Analysis of Oleoresin Capsicum (OC) Sprays

1 Scope

This procedure allows for the analysis of items suspected of having been exposed to oleoresin capsicum (OC) sprays. Typical items for analysis include the contents of OC spray canisters, clothing, swabs, or any item which may have been in contact with an OC spray.

This procedure applies to Chemistry Unit (CU) personnel that are qualified and authorized to examine evidence for the presence of OC spray chemicals.

2 Equipment/Materials/Reagents

- Common laboratory glassware and equipment
- Analytical balance
- Ultraviolet (UV) light source
- CrimeScope CS-16 light source
- Evaporator
- Time-of-flight mass spectrometer with direct analysis in real time ionization source (DART/TOFMS)
- Gas chromatograph/mass spectrometer (GC/MS) equipped with an electron impact ionization source and a 30 meter DB-5 column (or equivalent)
- GC/MS equipped with a chemical ionization source and a 30 meter DB-5 column (or equivalent)
- Liquid chromatography system with C18 column (or equivalent) coupled to a mass spectrometer (LC/MS) with electrospray ionization (ESI) (e.g., Thermo LTQ, Thermo LTQ OrbiTrap XL, Thermo Exactive OrbiTrap)
- Acetone
- Capsaicin
- 2-Chloroacetophenone (CN tear gas)
- Deionized water
- Dihydrocapsaicin
- Formic acid
- Methanol
- Orthochlorobenzalmalononitrile (CS tear gas)
- Polyethylene glycol (PEG, 550 average molecular weight)

3 Standards and Controls

3.1 Negative Control

A Negative Control will be prepared by mirroring the process used to prepare a sample from a questioned item. For example, use the same volume of methanol from the same source and lot and within a similar container used to extract a questioned item(s). If swabs are submitted as evidence, a blank swab (preferably from the same source as the evidence swabs) extracted in the same manner as the questioned item(s) will be used as a Negative Control. In some instances, an extract from a portion of the item(s) which does not contain a stain of interest may be selected for use as a Negative Control. It is left to the discretion of the examiner as to what constitutes an adequate Negative Control.

3.2 Positive Controls

All Positive Controls will be verified at the time of use. The amounts of materials indicated in this section may be scaled up or down as necessary.

3.2.1 Capsaicin Positive Control (100 ug/mL)

A capsaicin stock solution (1 mg/mL) is prepared by dissolving 10 mg of capsaicin in 10 mL of methanol. The capsaicin stock solution is then diluted 1:10 with methanol to prepare the capsaicin positive control solution. Store the solutions in glass containers in a freezer.

3.2.2 Dihydrocapsaicin Positive Control (100 ug/mL)

A dihydrocapsaicin stock solution (1 mg/mL) is prepared by dissolving 10 mg of dihydrocapsaicin in 10 mL of methanol. The dihydrocapsaicin stock solution is then diluted 1:10 with methanol to prepare the dihydrocapsaicin positive control solution. Store the solutions in glass containers in a freezer.

3.2.3 Capsaicin/Dihydrocapsaicin Positive Control (100 ug/mL each)

Prepared by combining aliquots of the capsaicin and dihydrocapsaicin stock solutions and diluting 1:10 with methanol. For example, mix 1 mL of capsaicin stock solution, 1 mL of dihydrocapsaicin stock solution, and 8 mL of methanol. Store the solution in a glass container in a freezer.

3.2.4 Orthochlorobenzalmalononitrile (CS Tear Gas) Positive Control (50 ug/mL)

Prepared by dissolving 5 mg of CS tear gas in 100 mL of methanol or acetone. Store the solution in a glass container in a freezer.

Alternatively, the MAAQ/CS (50 ug/mL) Positive Control from *Analysis of Bank Security Device Chemicals* (GenChem 2) may be used as a CS tear gas Positive Control.

3.2.5 2-Chloroacetophenone (CN Tear Gas) Positive Control (50 ug/mL)

Prepared by dissolving 5 mg of 2-chloroacetophenone in 100 mL of methanol or acetone. Store the solution in a glass container in a freezer.

4 Sampling

Typically, one or more samples (e.g., cuttings) are selected from the stained area(s) of the questioned item. When multiple samples are selected from the same item, the samples are typically combined prior to extraction.

Multiple items that are packaged together (e.g., swabs) or otherwise in contact with each other will typically be sampled as one collective item. For example, if two swabs are packaged together, one swab (or a portion of the swab) will typically be sampled as representative of the swabs. However, multiple swabs may be sampled and extracted together if the staining appears to be minimal.

Statistical sampling is performed according to the General Chemistry *Sampling Guidelines for Bulk Materials and Multi-Unit Populations* (GenChem 21).

When non-statistical sampling is utilized on a heterogeneous item, the results of examinations will be clearly limited to the sample(s) that were selected and analyzed.

5 Procedure

a. Perform a thorough visual examination of the item for the presence of stains. Record the color and location of any observed stains. The nozzle of a submitted OC spray canister will be inspected for the presence of stains. Items with no readily visible stains will be analyzed using the CrimeScope and UV light sources. Any stains that are subsequently visualized will be documented by photography, if possible.

b. Cut a small section (~ 1 cm^2) from the stained area of the item and transfer the cutting to a labeled test tube. Multiple cuttings from the same item, or items that were packaged together, may be combined. Use an empty, labeled test tube as a Negative Control.

c. If cutting is not practical, sample the area with a swab wetted with methanol and transfer the swab to a labeled test tube. Prepare a Negative Control swab wetted with methanol and transfer it to an empty, labeled test tube.

d. Add enough methanol (typically 1 to 2 mL) to the cuttings and/or swabs to submerge the sample(s). Use the same volume of methanol for the Negative Control(s) and each of the sample(s). Vortex mix then extract the Negative Control(s) and sample(s) for ~5 minutes by rotation/inversion.

e. Transfer the extracts to new, labeled test tubes. Filter any extracts that contain particulates with 0.2 um PTFE syringe filters that have been pre-rinsed with methanol. Collect the filtrates in new, labeled test tubes.

f. Concentrate the extracts under N_2 (g) flow at ~60 ºC to a final volume of ~150 to 200 uL. Do not allow the extracts to go to dryness as CS or CN tear gas residues may be lost.

g. If an OC spray canister was submitted as a comparison sample, obtain a sample of the contents by briefly spraying the canister into a wide mouthed glass or plastic container while in a fume hood. Use an empty, labeled container from the same supply as a Negative Control. Transfer an aliquot of the OC spray contents to a labeled test tube and prepare a dilution of ~ 1% in methanol. Add the same volume of methanol that was used to dilute the OC spray to the Negative Control container and sample the interior, then transfer the methanol to a labeled test tube.

h. Analyze extracts by DART/TOFMS in the positive ionization mode by sampling the extracts with the closed end of a glass capillary. Analyze the Negative Control(s), Positive Control(s), and PEG within the same data collection file. Negative ionization mode may also be used as deemed necessary.

i. Analyze the extracts and Positive Control(s) by GC/MS using electron impact (EI) ionization mode. Incorporate methanol blanks between all samples. Splitless injection mode may be necessary for weakly concentrated solutions.

j. If interferences for capsaicin and/or dihydrocapsaicin were observed by GC/MS and could not be mitigated with extracted ion chromatograms, analyze the applicable extracts by LC/MS (ESI). Incorporate methanol blanks between all samples.

k. If CS tear gas is suspected, analyze the applicable extracts and Positive Control(s) by GC/MS using negative ion chemical ionization (NICI) mode. Incorporate methanol blanks between all samples. [Note- GC/MS (NICI) should be performed even if analysis by DART/TOFMS and/or GC/MS (EI) was negative for CS tear gas since GC/MS (NICI) is more sensitive for CS tear gas.]

l. If CN tear gas is suspected, analyze the applicable extracts and Positive Control(s) by GC/MS using positive ion chemical ionization (PICI) mode. Incorporate methanol blanks between all samples. [Note- GC/MS (PICI) should be performed even if analysis by DART/TOFMS and/or GC/MS (EI) was negative for CN tear gas since GC/MS (PICI) is more sensitive for CN tear gas.]

6 Calculations

Not applicable

7 Measurement Uncertainty

Not applicable

8 Instrumental Conditions

Refer to *General Chemistry Instrument Parameters* (GenChem 34) for specific instrument settings and decision criteria that are not provided below.

The following instrumental conditions are not intended to be prescriptive nor exhaustive. Minor modifications to the conditions may be used as needed and without authorization, provided the same conditions are used for all applicable solvent blanks, control samples, and questioned items; and the Positive Control(s) provide acceptable data. The utilized conditions will be recorded and retained with the case notes.

8.1 Liquid Chromatography/Mass Spectrometry (LC/MS)

8.1.1 Liquid Chromatography Parameters

Mobile Phase Compositions		**Flow Parameters**			**Column Parameters**	
A: 0.05% v/v formic acid (aq)		total flow = 0.35 mL/min			type	C18
		time (min)	% A	% B	length	150 mm
B: Methanol		0	40	60	internal diameter	2.1 mm
		0.5	40	60	particle size	5 um
		5.5	10	90	temperature	30 ºC
Autosampler		6.5	10	90		
temperature	15 ºC	6.6	40	60		
injection volume	10 uL	total run time = 11 min.				

8.1.2 Mass Spectrometer Parameters

Duration = 9.00 min; Source parameters are set through the tune file and should be optimized on each instrument. Retain a copy of the tune parameters with the case notes.	
Scan Event #1	
Ionization mode	ESI (+)
Scan mode	Full scan MS
Scan range	150-550 *m/z*
Scan Event #2	
Ionization mode	ESI (+)
Scan mode	Product ion MS/MS
Precursor ion	306.7
Collision energy	11 %
Product scan range	80-340 *m/z*
Q	0.250
Time	30.000
IsoW	2.0
Scan Event #3	
Ionization mode	ESI (+)
Scan mode	Product ion MS/MS
Precursor ion	308.7
Collision energy	10 %
Product scan range	80-340 *m/z*
Q	0.250
Time	30.000
IsoW	2.0

9 Limitations

This procedure is primarily used for items that have visible stains (or stains that can be visualized using alternate light sources). The absence of visible stains on items may prevent the identification of capsaicinoids. Certain matrices may minimize the absorption of OC sprays upon exposure (e.g., Gore-Tex, nylon) or may interfere with the extraction and/or identification of OC sprays (e.g., plastics, heavily-stained items). The following conclusions apply to the analysis of OC sprays and/or comparisons involving OC sprays:

- Identification (i.e. identified)
- Consistent with

- Not identified
- Cannot be differentiated
- Excluded
- Inconclusive

Refer to *Chemistry Unit (CU) FBI Approved Standards for Scientific Testimony and Report Language for General Chemistry* (GenChem 32, ASSTR), *General Approach to Report Writing in General Chemistry* (GenChem 27), and *Department of Justice Uniform Language for Testimony and Reports for General Forensic Chemistry and Seized Drug Examinations* (GenChem ULTR) for examples of reporting examination conclusions and the associated limitations and decision criteria.

Refer to *General Chemistry Instrument Parameters* (GenChem 34) for instrumental limitations and decision criteria.

Refer to *General Chemistry Guidelines for Comparison of Mass Spectra* (GenChem 33) for mass spectra comparison decision criteria.

10 Safety

- Take standard precautions for the handling of all chemicals, reagents, and standards. Some of the chemicals may be carcinogenic. Refer to the *FBI Laboratory Safety Manual* for the proper handling and disposal of all chemicals. Personal protective equipment should be used when handling any chemical and when performing any type of analysis.
- OC, CS tear gas, and CN tear gas are lachrymators and should be handled carefully.

11 References

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Lewis K, Lewis RJ. An assessment of four solvents for the recovery of 2-chlorobenzylidene-malononitrile and capsaicins from "CS" and "pepper" type lachrymator sprays, and an examination of their persistence on cotton fabric. *J Forensic Sci* 2001; 46(2): 352-355.

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Sampling Guidelines for Bulk Materials and Multi-Unit Populations; FBI Laboratory Chemistry Unit – General Chemistry SOP (GenChem 21)

General Chemistry Instrument Parameters; FBI Laboratory Chemistry Unit – General Chemistry SOP (GenChem 34)

Guidelines for Comparison of Mass Spectra; FBI Laboratory Chemistry Unit – General Chemistry SOP (GenChem 33)

Chemistry Unit (CU) FBI Approved Standards for Scientific Testimony and Report Language for General Chemistry – General Chemistry SOP (GenChem 32)

General Approach to Report Writing in General Chemistry; FBI Laboratory Chemistry Unit – General Chemistry SOP (GenChem 27)

Department of Justice Uniform Language for Testimony and Reports for General Forensic Chemistry and Seized Drug Examinations (GenChem ULTR)

FBI Laboratory Safety Manual

Rev. #	Issue Date	History
3	05/04/20	Removed previous sections 1 (Introduction), 3 (Principle), 6 (Calibration), and 10 (Decision Criteria); added section 6 (Calculations); renumbered sections accordingly. Edited new section 1 for clarity and to include personnel; defined "CU". Changed lettered listing in new section 2 to bullets and revised the list. Edited new sections 3.1 and 3.2 to add detail; changed formatting; added CN tear gas and reference to MAAQ/CS positive control. Added content to section 4 (Sampling). Edited content of section 5 for clarity and added DART/TOFMS, GC/MS (PICI), and LC/MS (ESI) as instrumental techniques. Changed new section 7 title from 'Uncertainty of Measurement'. Section 8 edited to refer to GenChem 34 and added LC/MS (ESI) parameters. Section 9 edited to include conclusion statements and references to ASSTR, ULTR, etc. Updated references in section 11.
4	04/01/21	Section 1- added "and authorized". Section 2- added "coupled to a mass spectrometer (LC/MS)" and added acetone. Section 4, last sentence- added "on a heterogeneous item". Section 5- added line spacing for ease of reading (did not add change indicators for the spacing so changed content could be discerned). Section 5, steps (f), (h), (i), (j), (k), (l)- replaced "Negative Control(s) and extract(s) solutions" (and similar) with "extracts" for simplicity [as it is already clear that the "extracts" includes the Negative Control(s)]; steps (i), (k), (l)- added "Positive Control(s)". Sections 8 and 11-corrected GenChem 34 title to "Instrument". Section 9- added last sentence. Section 10- changed to bulleted format and added last bullet.

Approval

Redacted - Signatures on File

Chemistry Unit Chief: Date: 03/31/2021

General Chemistry
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