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Short communication

Solid-phase microextraction–gas chromatography–direct deposition infrared spectrometry as a convenient method for the determination of volatile compounds from living organisms

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Abstract

Gas chromatography–direct deposition infrared spectrometry (GC–DD-IR) permits coupling of GC to IR at a level of sensitivity of routine GC–MS coupling but the presence of ice resulting from living organisms limits the usefulness of the system. Headspace solid-phase microextraction (SPME) coupled to GC–DD-IR leads to a rigorous absence of water and can be applied to unknown volatiles trapped in situ in combination with SPME–GC–MS. For instance we succeeded in identification of the asparagus fly male pheromone and 1 min of an individual emission is sufficient to obtain a good IR spectrum in real time.

Keywords: Interfaces, GC–IR; *Platyparea poeciloptera*; Pheromones

1. Introduction

A direct deposition interface for gas chromatography–infrared spectrometry (GC–DD-IR) permits the coupling of GC to IR at a level of sensitivity of routine GC–MS coupling 1–2 orders of magnitude better than that of instruments using light-pipe interface [1]. Moreover the combination of GC–MS and GC–DD-IR is especially promising in the identification of unknown natural compounds in the picogram range [2].

In practice the system requires very careful operation and it appears to be very sensitive to the presence of water leading to the formation of ice on the cold (77 K) Zn–Se window normally transparent to IR.

This presence of ice resulting from the sample itself limits the usefulness of the system, specially in the spectral region 3700–3100 cm^{-1}.

In fact water, always abundant in the headspace of living organisms, limits the usefulness of GC–DD-IR in animal and plant odours studies and we tested various methods for isolation, transfer and concentration of natural samples: trapping on adsorbent, cryo-trapping followed by extraction, thermal desorption or solid-phase microextraction (SPME).

There are substantial differences between the techniques, and each has its strengths and weaknesses but SPME provides many advantages over conventional sample preparation techniques. SPME is very simple; it takes only a few minutes to complete and uses no solvent. It can be applied to flavour analysis of various samples [3]. This sample technique based on adsorption is used to preconcentrate trace compounds. We noticed by GC–MS that SPME on hydrophobic phase used in the headspace

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mode or even directly in an aqueous sample leads to a rigorous absence of water [4,5].

After preliminary experiments comparing many IR spectra obtained by SPME–GC–DD-IR with spectra obtained by KBr discs, as instruments using this cold interface can produce modified spectra [6], we applied this method to the identification of unknown volatiles trapped in situ.

The combination of GC–IR with GC–MS appears to be of interest in insect pheromone identification [7–9] and some positive experiments of trapping airborne pheromones by SPME have already been published [10]. We tried to identify the asparagus fly (*Platyparea poeciloptera*) male pheromone using SPME–GC–DD-IR and SPME–GC–MS.

2. Experimental

2.1. Instrumentation

2.1.1. Gas chromatography–mass spectrometry (GC–MS)

Chromatographic separations were performed using a Hewlett-Packard (Palo Alto, CA, USA) HP 5890 II instrument with a split–splitless injector equipped with a special liner (85 μl) adapted to SPME (no solvent) and a fused-silica capillary column (20 m×0.22 mm I.D.) with an HP-1 methylsilicone fiber (film thickness 0.33 μm).

The conditions were as follows: injector temperature, 200°C; carrier gas, helium; column flow-rate, 1.0 ml/min; splitless. Column temperature program, 40 to 200°C at 2°C/min.

Total ion chromatograms (TIC) and mass spectra were recorded using a Hewlett-Packard HP 5898 'mass engine' with an HP UX work-station in the electron impact (EI) ionisation mode at 70 eV or in the chemical ionisation (CI) mode using methane. The transfer line was maintained at 100°C, the source temperature at 200°C and the quadrupole temperature at 100°C.

2.1.2. Gas chromatography–IR spectrometry (GC–IR)

GC separations were performed using exactly the same instrument and the same conditions as for GC–MS.

The Gram-Schmidt reconstructed chromatogram (GSC) the functional group chromatograms (FGC) and the IR spectra were recorded using a Bio-Rad Digilab (Cambridge, MA, USA) FTS 45 A spectrometer equipped with a Digilab Tracer direct deposition interface for GC–DD-IR containing its own nitrogen-cooled MCT detector and with an SPC 3200 system for data acquisition and instrument control. The transfer line was maintained at 250°C and real-time spectra were obtained by addition of four scans.

In the direct deposition interface Tracer pure column effluent is condensed on a moving Zn–Se window transparent to IR radiation and cooled to 77 K. The width of the spots is about 0.1 mm at the limit of detection (about 100 pg) and the eluates can be scanned immediately by means of Fourier transform (FT) IR microscopy.

2.2. SPME of volatiles from living organisms

The individual pheromonal emission of 8 days-old males of *Platyparea poeciloptera* was sampled at mid-day when the males adopt a very special behaviour distending their two abdominal pouches [11].

During the headspace transfer, the fused-silica fiber coated with poly(dimethylsiloxane) (100 μm) was introduced by a hole (diameter: 1 mm) into the box (4 ml) containing the insect and exposed for 1 min in close proximity (5–10 mm) to it. Then the fiber was immediately inserted in the GC injector for desorption (2 min).

Reference spectra of pure compounds were obtained rapidly by opening commercial flasks and introducing the fiber to the neck of the flasks for approximately 10 s, more or less at a depth according to the relative supposed volatility of the compounds.

3. Results and discussion

The presence of solid ice in the window appears in the spectra as band broadening. This ice is introduced with the sample and also can result from insufficient conditioning of the system. Typically one day of high vacuum pumping is necessary to dry the

chamber after opening to atmosphere for routine maintenance.

Spectra obtained by the 'Tracer' are characteristic of a solid-phase sample in the amorphous state. We can, however, notice crystalline features such as band splitting (especially the CH_2-rocking doublet at 720 cm^{-1}) and fringing effects.

As well as exhibiting further evidence for crystallisation, C–O, C–N, O–H and N–H bonds are significantly broadened reflecting the strong influence of hydrogen bonding on the corresponding vibrations.

Spectra obtained by the 'Tracer' are in fact very similar with spectra obtained in KBr discs but some bands like C=O are shifted towards lower wavenumbers by about 10 cm^{-1} (Fig. 1). Spectra obtained by SPME–GC–DD-IR usually look more clean than those obtained by all other sampling methods without systematic band broadening due to the presence of ice. It is very fast and easy to use SPME–GC–DD-IR to obtain the spectrum of reference compound by simply opening a bottle without removing any sample.

For the example of the asparagus fly pheromone, the SPME fiber trapped the emission of a single individual during only 1 min exposure and this quantity is sufficient to obtain a good IR spectrum showing strong absorptions (Fig. 2) and good mass spectra even in CI (Fig. 3).

The mass spectrum in EI does not correspond to any spectrum in the library (Wiley 138 K Mass Spectral Database, Wiley, New York, USA) and the IR spectrum shows the presence of C=O, C–H, OH bonds but, for example, no unsaturation. The good peak shape on an apolar GC column is also incompatible with an acidic structure. The CI mass spectrum shows a molecular mass of 114 (Fig. 3b), corresponding to the functional groups identified by IR as a saturated cyclic hydrocarbon with ketonic and hydroxyl functional groups.

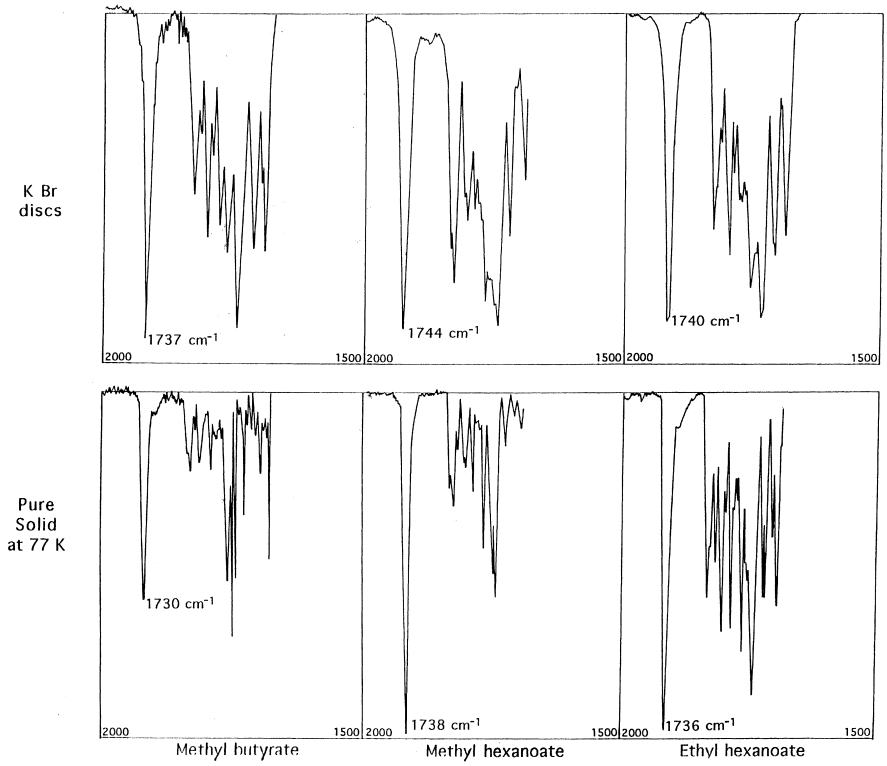

Fig. 1. Comparison of IR spectra (C=O region) obtained by KBr discs and direct deposition GC–IR (Digilab Tracer): (a) methyl butyrate, (b) methyl hexanoate, (c) ethyl hexanoate.

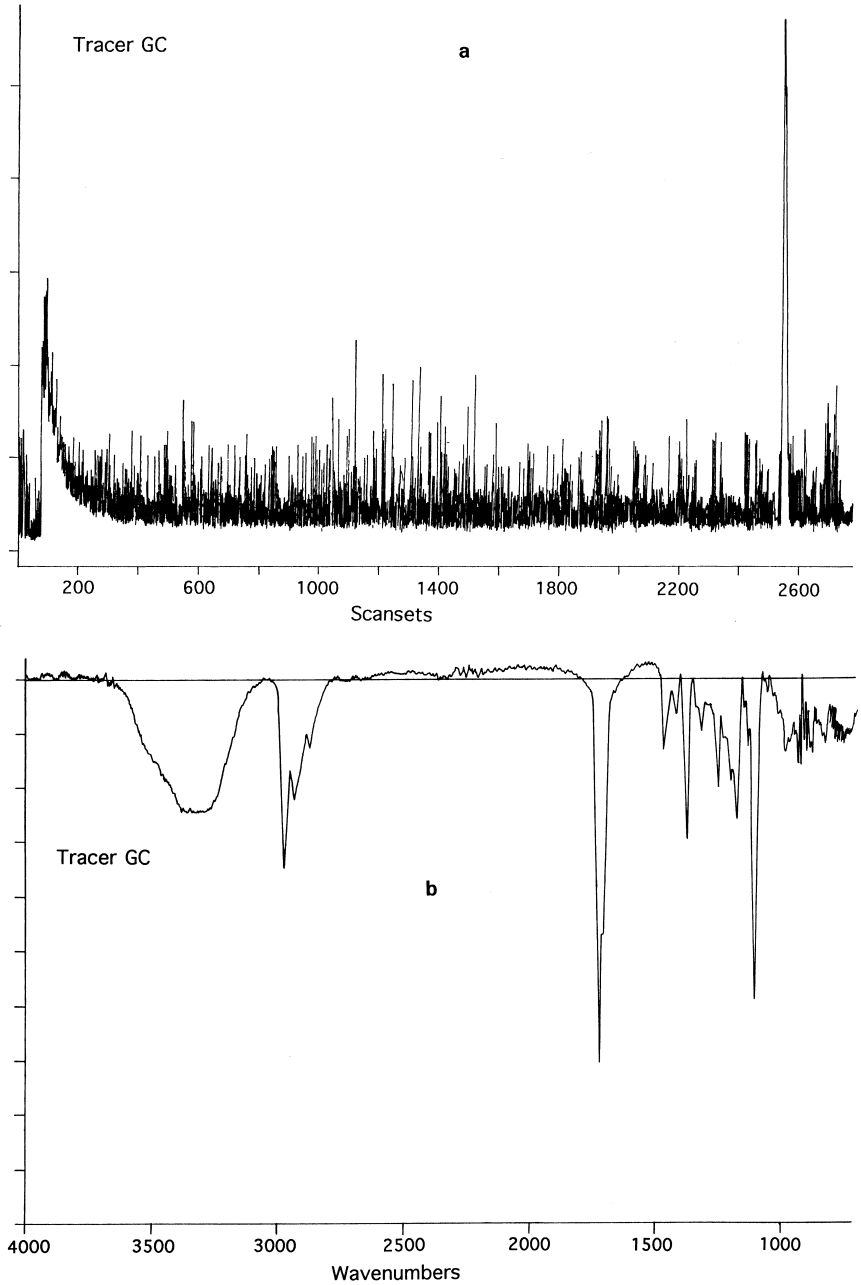

Fig. 2. (a) Gram-Schmidt reconstructed chromatogram and (b) IR spectrum of male *Platyparea poeciloptera* pheromone obtained by SPME–GC–DD-IR in one individual emission (Digilab 'Tracer'). For conditions, see Experimental.

Many isomeric structures are possible and we propose tentatively 1-hydroxy-ethyl cyclopropyl ketone because the fractionation of this structure leads to all the ions of the EI mass spectrum (Fig. 3a). The CH_2 stretches of this strained cyclic structure occur at frequencies of about 2980 cm^{-1}, higher than the corresponding frequencies of acyclic CH_2 stretches but, however, above those generally published for cyclopropanes [12].

The synthesis of this compound is now in progress

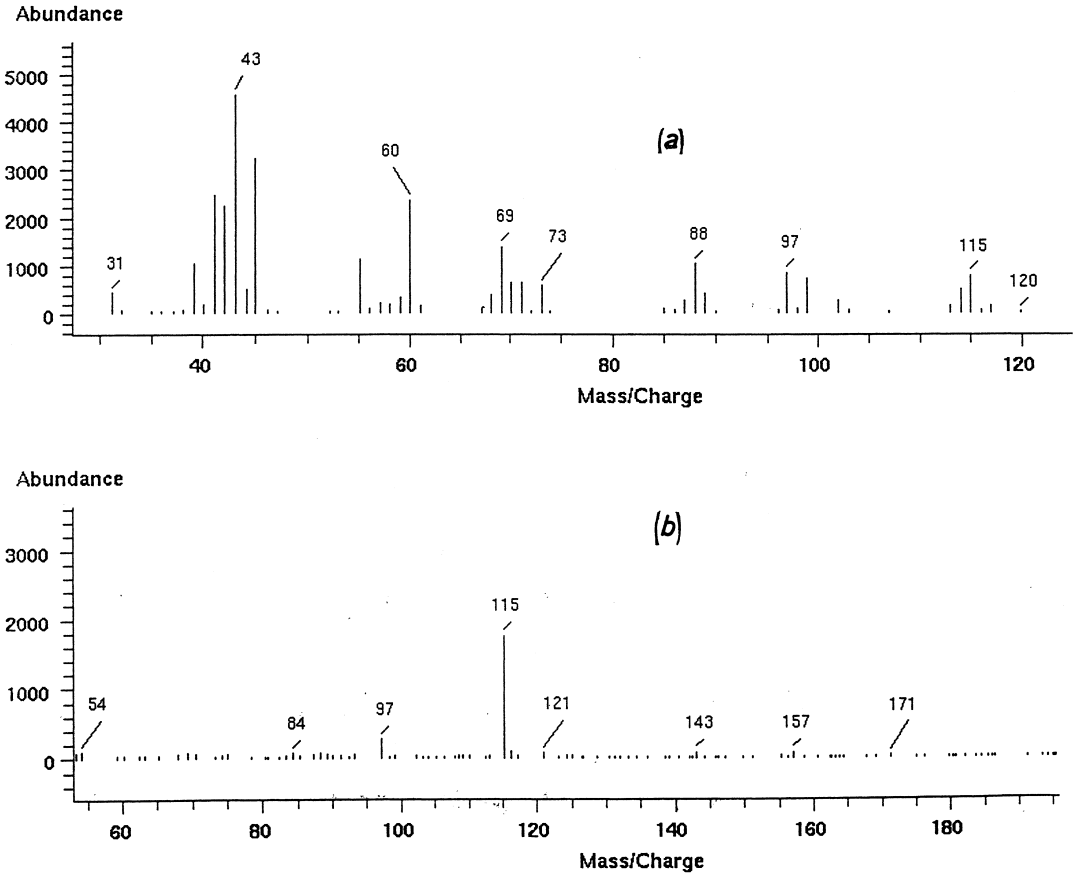

Fig. 3. Mass spectra of male *Platyparea poeciloptera* pheromone obtained by SPME–GC–MS coupling in one individual emission: (a) EI, (b) CI (HP 'mass engine' quadrupole).

and the first results indicate our choice to be good: one component of the synthetic mixture has the same IR and MS spectra and the same retention time using the above chromatographic conditions.

4. Conclusion

Coupling of SPME to GC–IR can permit the development of a GC–direct deposition–IR interface to-date limited by its water sensitivity. As SPME is a rapid sampling device and permits a significant reduction of the duration of GC analysis (solvent-free), SPME–GC is specially interesting for living organisms. For instance, the sensitivity of SPME–GC–DD-IR allows one to follow the kinetics of pheromonal emission of an individual insect 'on-line' [11]. In conclusion, this technique is a powerful and appropriate alternative to the now classical SPME–GC–MS techniques [13].

Acknowledgements

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References

[1] J. Auger, S. Ferary, J. Chromatogr. A 683 (1994) 87–94.
[2] S. Ferary, J. Auger, A. Touché, Talanta 43 (1996) 349–357.

[3] X. Yang, T. Peppard, J. Agric. Food Chem. 42 (1994) 1925–1930.
[4] S. Ferary, J. Auger, J. Chromatogr. A 750 (1996) 63–74.
[5] B. Jaillais, V. Bertrand and J. Auger, Talanta, in press.
[6] P. Jackson, G. Dent, D. Carter, D.J. Schofield, J.M. Chalmers, T. Visser, M. Vredenbregt, J. High Resolut. Chromatogr. 16 (1993) 515–521.
[7] C.A. Mc Daniel, J.O. Schmidt, R.W. Howard, J. Chem. Ecol. 18 (1992) 27–37.
[8] E. Thibout, S. Ferary, J. Auger, J. Chem. Ecol. 20 (1994) 1571–1581.
[9] W.S. Leal, M. Hasegawa, M. Sawada, M. Ono, J. Chem. Ecol. 20 (1994) 1705–1717.
[10] C. Malosse, P. Ramirez-Lucas, D. Rochat, J.P. Maurin, J. High Resolut. Chromatogr. 18 (1995) 669–670.
[11] E. Thibout, B. Jaillais and J. Auger, J. Chem. Ecol., submitted for publication.
[12] D. Lin-Vien, N.B. Colthup, W.G. Fateley, J.G. Grasselli, in: Handbook of Infrared and Raman Characteristic Frequencies of Organic Molecules, Academic Press, San Diego, CA, 1991, p. 503.
[13] F. Pelusio, T. Nilsson, L. Montanarella, R. Tilio, B. Larsen, S. Facchetti, J.O. Madsen, J. Agric. Food Chem. 43 (1995) 2138–2143.