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Identification and quantification of volatile organic compounds (VOCs) in vegetation leaves using headspace solid phase microextraction (HS-SPME) and gas chromatography–mass spectrometry (GC–MS)

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Nicolas Younes[1,2], Adam Carroll[3], Sophia L. Cain[1]

[1]Fenner School of Environment and Society, College of Science, The Australian National University, ACT, Australia.;
[2]ANU Bushfire Research Centre of Excellence, The Australian National University, ACT, Australia.;
[3]Join Mass Spectrometry Facility, College of Science, The Australian National University, ACT, Australia.

Nicolas Younes
Australian National University

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Abstract

Volatile organic compounds (VOC) are flammable compounds that are stored in the leaves and other organs of eucalypt trees. To assess the flammability and risk posed by eucalypts, it is important to detect the presence, and estimate the concentration, of VOCs. We escribe a SPME-GC-MS technique to identify VOCs from eucalypt leaf samples.

Guidelines

5 Laboratory Procedure

5.1 Preparation of standards – to be done in the laboratory
• Using methanol as diluent, prepare dilutions of all four standards individually (β-pinene, Eucalyptol, Cannabis Terpene Mix A, and Cannabis Terpene Mix B), at concentrations of 25, 50, 100, 250, 500 and 1000 ug/mL in individual 20 mL headspace vials.
• For each concentration of each standard, aliquot 10 µL to a new 20 mL labelled headspace vial.
• Store aliquotes and standards at -20°C until needed.
• Analyse standards and samples for terpenes using the standards as reference peaks for identification of terpenes in eucalyptus leaves.

5.2 Gas Chromatography–Mass Spectrometry (GC–MS) analysis
• Take samples out of the freezer and allow them to reach room temperature (approximately 10 minutes). Only select the samples to be processed on the day.
• Prepare/program the instrument operating conditions and method parameters as shown in Table 1.
• In the laboratory, seal at least four empty 20 mL headspace vials. These vials will be used as a “blank samples” for quality control purposes.
• Load standards and samples to the sampling rack in the following way:
  - 2 x “blank samples”
  - Standards, from lowest to highest concentration.
  - Randomly-selected samples, including field control samples. Random sampling helps to minimize the risk of inadvertently introducing systematic errors from the operator, or changes in the GC/MS instruments.
  - 2 x blank samples
  - Standards, from lowest to highest concentration.
• Total cycle time for one sample is approximately 86 min

Table 1: SPME GCMS method parameters

Head Space
Operation mode: Static headspace
Sample: 5 leaf discs, or small leaf, or branch section in 20 mL labelled headspace vial
Equilibration: 20 min at 80°C
SPME fibre: 20 min adsorption

GC parameters (Agilent 7890A)
Injection: Split injection, 200:1 split ratio
Carrier gas: Helium, 1mL/min, constant flow
Oven program: 40°C, hold for 10 min, 15°C/min to 200°C, 50°C/min to 325°C, hold for 3 min, Total GC run time 26 min, Cooldown time 10 min
Sterilize SPME fibre: 10 min

6 Metabolite identification and analysis

Specific steps will vary depending on the software used, but the overall process is summarised below, and more details can be found in (Agilent Technologies, 2010):

• Mass Hunter Quantitative Analysis version 10.0 can be used used to process all samples.
• All samples, standards and blanks are added to a quantitative analysis batch file.
• Create a new quantitative method was developed using the 'new method with acquired scan data' option; this method scans a data file for compound peaks and then uses the base peak (most abundant ion in the mass spectrum) as the ‘quant’ ion to run the quantification.
• Set number additional number of 'qualifier' ions; these additional ions help to confirm that the compound is correctly identified in samples (we commonly set this number to 4). Importantly, only the area of the ‘quant’ ion is used for quantification.
• Select a concentration (e.g. 500 µg/µL) for all standards (i.e. β-Pinene, Eucalyptol, Cannabis Terpene Mix A and Cannabis Terpene Mix B) and use this concentration for detecting the compounds in the GCMS data file; afterwards, manually screen the output for errors, detected products for which there are no know concentrations to ensure that only peaks associated with known compounds from the standards are included in the quantification method.
• To minimise the chance of other compounds being mistakenly quantified as one of the target compounds, and to reduce processing times, it is recommended to set a retention time window of approximately 0.1 minutes on the left hand side of the peak, and approximately 0.2 on the right hand side of the peak.
• The method to detect compounds will vary depending on the target vegetation species and the target compound. The ‘find by deconvolution’ method is commonly used.
• The calibration curves for each compound need to be added to the method file and each compound has to be linked to its corresponding calibration standard, so that the calibration curves are calculated by the software.
• Run the newly created method on the samples and export the outputs as needed.

Materials

- Leaf samples Sample
- Hole punch
- Markers and/or pens
- Self-adhesive labels for vials
- 20 mL amber headspace vials (e.g. Agilent Product Number 5188-6537) with septum-type caps.
- Gas Chromatograph system (GC): Agilent 7890A (or similar)
- Mass Spectrometer (MS), Agilent 5975C (or similar)
- GC Column: Agilent VF-5ms column, 30 m + 10 m EZ-Guard, 0.25 mm internal diameter and 0.25 µm film thickness,
- Carrier gas: Helium (>99.00% purity)
- Agilent Mass Hunter Quantitative Analysis software (version 10.0) or similar.
- Gerstel heating, shaking block, and Autosampler (e.g. Gerstel MPS, or similar)
- Supelco β-Pinene standard Product Number CRM40433 (Contains CAS-No: 67-56-1 and 127-91-3)
- Supelco Eucalyptol standard Product Number CRM40684 (Contains CAS-No: 67-56-1 and 470-82-6)
- Supelco Cannabis Terpene Mix B Product Number CRM40937 (Contains CAS-No: 67-56-1, 99-87-6, 87-44-5, 106-24-1, 138-86-3, 18172-67-3, 498-15-7, 78-70-6)
- Supelco Cannabis Terpene Mix A Product Number CRM40755 (Contains CAS-No: 67-56-1, 5989-27-5, 469-61-4, 106-22-9, 79-92-5, 89-82-7, 99-85-4, 99-86-5, 105-87-3, 13466-78-9, 7212-44-4, 80-56-8, 127-91-3)
- -20°C freezer to store samples and standards.
- Methanol ≥99.9%, suitable for HPLC analysis.
- Examination gloves (latex or other material).
- Standard computer capable of running the software required.

Troubleshooting

Sample collection

1 Remove the cap of a 20 mL headspace vial.

2 Using the hole punch, collect five punches from a leaf.

2.1 Ensure all hole punches are the same size.

2.2 Whenever possible, hole punches should be collected on either side of the main leaf vein, and along the longest axis of the leaf.

2.3 In case the leaves are too small for hole punches, a complete mature leaf can be weighed and put into the vial. Ensure 1) you weigh the leaf, 2) measure the leaf area, 3) the leaf is pushed to the bottom of the vial to prevent direct contact with the SPME fibre.

3 If you are collecting samples from spiny species such as *Ulex europaeus*, collect the distal third portion of a branchlet. Ensure that:

3.1 you weigh the cut portion,

3.2 measure the area of the cut portion,

3.3 put the whole cut portion into the vial and push it to the bottom of the vial to prevent direct contact with the SPME fibre.

4 Using the latex gloves, seal the 20 mL headspace vial and store it in a cool place sheltered from the sun (if possible at -20°C). To prevent potential contamination of the fibre, avoid touching the cap septa even with gloves.

5 Label the side of the vial using the adhesive labels and markers.

6 Collect a control sample from the same location as where the leaf samples are taken. To do this:

6.1 open a new vial,

6.2 move around the area to ensure surrounding air enters the vial,

6.3 using the latex gloves, seal the 20 mL headspace vial, and label it as “field control sample” of the site. At least one control sample per site per day is recommended.

6.4 store this vial it in a cool place sheltered from the sun (if possible at -20°C).

6.5 To prevent potential contamination of the fibre, avoid touching the cap septa even with gloves.

7 Once in the laboratory, store all samples at -20°C until needed.

8 Repeat these steps as necessary until all samples have been collected.

Laboratory Procedure

9 Using methanol as diluent, prepare dilutions of all four standards individually (β-pinene, Eucalyptol, Cannabis Terpene Mix A, and Cannabis Terpene Mix B), at concentrations of 25, 50, 100, 250, 500 and 1000 ug/mL in individual 20 mL headspace vials.

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13 Take samples out of the freezer and allow them to reach room temperature (approximately 10 minutes). Only select the samples to be processed on the day.

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16.2 Standards, from lowest to highest concentration.

16.3 Randomly-selected samples, including field control samples. Random sampling helps to minimize the risk of inadvertently introducing systematic errors from the operator, or changes in the GC/MS instruments.

16.4 2 x blank samples

16.5 Standards, from lowest to highest concentration.

17 Total cycle time for one sample is approximately 86 min

18 Refer to the SPME GCMS method parameters in Table 1.

GC parameters	*Agilent 7890A*
Injection	Split injection 200:1 split ratio
Carrier gas	Helium, 1mL/min, constant flow
Oven program	40 °C, hold for 10 min, 15 °C/min to 200 °C, 50 °C/min to 325 °C, hold for 3 min, Total GC run time 26 min Cooldown time 10 min
Sterilize SPME fibre	10 min

Table 1: SPME GCMS method parameters

Metabolite identification and analysis

19 Use Mass Hunter Quantitative Analysis version 10.0 to process all samples.

20 Add all samples, standards, and blanks to a quantitative analysis batch file.

21 Create a new quantitative method using the 'new method with acquired scan data' option. This method scans a data file for compound peaks and then uses the base peak (most abundant ion in the mass spectrum) as the 'quant' ion to run the quantification.

22 Set the number of additional 'qualifier' ions (commonly set to 4). These additional ions help to confirm that the compound is correctly identified in samples. Only the area of the 'quant' ion is used for quantification.

23 Select a concentration (e.g. 500 µg/µL) for all standards (β-Pinene, Eucalyptol, Cannabis Terpene Mix A, and Cannabis Terpene Mix B) and use this concentration for detecting the compounds in the GCMS data file. Afterwards, manually screen the output for errors and detected products for which there are no known concentrations to ensure that only peaks associated with known compounds from the standards are included in the quantification method.

24 To minimise the chance of other compounds being mistakenly quantified as one of the target compounds, and to reduce processing times, set a retention time window of approximately 0.1 minutes on the left hand side of the peak, and approximately 0.2 on the right hand side of the peak.

25 Select the method to detect compounds based on the target vegetation species and the target compound. The 'find by deconvolution' method is commonly used.

26 Add the calibration curves for each compound to the method file and link each compound to its corresponding calibration standard, so that the calibration curves are calculated by the software.

27 Run the newly created method on the samples and export the outputs as needed.

Time Taken

28 Standards Preparation – 1 hour
- Open vials and perform serial dilutions on each standard

29 Sample collection – approximately 20 samples in 1 hour
- Hole punch samples and seal in labelled headspace vials

30 Run GCMS – approximately 1.5 hours per sample
- Sample equilibration: 20 minutes per sample.
- Adsorption: 20 minutes per sample.
- GCMS: 26 minutes per sample.
- Fiber post bake: 10 minutes per sample.
- Oven cool-down period: 10 minutes per sample.

31 Compound recognition: 15 minutes per sample

Anticipated Results

32 Each sample, standard, and control vial should produce a chromatogram that can be used to identify each compound, alongside their corresponding retention times. In combination with the diluted standards, a concentration curve can be created for each compound and used to quantify the amount of each compound in a sample.

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