

Technical Procedure for Solid Phase Extraction of THC and THC-COOH for GC-MS Analysis

1.0 Purpose - This procedure specifies the required elements for the extraction of THC and THC-COOH using United Technologies Styre Screen Extraction Columns® for GC-MS Analysis.

2.0 Scope – This procedure applies to THC and THC-COOH extractions performed in the Toxicology Units of the State Crime Laboratory.

3.0 Definitions

- **Quality control (QC) check** – Periodic confirmation of the reliability of equipment, instrumentation, and/or reagents.

4.0 Equipment, Materials and Reagents

4.1 Equipment

- Centrifuge
- Mechanical Pipettes
- Class A Volumetric flasks
- Pressure manifold equipped with nitrogen
- Zymark TurboVap LV

4.2 Materials

- Test tubes (16 x 125, 13 x 100, 12 x 75)
- Test tube caps or stoppers
- Vortexer
- Pipette tips

4.3 Reagents

- Deionized water

4.4 Commercial Reagents

- Hexane, ACS grade
- Ethyl acetate, ACS grade
- Acetonitrile, ACS and HPLC grade
- Concentrated ammonium hydroxide, ACS grade
- Glacial acetic acid, ACS grade
- UCT Styre Screen® SSTHC Solid Phase Extraction Columns
- Nitrogen

4.5 Primary Reference Standards

- Delta-9-tetrahydrocannabinol (THC)
- 11-nor-delta-9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH)
- Delta-9-tetrahydrocannabinol (THC)-D₃
- 11-nor-delta-9-tetrahydrocannabinol-9-carboxylic acid(THC-COOH)-D₃

4.6 Critical Reagents

- Negative Blood
- BSTFA with 1 % TMCS (N,O-bis(trimethylsilyl)trifluoroacetamide with 1 % trimethylchlorosilane)

4.7 Prepared Reagents - Prepared reagents may be prepared by the Forensic Scientist in any amount provided that the component ratios are kept constant.

4.7.1 Cannabinoid Internal Standard Solution

4.7.1.1 Prepare a solution containing 1.0 µg/mL each of THC-D₃ primary reference standard and THC-COOH-D₃ primary reference standard in methanol.

4.7.1.1.1 Example: Add 1.0 mL of 100 µg/mL THC-D₃ and 1.0 mL of 100 µg/mL THC-COOH-D₃ to a 100 mL volumetric flask. Dilute to volume with methanol.

4.7.1.2 Lot number: Eight digit format year/month/day

4.7.1.2.1 Example: 20101231

4.7.1.3 Expiration: One year.

4.7.1.4 Refrigerate.

4.7.1.5 QC check: Successful calibration (see 5.1).

4.7.2 Cannabinoid Calibration Solution

4.7.2.1 Prepare a solution containing 1.0 µg/mL each of THC primary reference standard and THC-COOH primary reference standard in methanol.

4.7.2.1.1 Example: Add 0.100 mL of a 1.0 mg/mL THC primary reference standard and 1.0 mL of 100 µg/mL THC-COOH to a 100 mL volumetric flask. Dilute to volume with methanol.

4.7.2.2 Lot number: Eight digit format year/month/day

4.7.2.2.1 Example: 20101231

4.7.2.3 Expiration: One year.

4.7.2.4 Refrigerate.

4.7.2.5 QC check: Successful calibration (see 5.1).

4.7.3 Cannabinoid Verification Solution

4.7.3.1 Prepare a solution containing 500 ng/mL each of THC primary reference standard and THC-COOH primary reference standard in methanol.

4.7.3.1.1 Example: Add 50 µL of a 1.0 mg/mL THC primary reference standard and 500 µL of 100 µg/mL THC-COOH to a 100 mL volumetric flask. Dilute to volume with methanol.

4.7.3.2 Lot number: Eight digit format year/month/day

4.7.3.2.1 Example: 20101231

4.7.3.3 Expiration: One year.

4.7.3.4 Refrigerate.

4.7.3.5 QC check: Successful calibration (see 5.1).

5.0 Procedure

5.1 Calibrations

5.1.1 The Toxicology GC-MS Key Operator or designee shall calibrate the GC-MS CANSIM data analysis method upon preparation of a new lot of Cannabinoid Internal Standard Solution and after instrument maintenance that may affect the calibration as determined by the Toxicology GC-MS Key Operator.

5.1.2 Calibration Standards

5.1.2.1 Negative Cannabinoid Calibration Standard

5.1.2.1.1 In duplicate, prepare 1.0 mL of negative blood.

5.1.2.1.2 Prepare each duplicate as directed in 5.5.

5.1.2.2 10 ng/mL Cannabinoid Calibration Standard

5.1.2.2.1 In duplicate, add 10 µL of the Cannabinoid Calibration Solution to 1.0 mL of negative blood.

5.1.2.2.2 Prepare each duplicate as directed in 5.5.

5.1.2.3 15 ng/mL Cannabinoid Calibration Standard

5.1.2.3.1 In duplicate, add 15 µL of the Cannabinoid Calibration Solution to 1.0 mL of negative blood.

5.1.2.3.2 Prepare each duplicate as directed in 5.5.

5.1.2.4 20 ng/mL Cannabinoid Calibration Standard

5.1.2.4.1 In duplicate, add 20 µL of the Cannabinoid Calibration Solution to 1.0 mL of negative blood.

5.1.2.4.2 Prepare each duplicate as directed in 5.5.

5.1.2.5 25 ng/mL Cannabinoid Calibration Standard

5.1.2.5.1 In duplicate, add 25 µL of the Cannabinoid Calibration Solution to 1.0 mL of negative blood.

5.1.2.5.2 Prepare each duplicate as directed in 5.5.

5.1.2.6 50 ng/mL Cannabinoid Calibration Standard

5.1.2.6.1 In duplicate, add 50 µL of the Cannabinoid Calibration Solution to 1.0 mL of negative blood.

5.1.2.6.2 Prepare each duplicate as directed in 5.5.

5.1.2.7 100 ng/mL Cannabinoid Calibration Standard

5.1.2.7.1 In duplicate, add 100 µL of the Cannabinoid Calibration Solution to 1.0 mL of negative blood.

5.1.2.7.2 Prepare each duplicate as directed in 5.5.

5.1.3 Verification Standards

5.1.3.2 Negative Cannabinoid Verifier

5.1.3.2.1 Use 1.0 mL of negative blood.

5.1.3.2.2 Prepare as directed in 5.5.

5.1.3.3 10 ng/mL Cannabinoid Verifier

5.1.3.3.1 Add 20 µL of the Cannabinoid Verification Solution to 1.0 mL of negative blood.

5.1.3.3.2 Prepare as directed in 5.5.

5.1.3.4 25 ng/mL Cannabinoid Verifier

5.1.3.4.1 Add 50 µL of the Cannabinoid Verification Solution to 1.0 mL of negative blood.

5.1.3.4.2 Prepare as directed in 5.5.

5.1.3.5 75 ng/mL Cannabinoid Verifier

5.1.3.5.1 Add 150 µL of the Cannabinoid Verification Solution to 1.0 mL of negative blood.

5.1.3.5.2 Prepare as directed in 5.5.

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- 5.1.4** Chromatograph and quantitate the calibration standards on the GC-MS using the CANSIM GC-MS method as specified in the [Toxicology Gas Chromatography/Mass Spectrometry \(GC-MS\) procedure](#).
- 5.1.5** Update the calibration with the calibration standards.
- 5.1.5.1** The response at each level shall be the response of the calibration standard at that level.
- 5.1.5.2** The retention time for each component shall be the average retention time of the 50 ng/mL calibration standard duplicates.
- 5.1.5.3** The ion ratios for each component shall be the average ion ratios of the 50 ng/mL calibration standard duplicates.
- 5.1.6** The calibration curve shall be fitted to a linear model.
- 5.1.7** The calibration curves for each component must show a correlation of determination (r^2) of 0.985 or greater. If the calibration has a correlation of determination of less than 0.985, appropriate action (e.g., maintenance or new solution preparation) shall be taken and the calibration repeated.
- 5.1.8** The retention times of THC, THC-COOH, and the internal standards shall not differ by more than 2.0 % from the target value established in **5.1.5.2**.
- 5.1.9** The qualifier ion ratios of THC, THC-COOH, and the internal standards shall be within +/- 20 % of the target value established in **5.1.5.3**.
- 5.1.10** After each successful calibration, save the data analysis method according to the format "CANSIM" eight digit format year/month/day.
- 5.1.11 Calibration Verification**
- 5.1.11.1** Chromatograph the cannabinoid verification standards on the GC-MS using the CANSIM GC-MS method as specified in the [Toxicology Gas Chromatography/Mass Spectrometry \(GC-MS\) procedure](#) and quantitate with the data analysis method from **5.1.10**.
- 5.1.11.2** The verification standards shall meet the criteria below. If the standards do not meet the criteria below, appropriate action (e.g., maintenance or new solution preparation) shall be taken and the calibration and/or calibration verification shall be repeated.
- 5.1.11.2.1** THC and THC-COOH shall not be identified in the negative verifier.
- 5.1.11.2.2** The retention times of THC, THC-COOH and the internal standards shall not differ by more than 2.0 % from the target value.
- 5.1.11.2.3** The qualifier ion ratios of THC, THC-COOH and the internal standards shall be within +/- 20 % of the target value.

5.1.11.2.4 The quantitation results of each component shall be within +/- 20 % of the target value.

5.1.12 The calibration data shall be reviewed by another qualified Forensic Scientist and, if acceptable, approved in the Toxicology Unit section object repository in FA with the name of the data analysis method from **5.1.10**.

5.1.13 The calibration data packet shall include the following:

- Lot number of the cannabinoid calibration solution
- Lot number of the cannabinoid verification solution
- Lot number and expiration date of the cannabinoid internal standard
- Lot number of negative blood
- GC-MS sequence table
- GC-MS tune
- GC-MS method
- Quantitation reports for each calibration and verification standard
- Printed calibration table
- Each component r^2 value and the equation describing each calibration curve

5.2 Maintenance

5.2.1 Ensure that the pressure manifold is clean prior to use and clean after use.

5.2.2 Add water to the TurboVap if needed.

5.3 Sampling

5.3.1 Allow all solutions and samples to be analyzed to equilibrate to room temperature.

5.3.2 Ensure that all body fluids are homogenous by shaking and/or vortexing.

5.3.2.1 If a homogenous sample cannot be obtained, a notation will be made in the worksheet detailing the condition of the sample and its handling.

5.4 Standards and Controls

5.4.1 Each extraction batch must contain at minimum two positive and two negative controls. One positive and one negative control will be placed at the beginning and end of the analytical run. If necessary, add additional positive and/or negative controls so that the batch contains at least 10 % controls.

5.4.1.1 The positive and negative controls shall meet the criteria below for each extraction batch. If the positive and negative controls do not meet the criteria below, appropriate action (i.e., maintenance or new solution preparation) shall be taken and the batch shall be extracted again in accordance with the State Crime Laboratory [Procedure for Corrective Action and Non-Conformities](#). Note any problems in the GC-MS instrument log.

5.4.2 Positive controls

5.4.2.1 For each component to be identified, the corresponding internal standard (THC-d3 for THC and THCOOH-d3 for THCOOH) qualifier ion ratios must be within +/- 20 % of the target values.

5.4.2.2 For each extraction batch prepare two positive controls as directed in 5.1.3 with a mixture of 1.0 mL of negative blood and 50 and 150 µL of Cannabinoid verification solution respectively.

5.4.2.2.1 The positive control components to be quantitated must be within +/- 20 % of the target concentration.

5.4.2.2.2 The qualifier ion ratios of the positive control duplicate that was not used to set the target values must be within +/- 20 % of the target values (see 5.6.3).

5.4.3 Negative Control

5.4.3.1 Prepare a negative control for each extraction batch as directed in 5.5 with 1.0 mL of negative blood.

5.4.3.2 The corresponding internal standard (THC-d3 for THC and THCOOH-d3 for THCOOH) qualifier ion ratios must be within +/- 20 % of the target values for each component to be identified.

5.4.3.3 THC and THC-COOH must not be identified in the negative control. If either analyte is identified in the negative control, the extraction shall be repeated.

5.5 Extraction Procedure

5.5.1 To 1.0 mL of the blood to be analyzed add 100 µL of Cannabinoid internal standard solution.

5.5.2 Add 2.0 mL of cold acetonitrile (stored in freezer), slowly with mixing.

5.5.3 Mix and let stand for 5 minutes.

5.5.4 Mix and centrifuge for 10 minutes.

5.5.5 Decant liquid portion of the sample into a clean test tube and add 2.0 mL of deionized water.

5.5.6 Add sample to UCT Styre Screen® SSTHC solid phase extraction column with a flow rate of 5 mL/minute or less.

5.5.7 Mix water, acetonitrile and concentrated ammonium hydroxide in a ratio of 84:15:1. Add 1.0 mL of this mixture to the column with a flow rate between 1 and 15 mL per minute. Dispose of any unused portion of this mixture according to the State Crime Laboratory Safety Manual.

5.5.8 Dry the column with a nitrogen flow for 15 minutes or longer until the column is dry.

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- 5.5.9** Mix hexane, ethyl acetate and acetic acid in a ratio of 49:49:2. Mix well and collect the cannabinoids with 3.0 mL of this mixture with a flow rate of 5 mL per minute or less. Dispose of any unused portion of this mixture according to the State Crime Laboratory Safety Manual
- 5.5.10** Evaporate to dryness using a TurboVap.
- 5.5.11** Add 50 µL of BSTFA with 1 % TMCS and cap securely.
- 5.5.12** Mix and heat at 80 °C for 30 minutes.
- 5.5.13** Cool to room temperature.
- 5.5.14** Transfer to an insert in an auto-sampler vial and cap securely.

5.6 Post Extraction Procedure

- 5.6.1** Chromatograph on the GC-MS utilizing the CANSIM GC-MS method as specified in the [Toxicology Gas Chromatography/Mass Spectrometry \(GC-MS\) procedure](#).
- 5.6.2** Save the most current calibrated CANSIM GC-MS method with the initials of the Scientist and the date added to the end of the method name. The method shall correspond to the lot number of Cannabinoid Internal Standard Solution used to prepare the samples.
- 5.6.3** Use a positive control to replace the qualifier ion ratios and retention times of each component. The updated values are the target values.
- 5.6.4** Save the updated method.
- 5.6.5** Quantitate the remaining extracts, including the remaining positive control, using the updated CANSIM GC-MS data analysis method from **5.6.4**.
- 5.6.6** The Quality Control data packet shall be reviewed by a Forensic Scientist qualified to perform the [Technical Procedure for Solid Phase Extraction of THC and THC-COOH for GCMS Analysis](#) and, if acceptable, approved in the Toxicology Unit section object repository of FA with a file name beginning with “THCQC” (capitalization optional), followed by eight digit format year/month/day.
- 5.6.6.1** Example: THCQC20121004
- 5.6.7** The quality control data packet shall include the following:
- Lot number and expiration date of the cannabinoid internal standard solution
 - Lot number of the cannabinoid verification solution
 - Lot number of negative blood
 - Lot number of BSTFA with 1% TMCS
 - Lot number of UCT Styre Screen[®] SSTHC solid phase extraction column used
 - Completed extraction worksheet
 - GC-MS sequence list
 - GC-MS tune

- Updated CANSIM GC-MS method and calibration curves printed at the time of data analysis
- Quantitation reports of the positive control and negative controls

5.6.8 Control Charting

- 5.6.8.1** Complete the Toxicology Control Chart Form and submit to the Toxicology Technical Leader.

5.7 Identification of THC and/or THC-COOH

- 5.7.1** The internal standard area of the quantifier ion shall be greater than 50 % of the average of the area of the quantifier ion of the controls.

- 5.7.1.1** If the internal standard area is less than 50 %, the quantitation shall not be used. The case will be reanalyzed, sample volume permitting.

- 5.7.1.2** If there is insufficient sample volume remaining, the data may be reported qualitatively only if the acceptance criteria in **5.7.2**, **5.7.3**, and **5.7.4** are met.

- 5.7.2** The qualifier ion ratios of each component to be identified must be within +/- 20 % of the target value.

- 5.7.3** The quantifier ion retention time of each component to be identified must not differ from the target value by more than 2.0 %.

- 5.7.4** The quantitation result must be 10 ng/mL or greater to be identified.

5.8 Reporting

- 5.8.1** If the positive control fails for an analyte, the samples may be reported negative as long as the analyte is not present in the case sample.

- 5.8.1.1** If the analyte that failed in the positive control is present, the sample shall be re-extracted.

- 5.8.2** THC and THC-COOH identified by GC-MS analysis must also have a positive indication for Cannabinoids from an Immunoassay Drug Screen analysis to be reported. Refer to the Drug Chemistry Section Toxicology Unit [Toxicology Analysis](#) procedure for reporting of THC or THC-COOH.

5.9 Record the following in the case record:

- Approved CANSIM GC-MS method calibration data packet
- Approved THCQC data packet for the run
- Quantitation report of the sample

5.10 Calculations

- 5.10.1** Percent Difference Calculation:
$$|(\text{standard retention time} - \text{analyte retention time})| / (\text{standard retention time}) * 100$$

5.11 Uncertainty of Measurement – Currently being established.

6.0 Limitations

6.1 Store solid phase extraction columns in a closed container and protect from moisture.

6.2 Samples with THC or THC-COOH concentrations that exceed the upper level of the calibration curve may be reanalyzed after dilution with the proper matrix to bring them within the calibration range, or be recorded as “quantitation exceeded the 100 ng/ml upper limit of calibration.”

7.0 Safety

7.1 Refer to the [Laboratory Safety Manual](#).

7.2 BSTFA with 1 % TMCS shall be handled in a fume hood, with gloves and eye protection.

8.0 References

UCT Solid Phase Extraction Manual. United Chemical Technologies Inc. Bristol, PA., (2010): 39 - 40.

D’Asaro, John A. “An Automated and Simultaneous Solid-Phase Extraction of Δ^9 -Tetrahydrocannabinol and 11-Nor-9-Carboxy- Δ^9 -tetrahydrocannabinol from Whole Blood Using the Zymark Rapid Trace with confirmation and Quantitation GC-EL-MS.” *Journal of Analytical Toxicology*. 24: (2000), 289 – 295.

9.0 Records

- Calibration data
- Case record
- Toxicology Control Chart Form

10.0 Attachments- N/A

Revision History		
Effective Date	Version Number	Reason
09/17/2012	1	J-17 Conversion to ISO format
10/26/2012	2	5.1.4, 5.1.11.1, 5.6.1 - modified to include reference to GC-MS procedure; Correct numbering under 5.1.11 and subsequent lines; 5.1.13 - included additional requirements for Calibration data packet; 5.4.2.2 - corrected volumes used to create controls; 5.4.3.3 - inserted response to failed negative control; 5.4.4 - Removed control charting and moved to post extraction procedure (5.6); 5.5.4 - removed speed requirement; 5.5.10 - removed temperature portion of instruction added "using a TurboVap"; 5.6.3 and 5.6.5 - removed "duplicate"; 5.6.6 and 5.6.7 - created QC data packet requirements and review; 5.7.2 - removed each duplicate; 5.7.3 - clarified retention time; 5.7.4 - added criteria; 5.8 - added requirements for failed positive controls; 5.9 - updated calibration data packet language and removed information now contained within QC data packet, added QC data packet; 5.10.1 - removed rounding requirement; 6.2 - added reporting statement; 9.0 - added reference to new Toxicology Control Chart form
02/15/2013	3	2.0 - modified for procedure merge 5.1.14 and 5.4.2.2.3 - removed, redundant requirement-see 5.6.8.1 5.1.13 - updated to include GC-MS method
05/10/2013	4	4.7.1.2, 4.7.1.2.1, 4.7.2.2, 4.7.2.2.1, 4.7.3.2, and 4.7.3.2.1 - changed format of lot # and corresponding example 5.6.7 - inserted extraction worksheet into data pack requirements 5.11 - changed "N/A" to "Currently being established."