^+$	[5]
Methyl centralite (MC)	ESI pos., APCI pos.	240.1262	$[M+H]^+$	[5]

2 and 4-nitrodiphenylamine (2/4-NDPA)	ESI pos,. APCI pos.	214.0742	$[M+H]^+$	[5]
Nitroglycerin (NG)	ESI neg., APCI neg.	227.0024	[M+adduct]- dependent upon LC conditions	[12]
N-nitrosodiphenylamine (N-NODPA)	ESI pos., APCI pos.	198.0793	$[M+H]^+$	[11]
Nitrotoluenes (NTs)	ESI neg.	137.0476	[M-H]-	[13]
2,4,6-trinitrotoluene (TNT)	ESI neg.	227.0177	$[M-H]^-$	[2]

8. Identification of OGSR Compounds

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

8.2. The unknown samples should be run on the same instrument using the same method as the known reference standard.

8.3. Identification criteria is provided for the liquid chromatography and mass spectrometry:

8.3.1. Liquid Chromatography

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

8.3.2. Mass Spectrometry

8.3.2.1. The unknown and reference samples should have the same base peak and the same molecular ion, if present. The measured *m/z* value of the molecular ion should be within the instrument manufacturer's tolerance (mass accuracy) of the theoretical value of the target compound.

8.3.2.1.1. NOTE: If a collisional induced dissociation is included, then comparison of the *m/z* values and relative abundances of fragment ions between the questioned and reference samples can be used in the identification of a compound.

8.3.2.2. Isotopic ions present in the reference spectrum shall be present in similar proportions in the unknown sample spectrum; low abundance ions (less than 5% of the total spectral abundance) may be absent without precluding any identification.

8.3.2.3. Background subtraction may be necessary to remove any background contribution to the sample.

8.3.2.4. There shall be no unexplained extraneous ions that have a significant abundance.

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 should be analyzed, at minimum, at the beginning and the end of the analytical sequence on the instrument.

9.2.1.2. Quality control sample contains at least five compounds, as chosen by the individual laboratory, from the chemicals listed in Table 1.
NOTE: An example of a reference mixture is as follows: NG, EC, 2-NDPA, DPA and 2,4-DNT.

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.

9.2.2. Analyze a method blank with questioned extracts.

9.2.2.1. Prepare a method blank using the same items, procedure(s), reagents, and conditions for analysis as the questioned extracts.
NOTE: if using a filter to prepare questioned samples for analysis, then a negative control of one filter per manufacturer lot should be collected and analyzed prior to sample preparation to ensure that the filter is not contaminated.

9.2.2.2. Analyze a solvent wash blank between each sample.

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 peaks of interest 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 maintenance.

10.1.6. Maintain reports in accordance with laboratory policy and 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 analyzed 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 [2,3,12]

Parameter	Ref. [2]	Ref. [3]	Ref. [3]	Ref. [12]
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
Eluation 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

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