Report ref.	SRGET22-005
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Category	External
Subject	**Study review: "The Nose Knows; Chemical Scent Detection By a Protected Species Conservation Dog", Tegan Murrell**

Background

The goal of this document is to review the study in reference.

Reference of study

Murrell, T. (2022). The Nose Knows; Chemical Scent Detection By a Protected Species Conservation Dog (Thesis, Master of Science). University of Otago. Retrieved from http://hdl.handle.net/10523/12890

Reviewers

Quentin COURRIER	Maïwenn COMBES
	
• CTO Getxent • Master of Science (M.Sc.), Polymer Engineering, European School of Chemistry, Polymers & Material of Strasbourg (ECPM) • Lecturer: University of Strasbourg, University of Upper Alsace, ESTA School • Elite expert for global consulting companies (ex. Arthur D. Little) • 7 innovation awards in Europe • 4+ patents in polymer materials	• Project Leader & Analytical Manager Biodesiv • Master of Analytical Sciences for Bio-Industries (SA-BI), Faculty of Chemistry, University of Strasbourg • 1 publication in Nano Letters (organic chemistry) • 2+ years Analytical Engineer, Eurofins Analysis for Environment France • Development and optimization of analytical methods for environmental matrix • Analytical expertise in drafting standards for environmental analysis

Comments

Getxent tubes Authenticity:

Nor the author, nor the University of Otago belongs to Getxent customer database. Indeed Getxent is the only company which is allowed to produce and sell Getxent tubes in the world, the Getxent tubes used in this study might be counterfeit products.

As the products used cannot be tracked, it is not possible to make sure that they were:

- used before expiry date
- stored in proper conditions

Their performances can be strongly affected.

First Part – Frog Analysis

Impregnation conditions:

A study on set-ups for fibers has been performed by the author that proves that set-ups have a huge influence on the amount of odor collected by sorbents. Despite this information, the best set-up for fibers (2h, 1 frog, 450ml) has not been used with Getxent tubes (48h, 1 frog, 1000ml).

The volume used for the impregnation of Getxent tubes is 1000ml, 4x higher than with fibers, and far above our recommendations. The odor of the frog is diluted and the amount of VOCs collected by the Getxent tube is low.

Thus, the results obtained with Getxent tubes and fibers cannot be compared.

Packaging of sorbents:

After impregnation with frogs:

- the fibers were packed in aluminum foil and Kimax tubes
- the Getxent tubes were packed in sterile glass headspace vials with Teflon lined screw lids

The way the sorbents are packed has an influence on the quality of the odor released by the sorbents. Indeed, fibers and Getxent tubes cannot be compared because of the packaging differences, which can bring pollutions in the signal in the case of the fibers.

Fibers:

SPME fibers are made of:

- PDMS (adsorb and absorb VOCs)
- Carboxen (only adsorb VOCs)

Both materials are chemically apolar and will thus mainly collect apolar molecules.

However, odors are composed of many molecules both apolar and polar and polar molecules are not or poorly collected by apolar ad/absorbents like SPME fibers.

Getxent tubes, thanks to their polymeric structure with polar and apolar blocks, can collect all kind of molecules released by substances, including live animals.

No trials were performed to evaluate the ability of the dogs to detect SPME fibers impregnated with frogs' odor. Based on the previous explanations, lower performances than Getxent tubes are expected.

Extraction:

Getxent tubes absorb both polar and apolar molecules, but it is not the case of the fiber used in the study in reference (see Fibers). During GC-MS analysis VOCs from the Getxent tube are:

1. transferred to the fiber
2. transferred from the fiber to the GC-MS

Fiber plays a filter role as a filter, and it doesn't allow to show the real performance of the Getxent tubes.

For example, if we consider the fiber can collect 50% of the amount of VOCs and the Getxent tube 75% (random values):

- with a direct extraction of VOCs from fiber, max. 50% of the amount of VOCs is recovered
- with an indirect extraction of VOCs from the Getxent tube through the fiber, max. 50% x 75% = 37,5% of the amount of VOCs is recovered

Analytical method:

The intensities of the peaks on the chromatograms are very low (approx. 1x10^6 cps), it can be explained by the fact that:

- SPME fiber is made with PDMS/CAR coating that does not offer the best recovery compared to coating containing DVB (10.1515/revac-2017-0018)
- the split rate was set at 50 mL/min and the column flow rate at 1 mL/min. This means that only 1/50th of the VOCs extracted were injected into the column

The very low intensity of main peaks suggests that:

- Many VOCs might have not been detected
- Values obtained by integration of peaks are not accurate (Standard ISO 16017-1:2001, the signal / noise ratio must be at least 5:1)

Among identified peaks in chromatograms, at least 50% are deformed (co-elutions, shoulders, roughness of the baseline). A possible cause is the high diameter of the column that decreases the number of theoretical plates and thus generates less separation efficiency and low resolution of the peaks.

The column is bleeding (figure 17-B, between 5-15 minutes and after 30 minutes). It can be explained by the high thickness of the film in the column. The asperities of the baseline due to the bleeding can affect the shape of the peaks.

All these parameters make the peak integration not reliable in a qualitative and quantitative point of view, both for the analysis of the fibers and the tube.

Lower signal in impregnated Getxent tubes vs blank Getxent tubes:

As shown below, blank Getxent tubes are analytically clean (in Annex, you can find the method used).

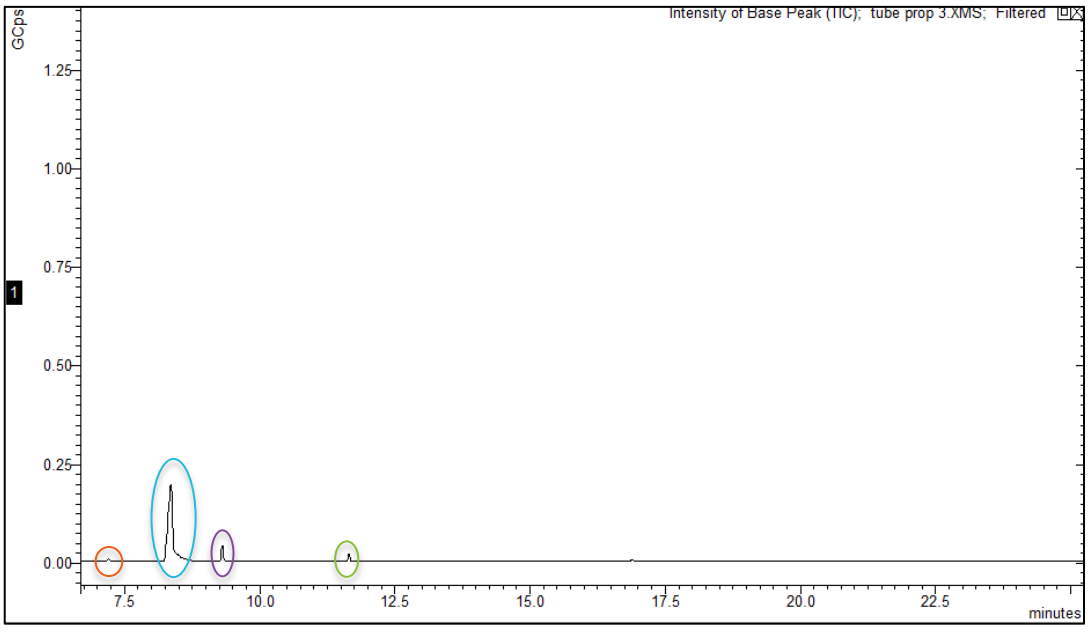

Figure 1: Chromatograms of not impregnated Getxent tube

The chromatogram of the blank Getxent tube shows 4 peaks:

- 7,20 min: octamethylcyclotetrasiloxane
- 8,30 min: water
- 9,30 min: decamethylcyclopentasiloxane
- 11,60 min: dodecamethylcyclohexasiloxane

Same peaks are also found when the analysis is performed with blank vials, without any sample. These signals come from the SPME fiber which is composed of siloxanes. The blank Getxent tube is therefore clean.

The fact that, in the study in reference, the blank Getxent tubes release higher signal than impregnated ones means that blank Getxent tubes have been polluted during their handling and/or storage.

The presence of this pollution can strongly decrease their performances.

Data process:

The intensity of signal is extremely low, and most peaks are in the same order of magnitude than the noise, peak integration is not reliable.

Thus, the peaks areas cannot be used as a reliable data to evaluate the performance of Getxent tubes nor to compare with fibers.

Comparison of chromatograms:

Only chromatograms of fibers impregnated with frogs' odor are shown, no chromatogram of Getxent tube impregnated with frogs' odor is shown.

Thus, the results obtained with Getxent tubes and fibers with frogs' odor don't allow to compare impregnations set-ups and VOCs concentrations.

Second Part – Chocolate Analysis

A Getxent tube impregnated with chocolate at room temperature (we assume 20-25°C) and pure chocolate heated up to 50°C have been compared. Chromatograms from chocolate impregnated Getxent tubes and pure chocolate are not comparable.

It is known that the increase of temperature strongly increases the amount of VOCs emitted (for example, amount of 2-ethylhexanol released from a plastic is 7x higher at 50°C vs 23°C ref. 10.1111/j.1600-0668.1997.t01-1-00007.x). In addition, the chocolate has been heated up above its crystallization temperature, increasing again its ability to release VOCs. It explains why the peaks from chocolate are far higher than the peaks from Getxent tubes impregnated with chocolate.

In addition, the chromatograms of Getxent tubes impregnated with chocolate is not flat meaning they collect VOCs. From a qualitative point of view, all the peaks identified in the chocolate are present in the impregnated Getxent tube. Other peaks are visible but come from the baseline noise or pollution during the handling of the tube because the tube is analytically clean (cf part "Lower signal in impregnated Getxent tubes vs blank Getxent tubes").

Main conclusion from the author:

The study in reference states "As these tubes [Getxent tubes] were unable to absorb representative VOC profiles from either Leiopelma or chocolate, it can be concluded that these tubes are ineffective as detection training aids."

It means that the absence of signal in GC-MS is a sufficient condition to prove that a sorbent cannot be used as detection training aid, i.e., training aid for detection dogs.

In accordance with the scientific community, one of the most renown university in the world in the field of dog detection stated the exact opposite: "Dogs provide a three order of magnitude more sensory capacity than most current diagnostic instruments" (10.3389/fvets.2015.00079). As dogs (trained with high sensitivity) are far more

sensitive than any analytical equipment, the lack of signal in GC-MS is not a sufficient condition to prove that a sorbent cannot be used as detection training aid, i.e., training aid for detection dogs.

Observations

Here are some general observations about the way the study has been performed decreasing the conclusions quality:

- a study has been performed on the fibers to define the best impregnation set-up (4 different set-up tested). For the Getxent tubes, only 1 set-up was tested, no study has been performed to define the best impregnation set-up.
- the absence of odor of the packaging used to store the fibers (aluminum foil + Kimax tubes) has not been assessed. It is known that many chemicals are used for the processing of aluminum foils, especially lubricants, partially released as VOCs. Kimax tubes have a cap in phenolic resin as well as rubber seal: both materials are known to emit VOCs. It is thus probable that the VOCs identified by GC-MS in fibers impregnated with frogs' odor come from the packaging and not from the frogs themselves.

Reviewer 1	Reviewer 2
Q. Courrier CTO 11.06.22	M. Combes Project Leader 11.06.22
	

Annex 1: GC-MS method

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MSWS 8.0.1 for SCION - Method Listing   Wed Jul 07 13:52:03 2021

Method: Tube.mth

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**********************
    CombiPAL AutoSampler
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Module Address: 24

CPAL Method:

                     Injection Mode: GC SPME
                     Read Bar Codes: Never
                   Required Syringe: SPME Fiber
                Agitator Temperature: 40.0 C
        Sample Pre-Incubation Time: 10 min. 0 sec.
     Pre-Incubation Agitator Speed: 250 rpm
    Pre-Incubation Agitation Cycle: 2 sec On, 4 sec Off.
          Extraction Agitator Speed: 250 rpm
            Fiber Depth From Bottom: 10 mm
                     Extraction Time: 50 min. 0 sec.
                            Injector: Front
                        Desorb Time: 2 min. 0 sec.
                Use Bakeout Station: No
    GC Cycle Time (for Prep Ahead): 36 min. 0 sec.

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    SCION Mass Spec
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Module Address: 40

Acquisition Method ==================

Acquisition delay 2.50 min.
No pre run macro.
No post run macro.
CID Gas off
Ion Source: EI
Data Type: Centroid

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    43X-GC  - Model 436-GC
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Module Address: 44

Valve Table
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    No Valves Used

 Front Injector Type S/SL
---------------------------------
             Oven Power: On
                Coolant: Off
     Enable Coolant at: 250.0 C
       Coolant Timeout: 20.00 min

      Temp     Rate       Hold      Total
       (C)    (C/min)    (min)      (min)
      -------------------------------
    250.0      0.0      20.00      20.00

       Time     Split     Split
       (min)    State     Ratio
       ----------------------
    Initial      On         10
       0.01     Off        Off
       2.00      On         50

 Front Injector EFC Type 21: Enabled
--------------------------
      Constant Column Flow: 1.00 ml/min
            Pressure Pulse: none

              No Backflush.

Column Oven
-----------
                Coolant: Off
     Enable Coolant at: 50.0 C
       Coolant Timeout: 20.00 min
    Stabilization Time: 2.00 min

      Temp     Rate       Hold      Total
       (C)    (C/min)    (min)      (min)
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