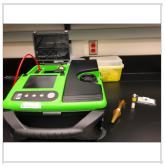

Version 2

Jan 21, 2021

Operating of the Torion® T-9 portable gas chromatograph-mass spectrometer (PerkinElmer) for the analysis of animal scent samples V.2

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1 Works for me dx.doi.org/10.17504/protocols.io.brpqm5mw

Alice Poirier

ABSTRACT

Chemosignals are mediators of social interactions in mammals, providing con- and hetero-specifics with information on fixed (e.g. species, sex, group and individual identity) and variable (e.g. social, reproductive and health status) features of the signaler. Yet methodological difficulties of recording and quantifying odor signals, especially in field conditions, have hampered studies of natural systems. We present the first use of the Torion® portable gas chromatography-mass spectrometry (GC-MS) instrument for *in situ* chemical analysis of primate scents. We collected and analyzed swab samples from the scent-glands and skin from 13 groups (57 individuals) of two sympatric species of wild emperor tamarins, *Saguinus imperator*, and Weddell's saddleback tamarins, *Leontocebus weddelli* (Callitrichidae). In total, 11 compounds of interest (i.e. probably derived from the animals) could be detected in the samples, with 31 of 215 samples containing at least one compound of interest. The composition of these 31 samples varied systematically with species, group, sex and breeding status. Moreover, we tentatively identified seven of the compounds of interest as methyl hexanoate, benzaldehyde, ethyl hexanoate, acetophenone, a branched C15 alkane, 4-methoxybenzaldehyde and hexadecan-1-ol. As the field of primate semiochemistry continues to grow, we believe that portable GC-MS instruments have the potential to help make progress in the study of primate chemosignaling in field conditions, despite limitations that we encountered. We further provide recommendations for future use of the Torion® portable GC-MS for *in situ* analyses.

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1 SAMPLE COLLECTION

1.1 Material:

- Individually numbered clean glass vials with screw-top and silicone septa lids in a cardboard box
- Bag of sterile cotton bud swabs
- Eppendorf tubes filled with distilled water
- Two Thermos® flasks filled with ice packs

1.2 Before each animal is processed, prepare:

- 20 vials on a tray
- 20 swabs ready to open
- One tube of distilled water

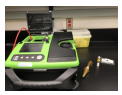
1.3 Sampling procedure:

1. Take the swab out of its wrapping
2. Wet swab it in distilled water
3. Rub swab against surface to sample (someone else must hold the animal for easier access to it), five times in an up-and-down movement
4. Open vial and insert swab, break the stem and close the lid; ensure swab does not touch any other surface before being stored in the vial, in which case the swab should be discarded and a new sample collected
5. Call out sample type and vial number
6. Store vial in Thermos® flask
7. Repeat sampling procedure for all glands and skin on each animal

2 INSTRUMENT SETUP

Torion® T-9
Portable Gas Chromatograph-Mass Spectrometer

Perkin Elmer **unk.**



2.1 Turn on generator (refill if necessary prior to starting it)

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2.2 Open helium tank valve (monitor helium level on gauge and write it down on the log)

2.3 On Torion® instrument:

1. Take off gas white cap from behind instrument
2. Check electrical connection
3. Take off side covers unless it is very cold and humid
4. Take off injector cover
5. Press start button and wait for start up
6. Open Chromion® software on touchscreen
7. Select 'GCMS' icon on touchscreen and wait until ready (establishes vacuum and oven temperature)
8. Connect computer with red interface cable

2.4 On computer:

1. Open Chromion® software
2. Connect to Torion® instrument, wait for connection to happen
3. GC and MS methods can be modified in Chromion®, then sent to instrument (see Methods presented in article main text)

3 PRE-RUN STEPS

3.1 System blank: always start with one or two system blanks to assess cleanliness of the instrument's column, injector and ion trap; on touchscreen select 'system blank' category

3.2 Bake run: if results of system blank show contamination of the column, start with baking injector and column, i.e. empty blank run at high temperature (320°C); a bake run method can be created in Chromion®

3.3 Fiber blank: to assess if SPME fiber is clean; on touchscreen select 'sample' category and inject fiber as if it were a normal sample; inspect resulting chromatogram, there should not be any important peaks and the baseline should be low

3.4 Fiber clean: if SPME fiber seems dirty, it can be inserted in injector for 15-30 sec where the heat will clean it; on touchscreen select 'sample' but ignore message for injection. This may contaminate the column as compounds will be liberated from the fiber and pass into the column, so it is important to run one or several fiber blanks afterwards, until the baseline on the chromatogram is clear

3.5 Performance validation: run once a day at startup, or less often if few samples are run; performance validation serves to verify instrument performance and calibrate peak detection. On touchscreen select 'performance validation' category and follow the steps. If performance validation fails, follow instructions given on the screen. It may be necessary to clean the instrument and SPME fiber further (repeat steps 2, 3 and/or 4), or to replace disposable material (e.g. injector septum). Always run a fiber blank after performance validation to clean the fiber

4 SAMPLE ANALYSIS (example fo a single sample)

Prepare material before taking samples out of the freezer:

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4.1
- Custodion® SPME syringe
- Sterile disposable syringe and needle
- Sterile paper wipes
- Pan of simmering water

4.2 Sample extraction procedure:

1. Tighten vial cap if necessary
2. Put vial on rack in simmering water; about half of the vial should be immersed in the water; leave for 2 min
3. Remove quickly from water bath and wipe water condensation off the lid
4. Pierce lid septum with syringe needle
5. Insert SPME needle, being careful not to touch the swab
6. Hold SPME syringe and vial in each hand under light, and carefully expose the fiber without it touching the swab or the vial edges; leave exposed for 2 min
7. Retract SPME fiber; ensure Torion®touchscreen is ready for run (category 'sample')
8. Press 'run' on touchscreen, remove SPME needle from the vial and insert it into injector following instructions on touchscreen; instructions indicate when to expose fiber inside the injector and when to remove it
9. Write down sample information in sample log (it is important to keep track of all runs)
10. When run is finished, another vial can be heated and extracted while data is still processed in instrument software
11. Run fiber blank every few samples to ensure fiber and column stay clean; bake fiber if necessary

5 POST-RUN STEPS

5.1 Backup all data from Torion®'s SD card onto computer

5.2 Close Chromion® software on touchscreen

5.3 Shut down Torion®

5.4 Close helium valve (do not do it until instrument is off, else it interrupts the vacuum system inside instrument)

5.5 Disconnect computer from Torion®

5.6 Shut down generator

5.7 Put back all covers

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5.8 Store SPME fiber in tight box protected from humidity and ambient air

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