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ORIGINAL PAPER

Application of mixed solvents for elution of organophosphate pesticides extracted from raw propolis by matrix solid-phase dispersion and analysis by GC-MS

[a]Kati Medina-Dzul, [a]David Muñoz-Rodríguez*, [b]Yolanda Moguel-Ordoñez, [a]Cristian Carrera-Figueiras

[a]*Cuerpo Académico de Química Fundamental y Aplicada, Facultad de Ingeniería Química, Universidad Autónoma de Yucatán, Periférico Norte km. 33.5, Tablaje catastral 13615, Colonia Chuburná de Hidalgo Inn, C.P. 97203, Mérida, Yucatán, México*

[b]*Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, CE Mocochá, km. 25 carretera Mérida-Motul, Mérida C.P. 97454, Mocochá, Yucatán, México*

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The excessive application of organophosphate pesticides (OPPs) in crop fields close to hives and its application in beekeeping practices are potential sources of contamination of propolis. Pesticides were extracted from raw propolis by matrix solid-phase dispersion. Because of the complex chemical composition of propolis, binary solvent mixtures based on acetonitrile and a relatively non-polar solvent in different proportions were tested for the selective elution of OPPs. The effect of mixed solvents on clean-up was evaluated by GC-MS and the main interfering compounds co-eluted with pesticides were identified. In addition, three volumes of the solvent mixture and the volume of the sample were evaluated in an experimental factorial design. The final experimental conditions were 1 mL of dissolved propolis and 8 mL of acetonitrile/dichloromethane ($\varphi_r = 25 : 75$). Mean recoveries ranged from 55 % to 96 % (relative standard deviation < 8 %) with an inter-day precision lower than 24 %. The proposed method was applied to the analysis of real samples obtained from local beekeepers.

Keywords: GC-MS, matrix solid-phase dispersion, propolis, pesticide residues, mixed solvents

Introduction

Organophosphate pesticides (OPPs) are extensively used for both agricultural and landscape pest control due to their relatively low toxicity and low persistence in the mammalian system compared with organochlorine pesticides (Kazemi et al., 2012). However, the excessive application of OPPs in crop fields close to hives can potentially contaminate propolis, wax, royal jelly and honey because the bees can transport the OPPs into the hive (Bogdanov, 2006). In addition, the organophosphate coumaphos is employed in beekeeping practices for the control of *Varroa destructor* (Oldroyd, 2007).

Propolis is a resinous substance that bees collect from the exudates of plants and use to seal holes in the hive (Marcucci, 1995). Because of its biological and pharmacological properties, propolis is extensively used in folk medicine, cosmetics and the food industry (Melliou et al., 2007). Hence, analytical methods are needed to protect the health of propolis consumers because the consumption of propolis contaminated with OPPs has an adverse impact on the human nervous system (Kazemi et al., 2012; Pope, 1999).

Pesticide analysis in raw propolis represents an analytical challenge due to the relatively high amount of resins (50 %), wax (30 %) and at least 300 organic compounds (Viuda-Martos et al., 2008) that can

*Corresponding author, e-mail: david.mr@uady.mx

be co-extracted with the analytes. Sample preparation is a key step in the analytical procedure since even small amounts of lipophilic compounds can act to the detriment of the chromatographic system and/or signal suppression. To protect the chromatographic system, different clean-up approaches and mixed solvents have been evaluated to extract pesticides from propolis. For the analysis of organochlorine pesticides by GC-electron capture detector, tandem solid-phase extraction (florisil-graphitised carbon cartridges) has been employed in the clean-up (Chen et al., 2009). Binary solvent mixtures based on hexane (hexane/ethylacetate (EtOAc); $\varphi_r = 1 : 1$ and $\varphi_r = 8 : 2$; hexane/dichloromethane (DCM) $\varphi_r = 8 : 2$)) were tested for pesticide elution. In addition, matrix solid-phase dispersion (MSPD) was applied to the extraction of four pesticides followed by GC-mass spectrometry detector (MSD). The sorbent was silica; DCM as well as mixed solvents (DCM/hexane ($\varphi_r = 1 : 1$ and $\varphi_r = 8 : 2$); DCM/EtOAc ($\varphi_r = 9 : 1$, $\varphi_r = 8 : 2$ and $\varphi_r = 7 : 3$)) were tested for pesticide elution. Both clean-up approaches reported the presence of endogenous compounds (unidentified) in extracts obtained with binary mixtures based on hexane.

A lipophilic sorbent (C_{18}) was used previously for the MSPD extraction of five OPPs followed by GC-MSD analysis (Acosta-Tejada et al., 2011). Pesticide elution was tested using acetonitrile (ACN) and EtOAc. However, a single solvent offered limited scope to improve the selectivity and recoveries of OPPs in MSPD extraction. In the current study, binary solvent mixtures based on ACN and a relatively non-polar solvent in different proportions ($\varphi_r = 85 : 15$ to $\varphi_r = 10 : 90$) were tested. The use of hexane was avoided due to its high affinity towards the lipophilic compounds (such as waxes) present in propolis. Hence, this work sought to evaluate the effect of binary mixtures on clean-up and to identify the main interfering compounds co-eluted with pesticides by GC-MS. The binary solvent mixtures that permitted the selective extraction of pesticides from propolis were used to study the effect of sample throughputs and mixed solvent volume in MSPD extraction applying experimental design. Finally, recoveries and inter-day precision were evaluated with the selected mixture.

Experimental

General

Solvents (ACN, isooctane (ISO), EtOAc, hexane, DCM, acetone, methyl isobutyl ketone (MIBK)) were obtained from Mallinckrodt Baker (USA). Octadecyl-functionalised silica gel was from Sigma–Aldrich (USA). Organophosphate pesticides standards (dichlorvos (DCV), diazinon (DZN), methyl parathion (MPT), malathion (MLT) and coumaphos (CMF)) were purchased from ChemService (USA). All standards were at least 98.5 % pure. Individual stock solutions were prepared in EtOAc and stored at –18 °C. Working standard solutions were prepared by diluting the stock solutions with EtOAc, as required.

Propolis samples for method development were obtained from an organic apiary (no detectable pesticide residues) in Tizimin (Yucatan, Mexico) and real samples were obtained from local beekeepers Mococha (Yucatan, Mexico).

Fortification of raw propolis

Spiked propolis samples were prepared by adding 1000 μL of a standard solution containing the five organophosphate pesticides (1 μg mL^{-1} each) to 1 g of propolis to obtain a spiked level of 1.0 μg g^{-1}. The spiked samples were left to stand for 40 min prior to MSPD extraction to allow the pesticides to be incorporated into the propolis matrix.

Extraction and clean-up

The final conditions were as follows: propolis (1 g), whether spiked or not, was dissolved in 10 mL of hexane; an aliquot (1 mL, 2 mL or 3 mL) of dissolved propolis was added to 1 g of C_{18} placed in a glass mortar. Then, C_{18} and the sample were homogenised with a pestle to obtain a mixture that was loaded into a polypropylene column (85 mm × 15 mm ID) and gently compressed to eliminate air pockets. Next, 4 mL, 6 mL or 8 mL of either ACN/EtOAc ($\varphi_r = 85 : 15$) or ACN/DCM ($\varphi_r = 25 : 75$) was added. Extracts were collected in a graduated vial and the solvents evaporated until dry with a low nitrogen flow-rate. The extract was reconstituted with ISO (1 mL) and frozen (< 10 °C) for at least 2 h to precipitate high molecular weight compounds. Finally, the extracts were immediately centrifuged (45 s, 10000 min^{-1}) and the supernatant was placed in vials for GC-MS analysis. Three replicate analyses were performed for each optimised condition.

Instrumentation and chromatographic conditions

GC-MS analyses were performed using an Agilent Technologies (USA) 6890N gas chromatograph coupled to a mass spectrometer 5973N (MSD) and a bonded fused-silica capillary column (30 m × 0.25 mm ID × 0.25 μm film thickness, 5 % diphenylsiloxane 95 % dimethylsiloxane) supplied by Supelco (USA). Helium (purity 99.999 %) was employed as carrier gas (1.0 mL min^{-1}).The oven temperature was programmed as follows: 120 °C for 3 min directly to 280 °C at 20 °C min^{-1} and held for 5 min. The solvent delay was 3 min. The injection port was maintained at 250 °C and 1 μL of the extract was injected in splitless mode (1.5 min). The eluent from the GC column was

Table 1. SIM programme for analysis of organophosphate pesticides in raw propolis

Group	Compound	t_R	Time window	SIM ions
		min		m/z
1	dichlorvos	5.56	3.00–8.00	220, 185[a], 109
2	diazinon	9.40	8.00–9.60	304[a], 199, 179
3	methylparathion	10.20	9.60–13.00	263[a], 125, 109
	malathion	10.40	–	173[a], 158, 125
4	coumaphos	14.73	13.00–20.00	362[a], 210, 109

a) Target ion; t_R – retention time.

Table 2. Factor definitions and coded levels

Factor	Coded levels	Definitions/mL
volume of hexanic tincture	+1	3
	0	2
	–1	1
volume of solvent mixture	+1	4
	0	6
	–1	8

In coded levels +1 indicates high level, –1 indicates low level and 0 represents central treatment.

transferred into the MSD via a transfer line (280 °C).

Electron impact (EI) mass spectra were obtained at 70 eV and monitored from 50 to 400 m/z on full scan-mode analysis (SCAN). For recovery evaluation, acquisition windows and ions (Table 1) were programmed in the mass spectrometer (selected ion monitoring mode; SIM). The temperatures of the quadrupole and ion source were 150 °C and 250 °C, respectively. The typical conditions of MSD were optimised through the autotune software option. The identification of the compounds was confirmed by the injection of matrix-matched standards and by comparison of the mass spectra.

Experimental design and statistical analysis

A 2^2 plus four replicates of the central treatment factorial design was applied according to a linear statistical model. (Montgomery, 2004). Two factorial designs were run, one for each solvent mixture (ACN/EtOAc; $\varphi_r = 85 : 15$ and ACN/DCM; $\varphi_r = 25 : 75$).

The factors evaluated for the MSPD extraction (Table 2) were the volume of dissolved propolis (low level 1 mL, high level 3 mL) and the volume of the solvent mixture (low level 4 mL, high level 8 mL). The experimental conditions for the central treatment were 2 mL of dissolved propolis and 6 mL of the solvent mixture. Chromatograms of the MSPD extracts were acquired by GC-MS-SIM and the variable response was the sum of the peak area of each pesticide.

Once the experimental conditions for each solvent mixture were defined, analysis of variance (ANOVA) with a design of two-way fixed effects (Montgomery, 2004) was performed to establish the effect between the solvent mixture (ACN/EtOAc; $\varphi_r = 85 : 15$, ACN/DCM; $\varphi_r = 25 : 75$) and pesticide recovery.

The extraction conditions (volume of dissolved propolis, volume and composition of solvent mixture) were chosen according to the better pesticide recovery; ANOVA was performed to establish the effect among days and pesticide recoveries. After each ANOVA, the multiple comparison of MSD was applied. (Montgomery, 2004). The "Statgraphic Plus 5.1" (Statpoint Technologies, USA) statistical computer package was used for the experimental design and statistical analysis. The significance level was $\alpha = 0.05$.

Pesticide recovery and calibration curve

The pesticide recoveries were evaluated using spiked propolis (1.0 μg g^{-1}) and external matrix-match calibration (0.025 μg mL^{-1}, 0.05 μg mL^{-1}, 0.2 μg mL^{-1}, 0.4 μg mL^{-1}, 0.6 μg mL^{-1}). Blank propolis was used to prepare the standard solutions for the matrix match calibration.

The limits of detection (LOD) and quantification (LOQ) of each pesticide were determined from the extracted ion chromatograms of the target ions. Using the data analysis software, the signal-to-noise ratio (S/N) was obtained for each pesticide peak in a matrix-match standard solution of 0.025 μg mL^{-1}. Based on that result, the concentration for S/N of 3 (LOD) and 10 (LOQ) was determined and expressed in μg kg^{-1}.

Results and discussion

GC-MS conditions

Pesticide resolutions were optimised in SCAN mode, using a standard solution (5 μg mL^{-1}) and varying the oven temperature and carrier gas flow-rates to achieve a good resolution between analytes and matrix peaks. In these experiments, one target

and two qualifier ions (Table 1), characteristic of each pesticide, were chosen for quantification and identification, respectively. The choice of the ions for the SIM acquisition mode was made to obtain the best S/N ratios.

Selectivity of binary solvent mixtures

The nature of the elution solvent is important in MSPD because the target analytes should be efficiently eluted with the minimum co-extraction of interfering compounds. Hence, the aim of this stage was to choose a binary solvent mixture to achieve a selective, simultaneous and quantitative elution of the analytes.

Accordingly, given that propolis is constituted of non-polar compounds (aliphatic hydrocarbons, diterpenes, sesquiterpenes, essential oils among others) (Mohammadzadeh et al., 2007) and the polarity of pesticides is intermediate to low, mixtures based on ACN and another solvent with relatively non-polar characteristics were tested. ACN was selected as the polar solvent to improve selectivity because it extracts many fewer lipophilic compounds (Hercegová et al., 2007). Also, ACN in combination with C_{18} as dispersant support helps obtain clean extracts due to the retention of lipophilic compounds in the sorbent (Lambropoulou & Albanis, 2007).

However, a relatively non-polar solvent is needed to elute the less polar pesticides. In contrast to previous reports, hexane was not included in binary mixtures due to its high affinity towards the waxes present in propolis. Therefore, EtOAc, acetone, MIBK and DCM were chosen for the solvent mixtures. The first two solvents provide acceptable high recoveries (70–110 %) of a wide range of pesticides (Hercegová, 2007). The other two solvents have also been applied to pesticide multi-residue analysis (dos Santos et al., 2008,

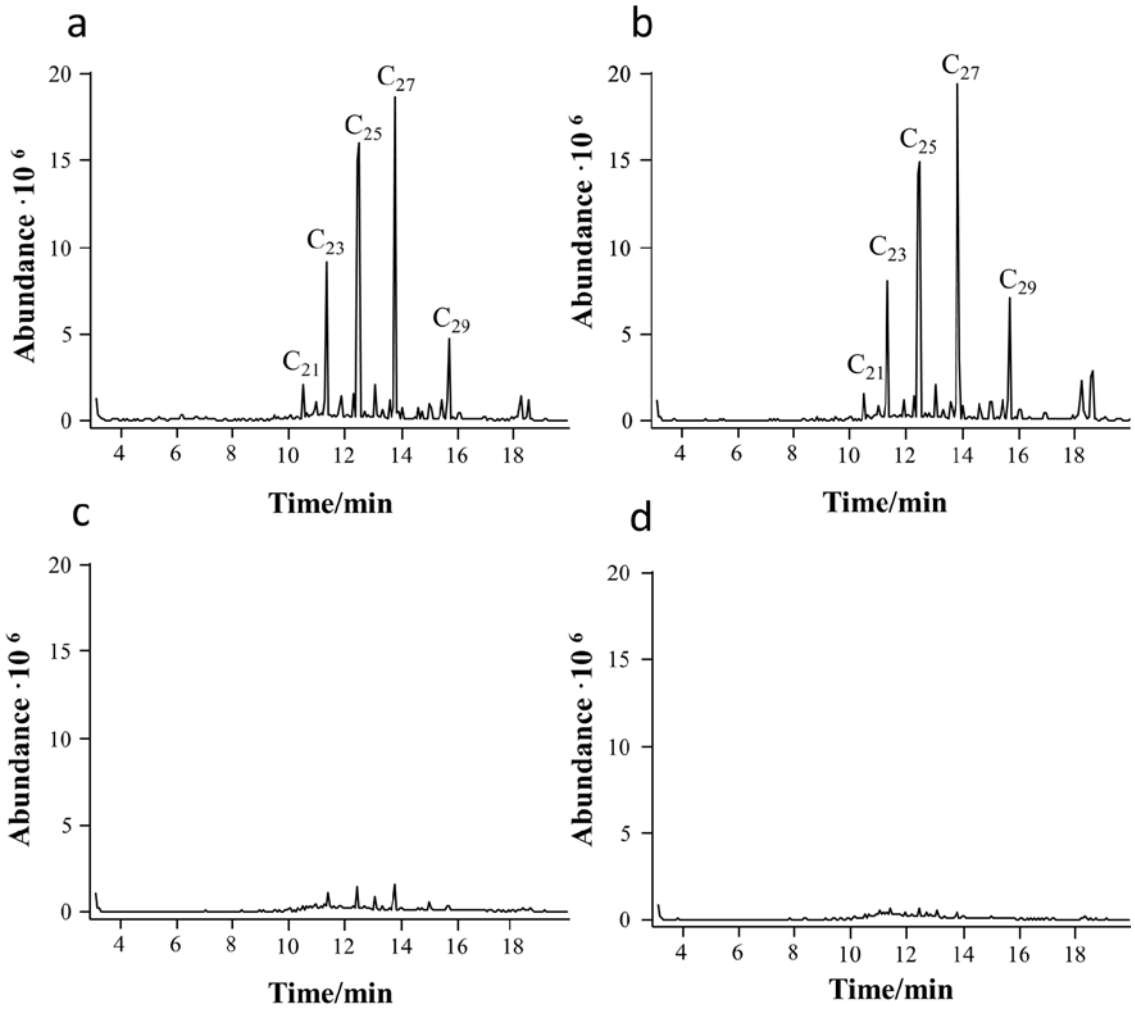

Fig. 1. Representative GC-MS-SCAN chromatograms of MSPD extracts obtained with binary mixtures ACN/MIBK ($\varphi_r = 55 : 45$) (a), ACN/acetone ($\varphi_r = 10 : 90$) (b), ACN/EtOAc ($\varphi_r = 85 : 15$) (c) and ACN/DCM ($\varphi_r = 25 : 75$) (d) and main co-extracted interfering compounds from propolis. MSPD extraction conditions: 1 mL of dissolved propolis, 1 g of C_{18} and 8 mL of each solvent mixture.

Lambropoulou & Albanis, 2007). Accordingly, the binary solvent mixtures ACN/MIBK, ACN/EtOAc, ACN/acetone and ACN/DCM were tested with the compositions (φ_r); 0 : 100, 15 : 85, 30 : 70, 45 : 55, 60 : 40, 75 : 25, 90 : 10, 100 : 0.

Lipophilic compounds co-eluted with pesticides in extracts obtained with all binary mixtures. C_{25}, C_{27} and C_{29} were identified (12–16 min) in GC-MS-SCAN chromatograms as the main co-eluting compounds (percentage of peak area up to 20 % for C_{25}, 42 % for C_{27} and 15 % for C_{29}). Peaks ascribed to C_{21}, C_{23} and C_{31} presented a percentage of area ranged from 0.52 % to 9.55 % in all binary mixtures. In addition, C_{24} and escualene were identified. The co-extractants identified have been reported as the constituents of propolis (Melliou, 2007; Walker & Crane, 1987; Sahinler & Kaftanoglu, 2005) and plants (Dove & Mayes, 2006) as the raw material for propolis.

The highest intensities of C_{25}, C_{27} and C_{29} were obtained with ACN/MIBK and ACN/acetone (Figs 1a and 1b). This accords with the results of Anastassiades et al. (2003) who reported the highest amount of co-extractives for vegetables and fruit extracts obtained with ACN/acetone and acetone in comparison with EtOAc and ACN extracts.

Additionally, extracts were obtained with pure ACN. The amounts of C_{21}, C_{23}, C_{25}, C_{27}, C_{29} and C_{31} were lower than 12 % of the area. This accords with the low capacity of ACN for the extraction of lipophilic compounds (Anastassiades et al., 2003). However, low peak areas of pesticides were acquired in GC-MS-SIM from MSPD extracts obtained from spiked propolis (1.0 μg g^{-1} of each pesticide).

The mixtures of ACN/EtOAc and ACN/DCM were used for the elution of pesticides from spiked propolis (1.0 μg g^{-1} of each pesticide). In general, these mixtures presented low amounts of C_{27}, C_{29} and C_{31}. Specifically, the binary mixtures selected were ACN/EtOAc (φ_r = 85 : 15) and ACN/DCM (φ_r = 25 : 75) because they exhibited low amounts of the main co-extractives and high peak areas for the five pesticides (Figs. 1c and 1d). In addition, the mixture ACN/DCM (φ_r = 25 : 75) was found to evaporate rapidly.

Volume of dissolved propolis and binary solvent mixture

To establish which factors have a significant effect on pesticide extraction efficiency, the volume of dissolved propolis added to C_{18} and the volume of solvent mixture were evaluated through a 2^2 plus four replicates of the centre treatment factorial design.

Two factorial designs were run, one for each solvent mixture (φ_r = 85 : 15 (ACN/EtOAc) and φ_r = 25 : 75 (ACN/DCM). To obtain a quantitative extraction of pesticides with the minimum volume of solvent mixture, three volumes were evaluated (8 mL, 6 mL and 4 mL). In addition, to increase the sample throughput, volumes of 1 mL, 2 mL and 3 mL of dissolved propolis were tested.

Table 3. Pesticide recoveries (R, %) from MSPD extracts and relative standard deviations (RSD, %) obtained under two sets of experimental conditions

Pesticide	% R (± RSD)	
	ACN/EtOAC (φ_r = 85 : 15)	ACN/DCM (φ_r = 25 : 75)
dichlorvos	$(41 \pm 20)^{a,\alpha}$	$(55 \pm 8)^{a,\beta}$
diazinon	$(65 \pm 20)^{b,c,\alpha}$	$(85 \pm 4)^{b,\beta}$
methylparathion	$(59 \pm 27)^{b,\alpha}$	$(96 \pm 5)^{g,\beta}$
malathion	$(76 \pm 44)^{c,\alpha}$	$(83 \pm 1)^{b,\beta}$
coumaphos	$(54 \pm 28)^{a,b,\alpha}$	$(85 \pm 7)^{b,\beta}$

Mean recovery (n = 3) with different Latin letters in the same column differs significantly (α = 0.05) among pesticides; mean recovery with different Greek letters in the same row differs significantly (α = 0.05) for each pesticide among pesticides; extraction conditions: 1 mL of hexanic tincture, 1 g of C_{18} and 8 mL of solvent mixture.

In general, most of the experiments revealed low values for peak areas which indicated that pesticides did not had not eluted from the MSPD column. The highest areas were obtained for φ_r = 25 : 75 (ACN/DCM) with: (i) 1 mL of dissolved propolis and 8 mL of mixture; (ii) 3 mL of dissolved propolis and 4 mL of the mixture. However, the first conditions (i) yielded a relatively clean extract and were chosen for pesticide recovery evaluation.

For φ_r = 85 : 15 (ACN/EtOAc), the highest area was obtained by adding 3 mL of dissolved propolis and eluting pesticides with 8 mL of solvent mixture.

Recovery evaluation

Recoveries obtained with ACN/EtOAc (φ_r = 85 : 15) ranged from 41 % to 76 % with RSD at least of 20 %. DCV and CMF had the lowest recoveries, 41 % and 54 %, respectively (Table 3). Recoveries of DZN, MPT and MLT were not markedly different (α = 0.05).

In contrast, recoveries from 55 % to 96 % and RSD lower than 10 % were obtained with ACN/DCM (φ_r = 25 : 75). The ANOVA indicated that recoveries of DCV and MPT were significantly different (α = 0.05) and also differed from DZN, MLT and CMF. However, recoveries did not differ between those three pesticides.

With both binary mixtures, DCV showed recoveries lower than 60 %. Because of its high volatility, low recoveries can be attributed to losses during the evaporation step. In agreement with these results, Shimelis et al. (2007) reported recoveries of dichlorvos ranged from 40 % to 67 % in recovery studies carried out with spiked samples (orange, orange juice and bacon).

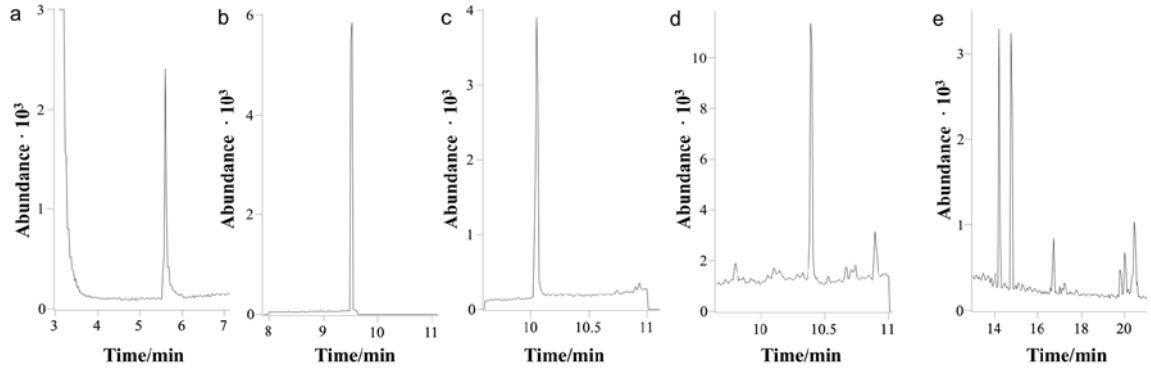

Fig. 2. Extracted ion chromatograms of DCV (m/z 185) (a), DZN (m/z 304) (b), MPT (m/z 263) (c), MLT (m/z 173) (d) and CMF (m/z 362) (e) in matrix-matched standard (0.6 μg mL^{-1}).

Table 4. Calibration data and values of LOD and LOQ

Pesticide	Calibration function	R^2	LOD	LOQ
			μg kg^{-1}	
dichlorvos	$A = 10370.82c - 54.80$	0.99947	33	110
diazinon	$A = 11410.61c - 69.59$	0.99918	10	35
methyl parathion	$A = 10872.61c - 40.05$	0.99978	43	143
malathion	$A = 22327.50c - 19.81$	0.99810	154	513
coumaphos	$A = 12217.98c + 57.61$	0.99956	1.4	4.8

A – analytical signal (peak area), c – pesticide concentration (μg mL^{-1}).

There was a statistical difference ($\alpha = 0.05$) in the pesticide recoveries obtained with both solvent mixtures. The mixture ACN/DCM ($\varphi_r = 25 : 75$) showed the highest recoveries for the pesticides. Those recoveries were higher than those reported in our previous work (Acosta-Tejada et al., 2011) (53.8–84.6 %; RSD: 3.1–14.6 %) with greater precision (RSD < 10 %). For CMF, the recovery was lower than that obtained in spiked ethanolic propolis tinctures (1.0 μg g^{-1}) (Pérez-Parada et al., 2011) with 2 g of $Al_2(SO_4)_3$ as solid support and 30 mL of EtOAc/DCM ($\varphi_r = 1 : 9$) as eluent (106 %; RSD: 12 %).

Table 5. Pesticide recoveries (R, %) in MSPD extracts and relative standard deviations (RSD, %) obtained with ACN/DCM ($\varphi_r = 25 : 75$) as eluting mixture on different days

Pesticide	Day 1	Day 2
	% R (± RSD)	
dichlorvos	$(55 \pm 8)^{a,\alpha}$	$(63 \pm 3)^{b,\alpha}$
diazinon	$(85 \pm 4)^{a,\beta}$	$(92 \pm 8)^{b,\beta}$
methyl parathion	$(96 \pm 5)^{a,\gamma}$	$(97 \pm 10)^{b,\gamma}$
malathion	$(83 \pm 1)^{a,\beta}$	$(106 \pm 7)^{b,\beta}$
coumaphos	$(85 \pm 7)^{a,\beta}$	$(126 \pm 12)^{b,\beta}$

Mean recoveries ($n = 3$) with different Latin letters in the same raw differ significantly ($\alpha = 0.05$) among days; mean recoveries with different Greek letters in the same column differ significantly ($\alpha = 0.05$) among pesticides.

Analytical performance of method

The typical extracted ion chromatograms of the pesticides in a matrix-matched standard (0.6 μg mL^{-1}) prepared from extracts of ACN/DCM ($\varphi_r = 25 : 75$) are shown in Fig. 2.

All the calibration functions (Table 4) presented acceptable determination coefficients ($R^2 > 0.995$). The intercepts were evaluated statistically and the results showed that they did not differ significantly from 0 (p value > 0.05). This shows that the intercept did not affect the accuracy of the method.

The LOD for the pesticides ranged from 1.4 μg kg^{-1} to 154 μg kg^{-1} and the LOQ from 4.8 μg kg^{-1} to 513 μg kg^{-1}. The LOD and LOQ values for DCV, DZN, MPT and CMF were lower than those reported in (dos Santos et al., 2008) for the analysis of pesticides in raw propolis. The exception was MLT (LOD and LOQ of 154 μg kg^{-1} and 513 μg kg^{-1}, respectively) which is ascribed to problems with the selectivity of the ion m/z 173.

The repeatability of pesticide extraction was evaluated for two days using spiked propolis (1.0 μg g^{-1}) and ACN/DCM ($\varphi_r = 25 : 75$) as eluting mixture (Table 5). On both the days of analysis, the recoveries were higher than 80 % with the exception of DCV (55 % and 63 %, respectively). The RSD values increased in the second day; however, they remained

below 15 % in agreement with accepted requirements (Hill & Reynolds, 1999).

Also, on the second day, CMF showed a recovery higher than the accepted range of 70–120 % recovery Hill and Reynolds (1999) which could be ascribed to elution of long-chain hydrocarbons or other non-polar compounds with the retention time and ions similar to CMF. Despite that, the statistical analysis (ANOVA) among pesticides on day 2 showed the same pattern as on the first day: recoveries of DCV and MPT differed significantly ($\alpha = 0.05$) and also from DZN, MLT and CMF. Recoveries among DZN, MLT and CMF did not differ statistically ($\alpha = 0.05$).

In addition, the ANOVA showed statistical differences ($\alpha = 0.05$) among the recoveries obtained on both days for the same pesticide (Table 5). This has been related to the variability and complex composition of propolis (dos Santos et al., 2008, Pérez-Parada et al., 2011). Thus, the inter-day precision (RSD) obtained here for DCV, DZN and MPT was lower than 10 %. In contrast, the RSD was 14 % and 23 % for MLT and CMF, respectively. Values ranging from 5.6 % to 12.1 % were reported by dos Santos et al. (2008) in the development of an analytical method for the analyses of pesticides from raw propolis. However, Pérez-Parada et al. (2011) reported relatively low inter-day precision (RSD 3.8–7.2 %) in the analysis of ethanolic propolis tinctures in comparison with the values reported here and by dos Santos et al. (2008). The differences between the studies can be explained by the fact that tinctures are of a less complex matrix because they are prepared in ethyl alcohol which does not dissolve hydrocarbons (C_{21}, C_{23}, C_{24}, C_{25}, C_{26}, C_{27} and C_{29}) that can be separated by filtration.

Real samples

The MSPD procedure developed was applied to the determination of pesticides in propolis. Four different samples of propolis were obtained from local beekeepers in Mococha, Yucatán, Mexico originating from conventional apiculture. No pesticide residues were found in these samples at concentrations above the detection limit.

Conclusions

Organophosphate pesticides were eluted from the MSPD column with four binary solvent mixtures (ACN/MIBK, ACN/EtOAc, ACN/acetone and ACN/DCM) at different compositions ($\varphi_r = 15 : 85$ to $\varphi_r = 90 : 10$) and the clean-up effect was evaluated by analysing the extracts by GC-MS.

The main co-eluting compounds (C_{21}, C_{23}, C_{24}, C_{25}, C_{26}, C_{27}, C_{29} and escualene) were identified by GC-MS-SCAN in propolis extracts obtained with the binary solvent mixtures. The volumes of the solvent mixture and of the dissolved propolis were also evaluated. The MSPD extraction conditions that yielded the cleanest extracts and recoveries from 55 % to 126 % (RSD < 15 %) were 1 mL of sample and 8 mL of ACN/DCM ($\varphi_r = 25 : 75$).

Good values of the determination coefficient ($R^2 > 0.998$) were obtained in the matrix-matched standards calibration. LOD and LOQ ranged from 1.4 μg kg^{-1} to 154 μg kg^{-1} and 4.8 μg kg^{-1} to 513 μg kg^{-1}, respectively. The inter-day precision (RSD) of the extraction was equal or lower than 23 %.

None of the OPPs studied were found when the method thus developed was applied to raw propolis.

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