

MSPD procedure for determining buprofezin, tetradifon, vinclozolin, and bifenthrin residues in propolis by gas chromatography–mass spectrometry

Tháise Fernanda Santana dos Santos ·
Adriano Aquino · Haroldo Silveira Dórea ·
Sandro Navickiene

Received: 27 October 2007 / Revised: 8 December 2007 / Accepted: 12 December 2007 / Published online: 13 January 2008
© Springer-Verlag 2007

Abstract A simple and effective extraction method based on matrix solid-phase dispersion (MSPD) was developed to determine bifenthrin, buprofezin, tetradifon, and vinclozolin in propolis using gas chromatography–mass spectrometry in selected ion monitoring mode (GC–MS, SIM). Different method conditions were evaluated, for example type of solid phase (C₁₈, alumina, silica, and Florisil), the amount of solid phase and eluent (*n*-hexane, dichloromethane, dichloromethane–*n*-hexane (8:2 and 1:1, *v/v*) and dichloromethane–ethyl acetate (9:1, 8:2 and 7:3, *v/v*)). The best results were obtained using 0.5 g propolis, 1.0 g silica as dispersant sorbent, 1.0 g Florisil as clean-up sorbent, and dichloromethane–ethyl acetate (9:1, *v/v*) as eluting solvent. The method was validated by analysis of propolis samples fortified at different concentration levels (0.25 to 1.0 mg kg⁻¹). Average recoveries (four replicates) ranged from 67% to 175% with relative standard deviation between 5.6% and 12.1%. Detection and quantification limits ranged from 0.05 to 0.10 mg kg⁻¹ and 0.15 to 0.25 mg kg⁻¹ propolis, respectively.

Keywords MSPD · Propolis · Pesticides · GC–MS

Introduction

Propolis is a complex resinous mixture of different plant exudates; these are gathered, modified, and used by honey-

bees as a general-purpose sealant and draught excluder in their hives. Because of its diverse biological and pharmacological activity, it has been used in folk medicine [1]. In general, the composition of propolis is directly related to that of bud exudates collected by honey bees from various trees [2–4]. Because of the versatile biological and pharmacological effects of propolis, it has wide applications in medicine, cosmetics, and the food industry [5]. Sources of contamination can be roughly divided into environmental and apicultural. Contaminants can reach the raw materials of bee products via air, water, plants, and soil and can then be transported into the bee hive by the bees. The most important contaminants are the substances used for control of bee pests [6]. Chemical protection of beehives is commonly carried out by treatment with different kinds of pesticide, for example as amitraz, cymiazole, bromopropylate, coumaphos, flumethrin, and fluvalinate [6]. Moreover, among the pesticides used in the northeastern part of the Brazil are tetradifon, vinclozolin, bifenthrin, and buprofezin. Consequently, after their application, pesticide residues may remain in this crop and constitute a health risk because of their toxicity. Therefore, monitoring of pesticide residues in propolis is of particular concern to consumer safety. The literature describes only a Brazilian chromatographic method for determination of pesticide residues, mainly organochlorine pesticides, namely aldrin, dieldrin, endrin and heptachlor, in propolis [7]. On the other hand, some methods for determining pesticide residues in honey have been described in the literature [8]. They include liquid–liquid extraction, matrix solid-phase dispersion, and headspace solid-phase microextraction procedures. Analyses have been performed by liquid chromatography (LC) with UV diode-array or MS detection, and gas chromatography with nitrogen–phosphorus or MS detection [9–16].

T. F. Santana dos Santos · A. Aquino ·
H. S. Dórea · S. Navickiene (✉)
Departamento de Química, Universidade Federal de Sergipe,
Av. Marechal Rondon s/n°, Jardim Rosa Elze,
Cep. 49100-000 São Cristóvão SE, Brazil
e-mail: sandnavi@ufs.br

This paper reports simple methodology for simultaneous determination of bifenthrin, buprofezin, tetradifon, and vinclozolin in propolis by matrix solid-phase dispersion (MSPD) and gas chromatography using mass-selective detection (GC–MS, SIM). Figure 1 shows the molecular structures of the pesticides studied.

Experimental

Standards, reagents and supplies

Dichloromethane, ethyl acetate, and *n*-hexane were nano-grade (Mallinckrodt Baker, Paris, KY, USA). Certified standards of bifenthrin, buprofezin, tetradifon, and vinclozolin were purchased from Dr Ehrenstorfer (Augsburg, Germany). All standards were at least 98.5% pure. Individual stock solutions of the analytes were prepared in dichloromethane at 500 mg mL⁻¹ and stored at -18 °C. Working standard solutions were prepared by diluting the stock solutions with dichloromethane, as required. Matrix-matched standards were prepared at the same concentration as calibration solutions by adding appropriate amounts of standards to the control matrix extract. Analytical-grade anhydrous sodium sulfate was supplied by Mallinckrodt

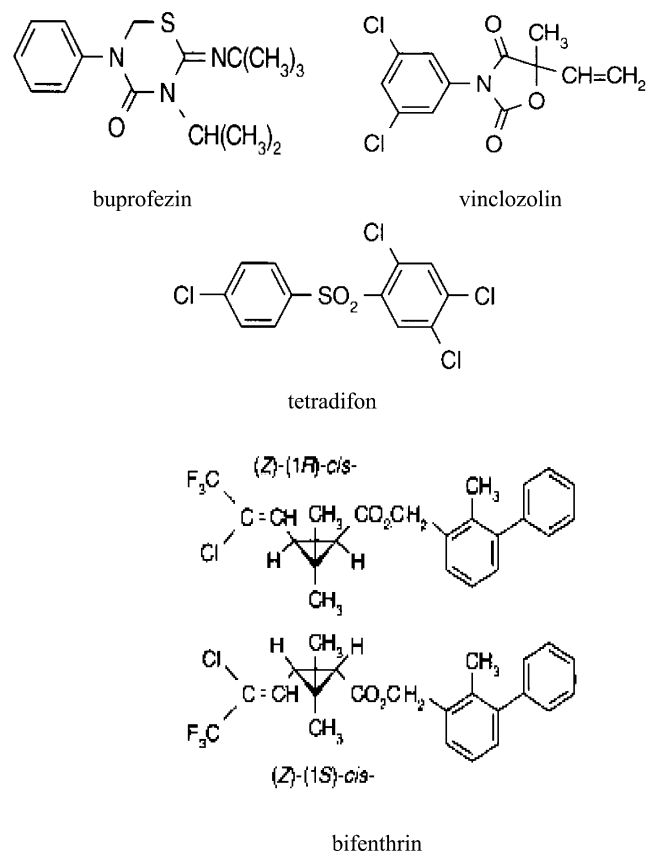


Fig. 1 Chemical structures of the pesticides studied

Baker. Research grade Florisil (80–100 mesh) was supplied by Sigma (Büchs, Switzerland). C₁₈-bonded silica (50 μm) was obtained from Phenomenex (Torrance, CA, USA), silica gel 60 (70–230 mesh) from Merck (Darmstadt, Germany), and neutral alumina (70–290 mesh, activity I) from Macherey–Nagel (Düren, Germany).

Instrumentation and chromatographic conditions

A Shimadzu system consisting of a QP-5050A mass spectrometer equipped with a GC-17A gas chromatograph with split/splitless injector was used for the quantification and confirmation of the pesticides studied. A fused-silica column DB-5MS (5% phenyl–95% polydimethylsiloxane; 30 m×0.25 mm ID, 0.25 μm), supplied by J&W Scientific (Folsom, CA, USA), was employed with helium (purity 99.999%) as carrier gas at a flow-rate of 1.8 mL min⁻¹. The column temperature was programmed as follows: 60 °C for 1 min and directly to 270 °C at 10° min⁻¹ and holding for 3 min. The solvent delay was 5 min. The injector port was maintained at 250 °C and 1 μL was injected in splitless mode (0.7 min). Data were acquired and processed by Shimadzu class 5000 software. The total analysis time was 28 min.

The eluent from the GC column was transferred via a transfer line held at 280 °C and fed into a 70-eV electron-impact ionization source. The analysis was performed in the selected ion monitoring (SIM) mode with the followed ions for the first acquisition window (5.00 to 19.00 min) *m/z* 84, 109 and 145 for vinclozolin, for the second acquisition window (19.00 to 23.00 min) *m/z* 122, 149 and 164 for buprofezin and *m/z* 174, 203 and 353 for bifenthrin, and for the third acquisition window (23.00 to 25.00 min) *m/z* 265 and 323 for tetradifon.

Sample preparation and fortification

The propolis samples were taken from hives from the Tapiti Apiary located in the city of Santo Amaro das Brotas, state of Sergipe, Brazil. Samples were collected in November 2006. A representative portion of the sample (800 g) was homogenized using a household blender and stored on jars at -18 °C until analysis. Fortified samples were prepared by adding 500 mL different standard solutions to 1 g propolis and mixing thoroughly in a blender, resulting in levels of 0.25 to 1.0 mg kg⁻¹. The fortified propolis aliquots were left to stand for a 30 min before extraction to allow the spike solution to penetrate into the matrix. Four replicate analyses were performed for each fortification level. The extraction procedure described below was followed.

Extraction procedure

An aliquot of propolis (0.5 g) was placed in a glass mortar (ca. 50 mL) and 1.0 g silica was added. The propolis was

then gently blended into the silica material with a glass pestle, until a homogeneous mixture was obtained (ca. 3 min). The homogenized mixture was introduced into a 100×20 mm ID polypropylene column packed with 0.1 g silanized glass wool at the bottom, 1.0 g anhydrous Na₂SO₄, and 1.0 g Florisil (upper layer). The column was gently tapped to remove air pockets. A 20-mL portion of dichloromethane–ethyl acetate (9:1, v/v) was added to the column and the sample was allowed to elute dropwise. The eluent was collected in a graduated conical tube, concentrated using a rotary vacuum evaporator (40 °C), and finally purged with nitrogen to a volume of 1 mL. A 1-μL portion of the extract was then directly analyzed by GC–MS.

Results and discussion

GC–MS conditions

For the purpose of measuring retention times and finding the best resolution between the pesticide peaks, experiments were carried out by varying the oven temperature and

carrier gas flow in full-scan mode (SCAN) using a standard solution (5 mg mL⁻¹). In these evaluations the characteristic ions were chosen for quantification of each pesticide. The choice of the ions for the selected ion monitoring (SIM) acquisition mode was made in order to obtain the best *S/N* ratios. Figure 2 shows the chromatograms obtained from a standard solution, a control propolis sample, and a propolis sample fortified with a standard solution of the pesticides studied. The total running time of GC–MS analysis was 25 min. On the other hand, matrix components can provide variation in the detector response to pesticides. So, the matrix effect was evaluated by comparing the detector response from pesticide standards prepared in dichloromethane with that from pesticide standards prepared in propolis extract. Preparation of standards by spiking blank propolis samples with known amounts of pesticides resulted in higher peak areas (approx. 30%) from the same pesticide concentrations. Consequently, quantification of pesticide residues was carried out using matrix-matched standards. The literature has reported the occurrence of enhancement or suppression of the chromatographic response owing to the matrix effect [14, 17].

Fig. 2 GC–MS (SIM) chromatograms obtained from: (a) standard solution at 1.0 μg mL⁻¹; (b) control propolis sample; (c) propolis sample fortified at 0.25 μg g⁻¹. Peaks: 1, vinclozolin; 2, buprofezin; 3, bifenthrin, 4, tetradifon

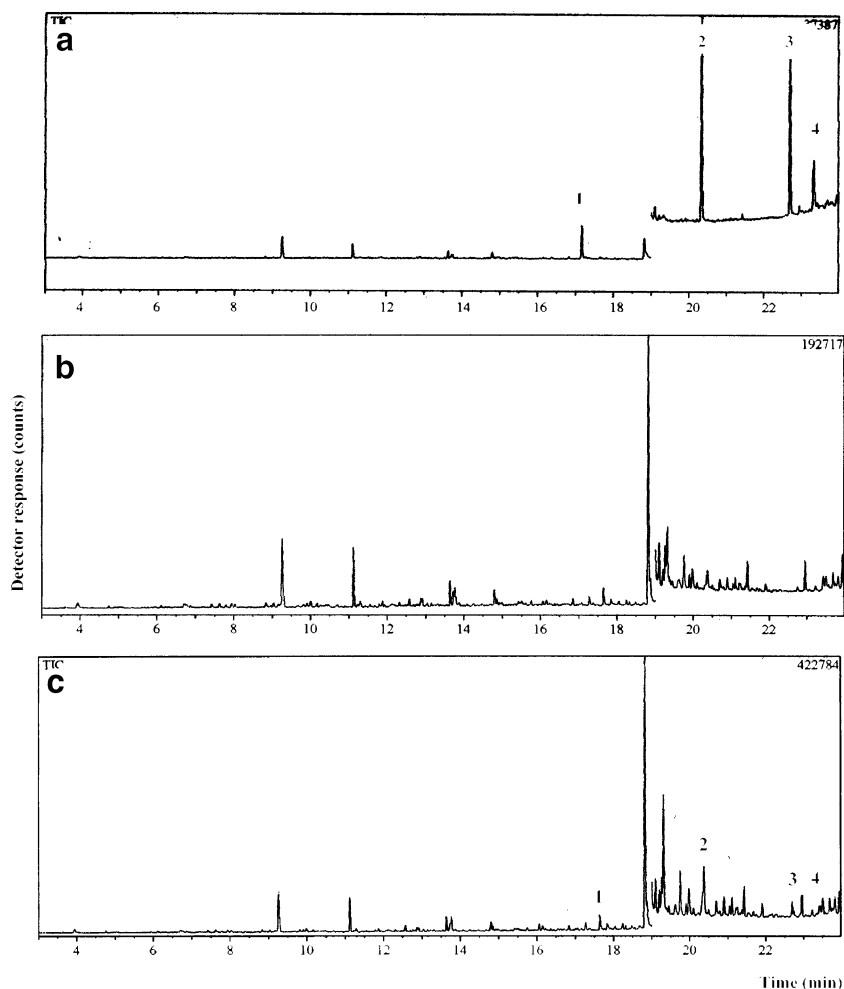


Table 1 Percentage recoveries and relative standard deviations obtained by MSPD procedure for propolis fortified with the pesticides studied ($n=4$)

Pesticide	Concentration (mg kg ⁻¹)	Mean recovery (%)	RSD (%)
Bifenthrin	0.25	67.0	9.0
	0.50	84.5	9.1
	1.00	94.2	10.8
Tetradifon	0.25	83.5	10.1
	0.50	84.9	5.7
	1.00	77.8	11.2
Vinclozolin	0.25	83.2	10.7
	0.50	82.2	5.6
	1.00	67.2	7.1
Buprofezin	0.25	175.0	8.4
	0.50	167.0	12.1
	1.00	106.0	10.6

MSPD procedure

The extraction method proposed is based on the MSPD procedure [18]. The most suitable extraction conditions were evaluated to achieve the highest recovery of vinclozolin, tetradifon, buprofezin, and bifenthrin from propolis. In these experiments, propolis samples spiked at 0.5 mg kg⁻¹ were used. Preliminary investigations were performed to choose the extraction solvent. Using silica as solid-phase sorbent and the solid-phase-to-propolis matrix proportion 1:2 (m/m), dichloromethane, two dichloromethane-*n*-hexane mixtures (8:2 and 1:1, v/v), and three dichloromethane-ethyl acetate mixtures (9:1, 8:2, and 7:3, v/v) were tested as elution solvent. When the data obtained were compared, rather different results were found for the solvents tested. Use of dichloromethane resulted in the cleanest extracts for pesticide extraction from propolis matrix, but the average recoveries were between 8.2 to 24.7%. On the other hand, elution of the MSPD column with the two dichloromethane-*n*-hexane mixtures (8:2 and 1:1, v/v) resulted in a higher background and more interfering peaks compared with

dichloromethane. Average recoveries of the pesticides were between 12.0 and 54.0%. Dichloromethane-ethyl acetate mixtures (9:1, 8:2, and 7:3, v/v) were therefore evaluated as eluting solvent, and two volumes (20 and 40 mL) were tested for each dichloromethane-ethyl acetate mixture. The best results were obtained with 20 mL dichloromethane-ethyl acetate (9:1, v/v), because recovery increased slowly with increasing eluent volume. So, the eluent volume used for subsequent studies was, therefore, 20 mL. Based on these experiments addition of another adsorbent to minimize co-extraction of matrix interferences was investigated. In addition, different silica-to-propolis matrix ratios (1:1, 2:1, and 3:1 m/m) were evaluated in attempts to optimize the MSPD method. The results obtained showed that the best recoveries were obtained by using 1.0 g silica-0.5 g propolis matrix (2:1, m/m). Moreover, increasing the amount of silica did not improve the recovery of the pesticides studied. Finally, elution of the pesticides in propolis from a silica-propolis matrix using 20 mL of dichloromethane-ethyl acetate (9:1, v/v) resulted in high recoveries ranging from 119% to 251% for these compounds. These values can be attributed to the presence of interfering endogenous compounds. To ensure removal of these interfering compounds addition of another adsorbent packed below the silica-blended sample in the MSPD column was investigated. Florisil and C₁₈ were separately tested as the clean-up layer in the extraction column. The results demonstrated that Florisil was more effective than C₁₈ at removing interfering compounds, with values ranging from 67% to 127%. All analyses were carried out in duplicate. On the basis of these overall results, the combined effect of silica as solid-phase, Florisil as clean-up layer, and dichloromethane-ethyl acetate (9:1, v/v) as elution solvent makes this extraction column suitable for determining bifenthrin, buprofezin, vinclozolin, and tetradifon in a complex matrix such as propolis. Once the factors that affect the MSPD procedure, namely type and amount of solid-phase (1 g silica-C₁₈), co-column (1 g Florisil), and eluting solvent mixture (40 mL dichloromethane-ethyl acetate (9:1, v/v)), had been optimized, validation of the method was performed.

Table 2 Retention times, calibration data, and LOD and LOQ of the pesticides analyzed by GC-MS

Pesticide	Retention time (min)	Calibration data		LOD (mg kg ⁻¹)	LOQ (mg kg ⁻¹)
		Equation	Correlation coefficient		
Vinclozolin	17.19	$y=42338x - 2201$	0.9982	0.10	0.25
Buprofezin	20.37	$y=174413x - 11636$	0.9973	0.05	0.15
Bifenthrin	22.79	$y=10255x - 41595$	0.9961	0.05	0.15
Tetradifon	23.37	$y=664063x - 16309$	0.9986	0.10	0.25

Recovery study

A study of recovery of the pesticides from the propolis matrix at three concentration levels was performed in order to assess the extraction efficiency of the proposed method. For this purpose untreated propolis was fortified at three concentrations (0.25, 0.50, and 1.0 mg kg⁻¹) and processed as described above. Fortification levels were selected to reach the maximum residue levels (MRLs) established by European Union legislation, which range from 0.2 mg kg⁻¹ to 1.0 mg kg⁻¹ for the pesticides in honey, because maximum limits of pesticide residues in propolis are not established in the Brazilian regulations [19]. Residues were quantified by using the external standard method. Four sample replicates spiked at each fortification level were assayed. Average recoveries ranged from 67.0 to 175.0%. Recovery data were calculated by comparison with the appropriate working standard solutions. Recoveries higher than acceptable values (70–120%) were obtained for buprofezin in propolis (106–175%). This enhancement of recovery could be attributed to excessive presence of endogenous compounds at the same retention time when the extraction procedure was performed. Because propolis is a very complex matrix, containing non-volatile compounds, final pesticide recoveries were not reproducible. Accuracy was calculated as the ratio (%) of found to known concentrations and precision was determined as the coefficient of variation (RSD, %), which is the ratio of the standard deviation to the average concentration found. Standard solutions were injected after every ten samples to monitor changes in chromatographic conditions. The repeatability of the complete method was determined by replicate analysis of fortified samples on different days. The repeatability of the method, expressed as RSD, ranged from 5.6 to 12.1%, as can be seen in Table 1.

Linearity, and detection and quantification limits

The detector response was linear over the range of concentrations studied. Linearity was determined for all the compounds using blank propolis samples fortified at concentrations of 0.05, 0.1, 0.2, 1.0, 2.0, 5.0, and 10.0 µg mL⁻¹, in duplicate. The slope and intercept values, and their standard deviations, were determined by regression analysis. Good coefficients of determination were obtained from the pesticides, ranging from 0.9961 to 0.9986.

The limits of detection (LOD) for the pesticides studied ranged from 0.05 to 0.10 mg kg⁻¹, which were calculated on the basis of the standard deviation of the noise (a value seven times the standard deviation of the blank) and the slope of the regression line. The limits of quantification

(LOQ) were determined as the lowest concentration giving a response of ten-times the average of the baseline noise defined from seven unfortified samples. LOQ values for these compounds ranged from 0.15 to 0.25 mg kg⁻¹ [20]. Table 2 summarizes these data for the pesticides studied.

Real samples

The developed MSPD procedure was applied to the determination of pesticides in propolis samples. Two different samples of propolis obtained from a local market were analyzed using this procedure. No residues above the detection limits of the pesticides studied were found in these samples.

Conclusions

The proposed MSPD procedure followed by GC–MS (SIM) was successfully applied to the extraction of bifenthrin, buprofezin, tetradifon, and vinclozolin from propolis. The method uses a silica gel-based MSPD column, a Florisil co-column, and dichloromethane–ethyl acetate (9:1, v/v) as elution solvent. The results demonstrate that the accuracy, precision, and selectivity of the proposed method are acceptable for multiresidue analysis of pesticides. The method also requires a small sample and offers considerable savings in terms of solvent consumption, cost of materials, sample manipulation, and analysis time. In addition, analysis of these pesticides in propolis could be accomplished in <50 min upon sample receipt, after suitable adjustment of the chromatographic conditions.

Acknowledgements The authors wish to thank CNPq/MCT-FAPITEC-SE/FUNTEC (process n° 35.0073/2004-0) for a fellowship and for financial support of this study.

References

1. Alencar SM, Oldoni TLC, Castro ML, Cabral ISR, Costa-Neto CM, Cury JA, Rosalen PL, Ikegaki M (2007) *J Ethnopharmacol* 113:278–283
2. Sforzin JM (2007) *J Ethnopharmacol* 113:1–14
3. Kumazawa S, Ueda R, Hamasaka T, Fukumoto S, Fujimoto T, Nakayama T (2007) *J Agric Food Chem* 55:7722–7725
4. Huang WJ, Huang CH, Wu CL, Lin JK, Chen YW, Chuang SE, Huang CY, Chen CN (2007) *J Agric Food Chem* 55:7366–7376
5. Popova MP, Barkova VS, Bogadov S, Tsvetkova I, Naydenski C, Marcazzan GL, Sabatini AG (2007) *Apidologie* 38:306–311
6. Bogdanov S (2006) *Apidologie* 37:1–18
7. Polese L, Navickiene S, Jardim EFG, Malaspina O, Palma MS, Ribeiro ML (2001) *Pest Rev Ecotoxic Meio Ambiente* 11:35–44
8. Rial-Otero R, Gaspar EM, Moura I, Capelo JL (2007) *Talanta* 71:503–514

9. Herrera A, Arquillue C, Conchello P, Bayarri S, Lazzaro R, Yague C, Arino A (2005) *Anal Bioanal Chem* 381:695–701
10. Pirard C, Widart J, Nguyen BK, Dleuze C, Heudt L, Haubruge E, De Pauw E (2007) *J Chromatogr A* 1152:116–123
11. Baratak AA, Badawy HMA, Salama E, Attallah E, Maatook G (2007) *J Food Agric Environ* 5:97–100
12. Erdogrul O (2007) *Food Control* 18:866–871
13. Pang GF, Fan CL, Liu YM, Cao YZ, Zhang JJ, Fu BL, Li XM, Li ZY, Wu YP (2006) *Food Addit Contam* 23:777–810
14. Georgakopoulos P, Foteinopoulou E, Athanasopoulos P, Drosinos E, Skandamis P (2007) *Food Addit Contam* 24:360–368
15. Rissato SR, Galhiane MS, Almeida MV, Gerenutti M, Apon BM (2007) *Food Chemistry* 101:1719–1726
16. Kamel A, Al-Ghamdi A (2006) *J Environ Sci Health B* 41:159–165
17. Poole CF (2007) *J Chromatogr A* 1158:241–250
18. Barker SA (2000) *J Chromatogr A* 880:63–68
19. Council regulation n° 2377/90EEC (1990) Community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin
20. European Union (2003) Quality control procedures for pesticide residues analysis. Document SANCO/10476/2003