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Full Paper

Influence of Adsorbent Nature on the Dynamic Headspace Study of Insect Semiochemicals

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In chemical ecology studies (insect–insect, insect–plant relationships), it is important to choose the appropriate sampling methods and the correct optimization of sampling by using dynamic systems. In the present work, different adsorbents were evaluated in a dynamic system that presents a stream of purified air flowing through an aeration chamber containing a mixture of volatile organic compounds, mainly insect semiochemicals such as α-pinene, sulcatone, β-linalool, menthone, isomenthone, methyl salicylate, grandlure I, grandlure II, grandlure III, grandlure IV, eugenol, and α-ionone. Traditional adsorbents such as Tenax TA, Porapak Q, Hayesep Q, and activated charcoal were evaluated; further, alternatives such as Porapak Rxn RP, HLB, SCX, and silica gel, among others were proposed owing to their lower cost. The results demonstrated that Porapak Q and Porapak Rxn RP, despite their different chemical composition, were able to produce similar ratios of compounds to that of the reference solution and, moreover, with the highest recovery yields. However, it is important to emphasize the adsorption selectivity provided by SCX for eugenol and α-ionone. When Porapak Rxn RP was used in the analysis of *Eucalyptus globulus* volatiles, excellent results were obtained, and these agree with reported data from a hydrodistillation method.

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Introduction

Some living beings such as certain insects or plants modulate the emission of volatile compounds as communication strategies. To understand the communication between them and in order to interfere by enhancing their attractive or repulsive action, it is important to study the nature of this volatile emission of semiochemicals. Therefore, it is necessary to collect these volatile compounds in sufficient amounts to determine both their chemical structures and relative quantities of the behaviourally active compounds.[1] The techniques commonly used in chemical ecology for identification and quantification of volatile semiochemicals include preconcentration techniques for headspace analysis in gas chromatography, modified specifically according to the needs of the living individuals being studied.[2] Headspace analysis is generally defined as a vapour-phase extraction, involving the partitioning of analytes between a non-volatile liquid or a solid phase and the vapour phase above the liquid or solid.[3] Headspace techniques can be classified into dynamic and static headspace analysis. In dynamic headspace analysis, the sample is confined in an all-glass aeration chamber and a purified airstream (carrier gas) is passed over the sample. The volatile chemicals released by the sample are carried by the airstream through a solid trap, usually a porous organic polymer such as Porapak Q, Tenax TA or activated charcoal, where the analytes are adsorbed and preconcentrated (Fig. 1). Analyte desorption from the solid trap for gas chromatography analysis can be achieved by either elution with a solvent (solvent desorption) or rapid heat treatment (thermal desorption).[4] In static headspace analysis (used less often), the sample is tightly enclosed in a vessel, where it comes into equilibrium with its vapour at room temperature. The headspace can be sampled using a syringe or a similar device and injected directly into the gas chromatograph.[3] The main limitation of classical static headspace analysis is that sensitivity is lower than that of dynamic techniques. Solid-phase microextraction (SPME) has been developed as a rapid and solvent-free technique. This technique uses a fine fused silica fibre with a polymeric coating to extract organic compounds from their matrix and directly transfer them into the injector of a GC for thermal desorption and analysis.[5] The limitation of this technique is that the sample cannot be stored and reanalysed. Also, it is necessary to properly select the fibre coating to fit the polarity and volatility of the compounds being assayed.[6] Generally, microchemical reactions that need samples in solution are necessary for the final identification of volatile compounds.[2]

The experiment design consists of groups of insects (separated by sex) with or without food sources, which is a complex

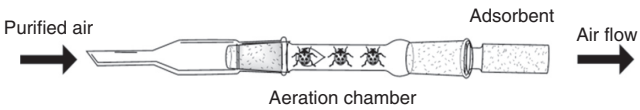

Fig. 1. Aeration chamber schematic representation.

system to study. For this reason, careful selection of extraction techniques is important to minimize the loss of compounds during their collection and a large number of samples is often needed for statistical calculations to serve as the basis for general conclusions.

There are few reports about systematic studies concerning the optimization of this technique for the analysis of volatile compounds frequently found in insect aerations. Considering these facts, the goal of the present research was to evaluate the scope of dynamic headspace analysis for a sample composed of different semiochemicals, paying particular attention to the efficiency of the adsorption step, using typical adsorbents and, further, proposing some less expensive alternatives available in any organic laboratory.

Materials and Methods

Volatile Standards

Most of the reagents used, α-pinene ((1*R*)-2,6,6-trimethylbicyclo[3.1.1]hept-2-ene, 98 % purity), sulcatone (6-methyl-5-hepten-2-one, 99 % purity), β-linalool (1,6-octadien-3-ol, 97 % purity), α-ionone (4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3-buten-2-one, analytical standard), menthone (5-methyl-2-(1-methylethyl)-cyclohexanone, mixture with isomer isomenthone), methyl salicylate (99 % purity), and eugenol (4-allyl-2-methoxyphenol, 99 % purity), were purchased from Sigma–Aldrich (Buenos Aires, Argentina). Grandlures I, II, III, and IV were purchased from ChemTica Internacional (Heredia, Costa Rica).

Adsorbents

Tenax TA, Porapak Q, Hayesep Q, silica gel, and activated charcoal were purchased from Sigma–Aldrich.

In Table 1, the adsorbents used in the present work and their chemical composition are summarized. The polymeric adsorbents were conditioned by passing 10 mL of methanol and 10 mL of hexane (both HPLC grade) through them before their use.

Solid-phase extraction (SPE) Supra-Clean strong cation exchange (SCX, 200 mg in a 3 mL cartridge) cartridges were obtained from PerkinElmer (Buenos Aires, Argentina). Porapak Rxn RP (40 mg in a 6 mL cartridge) and Oasis HLB (60 mg in a 3 mL cartridge) SPE cartridges were purchased from Waters (D'amico Sistemas SA, Buenos Aires, Argentina).

Collection and Analysis of the Volatile Compound Mixture

A standard solution at a final concentration of 1 μg mL^{-1} of each volatile compound was prepared in dichloromethane. One millilitre of this solution was placed in one all-glass aeration cylindrical chamber (30-cm height × 6 cm outside diameter). Headspace volatiles were collected for 24 h and trapped on glass columns (15-cm height × 0.5 cm diameter) with 50 mg of adsorbent or an SPE cartridge. Charcoal-filtered humidified air was pushed through the aeration system (1.0 L min^{-1}) in order to emulate a typical insect pheromone collection.[7] Adsorbed aeration volatiles were eluted with 1 mL of HPLC-grade hexane (Sintorgan SA, Buenos Aires, Argentina) and stored at −20°C in a freezer until their analysis.

Extracts were analysed by gas chromatography–mass spectrometry (GC-MS) using a Thermo Scientific Focus GC coupled with a DSQII electron ionization mass detector. The GC was operated in the splitless mode. A TR-5MS capillary column (30 m × 0.25 mm × 0.25 μm) (Thermo Fisher Scientific) was used under the following analytical conditions: initially, the column temperature was kept at 50°C for 5 min, and then increased at a rate of 7°C min^{-1} to a final temperature of 250°C, and then kept at this temperature for 10 min. Additionally, a chromatograph GC Konik 3000 series equipped with a ZB-5 capillary column (30 m × 0.25 mm × 0.25 μm) (Phenomenex, Inc., Torrance, CA, USA) and a flame ionization detector (FID) was used. The column oven was kept at 50°C for 4 min, the temperature increased at a rate of 7°C min^{-1} 250°C, and then kept constant for 10 min. The ratios of compounds were calculated from GC peak areas, so that the total peak areas of interest equalled 100. Quantification of compounds was achieved using known amounts of volatile compounds as external standards using the Konik GC.

Volatile Compounds from a Natural Source

The methodology described above was trialled on a natural source. Volatile compounds from *Eucalyptus globulus* aerial parts (leaves) were analysed using Porapak Rxn RP as adsorbent. The plantation was located in Santiago del Estero Capital, Argentina (27°45′S, 64°18′W, 170 m above sea level). Sample preparation and volatiles collection were carried out under the same conditions as for the standard mixture tested.

Statistical Analysis

All assays were carried out in triplicate. The effects of the different adsorbents used on the relative ratios and quantities of test compounds were compared using the *t*-test for means of paired samples ($P < 0.01$).

Results and Discussion

Table 2 shows the volatile organic compounds assayed in the current study and some of their properties are described. In order

Table 1. Summary of the sorbents and their properties as reported by the manufacturer
NA, not available

Name	Composition	Size [μm]	Maximum operational temperature T_{max} [°C]
Tenax TA	Polydiphenylene oxide	60–80	350
Porapak Q	Divinylbenzene/ethylvinylbenzene	80–100	250
Hayesep Q	Divinylbenzene	80–100	275
Silica gel	Silicon dioxide	60–100	NA
Activated charcoal	Carbon	100	NA
SCX	Benzenesulfonic acid/silica	60–100	NA
Porapak Rxn Rp	Reverse phase	60–100	NA
Oasis HLB	Divinylbenzene/vinylpyrrolidone	60–100	NA

to show the biological significance of these molecules, the main references where their activities as pheromones were reported are presented (more details of the semiochemical compounds in the present study are available at www.pherobase.com). Their chemical structures are shown in Fig. 2, highlighting the presence of different functional groups such as ketone, aldehyde, ester, alcohol, aromatic rings, and double bonds, among others important ones.

Fig. 3 shows the chromatogram obtained from the standard solution of compounds. The compounds have different responses at the same concentration ($1\,\mu g\,mL^{-1}$) under the chromatographic conditions used. It can be seen that **9**, **10**, and **12** have a rather low signal intensity compared with the others compounds. The best response was observed for **2**. The parameters derived from the repetition of this experiment were established for use as controls or standards.

One important factor that modifies insect behaviour (insect receiving the message) is the ratio between semiochemicals released by biological samples under study (insects or plants sending the message); for this reason, how the nature of the adsorbents modified the ratio of compounds was analysed first (Table 3). First, when the adsorbent studied was SCX (cationic exchange), compounds **1**, **3**, **7**, **8**, **9**, and **10** were not detected. In the case of activated charcoal, only **1** was not detected. Further, **9**, **10**, **11**, and **12** were not detected using silica gel as adsorbent, even when acetone and methanol were used in the extraction step. For this reason, these three adsorbents were excluded for the statistical comparison of ratios of volatile molecules recovered. The other compounds were adsorbed and quantified in the experiments.

Porapak Q, Hayesep Q, and Porapak Rxn RP were the adsorbents with the best correlation ($P \leq 0.01$) between the ratios determined for each volatile compound and the amounts present in the standard solution. The main differences were found in the ratios of the most and least volatile compounds (**1**, **2** and **11**, **12** respectively). However, the other adsorbents allowed detection of the majority of the compounds under study. Silica gel could be used in preliminary assays in the identification of volatiles and SCX would be useful to concentrate (or isolate) a range of polar compounds.

Table 2. Volatile compound information

Compound	RI[A]	M_w [g mol^{-1}]	Boiling point [°C][B]	Ref. for pheromone[C]
1. α-Pinene	940	136	155	[8]
2. Sulcatone	991	126	73	[7, 9]
3. β-Linalool	1110	154	194	[10]
4. Menthone	1178	154	207	[11]
5. Isomenthone	1187	154	207	[11, 12]
6. Methyl salicylate	1221	152	222	[13]
7. Grandlure I	1228	154	210	[14]
8. Grandlure II	1249	154	212.7	[15]
9. Grandlure III	1281	152	226.6	[15, 16]
10. Grandlure IV	1289	152	226.6	[15, 16]
11. Eugenol	1377	164	254	[17]
12. α-Ionone	1443	192	259	[18]

[A]Experimental GC linear retention index.
[B]Data obtained from Sigma–Aldrich (Argentina) or Angene International Limited (China).
[C]References where the compound acts as an insect pheromone.

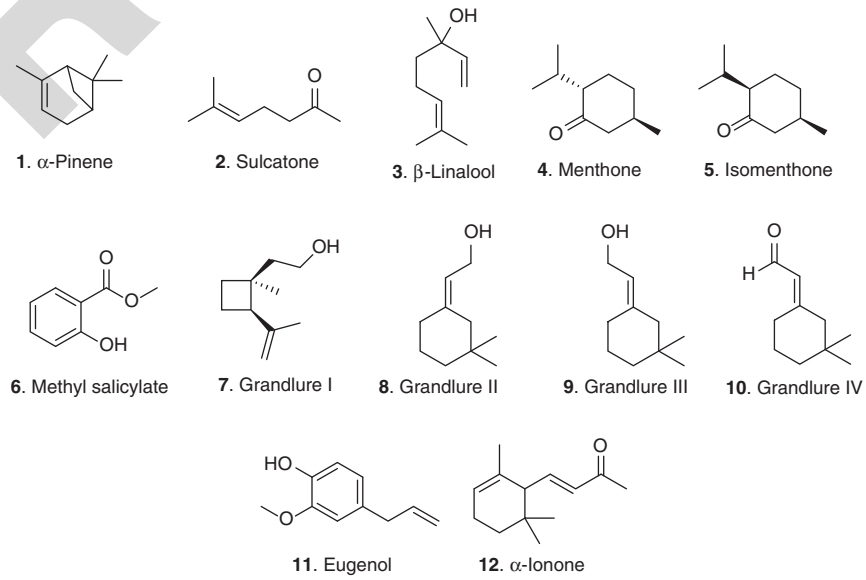

Fig. 2. Pheromone structures.

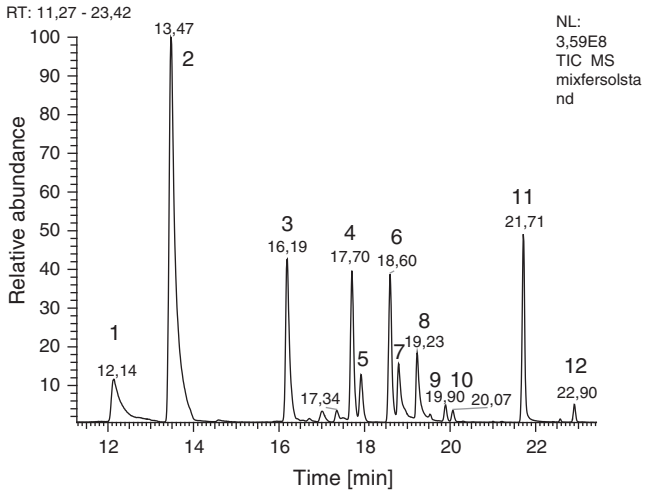

Fig. 3. GC chromatogram of the volatile organic compound mixture.

A previous report did not find differences in the ratios of α-pinene and sulcatone using TENAX TA, Porapak Q, and Hayesep Q under static headspace conditions and after 1 h of equilibrium between the sample and its vapour, a vacuum pump having then been used to extract the gaseous phase through the adsorbent for 30 s.[19] This protocol differs drastically from that used in the present work mainly in the fact that a dynamic headspace was used for 24 h in which the extraction of compounds is continuous. When aeration is carried out for long periods of time, a adsorption–desorption equilibrium due to air pressure is established. The equilibrium favours the adsorption of less-volatile compounds but desorption of more volatile molecules can take place. These facts produce great differences between the adsorbents assayed in their response to the volatility of compounds.[20]

In the study of biological samples (for example, insects), it is generally necessary to have sampling systems that allow reliable data to be obtained over long periods of time. As usually the variables that affect volatile emissions from the sample are numerous and unknown, it is important to fix the temperature and humidity ranges, age of insects, and maturity, among others.

Fäldt et al.[6] reported the sampling of α-pinene, linalool, and other volatile compounds in a dynamic system (30 min) using Tenax TA, Porapak Q, and charcoal, with similar results to those observed in the present work. However, as they used SPME sampling, they concluded that it is difficult to quantify these molecules owing to the large coefficients of variation in the determinations.

The main advantages associated with solvent desorption are that the liquid sample obtained can be stored in the freezer, sealed under nitrogen in glass ampules, and used several times when replication is required. Quantification is also facilitated by having a liquid sample to which an internal standard can be readily added, although if the objective of the investigation is the identification of the volatile molecules, the solvent peak can mask compounds with short retention times.

Another important parameter to describe an absorbent is the total amount (percentage recuperation) of analyte extracted (Fig. 4). Only with Porapak Q and Porapak Rxn RP could α-pinene, the most volatile compound in this study, be extracted, with >50 % of recuperation. Moreover, Oasis HLB, Tenax TA, and Hayesep Q gave recoveries lower than 10 %. However, with SCX, activated charcoal, and silica gel as adsorbents, α-pinene was only detected at trace levels.

A similar result was seen for sulcatone, but in this case, it could also be extracted with SCX, carbon, and silica gel but in low quantities.

Tenax TA, Porapak Q, Hayesep Q, and Porapak Rxn Rp had good to very good recuperation percentages for the other compounds tested, the best yields being obtained using Porapak Q. Oasis HLB and activated charcoal allowed recovery of the compounds but in general in a lower percentage.

Compounds **11** and **12** were concentrated with a high selectivity on SCX (cationic exchange polymer). However, when the adsorbent was silica gel, poorer yields were obtained for all compounds.

Also, Tenax TA and Porapak Q were compared in recent work, using other volatile compounds; differences were found in the capacity of extraction between them.[21]

Moreover, reverse-phase adsorbents (C18 RP, C8 RP, C4 RP) alone and mixed with Celite were assayed, although the results were not promising.

In order to evaluate the adsorbent capacity of Porapak Rxn RP for volatile compounds released from a natural source, *Eucalyptus globulus* leaves were assayed under the same conditions as the standard mixture. *Eucalyptus* species are well known as a source of essential oils, which are generally obtained by hydrodistillation.[22,23] Ten compounds were identified in the headspace of *E. globulus* leaves: **1**, β-myrcene, α-phellandrene, limonene, eucalyptol, γ-terpinene, (−)-terpinen-4-ol, α-terpineol, α-copaene, and α-gurjunene (Table 4).

The compounds identified are in good agreement with those reported in the scientific literature, which were extracted by hydrodistillation.[24] Also, the relative ratios among the volatile molecules in this plant extract are consistent with data previously described,[25] and vary according to the extraction technique used, *Eucalyptus* age, and period of year of sampling. It is important to note that all the main compounds were extracted. Essential or volatile oils are key components of many plant organs responsible for ecological signalling besides other physiological roles.

Adults of both specialist and generalist herbivore insects can detect a wide range of plant odours and they even perceive some volatiles beyond the plant species they normally colonize. It was recently reported that *Eucalyptus* essential oils inhibit the attraction of *Plutella xylostella* males (*Brassicaceae* insect plague) towards the female pheromone.[26] Further, the great importance of *Eucalyptus* spp. essential oils and their toxics effects on *Aedes aegypti* larvae have been reported.[27] These are simple examples that give to our adsorbent proposal experimental relevance.

Conclusions

In chemical ecology studies (insect–insect, insect–plant relationships) is important to consider the necessity of choosing the appropriate sampling methods and the correct optimization of sampling by using dynamic systems. In the present work, it was found that, among the traditional adsorbents used, Porapak Q exhibited the best recovery yields and the best performance as adsorbent, producing results close to the standard solution composition. Further, the SPE cartridge Porapak RP Rxn, a low-cost alternative, showed similar results to Porapak Q. HLB allowed the identification of all volatile compounds, similarly to activated charcoal, a non-polymeric adsorbent, whereas SCX gave mainly

Table 3. GC peak area percentage (mean ± s.e.) of volatile organic compounds (n=3)
nd, not detected

Compound	Solution standard	Tenax TA	Porapak Q	Hayesep Q	SCX	Porapak Rxn RP	Oasis HLB	Activated charcoal	Silica gel
1. α-Pinene	5.43 ± 0.18	0.17 ± 0.02	2.30 ± 0.11	0.16 ± 0.02	nd	5.02 ± 0.49	2.42 ± 0.15	nd	nd
2. Sulcatone	41.43 ± 1.09	2.58 ± 0.29	45.88 ± 0.95	24.87 ± 0.72	0.78 ± 0.17	27.39 ± 2.57	16.19 ± 1.24	3.21 ± 0.31	18.32 ± 1.04
3. β-Linalool	12.2 ± 0.3	11.73 ± 0.34	14.39 ± 0.36	24.55 ± 0.21	nd	12.59 ± 1.13	15.07 ± 1.23	9.51 ± 0.40	36.81 ± 3.11
4. Menthone	9.00 ± 0.18	6.51 ± 0.99	9.88 ± 0.18	17.44 ± 0.31	3.44 ± 0.13	14.2 ± 1.02	4.71 ± 0.43	5.35 ± 0.51	30.21 ± 2.48
5. Isomenthone	2.93 ± 0.07	2.07 ± 0.31	3.08 ± 0.05	5.38 ± 0.12	1.04 ± 0.09	4.09 ± 0.37	2.93 ± 0.27	1.89 ± 0.11	10.82 ± 0.46
6. Methyl salicylate	8,72 ± 0.13	27.81 ± 0.45	8.99 ± 0.18	16.63 ± 0.11	5.16 ± 0.47	12.2 ± 1.69	4.49 ± 0.26	13.87 ± 1.42	2.95 ± 0.26
7. Grandlure I	4.44 ± 0.14	13.23 ± 0.06	4.88 ± 0.06	3.16 ± 0.03	nd	4.38 ± 0.35	10.34 ± 0.86	18.44 ± 1.17	0.43 ± 0.19
8. Grandlure II	4.50 ± 0.27	12.72 ± 0.13	5.03 ± 0.14	3.26 ± 0.02	nd	4.93 ± 0.04	8.92 ± 0.55	15.16 ± 0.54	0.69 ± 0.42
9. Grandlure III	0.80 ± 0.01	2.61 ± 0.03	0.87 ± 0.03	0.56 ± 0.02	nd	1.39 ± 0.09	6.98 ± 0.21	3.82 ± 0.34	nd
10. Grandlure IV	0.55 ± 0.004	2.38 ± 0.03	0.72 ± 0.05	0.47 ± 0.01	nd	1.09 ± 0.03	4.41 ± 0.09	4.2 ± 0.36	nd
11. Eugenol	9.22 ± 0.12	15.79 ± 0.18	3.40 ± 0.12	2.62 ± 0.23	78.61 ± 0.62	11.68 ± 0.63	16.14 ± 1.38	19.98 ± 1.56	nd
12. α-Ionone	0,83 ± 0.01	2.40 ± 0.05	0.58 ± 0.02	0.89 ± 0.04	10.99 ± 0.28	1.06 ± 0.06	7.38 ± 0.32	4.57 ± 0.16	nd

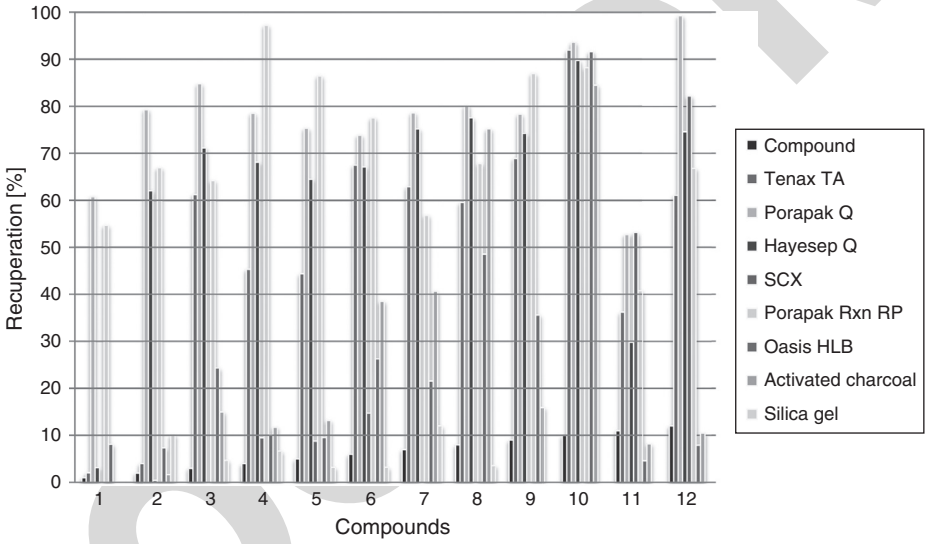

Fig. 4. Recuperation percentage of compounds as function of adsorbent nature ($n = 3$).

Table 4. GC peak area percentage (mean ± s.e.) of volatile organic compounds identified in *Eucalyptus globulus* leaves (n=3)

Compounds	Area (%)
1. α-Pinene	0.81 ± 0.03
β-Myrcene	3.89 ± 0.05
α-Phellandrene	2.05 ± 0.02
Limonene	15.8 ± 0.2
Eucalyptol	58.5 ± 0.9
γ-Terpinene	2.39 ± 0.03
(−)-Terpinen-4-ol	5.3 ± 0.1
α-Terpineol	8.6 ± 0.4
α-Copaene	1.59 ± 0.06
α-Gurjunene	1.06 ± 0.02

the concentration of eugenol and α-ionone. Silica gel had the poorest adsorbent power among the materials assayed.

When a natural source of volatiles, *Eucalyptus globulus* leaves, was assayed, excellent results were obtained, and these agree with reported data.

In conclusion, efficient alternative adsorbents to do chemical ecology investigations open the possibility of using other non-traditional adsorbents in SPE cartridges.

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