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Application of a novel sol–gel polydimethylsiloxane–poly(vinyl alcohol) solid-phase microextraction fiber for gas chromatographic determination of pesticide residues in herbal infusions

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Abstract

A simple and environmentally friendly methodology for headspace solid-phase microextraction (HS-SPME) using a new fiber coated with polydimethylsiloxane–poly(vinyl alcohol) (PDMS/PVA) is reported for the trace determination of organochlorine (OCP) and organophosphorus (OPP) pesticides in herbal infusions of *Passiflora* L. by GC-ECD. The capacity of the PDMS/PVA coating for the pesticides was compared to that of commercial PDMS fibers, with advantageous results. The effects of parameters such as the sample ionic strength, dilution of the infusion, extraction temperature and time were investigated. The optimized conditions for the determination of OCP and OPP in *Passiflora* L. infusions were extraction time and temperature, respectively, of 38 min and 67.5 °C, with 5 min of sample/headspace equilibration time. The analytical curves for the range between 0.04 ng mL^{-1} to 6 ng mL^{-1} of each compound presented a good quality (correlation coefficients of 0.921 or better). The detection limits for the OCP and OPP in these matrices varied from 0.01 ng mL^{-1} (β-endosulfan) to 1.5 ng mL^{-1} (malathion). The sensitivity of studied methodology was adequate, as well as its accuracy (78.7–91.5%) and precision (R.S.D. = 1.2–14.2%).

Keywords: *Passiflora* L.; Phytomedicines; Pesticides

1. Introduction

Solid-phase microextraction (SPME) is a relatively recent technique that has been used for the extraction and preconcentration of a wide range of analytes in a variety of matrices [1]. SPME is based on the equilibrium of target analytes between a fused silica fiber coated with a thin film of sorbent and the sample matrix [2,3]. A number of sorbents is available commercially as coatings for SPME fibers but some analytical methodologies might demand specific properties for extraction of selected compounds, i.e., special coatings that have a particular volume and a selectivity towards particular analytes [4]. In addition to the most common liquid polymeric coatings such as polydimethylsiloxane (PDMS), some experimental coatings have been prepared and studied for several applications [5]. For example, fibers coated with alternative materials can be successfully prepared using sol–gel synthetic routes, resulting in many advantages such as the strong adhesion of the prepared coatings to the fused silica substrate due to chemical bonding, high thermal stability, porous structure and large surface area [6,7]. In this way, a novel sol–gel PDMS/poly(vinyl alcohol) (PDMS/PVA) coating fiber was recently developed and proved to be highly efficient to extract non-polar compounds from aqueous samples [8].

The PDMS/PVA fiber was applied to isolate different non-polar and polar pesticides from a complex sample—infusions of *Passiflora* L. These infusions have been extensively used as phytomedicines and studied in our research group [9–12]. Some species of *Passiflora* are employed in folk medicine due to their anxiolytic, hypnotic, antispasmodic and anodynic properties. *Passiflora incarnata* L. is the official species in European Pharmacopoeia [13] while *Passiflora alata* Dryander is the Brazilian official one [14]. Although the pharmaco-

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logical effects of *P. alata* leaves are reported in the Brazilian Pharmacopoeia, this species is often replaced by *Passiflora edulis* Sims, which is more commonly available owing to its extensive use in the fruit juice industry and the fresh fruit market [10]. Plant infusions are complex samples, containing several endogenous compounds extracted from the leaves by the hot water during their preparation. Also, it is possible to find a wide range of different pesticide residues on these infusions, depending on the procedence of a particular sample; when present, these analytes are usually found on extremely low levels ($\mu g\,L^{-1}$ or less). Therefore, the procedures for the chromatographic detection and quantitation of organochlorine (OCP) and organophosphorus (OPP) on *Passiflora* infusions must incorporate selective, robust and effective clean-up and extraction steps [15].

Concerning the presence of toxic compounds in medicinal plants such as pesticides, some of the most recent Pharmacopoeias, such as the British [16], American [17], and European ones [13], included methods for the determination of these compounds in herbal drugs. However, most of the official analytical methodologies proposed by pharmaceutical codes are based on traditional liquid–liquid or liquid–solid extraction, being time-consuming, costly and requiring larger samples and greater volumes of hazardous solvents. Moreover, *Passiflora* L. is extensively consumed mainly in the form of infusions of their dried leaves and alternative analytical methodologies for the determination of pesticides in aqueous matrices have not been established by such codes. Actually, there are only few studies based on modern sample preparation methods as SPME, SBSE (stirring bar sorptive extraction) and SFE (supercritical fluid extraction) focusing on the determination of pesticides in phytomedicines (herbal drugs, infusions, tinctures, dried extracts, etc.) [11,12,18,19].

Nowadays, the trend in analytical technologies is the miniaturization, automation, hyphenation, simplification and reduction of processing time and consumption of toxic solvents. So, in this work a simple and environmentally friendly methodology for the determination of OCP and OPP residues in *Passiflora* infusions (*P. alata*, *P. edulis* and *P. incarnata*), using headspace SPME with a new PDMS/PVA fiber coupled to gas chromatography with electron capture detection (GC-ECD) is presented.

2. Experimental

2.1. Materials

The PDMS/PVA fibers ($d_f \sim 5\,\mu m$) used in this work were prepared according to Lopes and Augusto [8]. Commercial SPME fibers coated with 30 μm PDMS (P30) and fitted in an appropriate holder (Supelco Inc., Bellefont, PA, USA) were also used. Prior use, the fibers were conditioned at 280 °C for approximately 6 h in a GC injection port under flow of the carrier gas (He); the PDMS fibers were conditioned according to the supplier's instructions.

P. alata, *P. edulis* and *P. incarnata* leaves were obtained from cultivated specimens grown in the cities of Ribeirão Preto and Botucatu (State of São Paulo, Brazil). The plant material was dried at 35 °C for 24 h, powdered, sieved (1–2 mm) and stored in sealed glass flasks protected from humidity, heat and light. Infusions for each different specimen were prepared in the same day of use by suspending 1.00 ± 0.05 g of dried leaves in 100 mL of boiling water for 5 min; the infusions were filtered before use.

The pesticides studied were selected according a preliminary field survey, which revealed the most common compounds used in *Passiflora* cultivation: chlorothalonil, methyl parathion, malathion, α-endosulfan and β-endosulfan. Individual $0.1\,g\,L^{-1}$ stock solutions of the OCP and the OPP were prepared in methanol from the corresponding pure (≥99%) substances (ChemService, Westchester, PA). A methanolic working solution with adequate concentrations for each analyte (from $0.2\,ng\,mL^{-1}$ for endosulfan to $6\,ng\,mL^{-1}$ for malathion) 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, IL, USA) were used. During the extractions, the vials were thermostatized using in water from a heated circulating bath (Polystat, USA).

2.2. Chromatographic analysis

The chromatographic analysis were performed using an AutoSystem XL GC (Perkin–Elmer, Norwalk, CT) equipped with an electron capture detector and fitted with a HP-1 column (30 m × 0.32 mm × 0.25 μm), in the splitless mode. Injector and detector temperature: 260 °C and 320 °C, respectively. The GC-ECD conditions were: oven temperature program: from 140 °C (1 min) to 200 °C at $8\,°C\,min^{-1}$ (2 min), then to 280 °C at $15\,°C\,min^{-1}$ (1 min). Helium (UP grade) was used as carrier gas at a flow rate of $1.3\,mL\,min^{-1}$.

2.3. Method development

The operational conditions for the HS-SPME method were studied using *Passiflora* infusions spiked with $0.5\,ng\,mL^{-1}$ of chlorothalonil, $1.5\,ng\,mL^{-1}$ of methyl parathion, $3\,ng\,mL^{-1}$ of malathion, $0.1\,ng\,mL^{-1}$ of α-endosulfan and $0.1\,ng\,mL^{-1}$ of β-endosulfan. The parameters investigated were the ionic strength of the samples, the diluting matrix factor, the temperature and time of extraction. In the general procedure adopted through all experiments, 5 mL of sample spiked with pesticides were 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 an adequate period of time. The extracted analytes were immediately desorbed at 260 °C and the fiber was kept in the GC injector for 15 min, which was enough to ensure total desorption and no memory effects. All extractions were performed in triplicate.

The effect of ionic strength was assessed using as samples infusions containing varied concentrations of NaCl (up to saturation). The effect of infusion dilution on the extraction efficiency was studied using as samples infusion previously saturated with NaCl and diluted with aqueous saturated NaCl solution in the proportions 75:25, 50:50, and 25:75 (v/v infusion: aqueous saturated NaCl).

The optimization of extraction time, t_{ext}, and temperature, T, was performed simultaneously through a multivariate approach (2^2 factorial designs). The upper and lower levels for the variables in the initial experiments were 50 °C and 70 °C (T) and 20 min and 40 min (t_{ext}). After the initial factorial design, five additional experiments using T ranging from 57 °C to 78 °C and t_{ext} ranging from 17 min to 38 min were performed. The results were fitted to a proper response surface, which was employed to find values of T and t_{ext} where the extraction efficiency was maximized.

2.4. Headspace SPME procedure

After the experiments above described, the optimized method (50:50 diluted infusion saturated with NaCl as sample, t_{ext} = 38 min and T = 67.5 °C) was applied to estimate analytical curves for the studied OCP and OPP (0.04–6.0 ng mL^{-1}).

3. Results and discussion

3.1.1. Effect of ionic strength and infusion dilution on extraction efficiency

We choose to perform the extraction of the target analytes using the headspace mode, instead of direct immersion of the fiber in the infusion samples, to avoid contamination of the fiber surface by non-volatile matrix components, preserve its integrity and to minimize introduction of possible intererferents in the chromatographic system. Previous studies [20,21] already pointed the feasibility of application of headspace instead of direct immersion extraction for SPME determinations of OPP and OCP in similar samples.

In the study of the effect of ionic strength, it was observed that the addition of NaCl enhanced considerably the extraction efficiency. The best results for were obtained with addition of NaCl to the infusions until their saturation. In practice, the control of NaCl concentration was used to enhance extraction based on a similar principle employed for solvent extraction methods, i.e., the presence of dissociated ions in the tea solutions decreases the solubility of the OCP and OPP, which then partition more rapidly into the headspace. Considering these observations, all further experiments were conducted using infusions saturated with NaCl.

The effect of the sample dilution on the extraction efficiency can be seen on Fig. 1. To facilitate the interpretation of the data, the peak areas for all analytes extracted from diluted samples were normalized in relation to the corresponding peak areas for undiluted samples (normalized area = peak area for diluted sample/peak area for undiluted sample). For chlorotalonil, methyl parathion and malathion, the extraction efficiencies increase with infusion dilutions to 50:50. As for α- and β-endosulfan, the increment was observed even for 25:75 infusion dilution, but there is also a significant decrease on the precision of the data, as indicated by the larger R.S.D. values. This effect was already been observed and discussed in the literature [20] and it was attributed to the binding of the pesticides to matrix components such as pectin and flavonoid compounds. Most of the pesticides present in the infusions are bound to these heavy substances present in the infusions. When the sample is diluted, the corresponding binding equilibrium is affected and bound pesticides are released, increasing the concentration of free extractable species and the headspace extraction efficiency. For the continuing experiments, a 50:50 dilution of the sample was adopted as being the best compromise between extraction efficiency and overall precision for all analytes.

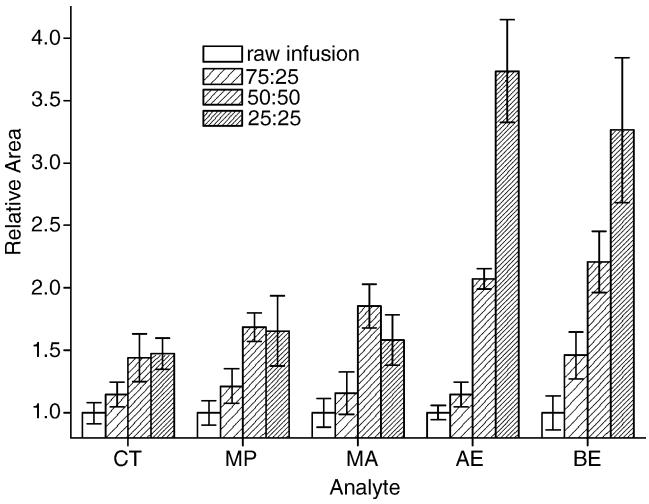

Fig. 1. Normalized peak areas as function of sample dilution; error bars represent the R.S.D. of the data (n = 3). Analytes: CT, chlorothalonil; MP, methyl parathion; MA, malathion; AE, α-endosulfan and BE, β-endosulfan.

3.1.2. Optimization of extraction time and temperature

The multivariate approaches here employed were adopted to minimize the number of assays necessary to find out the best working values for these variables. Also, these procedures allow the optimization of the variables even when their effect on the response is interdependent and not merely addictive [22]. The values for T and t_{ext} determined for maximization of the extraction efficiency after the multivariate experiments were, respectively, 67.5 °C and 38 min.

3.2. Comparison of PDMS/PVA and PDMS fibers

According to Lopes and Augusto [8], the morphology of the sol–gel PDMS/PVA fiber was investigated by the scanning electron microscopy technique, suggesting that the

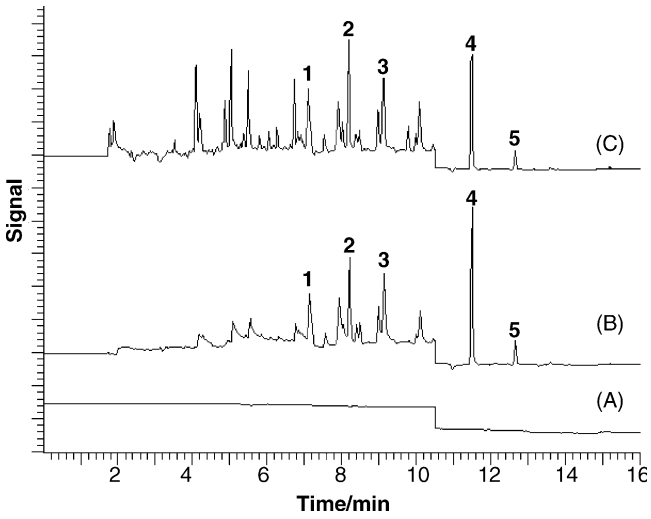

Fig. 2. Chromatograms for 30 min HS-SPME at room temperature from raw *P. alata* infusion spiked with the OPP e OCP: (A) blank run for the PDMS/PVA fiber; (B) 30 μm commercial PDMS fiber and (C) PDMS/PVA fiber. Peak identification: (1) chlorothalonil (0.5 ng mL^{-1}); (2) methyl parathion (1.5 ng mL^{-1}); (3) malathion (3 ng mL^{-1}); (4) α-endosulfan (0.1 ng mL^{-1}) and (5) β-endosulfan (0.1 ng mL^{-1}). The bump observed for t = 10.5 min corresponds to a software-programmed increase on the signal attenuation.

coating is a highly porous structure with an estimated average film thickness of 5 μm. This porous structure provided high surface area, allowing very fast and effective extraction. Also, the thermogravimetric data for the PDMS/PVA coating showed that this material is more stable towards heating than conventional PDMS, supporting temperatures higher than 300 °C without appreciable decompositon. As a result, the PDMS/PVA fibers have a remarkable long lifetime—a single PDMS/PVA fiber was recorded to be used for more than 300 extractions during a time range of more than 5 months before degradation of the coating.

Fig. 2 shows chromatograms from extractions of undiluted, unsalted *P. alata* infusions spiked with the analytes after extractions with the PDMS/PVA and with a commercial 30 μm PDMS fiber, obtained during preliminary exploratory experiments. A chromatogram for a blank run of the PDMS/PVA fiber obtained in the same conditions is also shown. Larger amounts of α-endosulfan and chlorotalonil were extracted by the 30 μm PDMS (as reflected by the slightly larger peaks); PDMS/PVA was more effective for methyl parathion and malathion, and for β-endosulfan the extracted amounts were similar. However, it should be considered that the PDMS/PVA fiber has a coating thickness of approximately 5 μm, which leads to a volume of extracting phase of ∼46 μm^3, almost 1/3 of the coating volume of the 30 μm PDMS commercial, 132 μm^3 [8]. Therefore, the PDMS/PVA coating has a larger affinity towards the analytes than regular PDMS. The fiber here employed was coated with three layers of sol–gel PDMS/PVA polymer. Thicker PDMS/PVA fibers can be easily prepared increasing the number of coating layers, if a fiber with higher absolute extraction capacity is necessary. However, one of the most attractive features of sol–gel coatings for SPME is the thickness of the coatings: it is possible to obtain extremely thin films of sorbents with large extractive power [23], such as for the PDSM/PVA here presented. Such thinner coatings allows faster extractions and easier thermal desorption when compared to conventionally coated fibers.

Other interesting aspect of Fig. 2 is related to the section of the chromatograms up to 7 min. Several large peaks appear in this region of the chromatogram for the PDMS/PVA

Table 1
Concentration range in ng mL^{-1}, slopes S in 10^{-6} area counts ng^{-1} mL^{-1}, intercepts I in 10^{-4} area counts and correlation coefficients r for the analytical curves, limits of detection LD and of quantitation LQ in ng mL^{-1}, and R.S.D. for OCP and OPP in the *Passiflora* infusions using the optimized HS-SPME method with the PDMS/PVA fiber

Species	Analyte	Range	S	I	r	LD[a]	LQ[b]	RSD[c]
P. alata	CT[d]	0.2–1.0	1.3 ± 0.1	6	0.983	0.13	0.45	12.1
	MP	0.6–3.0	1.10 ± 0.04	2	0.995	0.23	0.77	2.8
	MA	1.2–6.0	0.41 ± 0.04	7	0.974	1.00	3.36	3.7
	AE	0.04–0.2	33 ± 4	22	0.954	0.05	0.15	1.3
	BE	0.04–0.2	4.0 ± 0.2	4	0.994	0.01	0.05	1.4
P. edulis	CT	0.2–1.0	1.3 ± 0.1	5	0.979	0.15	0.50	1.2
	MP	0.6–3.0	0.51 ± 0.08	33	0.980	0.85	2.83	3.9
	MA	1.2–6.0	0.43 ± 0.06	3	0.993	1.50	5.10	1.4
	AE	0.04–0.2	37 ± 6	10	0.921	0.06	0.20	1.5
	BE	0.04–0.2	9.7 ± 0.9	4	0.975	0.03	0.11	4.7
P. incarnata	CT	0.2–1.0	1.5 ± 0.2	3	0.969	0.20	0.69	3.8
	MP	0.6–3.0	1.2 ± 0.1	3	0.978	0.47	1.50	7.9
	MA	1.2–6.0	0.44 ± 0.05	1	0.967	1.10	3.90	1.5
	AE	0.04–0.2	41 ± 7	6	0.975	0.06	0.20	1.3
	BE	0.04–0.2	10.5 ± 0.8	0	0.988	0.03	0.09	8.3

[a] LD = 3σ/S.
[b] LQ = 10σ/S.
[c] R.S.D. measured at the highest concentration level of the analytical curves (n = 3).
[d] CT: chlorothalonil; MP: methyl parathion; MA: malathion; AE: α-endosulfan and BE: β-endosulfan.

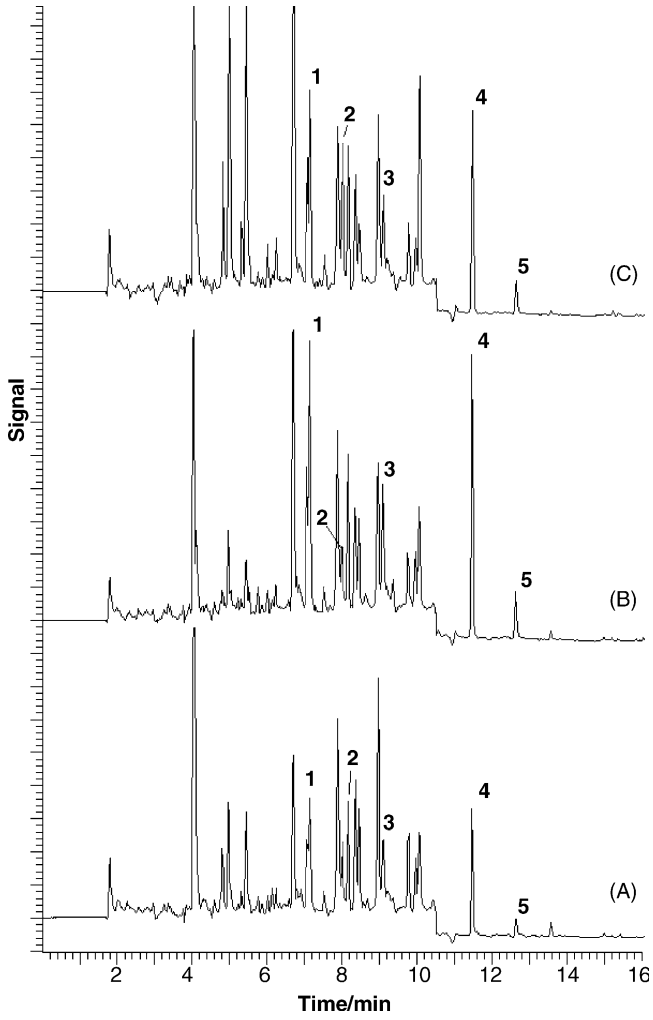

Fig. 3. Chromatograms of (A) *P. alata* (B) *P. edulis* and (C) *P. incarnata* spiked samples. (1) Chlorothalonil (0.2 ng mL^{-1}), (2) methyl parathion (0.6 ng mL^{-1}), (3) malathion (1.2 ng mL^{-1}), (4) α-endosulfan (0.04 ng mL^{-1}), (5) β-endosulfan (0.04 ng mL^{-1}) obtained with the optimized HS-SPME method and the PDMS/PVA fiber. See text for experimental conditions.

fiber, which either are not visible or are considerably smaller on the PDMS chromatograms. Also, the chromatogram for the PDMS/PVA fiber blank obtained in the same conditions shows only a clean baseline, precluding fiber bleeding as the source of these peaks. These chromatograms indicate that the PDMS/PVA can extract efficiently compounds covering a wider range of physico-chemical, properties than PDMS, specially more volatile (and less retained) analytes. This is a very desirable and interesting feature for a SPME coating, since can potentially allow detection and quantitation of larger number species with varied characteristics using the same fiber.

3.3. Analytical method validation

The Table 1 summarizes the results of method validation for the analytes in the *Passiflora* infusions; Fig. 3 shows typical chromatograms of spiked *P. alata*, *P. edulis*, and *P. incarnata* samples obtained with the PDMS/PVA fiber. The increase on the overall extraction efficiency is easily noticed when comparing the chromatograms on Figs. 2 and 3, which are plotted using the same signal scale. The peaks on Fig. 3A, corresponding to extractions of *P. alata* infusions under optimized conditions are much more intense than those in Fig. 2, corresponding to the same sample extracted before the multivariate optimization of the SPME procedure—even considering that the spiked concentration of on Fig. 2 is larger (e.g., 0.5 ng mL^{-1} versus 0.2 ng mL^{-1} for chlorotalonil).

The analytical curves were constructed with six concentration levels for each analyte and, in general, they presented fair to good precision (r from 0.921 to 0.995). Detection and quantification limits (LD and LQ, respectively) were determined correlating the residual standard deviation of the intercepts of regression lines and the slope of the analytical curves (S) [24] and ranged from 0.01 ng mL^{-1} (β-endosulfan) to 1.5 ng mL^{-1} (malathion), which are considered satisfactory for the analytical purposes [11]. The slopes of the analytical curves for some analytes are different for each *Passiflora* species: e.g., for β-endosulfan, the slope of the curve obtained from *P. incarnata* infusion is approximately 2.5 times greater than the corresponding parameter from the analytical curve for the same pesticide in *P. alata*. This is an indication of strong matrix effect in these samples, which is different for each studied species as reported before [10].

To evaluate the repeatability and reproducibility of the measurements, analyses of the *Passiflora* samples used for calibration curves were considered. The R.S.D. data on Table 1 illustrates the repeatability obtained for the highest concentration levels studied for the pesticides in all samples. The relative standard deviations (R.S.D.) of the peak areas found for all analytes in *Passiflora* samples were from 0.1% to 14.2%, with 13 out of the 15 R.S.D. values minor than 5.0%. It is important to emphasize that the results found after 1 month were very similar to the data obtained previously, which show a good reproducibility for the proposed methodology (R.S.D. < 19.3%).

In addition, accuracy of the method was determined calculating the recovery in the extraction of *P. alata* samples (n = 3) spiked at the same concentration level used for the repeatability study. Mean recoveries were 78.7% for chlorothalonil (R.S.D. = 5.4), 89.6% for methyl parathion (R.S.D. = 4.4), 91.2% for malathion (R.S.D. = 12.1), 89.5% for α-endosulfan (R.S.D. = 2.0) and 91.5% for β-endosulfan (R.S.D. = 4.8). These results indicate that quantitative analysis of *Passiflora* samples in the 0.04–6 ng mL^{-1} range of OCP and OPP is possible using external standardization, showing the applicability of this methodology for routine analysis.

4. Conclusions

A novel SPME fiber coated with PDMS/PVA co-polymer was successfully employed to determine residues of OCP

and OPP in *Passiflora* infusions by HS-SPME and GC-ECD. The resulting procedure was shown to be a good alternative methodology for the determination of selected OCP and OPP residues in *Passiflora* samples, being a simple, fast, reproducible, effective and also an environmentally friendly analytical method.

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