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Multiresidue determination of pesticides in honey samples by gas chromatography–mass spectrometry and application in environmental contamination

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Abstract

A simple and fast multiresidue method has been developed to determine 48 pesticides within the major groups of pesticides (organohalogen, organophosphorous, pyrethroids and organonitrogen) in representative samples of locally produced honey, in Bauru (State of São Paulo, Brazil) during 2003–2004. The recovery results found ranged from 76% to 95% and the limits of detection were lower than 0.01 mg/kg for gas chromatography with electron impact mass spectrometric detection in the selected ion monitoring mode (GC–MS-SIM). The results indicated that most pesticides found in the samples belonged to the organohalogen and organophosphorous groups and lower levels of residues of some organonitrogen and pyretroids were also detected. Malathion residues were detected in all the samples, in a high concentration, owing to its applications to control dengue mosquitoes in the area studied.

Keywords: Pesticides residue analysis; Capillary gas chromatography–mass spectrometric detection with selective ion monitoring; Honey; Environmental contamination

1. Introduction

Great productivity gains can be achieved in agriculture, by using the adequate pesticides. Indeed, they are needed to meet the world's demand on foodstuffs and no other alternative can compete to be used in such a large scale. Slow degradation of pesticides, in the environment, and extensive or inappropriate use by farmers, can lead to environmental contamination of the water, soil, air, several types of crops and, indirectly, humans (Hamilton & Crossley, 2004; Olkowski, 1991).

As a result, consumers are exposed to pesticides, usually in minute quantities, in several food groups including fruits, juices, honey and vegetables and the monitoring pesticide residues in honey, helps to assess the potential risk of this product to consumers' health, providing information on the pesticides which have been used in the field crops, surrounding the hives.

Honeybees (*Apis mellifera*), perform the vital task of pollinating agricultural crops and native species and are important to the commercial production of honey and beeswax. Every day, 10,000–25,000 honeybee workers make an average of 10 journeys to explore roughly 7 km^2 in the area near their hive, gathering nectar, water, and pollen from flowers. During this process, various microorganisms, chemical products, and particles, suspended in the air, are intercepted by these workers and retained in the hair of their body surface, or inhaled and attached to their trachea (Devillers & Pham-Delegue, 2002). Thus, these

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easy-to-breed, almost ubiquitous organisms, with modest food requirements, are highly sensitive to biological, chemical, and physical factors, such as parasites, industrial contaminants, or pesticides and may be used as one bio-indicator to monitor the environmental stress (Celli & Maccagnani, 2003; Fernández, Pico, & Mañes, 2002; Kevan, 1999). Furthermore, the contact of honey bees with almost all environmental sectors (soil, vegetation, water, air) provides numerous indicators (through foraging), for each season. Finally, a variety of materials is taken into the hive (nectar, pollen, honeydew, propolis and water) and stored (Winston, 1991).

The maximum concentration of pesticide residues (MRLs), legally permitted in honey, has been established by different national regulations. Germany, Italy, and Switzerland have set MRLs for amitraz, bromopropylate, coumaphos, cyamizole, flumetrine, and fluvalinate, which oscillate between 0.01 and 0.1 mg/kg in Germany, between 5 and 500 mg/kg in Switzerland, and are of 10 mg/kg in Italy (Bogdanov, 1999). Up to date, the maximum limits of pesticide residues in honey are not included in the *Codex Alimentarius* (Codex Alimentarius, 1998). The European Union (EU) legislation has regulated the MRLs for three acaricides: amitraz, coumaphos, and cyamizole, which are 0.2, 0.1, and 1 mg/kg, respectively (Commission Regulation (EC), 1999) and the US Environmental Protection Agency (Food & Drug Administration, 2003) has established MRLs for amitraz (1 mg/kg), coumaphos (0.1 mg/kg), and fluvalinate (0.05 mg/kg).

Pesticide residues programs for monitoring honey concentrate in the determination of residues of acaricides that are used to control *Varroa jacobsoni*, a parasitic mite that affects honeybee colonies (Fernández-Muiño et al., 1997; Menkissoglu-Spiroundi, Tsigouri, Diamantidis, & Thrasyvoulou, 2001; Wallner, 1999). Only a few studies have focused on pesticides used for crop protection and introduced into hives by contaminated bees and wax (Al-Rifai & Akkel, 1997; Anju, Beena, Gahlawat, Sihag, & Kathpal, 1997; Driss, Zafzouf, Sabbah, & Bouguera, 1994).

A multiresidue method, able to detect and quantify pesticides, in a relatively short period, comprising minimum extraction and clean-up steps, is crucial for an efficient monitoring program (Bogdanov, 1999). Pesticides in honey are usually extracted by treating the sample with an organic solvent (Fernández et al., 2002; Porrini et al., 2003; Tsipi, Triantafyllou, & Hiskia, 1999), or in a solid phase, by the passage through octadecylsilane cartridges (Albero, Sanchez-Brunete, & Tadeo, 2004; Blasco et al., 2003; Martel & Zeggane, 2002; Tsipi et al., 1999), after dilution of the honey sample with water. Clean-up is necessary in order to reduce the detection limits of methods and/or to avoid interferences from the matrix. Extensive clean-up of extracts may result in the partial loss of some compounds as well as in increased labor and cost demands, but inadequate clean-up, can lead to adverse effects related to the quality of the generated data, such as masking of the residue peaks by co-eluting matrix components, the occurrence of false positives and inaccurate quantification. The most common interferences that are present in apiarian extracts are lipids, pigments, and carbohydrates. Sample clean-up techniques include gel permeation chromatography on Bio Beads SX3 (Dalpero et al., 2001), liquid–liquid partitioning (Herrera et al., 2005), solid-phase extraction (SPE) (Blasco et al., 2003; Fernández et al., 2002; Rissato, Galhiane, Knoll, & Apon, 2004) and adsorption chromatography (on silica, Florisil, active carbon, alumina, silica-gel/charcoal) (Fernández et al., 2002).

Many analytical methods for pesticide determination in honey are limited to the analysis of a few compounds such as organochlorines (Blasco et al., 2004), organonitrogens (Rezić, Horvat, Babić, & Kaštelan-Macan, 2005), acaricides and insecticides that are used in honey beehives (Jimenez, Bernal, Toribio, del Nozal, & Martín, 2002; Korta et al., 2001) or multiresidue methods (Herrera et al., 2005) which analyze some classes, using at least two different detectors. On the other hand, honey production specialists, even researchers, mainly focus on variables in which bees are concerned. An analytical method to effectively contribute to this issue, should cover a great number of analytes, being suitable to use and applicable to environmental contamination.

This work aimed at developing a rapid and simple multiresidue method, so as to, simultaneously, determine and confirm 48 pesticides of different classes: organohalogen, organophosphorous, organonitrogen and pyrethroids in honey samples by gas chromatography–mass spectrometry. The method was applied to analyze these compounds in the samples collected in an apiary localized in an ecological reserve in Bauru (State of São Paulo, Brazil) and temporal trends on pesticide contamination were evaluated.

2. Experimental

2.1. Chemicals

2.1.1. Pesticides standard

Pesticide standards were purchased from Dr. Ehrenstorfer (Augsburg, Germany) and most of them were of >99% certified purity. Concentrations of standard solutions were corrected by the certified purity of the standards, whenever below 99%. Individual stock standard solutions of pesticides were prepared by dissolving 20–50 mg of each compound in 25 mL of acetone and stored in glass flasks at −20 °C. Mixed compound calibration solutions, in acetone, were prepared from the stock solutions and used as spiking solutions as well. Matrix-matched standards were prepared in the same concentration as that of calibration solutions, by adding appropriate amounts of standards to the control matrix.

2.1.2. Organic solvents and reagents

Acetone, *n*-hexane, acetonitrile, ethyl acetate and dichloromethane, of special grade for pesticide residue analysis were purchased from Mallinkrodt, Merck. BAK-

ERBOND Octadecyl (C_{18}), Florisil and alumina (3 mL, 500 mg) were purchased from J.T. Baker.

2.2. Sample collection

An uncontaminated honey sample (control) was selected to be used in the optimization and validation experiments. The honey samples represented the locally produced honey, i.e. in the apiary in Bauru (State of São Paulo). These samples were collected during the harvesting of honey (September/December, 2003/2004). The samples, weighing between 500 and 1000 g, were stored in their original containers, at 10 °C, in a dark place, until their analysis.

2.3. Extraction

In order to analyze a large number of pesticides from different classes, a simple method has been developed to expand the range applicability of a previously tested multiresidue method for pesticide analysis in honey samples (Rissato et al., 2004). A 10 g portion of honey sample was weighed in an Erlenmeyer flask and spiked when required with the pesticide standard solution, being mixed with 5 mL water and homogenized by shaking, to reduce its viscosity and facilitate its handling. The sample was mixed with 50 mL of the solvents tested (acetonitrile, acetone, ethyl acetate and dichloromethane) and submitted to extraction, by agitating, for 20 min. Then, the organic phase was separated by centrifugation at 2500*g*, for 10 min, the supernatant was collected and the residue was re-extracted with 40 mL of solvent. The two portions collected were combined and the solvent was evaporated in a rotary evaporator, under reduced pressure at 65 °C and dried under a gentle stream of pure nitrogen. Finally, the residue was dissolved in 5 mL of ethyl acetate and passed through a 0.50 μm sized pore PTFE filter.

For honey fortification, 10 g of the control sample were heated in a water bath at 40 °C, for 20 min, being spiked by adding an appropriate volume of the standard working solution to reach the concentrations of 0.02, 0.20 mg/kg. The mixture was mechanically stirred in a blender, so as to ensure homogenization, and then submitted to the extraction step.

2.4. Clean-up

The clean-up of the samples was performed by means of a Supelco VISIPREP-12 manifold using alumina, Florisil and C_{18} cartridges which were conditioned with approximately 5 mL of acetone. The extract was added to the column and eluted under gravity with two portions of 10 mL for each of the tested mixtures of hexane/ethyl acetate, at several ratios (80:20, 70:30, 60:40; 50:50, v/v). Once elution was completed, the collected extracts were concentrated under a gentle N_2 stream and the residue was dissolved in 1 mL of ethyl acetate and submitted to analysis by GC/MS.

2.5. GC/MS

Confirmatory run analysis was done on a Hewlett–Packard Model 5890 Series II gas chromatograph with a HP 5972 mass selective ion detector (quadrupole) and a fused-silica capillary column LM-5-5% phenyl 95% dimethylpolysiloxane (35 m × 0.25 mm i.d., film thickness 0.25 μm). GC operated under the following conditions: initial temperature 60 °C increased at 25 °C/min to 150 °C, held for 1 min, increased at 3 °C/min to 200 °C, held for 1 min and 8 °C/min to 290 °C being held for 8 min.

The carrier gas (helium) flow rate was in constant flow mode at 1.0 ml/min. Splitless injection of a 1 μL volume was carried out at 250 °C with the purge valve on at 2 min. The liner used was amino deactivated single gooseneck from Restek (Bellefonte, USA).

The mass spectrometer was operated in electron ionization mode with impact ionization voltage 70 eV, a transfer line temperature of 290 °C, ion source 230 °C, electron multiplier voltage 1200 V, solvent delay 2.9 min and selected ion monitoring (SIM) mode. Dwell time was adjusted so that the number of cycles per second was 1.4 throughout the chromatographic run, providing a sufficient number of chromatographic points for all compounds.

2.6. Limits of detection

Detection limits (LOD) of the GC/MS were determined for each pesticide by the successive dilution of the standard mixed pesticide solution, followed by injection into the GC-column several times. Serial dilution experiments provided the necessary information to calculate the detection limits (Boyd-Boland & Pawliszyn, 1995; Lehotay & Valverde-Garcia, 1997).

2.7. Quality control

The quality control for the analysis of pesticides in honey, consisted of five honey samples, one honey spike, one water blank, one water spike, eight calibration standards (ranging from 0.010 to 2.00 mg/L of mixed pesticide solution standards), a calibration check standard, and ethyl acetate rinses. The honey spike was selected from a set of several free pesticides samples and consisted in fortifying the honey with a mixed pesticide spike standard. The honey and water samples were fortified at 0.020 mg/L and analyzed as previously described. Acceptable spike recoveries ranged from 60% to 130% and the positive results, in the honey samples, were confirmed by comparing the retention time, identifying the main ions, in relation to those of a pesticide standard. Retention times were within ±0.20 min of the expected retention times. The water blanks and spikes were analyzed in order to account for any residual interference or possible contamination sources, such as glassware, handling and others. The presence and confirmation of pesticides or pesticide residues in the water blanks resulted in the extraction and analysis of the entire

batch. After completion of the standards, blanks, spikes, sample extracts, and rinses, a 0.200 mg/L calibration standard was analyzed to account for any differences or variations during the entire batch analysis. Any deviation beyond 15% required a new injection or analysis of the entire batch to be repeated. The quantitation of any pesticide(s) present in the honey extract was determined as previously described.

3. Results and discussion

3.1. Method development

In order to minimize the effect of honey co-extractives on the determination of the analytes studied, as well as to improve recoveries, various solvents have been tested: acetonitrile, acetone, dichloromethane and ethyl acetate.

Acetonitrile showed low recovery results for some organohalogens. Dichloromethane showed to be inappropriate for quantitative extraction of moderately polar and polar pesticides as well as acetone that presented high recovery results for some studied pesticides (>160%). In short, ethyl acetate was the most suitable solvent for the extraction of multiresidue pesticides.

Since high molecular-weight compounds present in the samples can be co-extracted with the analyzed pesticides, a clean-up step is recommended to diminish the interferences in the final extract, which can damage the capillary column as well as result in a matrix enhancement effect (Stajnbaher & Zupancic-Kralj, 2003).

Preliminary clean-up experiments were carried out in order to find the best sorbent for the solid-phase extraction, being Florisil, alumina and C_{18} commercial cartridges tested. The elution solvent should be of a medium polarity to elute the less polar to polar residues, leaving high molecular weight compounds in the cartridge, although for a more efficient elution of more polar organophosphorous pesticides, a more polar solvent mixture should be tested. Therefore, mixtures of hexane/ethyl acetate at several ratios (80:20, 70:30, 60:40; 50:50, v/v) were evaluated. For Florisil, low recoveries were obtained with 80:20, 70:30 tested mixtures. It was observed that by increasing the eluting solvent polarity (60:40; 50:50), greater recoveries were obtained. Thus, the mixture of hexane/ethyl acetate 50:50 was chosen as an elution solvent for the 48 pesticides, presenting recoveries ranging from 81% to 103%.

As for alumina as an adsorbent, the results were acceptable (ranging from 60% to 85 %) for the majority of the target pesticides, and C_{18} presented an unacceptable recovery for more than 50% of the same compounds. Florisil was the most adequate adsorbent for the clean-up of the compounds studied.

3.2. GC–MS/SIM, sensitivity, and linearity

The samples were analyzed by GC–MS/SIM, according to the conditions listed in Section 2.5. Chromatograms of an injected extract from a control and spiked (0.20 mg/kg) honey are shown in Fig. 1. The compounds were identified by their retention time, fragment ions (m/z), regarding the pesticide standards listed in Tables 1 and 2.

The limit of detection (LOD) of each pesticide listed in Table 1 was determined from injection of the standards and was defined as approximately three times the standard deviation. Of these pesticides, 45 had LODs less than 0.005 mg/L, with 28 pesticides having LODs equal to or less than 0.001 mg/L. Linearity was obtained for pesticides by using standards ranging from 0.010 to 2.0 mg/L, and 48 compounds have $r^2 > 0.997$.

3.3. Spike recoveries

Spike recoveries were determined by adding the pesticides to a control honey sample at a final concentration of 0.02 or 0.20 mg/kg and analyzing the spiked honey using the developed method (Table 1). For the high spike concentration (0.20 mg/kg), spike recoveries greater than 82% were found for 29 of the studied pesticides (out of 48 total pesticides) and recoveries of 12 compounds higher than 90% were observed for extracted honey spike. The data also showed that seven pesticides had spike recoveries below 80%. These numbers are similarly reflected for honey spiked at the low-spiked concentration (0.02 mg/kg).

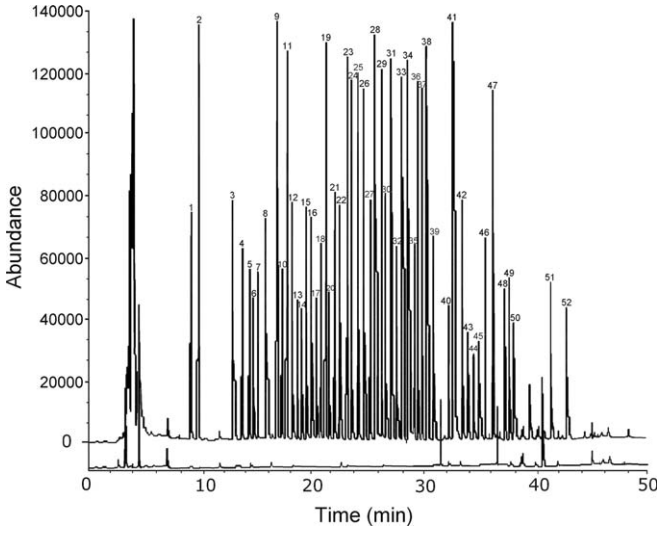

Fig. 1. GC–MS-SIM chromatogram of the extracts from a control and a spiked (0.20 mg/kg) honey (see conditions in Section 2.4). 1: Dichlorvos; 2: linuron; 3: trifluralin; 4: hexachlorobenzene; 5: simazine; 6: atrazine; 7: lindane; 8: terbuthylazine; 9: diazinon; 10: chlorothalonil; 11: metribuzin; 12: parathion methyl; 13: alachlor; 14: promethrin; 15: dicofol; 16: fenitrothion; 17: pirimiphos-methyl; 18: aldrin; 19: malathion; 20: metolachlor; 21: fenthion; 22: chlorpyrifos; 23: triadimefon; 24: imazalil; 25: pendimathalin; 26: phentoate; 27: procymidone; 28: methidathion; 29: endosulfan alfa; 30: profenophos; 31: cypropconazole; 32: endosulfan beta; 33: ethion; 34: benalaxyl; 35: endosulfan sulfate; 36: hexazinone; 37: bromopropylate; 38: propiconazole; 39: g-cyhalothrin; 40: pyrazophos; 41: tebuconazole; 42: prochloraz; 43, 44, 45: cyfluthrin; 46: metoxychlor; 47: tetradifon; 48, 49, 50: cypermethrin; 51, 52: fluvalinate.

Table 1
Retention times (t_R), limits of detection (LOD, mg/L), limits of quantification (LOQ, mg/L), recovery results (%) (high and low spike) and determination coefficient of the pesticides studied

Pesticides	t_R (min)	LOD (mg/L)	LOQ (mg/L)	Recovery % (RSD) high spike	Recovery % (RSD) low spike	Determination coefficient (r^2)
Organohalogen						
Aldrin	20.91	0.0020	0.0080	95 (4.5)	93 (6.6)	0.999
Bromopropylate	30.03	0.0005	0.0020	88 (3.8)	90 (5.3)	0.998
Chlorothalonil	17.13	0.0016	0.0050	79 (5.2)	80 (4.4)	0.999
Dicofol	19.63	0.0010	0.0040	82 (6.1)	79 (5.8)	1.000
Endosulfan Alfa	26.11	0.0004	0.0015	91 (6.4)	93 (7.2)	0.997
Endosulfan Beta	27.31	0.0015	0.0050	83 (5.6)	80 (6.4)	0.999
Endosulfan Sulfato	28.74	0.0020	0.0080	90 (3.9)	86 (4.1)	0.998
Hexachlorobenzene	13.67	0.0015	0.0060	87 (4.4)	84 (7.0)	0.998
Lindane	15.19	0.0015	0.0050	84 (4.9)	88 (5.2)	1.000
Metoxychlor	35.62	0.0015	0.0060	88 (3.8)	82 (6.3)	1.000
Tetradifon	36.20	0.0005	0.0020	92 (6.0)	90 (5.5)	0.999
Organonitrogen						
Alachlor	18.73	0.0040	0.0150	83 (6.3)	79 (7.2)	0.998
Atrazine	14.71	0.0050	0.0180	78 (4.8)	81 (6.7)	1.000
Benalaxyl	28.35	0.0002	0.0010	81 (7.8)	80 (5.8)	0.997
Cyproconazole	26.96	0.0002	0.0008	79 (5.4)	77 (6.3)	0.999
Hexazinone	29.37	0.0005	0.0015	90 (5.9)	93 (7.4)	0.999
Imazalil	23.81	0.0005	0.0015	82 (6.5)	80 (6.0)	0.998
Linuron	9.86	0.0002	0.0010	93 (7.2)	91 (5.3)	1.000
Metolachlor	21.84	0.0050	0.0180	82 (6.6)	80 (4.3)	0.999
Metribuzin	17.52	0.0003	0.0012	80 (7.1)	76 (6.9)	0.998
Pendimethalin	24.28	0.0005	0.0015	77 (6.4)	79 (4.0)	0.997
Prochloraz	33.54	0.0010	0.0030	89 (5.8)	86 (6.1)	0.999
Procymidone	25.19	0.0010	0.0040	89 (6.8)	84 (5.9)	1.000
Prometryn	19.22	0.0040	0.0120	101 (6.5)	96 (5.2)	0.999
Propiconazole	30.57	0.0002	0.0010	91 (7.2)	94 (4.8)	0.998
Simazine	14.23	0.0015	0.0050	86 (4.9)	83 (6.6)	0.997
Tebuconazole	32.91	0.0005	0.0020	87 (4.9)	81 (7.2)	1.000
Terbuthylazine	15.79	0.0013	0.0045	88 (6.3)	85 (7.3)	0.998
Triadimefon	23.25	0.0005	0.0020	85 (4.9)	88 (6.2)	1.000
Trifluralin	12.88	0.0010	0.0040	89 (5.5)	91 (5.8)	0.999
Organophosphorous						
Chlorpyrifos	22.83	0.0008	0.0030	103 (6.2)	112 (4.9)	0.999
Diazinon	16.18	0.0002	0.0010	93 (7.2)	90 (6.5)	0.999
Dichlorvos	9.02	0.0010	0.0040	84 (6.8)	86 (7.1)	0.997
Ethion	27.93	0.0002	0.0010	79 (5.5)	78 (6.0)	0.998
Fenitrothion	20.08	0.0010	0.0040	77 (7.2)	80 (5.2)	1.000
Fenthion	22.45	0.0008	0.0030	110 (4.9)	105 (7.3)	0.999
Malathion	21.36	0.0002	0.0010	87 (5.7)	89 (5.8)	0.998
Methidathion	25.61	0.0002	0.0010	81 (6.2)	79 (6.9)	0.997
Parathion-methyl	18.25	0.0010	0.0035	76 (4.9)	80 (5.6)	0.999
Phentoate	24.72	0.0006	0.0025	91 (5.2)	95 (7.4)	0.999
Pirimiphos-methyl	20.44	0.0050	0.0200	86 (7.3)	83 (5.9)	0.998
Profenophos	26.39	0.0008	0.0030	85 (4.2)	81 (5.1)	1.000
Pyrazophos	32.46	0.0040	0.0150	94 (6.9)	94 (7.2)	0.999
Pyrethroids						
λ-Cyhalothrin	31.24	0.0015	0.0050	88 (3.3)	92 (4.6)	1.000
Cyfluthrin	33.90	0.0012	0.0040	109 (4.0)	119 (5.8)	0.999
	34.25					0.999
	34.82					1.000
Cypermethrin	37.12	0.0018	0.0060	89 (4.3)	85 (6.7)	0.999
	37.53					0.998
	38.01					0.999
Fluvalinate	41.46	0.0025	0.0080	91 (3.5)	88 (4.9)	1.000
	41.98					1.000

The accuracy of the technique was evaluated in terms of repeatability (within the day, by relative standard deviation, RSD) by the analysis of three replicate spiked honey samples at 0.02 and 0.2 mg/kg. The precision can be considered optimal, taking into account, a non-automated procedure, having a repeatability of

Table 2
Main ions of pesticides detected by GC/MS

Pesticides	Main ions (*m*/*z*)
Organohalogen	
Aldrin	263; 293; 329
Bromopropylate	149; 167; 279
Chlorothalonil	263; 293; 329
Dicofol	111; 139; 251
Endosulfan	237; 265; 339
Hexachlorobenzene	214; 249; 284
Lindane	181; 183; 109
Metoxychlor	227; 274; 374
Tetradifon	159; 229; 356
Organonitrogen	
Alachlor	160; 188; 143
Atrazine	215; 200; 173
Benalaxyl	148; 206; 91
Cyproconazole	222; 139; 73
Hexazinone	171; 128; 83
Imazalil	173; 215; 296
Linuron	61; 160; 248
Metolachlor	162; 211; 238
Metribuzin	198; 144; 182
Pendimethalin	252; 281; 220
Prochloraz	180; 266; 308
Procymidone	283; 285; 96
Prometryn	241; 184; 226
Propiconazole	173; 221; 259
Simazine	201; 186; 173
Tebuconazole	125; 250; 307
Terbuthylazine	214; 229; 173
Triadimefon	57; 208; 293
Trifluralin	263; 306; 335
Organophosphorous	
Chlorpyrifos	97; 197; 314
Diazinon	88; 179; 304
Dichlorvos	109; 185; 220
Ethion	231; 384; 153
Fenitrothion	277; 125; 109
Fenthion	278; 125; 109
Malathion	173; 127; 125
Methidathion	145; 85; 302
Parathion-methyl	263; 125; 109
Phentoate	274; 246; 125
Pirimiphos-methyl	276; 305; 290
Profenophos	208; 339; 139
Pyrazophos	221; 232; 373
Pyrethroids	
λ-Cyhalothrin	181; 197; 199
Cyfluthrin	163; 206; 226
Cypermethrin	163; 181; 209
Fluvalinate	250; 181; 252

less than 8% for the great majority of the analytes (Table 1).

The influence of matrix co-extractives on the response of analytes is a well-known phenomenon in pesticide residue analysis, which can result in either a decreased detection response or an increased analytical sign (Molins et al., 1998). Excessively high recovery values were previously observed and explained by the phenomenon known as ''matrix-induced chromatographic response enhancement'' which can occur for particular pesticides, matrix types, depending on the status of the capillary column (Erney, Pawlowski, & Poole, 1997).

The spike recovery data shown in Table 1 suggests that the combination of the eluting solvents (hexane/ethyl acetate) and the cleanup cartridge may have been effective in minimizing any matrix enhancement effects. In the present work, we utilized a Florisil cartridge for clean-up, a stronger non-polar solvent mixture consisting of ethyl acetate and hexane, and preparation of standards and the honey extracts in ethyl acetate. The data at the low-spike concentrations (0.02 mg/kg) revealed that only 4 of the 48 pesticides tested have recoveries greater than 100%, suggesting that the combination of these methods can be used to minimize possible matrix enhancement.

Fig. 1 reports a GC/MS chromatogram, accomplished in the selected ion monitoring mode, relevant to a control honey extract fortified at 0.20 mg/kg level. Control samples revealed the absence of pesticides residues, naturally contaminating the analyzed samples, as well as the absence of matrix components co-eluting with the target analytes. This was a clear sign of the high selectivity of the developed method.

3.4. Monitoring program

A few works on the monitoring of pesticide residue levels in honey have been previously published in the literature. The methods are limited to determine acaricides and insecticides widely used in hives (Blasco et al., 2004; Jimenez et al., 2002; Martel & Zeggane, 2002) or only to evaluate one pesticide class (Blasco et al., 2004). A recent multiresidue pesticide method, developed for 15 organohalogen pesticides (OCPs), six polychlorinated biphenyls (PCBs), and seven organophosphorous pesticides (OPPs), is implemented for routine determinations of residues in honey, showing low levels of pesticides in real honey samples (Herrera et al., 2005).

In present work, the applicability of the method to the monitoring program was assayed during a pilot study in which real honey samples, taken from an ecological reserve near an intensive horticulture area in Bauru, State of São Paulo, Brazil, were collected and analyzed in the period of 2003/2004.

The main goal was to identify the pesticides that were contaminating the honey, their quantities and the reason why the pesticide was there. Pesticide concentrations, detected in honey, collected over a 2-year period, are reported in Table 3. This work does not report on concentration results below the quantification limit or pesticide identity which could not be confirmed.

Residues of organohalogen (endosulfan sulfate, hexachlorobenzene and tetradifon) and organophosphorous (atrazine, simazine and tebuconazole) pesticides were found in most samples studied. The presence of residues of such compounds, in the samples analyzed, can be attributed to arbitrary applications in adjacent horticultural properties where vegetables and grain crops are grown.

Despite the extensive use of pesticides to control most agricultural pests, residues of this group were not com-

Table 3
Pesticide concentrations in mg/kg of honey samples obtained in a monitoring program carried out in the ecological reserve in Bauru (State of São Paulo, Brazil), over a 2-year period

Pesticides	Residue (mg/kg)	
	2003	2004
Organohalogen		
Aldrin	ND	0.020
Bromopropylate	ND	ND
Chlorothalonil	<LOD	<LOD
Dicofol	<LOD	<LOD
Endosulfan Alfa	ND	<LOD
Endosulfan Beta	ND	ND
Endosulfan Sulfato	0.027	0.024
Hexachlorobenzene	0.018	0.016
Lindane	ND	ND
Metoxychlor	ND	ND
Tetradifon	0.008	0.010
Organonitrogen		
Alachlor	ND	ND
Atrazine	ND	0.081
Benalaxyl	ND	ND
Cyproconazole	ND	ND
Hexazinone	ND	ND
Imazalil	ND	ND
Linuron	ND	ND
Metolachlor	ND	ND
Metribuzin	ND	ND
Pendimethalin	ND	ND
Prochloraz	ND	<LOD
Procymidone	ND	ND
Prometryn	ND	ND
Propiconazole	ND	<LOD
Simazine	0.017	0.015
Tebuconazole	0.003	0.005
Terbuthylazine	ND	ND
Triadimefon	ND	ND
Trifluralin	ND	ND
Organophosphorous		
Chlorpyrifos	0.010	0.015
Diazinon	ND	ND
Dichlorvos	ND	ND
Ethion	ND	ND
Fenitrothion	ND	ND
Fenthion	ND	ND
Malathion	0.243	0.209
Methidathion	ND	ND
Parathion-methyl	ND	ND
Phentoate	ND	ND
Pirimiphos-methyl	ND	ND
Profenophos	ND	ND
Pyrazophos	ND	ND
Pyrethroids		
λ-Cyhalothrin	ND	ND
Cyfluthrin[a]	ND	ND
Cypermethrin [a]	ND	0.092
Fluvalinate [a]	ND	ND

[a] Qualification performed by the sum of the peak areas of isomer forms.

monly detected in the honey samples analyzed. Nevertheless, in 2003–2004, a very noxious episode of malathion contamination took place. This fact can be related to the application of this pesticide in the control of the dengue vector mosquitoes by Health Officials. No maximum residue levels (MRLs) have been established for these pesticides in honey, so far. Fig. 2 represents a real sample where the chromatographic peaks obtained in SIM (single ion monitoring) are shown.

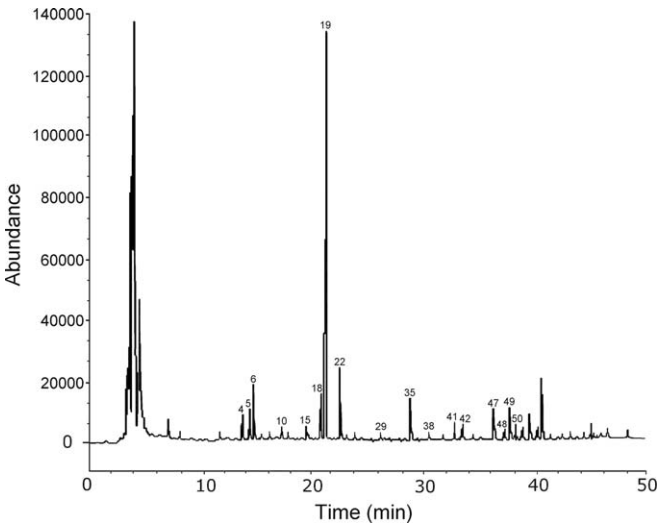

Fig. 2. GC–MS-SIM chromatogram of a real honey sample in the year 2004 (see conditions in Section 2.4).

The contamination of the area surrounding bee colonies markedly influences the kind and concentration of contaminants found in the honey samples. The distance between the colonies and the horticulture areas, in this case, is about one and a half miles, even though the present study has been carried out in an ecological reserve, one can realize how the neighboring areas can affect, in a substantial manner, the quality of the honey produced.

4. Conclusions

With the development of this multiresidue method, 48 pesticides were analyzed in honey. This methodology showed to be very simple and rapid, requiring small sample sizes, minimizing solvent consumption and hazardous waste. The utilization of mass spectrometric detection provided both quantitative information and confirmation of pesticide residues in honey.

Furthermore, the method was successfully applied to a monitoring program during the period of 2003–2004, using honey as a sign of pesticide use and its concentration in the region studied.

The results obtained, following the application of the method in real honey samples, indicated a low level of contamination by pesticide residues, nevertheless, malathion was detected in higher concentrations, as compared to others, due to applications of this pesticide in the control of dengue mosquitoes.

Since honeybees travel long distances and come close to many plants, honey may be an easily accessible environmental pollution indicator. On the other hand, from an ecological point of view, the vectors are preserved, being possible their settlement near possible contamination sources.

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