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Fiber introduction mass spectrometry: determination of pesticides in herbal infusions using a novel sol–gel PDMS/PVA fiber for solid-phase microextraction

Rogério Cesar da Silva,[1] Vânia Gomes Zuin,[2] Janete Harumi Yariwake,[2] Marcos Nogueira Eberlin[1]* and Fabio Augusto[3]

[1] ThoMSon Mass Spectrometry laboratory, Institute of Chemistry, State University of Campinas, 13084-971, Campinas, SP, Brazil
[2] Institute of Chemistry of São Carlos, São Paulo University, 13560-970, São Carlos, SP, Brazil
[3] Gas Chromatography Laboratory, Institute of Chemistry, State University of Campinas, 13084-971, Campinas, SP, Brazil

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An application of the direct coupling of solid-phase microextraction (SPME) with mass spectrometry (MS), a technique known as *fiber introduction mass spectrometry* (FIMS), is described to determine organochlorine (OCP) and organophosphorus (OPP) pesticides in herbal infusions of *Passiflora* L. A new fiber coated with a composite of poly(dimethylsiloxane) and poly(vinyl alcohol) (PDMS/PVA) was used. Sensitive, selective, simple and simultaneous quantification of several OCP and OPP was achieved by monitoring diagnostic fragment ions of *m/z* 266 (chlorothalonil), *m/z* 195 (α-endosulfan), *m/z* 278 (fenthion), *m/z* 263 (methyl parathion) and *m/z* 173 (malathion). Simple headspace SPME extraction (25 min) and fast FIMS detection (less than 40 s) of OCP and OPP from a highly complex herbal matrix provided good linearity with correlation coefficients of 0.991–0.999 for concentrations ranging from 10 to 140 ng ml^{-1} of each compound. Good accuracy (80 to 110%), precision (0.6–14.9%) and low limits of detection (0.3–3.9 ng ml^{-1}) were also obtained. Even after 400 desorption cycles inside the ionization source of the mass spectrometer, no visible degradation of the novel PDMS/PVA fiber was detected, confirming its suitability for FIMS. Fast (*ca* 20 s) pesticide desorption occurs for the PDMS/PVA fiber owing to the small thickness of the film and its reduced water sorption. Copyright © 2007 John Wiley & Sons, Ltd.

KEYWORDS: solid-phase microextraction (SPME); fiber introduction mass spectrometry (FIMS); PDMS/PVA fibers; *Passiflora* L. infusion; pesticides

INTRODUCTION

Solid-phase microextraction (SPME) is a relatively new and very successful sample concentration technique that has been largely employed for the extraction of many types of volatile and semivolatile organic compounds mainly from environmental, biological and food sample matrices.[1,2] The main SPME features include simplicity, high sensitivity, reliability, portability and easy of automation. It represents an environmentally friendly alternative to traditional sample preparation techniques since it eliminates the use of undesirable organic solvents.[3] In SPME, a small piece of fused silica is coated with a thin film of polymeric phase (e.g. poly(dimethylsiloxane) (PDMS), polyacrylate) or dispersions of solid adsorbents in polymers (e.g. PDMS/divinylbenzene (PDMS/DVB), PDMS/Carboxen). This film has the ability to both sorb and concentrate the organic analyte.[4] The limited variety of commercial SPME sorbents demands, however, the development of new experimental fiber materials. These materials could be prepared by sol–gel routes, offering many advantages compared to commercially available alternatives, such as the strong adhesion of the prepared coatings to the fused silica substrate due to chemical bonding, different ranges of selectivity, highly porous structure, large surface area and remarkable thermal stability.[5,6] We recently developed a novel sol–gel PDMS/poly(vinyl alcohol) (PDMS/PVA) coated fiber and showed it to be highly efficient to extract nonpolar and semipolar compounds from aqueous samples.[7,8]

Recently, we have also described a new technique named *fiber introduction mass spectrometry* (FIMS) by which the full direct coupling of SPME and MS without chromatographic separation was achieved.[9] FIMS is an attractive approach integrating the convenience of SPME as a sample preparation technique and the speed, sensitivity and selectivity of MS as the detection and quantitation technique.[10] In this hyphenated technique, the SPME fiber, containing analytes sorbed (extracted) from a sample, is directly introduced into the ionization chamber of a mass spectrometer and placed between the two electron ionization (EI) filaments. The combination of the heating promoted by irradiation from the filaments and the high vacuum causes desorption

*Correspondence to: Marcos Nogueira Eberlin, ThoMSon Mass Spectrometry Laboratory, Institute of Chemistry, State University of Campinas, 13084-971 Campinas, SP, Brazil.
E-mail: eberlin@iqm.unicamp.br

of the extracted species, directly in the region of maximum ionization power of the MS. Monitoring of diagnostic ions of each analyte allows fast, selective and sensitive quantitation. A possible drawback to FIMS is that the lifetime of the fiber can be considerably reduced owing to the stress imposed during desorption. Riter *et al*.[11] used a variation of the PDMS-FIMS device coupled to a portable mass spectrometer to determine several volatile organic compounds in air and the headspace over aqueous solutions with limits of detection (LOD) in the low nanogram per milliliter range. The use of the two methods in tandem, single-sided membrane introduction mass spectrometry (SS-MIMS) and FIMS, as a technique for field analysis has been described by Cotte-Rodriguez and collaborators.[12] FIMS has also been employed to determine explosive emulator *ortho*-nitrotoluene and the chemical warfare agent emulator methyl salicylate in air with LOD in the nanogram per milliliter level. The application of FIMS to real matrices was reported by Van Hout *et al*.[13], who quantified levels down to 1 ng ml^{-1} of lidocaine in urine, after 1 min of extraction with 30 μm PDMS fibers, totaling 3 min for the overall analysis. We have also applied FIMS with the PDMS/DVB fiber to perform simple extraction and MS detection of phthalates in mineral water with good linearity and precision with LOD below the maximum phthalate concentration allowed by the U. S. Environmental Protection Agency (USEPA) for drinking water.[14]

Infusions of *Passiflora* L. leaves are extensively consumed as phytomedicines owing to their sedative and antianxiolytic properties. In Brazil, owing to its large use in the fruit juice industry and the fresh fruit market,[15,16] *Passiflora edulis* Sims. f. *flavicarpa* Deg. is one of the most common species available. In general, pesticides are not allowed in medicinal plant cultivation, but in species such as *Passiflora edulis* Sims. f. *flavicarpa* Deg. traces of these compounds can be found in the dried leaves since they are used for fruit crop protection. This possibility makes trace determination of pesticides in phytomedicines prepared from *Passiflora* L. a major analytical task.[17] Official methods to assess pesticide contamination in phytopharmaceuticals, such as given in the European[18] or the British Pharmacopoeias,[19] require the use of outdated sample preparation approaches such as liquid–liquid extraction (LLE) or solid–liquid extraction (SLE). In addition to the use of large amounts of toxic solvents, these traditional extraction methods are time consuming and involve labor-intensive clean-up procedures. After the steps of extraction and clean-up, the detection and quantitation of the organochlorine pesticides (OCP), organophosphorus pesticides (OPP) and pyrethroid pesticides should be performed by gas chromatography (GC) with a nitrogen/phosphorus detector (NPD), electron capture detector (ECD) or even an atomic emission spectrometry detector (AED).[18]

To improve trace pesticide determination in herbal infusions in regard to superior selectivity, sensitivity, speed, simplicity and robustness, we tested FIMS using a novel and thermally stable sol–gel PDMS/PVA SPME fiber. We tested the method for its ability to extract and quantify traces of OCP and OPP in herbal infusions of *Passiflora* L.

EXPERIMENTAL

Samples

Leaves of *P. edulis* Sims. f. *flavicarpa* Deg. were obtained from cultivated specimens grown in the city of Ribeirão Preto (State of São Paulo, Brazil). The plant material was dried at 35 °C for 24 h, powdered, sieved (1–2 mm) and stored in glass flasks protected from humidity, heat and light. Infusions were prepared on the same day of use by suspending (1.00 ± 0.05) g of dried leaves in 100 ml of boiling water for 5 min.

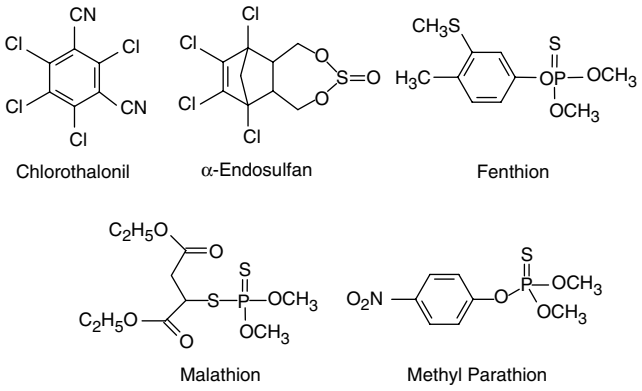
Materials

The PDMS/PVA fibers (5 μm) were obtained as described previously.[7] Commercial SPME fibers coated with 65 μm PDMS/DVB and fitted in an appropriate holder (Supelco, Bellefonte, USA) were also used. Prior to use, the PDMS/PVA fibers were conditioned at 280 °C for approximately 6 h in a GC injection port under the flow of the carrier gas (He); the PDMS/DVB fibers were conditioned according to the supplier's instructions.

The pesticides studied (Scheme 1) were selected among the most common ones used in *Passiflora* L. cultivation, on the basis of a field survey in Brazil. Individual 0.1 g l^{-1} stock solutions of the OCP and the OPP were prepared in methanol from the pure (≥99%) compounds (ChemService, Westchester, USA). A methanolic working solution with adequate concentrations for each analyte was used to spike infusion samples for method optimization and validation. For all extractions, 16 ml glass vials capped with Teflon/silicone septa (Pierce, Rockford, USA) were used. During the extractions, the vials were thermostatted in water from a heated circulating bath (Polystat – Cole Parmer, Vernon Hills, IL., USA).

HS-SPME procedure

In the HS-SPME procedure with the PDMS/PVA fiber,[8] 5 ml of aqueous sample saturated with NaCl (2.5 ml of *Passiflora edulis.* infusion plus 2.5 ml of Milli-Q water) was enclosed in the vials and magnetically stirred (1200 rpm) for 5 min for sample/headspace equilibration. Then, the PDMS/PVA fiber was exposed to the sample headspace for 20 min at 67.5 °C. Using a FIMS device, the extracted analytes were immediately desorbed from the fiber inside the ionization region of the MS for 40 s, a period long enough to ensure



Scheme 1. The compounds studied.

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total desorption and no memory effects. All extractions were performed in triplicate.

FIMS procedure

The SPME (PDMS/PVA) determination of OCP and OPP pesticides in *Passiflora* L. infusions was previously studied and optimized by us using a GC-ECD system.[8] All FIMS measurements described here were carried out using an Extrel (Pittsburgh, USA) mass spectrometer fitted with a high-transmission 3/4-in. quadrupole and a EI/CI ion source, the details of which are given elsewhere.[20] The coated fiber was placed right between the two parallel MS filaments for uniform heating, efficient desorption and 70 eV EI of the analytes. The operating conditions for FIMS for the analysis with the PDMS/PVA fiber were studied using *P. edulis* infusions spiked with 100 ng ml^{-1} of chlorothalonil, methyl parathion, malathion, α-endosulfan and fenthion. The extracted analytes were immediately desorbed inside the MS after the extraction. The desorption time was 40 s for all experiments and no carryover between runs was observed with this condition. The MS gain and electron multiplier voltages were 1×10^{11} and 1200 V, respectively. Detection and quantitation of the pesticides were performed by selective ion monitoring (SIM) of diagnostic fragment ions of m/z 266 for chlorothalonil, m/z 195 for α-endosulfan, m/z 278 for fenthion, m/z 263 for methyl parathion and m/z 156 for malathion. Analytical curves obtained for the concentration range between 10 and 140 ng ml^{-1} allowed assessment of quantitative figures of merit of the FIMS method. The LOD and the limits of quantitation (LOQ) were calculated from signal-to-noise ratios (S/N) of 3 and 10, respectively, estimated from data collected from extractions of 10 ng ml^{-1} for the OCP and OPP in *Passiflora* L. infusions. Recovery studies were performed with infusion samples spiked with 100 and also 20 ng ml^{-1} of each pesticide.

RESULTS AND DISCUSSION

The PDMS/PVA and PDMS/DVB fibers were preconditioned in a GC injector, as well as by repeated introductions (*ca* 5–6 times) into the FIMS system and exposure of their coatings to the EI filaments up to 1 min. After the preconditioning, a clean baseline was observed (Fig. 1(A) for the PDMS/PVA fiber), which facilitates the identification of both OCP and OPP. Deterioration of the coatings of the PDMS/PVA and PDMS/DVB fibers were noted only after *ca* 400 and 150 desorption cycles, respectively. The improved stability of the PDMS/PVA fiber was attributed to the incorporation of poly(vinyl alcohol) as the organic modifier, which results in cross-linked coatings that imparts higher porosity and extractive capacity compared to pure sol–gel PDMS.[7]

Figure 1(B) presents the EI-FIMS obtained for unspiked *Passiflora* L. infusion after PDMS/PVA extraction. No significant ions of m/z higher than 150 are detected in this spectrum. The ions at m/z 149 and below probably resulted from volatile endogenous matrix compounds, such as terpenic hydrocarbons, as well as other nonpesticide contaminants such as phthalic acid esters. Since the diagnostic ions of all pesticides display m/z higher than 150 (Fig. 2), adequate

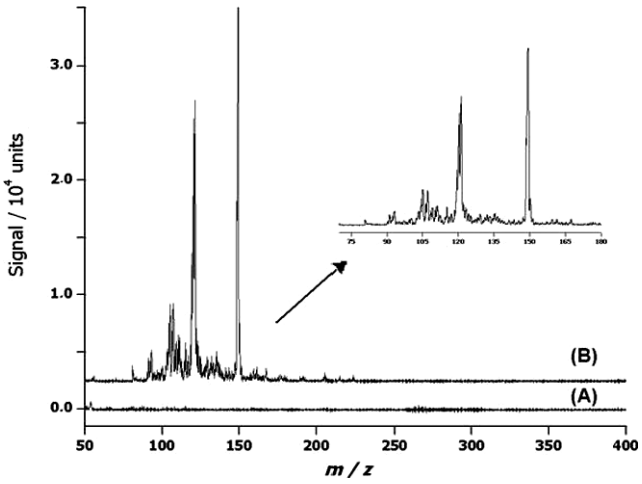

Figure 1. 70 eV EI-FIMS using the PDMS/PVA fiber for (A) blank spectrum (B) *Passilora* L. infusion acquired after nearly 1 min of the desorption period.

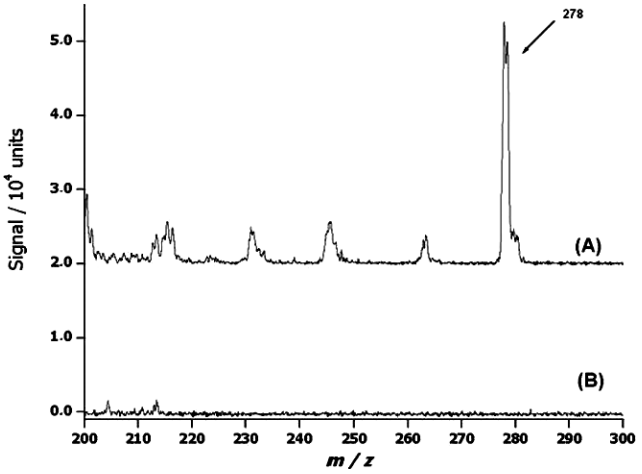

Figure 2. (A) 70 eV EI-FIMS using the PDMS/PVA fiber of infusions spiked with 100 ng ml^{-1} of fenthion and (B) blank extraction.

OCP and OPP determination in such complex samples can be performed without major matrix interferences.

Figures 3 and 4 show typical SIM-FIMS profiles for chlorothalonil and fenthion using either PDMS/PVA or PDMS/DVB fibers. Note that time 'zero' on these graphs corresponds to the instant of exposure of the fiber to the MS ionization chamber. The absolute extracted amounts with PDMS/DVB fiber is larger than those with the PDMS/PVA fiber (Figs 3 and 4), which is expected considering that the PDMS/DVB fiber has a thicker sorptive coating: 65 *vs* 5 μm. PDMS/PVA provides, however, faster desorption and a more symmetric SIM peak for both analytes. Furthermore, the different polarities of the fibers influence the amount of water that is sorbed by the fibers. The more polar PDMS/DVB fiber sorbs much more water than PDMS/PVA; therefore when inserted in the ion source, rapid desorption of a large number of water molecules increases the pressure and chemical noise which raises the base line, producing a 'ghost' peak at 4 s even when using SIM (for the ions of m/z 266 and 278). The analyte FIMS peaks reach a maximum

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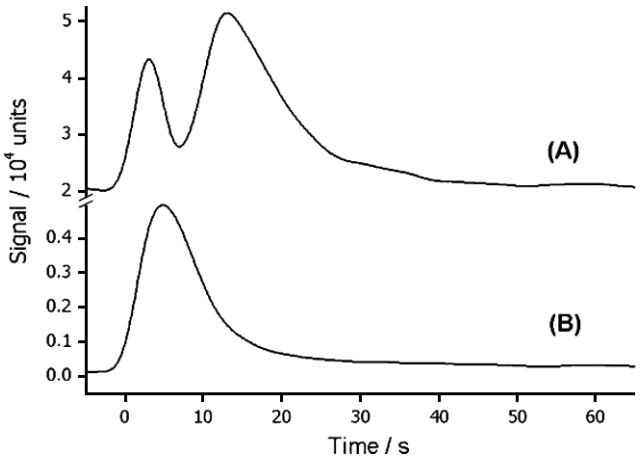

Figure 3. SIM-FIMS profiles for 100 ng ml^{-1} of chlorothalonil (*m/z* 266) in *Passiflora* L. infusions using (A) PDMS/DVB and (B) PDMS/PVA fibers.

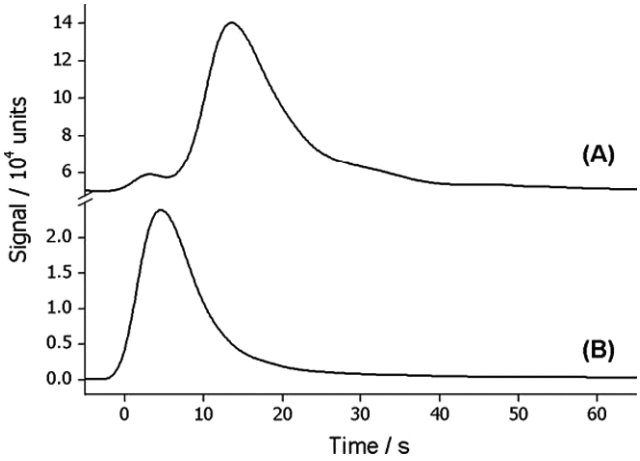

Figure 4. SIM-FIMS profile for 100 ng ml^{-1} of fenthion (*m/z* 278) in *Passiflora* L. infusions using (A) PDMS/DVB and (B) PDMS/PVA fibers.

at approximately 19 s. For the PDMS/PVA fiber with a much thinner and less polar coating, no 'water peak' is observed, and the analyte is detected at 8 s as a nearly ten times less intense peak. The PDMS/PVA fiber sorbs little or no water, and also has an extended lifetime (400 SPME-FIMS desorption cycles *vs* 150 for PDMS/DVB); hence it has an excellent combination of properties for FIMS. One of the most attractive features of sol–gel coatings for SPME is the thickness of the coatings; hence it is possible to obtain extremely thin films of sorbents with large extractive power[21] such as for the PDMS/PVA fiber presented here. The FIMS results using the 5 μm PDMS/PVA fiber confirm such characteristics, allowing faster extractions and better thermal desorption when compared with the conventional PDMS/DVD fiber.

Analytical method validation

Table 1 shows the validation results for the multiresidue procedures for OCP and OPP in the *Passiflora* L. infusions. The analytical curves were constructed with six concentration levels for each analyte. LOD and LOQ ranged from 0.3 to 3.9 ng ml^{-1} and 1 to 13 ng ml^{-1}, respectively, and could be considered adequate for the analytical purposes.[16] Good correlation was obtained among the curves with coefficient between 0.991 to 0.999; also, the *F*-test results indicate that the analytical curves can be considered as linear with 95% confidence. The sensitivity, expressed as the inclination of the analytical curves, increases in the order fenthion > α-endosulfan > methyl parathion > chlorothalonil > malathion.

Accuracy of the multiresidue method was also determined by calculating the recovery for extractions of *Passiflora* L. infusions ($n = 3$) spiked with 20 and 100 ng ml^{-1} of the analytes. Average recoveries ranged from 80% for α-endosulfan (RSD = 14.9%) to 110% for methyl parathion (RSD = 1.8%), which is adequate for the quantitation of the selected OCP and OPP in *Passiflora* L. infusions within the concentration range studied.

CONCLUSIONS

FIMS using a new fiber coated with a PDMS/PVA composite has been shown to provide an effective and environmentally friendly alternative to determine pesticides in herbal infusions, as demonstrated here for OCP and OPP residues in *Passiflora* L. infusions. Simplicity, speed, selectivity as well as robustness are the main advantages found for the (PDMS/PVA)-FIMS method. Low LOD and LOQ were obtained for the pesticides in a much shorter time of analysis when compared to official methods. Faster detection

Table 1. Slopes *S*, intercepts *I* and correlation coefficients *r* for the analytical curves obtained for multiresidue determination, limit of detection (LOD) and limit of quantitation (LOQ) in ng ml^{-1}, *F*-test parameter, recovery (*R*) and RSD for OCP and OPP in *P. edulis* infusions using FIMS with the PDMS/PVA fiber

Analyte[e]	S	$I/10^3$	*R*	LOD	LOQ	*F*[a]	*R*[b] (%)	*R*[c] (%)	RSD[d] (%)
MA	23 ± 1	1.24 ± 0.09	0.994	3.9	13	318	–	104	3.6
AE	189 ± 6	1.2 ± 0.5	0.998	0.9	3	908	80	105	5.3
MP	90 ± 3	0.1 ± 0.2	0.998	0.9	3	1125	110	103	6.1
CT	27 ± 2	0.3 ± 0.1	0.991	2.8	9	232	85	88	4.7
FT	546 ± 14	1 ± 1	0.999	0.3	1	1532	91	96	4.4

[a] F = critical value, 95% confidence: $F_{2,4} = 6.94$. [b] Sample spiked with 20 ng ml^{-1}. [c] Sample spiked with 100 ng ml^{-1}. [d] RSD = average RSD for all triplicate signal measurements. [e] MA, malathion; AE, α-endosulfan; MP, methyl parathion; CT, chlorothalonil and FT, fenthion.

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(20 s) and better signal resolution is obtained with the 5 μm PDMS/PVA fiber as compared to a PDMS/DVB commercial fiber owing to smaller film thickness and consequently the extraction of reduced amounts of interfering water.

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