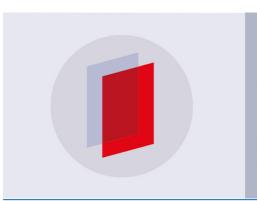# Recovery evaluation of organophosphorus pesticides from bee pollen by matrix solid-phase dispersion extraction using sorbents based on silica and titania

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Recovery evaluation of organophosphorus pesticides from bee pollen by matrix solid-phase dispersion extraction using sorbents based on silica and titania

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Abstract. This work focused on the evaluation of the recovery of organophosphorus pesticides from bee pollen after matrix solid phase-dispersion extraction (MSPD). Materials based on silica, titania and titania modified with polivylnylimidazole or polyestirene were used as adsorbents for the extraction of pesticides. Small amounts of fortified pollen (0.1 g, at 1 micro-g/g of pesticides), adsorbent (0.4 g) and solvent elution (1 mL de acetonitrile - ACN) were used in the extractions. For recovery evaluation, pollen extracts were analyzed by gas chromatography coupled with mass spectrometry.

1. Introduction

Matrix solid-phase dispersion (MSPD) is a sample preparation technique applied widely to solid, semisolid or viscous samples [1]. It has been employed for the extraction of organic contaminants [2] from many different samples such as propolis [3], pollen [4] and honey bees [5]. The steps for MSPD involve mixing the sample in a glass mortar in the presence of a solid support (adsorbent), packing the mixture in a glass (or polypropylene column) and eluting the analytes from the column with an appropriate solvent [1]. In the last step, interfering compounds are retained selectively on the solid phase. An alternative is the elution of interferences with a suitable solvent (wash step) followed by the elution of analytes retained in the MSPD column with another solvent [6].

The factors that affect the efficiency of extractions by MSPD are (1) the physicochemical properties of both the solid support and the bonded phase, (2) nature of matrix sample, (3) matrix modification, (4) solvent elution and its sequence addition [1]. Among these factors, the former affects the retention/elution of the analytes and the dispersion of the solid matrix sample onto the solid support. Thus, the solid supports typically reported in MSPD are commercial. These are based in

underivatized silica or derivatized with organic groups such as octadecyl (-C18), cyanopropyl (-$(CH_2)_3CN$) and aminopropyl (-$(CH_2)_3NH_2$) [7]. However, even though materials-science provides the tools for the development of new solid supports and organic phases, there are few reports on the development and application of "home-made" solid supports [8]. Thus, titania (TiO_2) organic-inorganic hybrid materials have not been employed as the solid phase in MSPD. In comparison with silica based materials, the main advantages of TiO_2 materials are its stability at pH 1-14 and its mechanical strength.

The aim of this work was the evaluation of new solid supports for the extraction of organophosphorous pesticides from bee pollen. One of the solid supports tested was commercially available and is based on silica (Florisil). The others were synthesized in the laboratory and are based on titania (TiO_2) and titania modified with polyvilnylimidazole (TiO_2-PVI) or polystyrene (TiO_2-PST). The bee pollen was selected as sample due to its complex chemical composition [9] and pesticides (dichlorvos, diazinon, methyl parathion, malathion and coumaphos) because of its wide use against pests in crop fields and bee hives.

2. Experimental

2.1. Chemicals and materials
Florisil was obtained from Sigma-Aldrich (St. Louis, MO, USA). Analytical grade anhydrous sodium sulfate (Na_2SO_4) and solvents (acetonitrile, isooctane, ethyl acetate) were purchased from J. T. Baker (Mallinckrodt Baker, Phillipsburg, NJ, USA). Pesticide standards (at least 97% pure) [dichlorvos (DCV), diazinon (DZN), methyl parathion (MPT), malathion (MLT) and coumaphos (CMF)] were obtained from ChemService (West Chester, PA, USA). Individual stock solutions were prepared in ethyl acetate and stored at -18ºC. Working standard solutions were prepared by diluting the stock solutions with ethyl acetate.

2.2. Fortified bee pollen samples
Bee pollen was spiked by the addition of pesticide standard solution in ethyl acetate (100 µL at 1 µg/mL) to 0.1 g of pollen to obtain a concentration of 1 µg/g. Then, the sample was left to stand (60 min) to allow pesticides to incorporate into the pollen.

2.3. Experimental design
In this stage of methodology, Florisil was used as solid support in MSPD. An unreplicated 2^4 factorial design [10] was used to study the experimental factors that affect the efficiency of MSPD extraction: (1) mass of solid support (A: 0.2 g, 0.4 g), (2) conditioning solid support (B: no, yes), chemical nature of elution solvent (C: ethyl acetate, acetonitrile) and solvent volume (D: 1 mL, 2 mL). The recovery percent (%R) of pesticides in pollen extracts was the variable response.

2.4. MSPD extraction
Bee pollen (0.1g) and solid support (Florisil, TiO_2-PVI, TiO_2-PST or TiO_2) were placed in a mortar. Then, both were dispersed with a pestle to obtain a homogeneous mixture which was packed in a polypropylene column with a filter paper disc at the bottom. Next, it was compressed softly to eliminate air pockets. The pesticides were eluted with organic solvent which was evaporated to dryness with a gentle air flow. The extract was reconstituted with isooctane and Na_2SO_4 was added (0.2 g). The extract was frozen (-3°C, 60 min) and centrifuged (50 min, -5°C) to precipitate high molecular weight compounds. Finally, the supernatant was analyzed by GC/MSD.

2.5. Instrumentation and chromatographic conditions
An Agilent Technologies 6890N gas chromatograph coupled with a mass spectrometer 5973N (GC/MSD) was used. A fused-silica column Equity-5 (5% phenyl-95% polydimethylsiloxane; 30 m × 0.25 mm ID, 0.25 µm), supplied by SUPELCO (Bellefonte, PA, USA) was employed with He (purity

99.999) as carrier gas (flow rate: 1.0 mL·min^{-1}). The oven temperature was programmed as follows: 120ºC for 3 min directly to 280ºC at 20ºC·min^{-1} and holding for 5 min (solvent delay: 3 min). The inlet temperature was 250ºC and 1 μL of the extract was injected in a splitless mode (1.5 min). The eluent from the GC column was transferred into the MSD via a transfer line (at 280ºC). Typical conditions were optimized through the autotune software option. The electron impact mode (70eV) was used as ionization source (250ºC) and masses were monitored between 50-400 m/z (SCAN mode). The temperature of the quadrupole was 150ºC. The analysis was performed in selected ion monitoring (SIM). The mass-charge ratios (m/z) for the ions for each pesticide were 109, 185, 220 for DCV; 179, 199, 304 for DZN; 109, 125, 263 for MPT; 125, 158, 173 for MLT and 226, 334 and 362 for CMF.

2.6. Efficiency of the MSPD extraction with SiO$_2$-poly (vinyl-imidazole)
Recovery experiments were done at three fortified levels (0.1, 0.5 y 1.0 μg/g) to evaluate the efficiency of the solid supports in MSPD. Recoveries were calculated through external calibration and pesticide standards were prepared by dilution with bee pollen extracts to minimize matrix effect.

3. Results and discussion
Recoveries of 1-110% were obtained by MSPD using Florisil as the solid support. The lowest recovery was for DCV and the highest for DZN. Recoveries higher than 120% were obtained for MPT, MLT and CMF in experiments where ethyl acetate (1 mL) was used as the eluent. This is ascribed to the elution of interfering compounds having the same retention time of MPT, MLT and CMF. It is reported that ethyl acetate elutes large amounts of non-polar interfering compounds [11]. In contrast, lowest recoveries (<60%) were obtained with acetonitrile (2 mL) since we believe that the analytes may evaporate during the increased time required to evaporate the large volume of solvent.

The experimental conditions that enabled good recovery percent for all of the pesticides studied were as follows: 0.4 g of Florisil without conditioning and 1 mL of acetonitrile. These conditions were applied for the extraction of fortified bee pollen at 0.1, 0.5 and 1.0 μg/g of fortification. At the lowest level, DCV and MPT were not detected and recoveries for MLT were lower than 11%. However, DZN showed a good recovery (105%). At 0.5 and 1.0 μg/g of fortification, recoveries for DCV were 69% and 48%, respectively. Nevertheless, recoveries for DZN, MPT and MLT ranged between 76 to 114%.

We evaluate the efficiency of TiO$_2$-PVI, TiO$_2$-PST or TiO$_2$ using 0.4 g of those materials as solid support instead of Florisil and 1.0 mL of acetonitrile. Recoveries are presented in table 1.

Table 1. Pesticide recoveries (%R) using sílica and titania based materials as the solid support and spiked pollen at 1.0 μg g^{-1} (n=3).

Pesticide	Material	%R	% RSD
Dichlorvos	Florisil	48	5
	TiO$_2$-PVI	33	36
	TiO$_2$-PST	32	10
	TiO$_2$	22	11
Diazinon	Florisil	96	4
	TiO$_2$-PVI	79	8
	TiO$_2$-PST	73	8
	TiO$_2$	86	22
Malathion	Florisil	77	14
	TiO$_2$-PVI	84	49
	TiO$_2$-PST	89	15
	TiO$_2$	99	16

Two pesticides (MPT and CMF) were not detected in chromatograms probably due to its retention into the TiO_2 materials. For DZN and MLT, recoveries were higher than 70% with the three materials based in TiO_2. However, recoveries obtained with TiO_2 were relatively higher than those obtained with TiO_2-PVI or TiO_2-PST. The lowest recoveries were for DCV (%R <35).

The recoveries for DZN and MLT with TiO_2 materials were similar to those achieved with Florisil. DCV recoveries obtained with both kinds of material were lower than 50%. This pesticide has a high vapor pressure and it can be lost during the solvent evaporation step [12].

For the three pesticides extracted with TiO_2-PST the precision (%RSD) was relatively higher than those obtained with Florisil, but it was equal to or lower than 15% as required for pesticide residue analysis [13]. Finally, precision of recoveries for MLT was close to %RSD obtained with Florisil. %RSD values of DCV, DZN and MLT were 20% higher than those obtained with TiO_2 or TiO_2-PVI.

4. Conclusions

Solid phases based on TiO_2 were used in MSPD to extract organophosphorous pesticides from spike bee pollen and pesticide recoveries were determinated.

The experimental conditions that yield pesticide recoveries from 70 to 120% were 0.4 g of Florisil (no conditioning) and acetonitrile (1 mL). Recoveries obtained for DZN, MPT and MLT with Florisil in spiked bee pollen (0.5 and 1.0 µg/g) were 76 to 114%. DCV presented recoveries from 47 to 69%. Low recoveries (<11%) were obtained at 0.1 µg/g or signals were not detected for pesticides.

With titania materials, DCV, DZN and MLT were detected in spiked pollen extracts. The recoveries for the first pesticide were lower than 35%. The last two pesticides presented recoveries higher than 70%. MPT and CMF were not eluted from the MSPD column.

Recoveries for DZN and MLT with the three kind of TiO_2 materials were similar to those obtained with Florisil. Precision of pesticide recoveries was acceptable (RSD≤15%) with TiO_2-PST.

5. Acknowledgements

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