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Simultaneous determination of organophosphorous insecticides in bean samples by gas chromatography - flame photometric detection

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ABSTRACT. The indiscriminate use of organophosphorous pesticides (OPPs) in crops may leave residues in food and may cause poisoning in the applicators. A method was developed for the determination of five OPPs in bean samples by Gas Chromatography-Flame Photometric Detection (GC-FPD). Validation parameters comprised linearity between 0.24 and 8.56 μg g^{-1} (r = 0.9985) for diazinon; 0.23 and 8.14 μg g^{-1} (r = 0.9959) for methyl parathion; 0.28 and 10.25 μg g^{-1} (r = 0.9987) for methyl pirimiphos; 0.52 and 18.87 μg g^{-1} (r = 0.9955) for malathion; 0.86 and 13.67 μg g^{-1} (r = 0.9919) for ethion. The limits of quantification (equal to those of detection) were the lowest rates of ranges mentioned above for each compound. The extraction method showed approximately 95% recovery, with CV% < 15%. Although twenty-eight bean samples obtained in the southern region of the state of Minas Gerais, Brazil, were analyzed, they failed to match any of the OPPs under analysis. The absence of OPPs in the samples could be due to the degradation that occurred between the use of OPPs and bean commercialization, levels below the detection /quantification limits and the non-use of OPPs in bean cultivation.

Keywords: GC-FPD, organophosphorous insecticides, beans.

Determinação simultânea de pesticidas organofosforados em feijão por cromatografia gasosa com detecção fotométrica de chama

RESUMO. O uso indiscriminado de pesticidas organofosforados (POFs) em lavouras pode deixar resíduos no alimento, além de provocar intoxicações nos aplicadores. Desta forma, foi desenvolvido um método para a determinação de cinco POFs em amostras de feijão por cromatografia gasosa com detecção fotométrica de chama (GC-FPD). Alguns parâmetros de validação obtidos foram: linearidade entre 0,24 e 8,56 μg g^{-1} (r = 0,9985) para a diazinona, 0,23 e 8,14 μg g^{-1} (r = 0,9959) para o paration metílica, 0,28 e 10,25 μg g^{-1} (r = 0,9987) para o pirimifós metílico, 0,52 e 18,87 μg g^{-1} (r = 0,9955) para o malationa e 0,86 e 13.67 μg g^{-1} (r = 0,9919) para o etion. Os limites de quantificação (iguais aos de detecção) foram os menores valores dos intervalos acima citados para cada composto. O método de extração apresentou recuperação em torno de 95% com %CV < 15%. Foram analisadas 28 amostras de feijão adquiridas na região sul de Minas Gerais, mas não se encontrou nenhum dos POFs estudados. A ausência destes POFs nas amostras pode ser atribuída à degradação ocorrida entre o uso dos POFs e a comercialização do feijão, à presença de níveis abaixo dos limites de detecção/quantificação do método, como também à não utilização de POFs no cultivo de feijão.

Palavras-chave: GC-FPD, inseticidas organofosforados, feijão.

Introduction

Extensive use of pesticides in agriculture and environment pollution caused by industrial emissions during production have produced residues of chemicals and their metabolites in food commodities, water and soil (KAMPIOTI et al., 2005). So that a high yield of food grains could be generated, several pesticides have been developed, which in turn has facilitated the prosperity of populations and brought relief to farmers. In fact, pesticide residues have been focused by many environmental studies. Each class has a different target and their physical properties and diversity limit the coverage of a single analytical method. It is also impossible to monitor all the pesticides used against one particular type of food since several methods are employed. Thus, analytical laboratories require fast and efficient multiresidue methods to maximize the coverage of their monitoring activities. Researchers have actually reported many multiresidue analytical methods (COOK et al., 1999; FILLION et al., 2000; UENO et al., 2004; MARIANI et al., 2010).

The determination of organophosphorus pesticides (OPPs) in food and vegetables is a matter of public concern since these pesticides are widely applied and

their residues constitute a potential risk to human health. Measurement of pesticide residues in different matrices involves two basic steps, namely, sample preparation (extraction and clean up) and instrumental analysis. Generally, trace analysis of complex samples requires the preparation of good samples to reduce matrix interference and enrich the analytes. A sample preparation should ideally be rapid, simple, cheap, environment friendly and must provide clean extracts (STAJNBAHER; ZUPANCIC-KRALJ, 2003).

Beans (*Phaseolus vulgaris* L.) are mainly consumed in the northeastern and southeastern regions of Brazil. The food is one of the most traditional in Brazilian diet as it provides nutrients, such as protein, iron, calcium, magnesium, zinc, carbohydrates, fiber and vitamins, especially B-complex, essential to humans. Since beans rank third among the food consumed and contain 11.2% of total calories ingested, they are the main source of protein and calories ingested by low-income populations.

Therefore, current investigation proposes the development and validation of a simple and economical GC-FPD method for the simultaneous determination of diazinon, ethion, malathion, methyl parathion and methyl pirimiphos (Figure 1) in bean samples from the southern region of the state of Minas Gerais, Brazil.

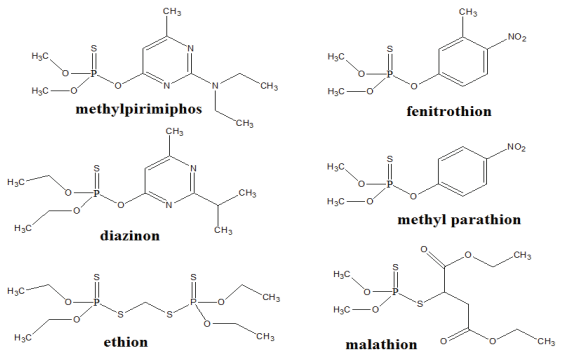

Figure 1. Molecular structures of diazinon (1), methyl parathion (2), methyl pirimiphos (3), fenitrothion (I.S) (4), malathion (5) ethion (6).

Material and methods

Experimental

Standard solutions and chemicals

Diazinon (98.7%), ethion (99%), fenitrothion (98%, internal standard – I.S.), malathion (99%), methyl parathion (99%) and methyl pirimiphos (99.5%) were obtained from Chem Service (West Chester, USA). Stock standard solutions of OPPs were prepared by dissolution of each drug in ethyl acetate and stored at -20°C, in the absence of light.

All chemicals were of analytical-grade and highest purity available. Acetone, dichloromethane, hexane and methanol pesticide-grade were obtained from Grupo Química (Rio de Janeiro State, Brazil). Water was distilled and purified using a Millipore Milli-Q Plus system (Bedford, USA). HPLC-grade ethanol and acetonitrile were purchased from Merck (Darmstadt, Germany).

Instrumentation and analytical conditions

A GC 1000 equipped with a flame photometric detector (FPD, Ciola Gregori Ltda., São Paulo State, Brazil), a splitless injector inlet liner interfaced to a PC with DANI DS 1000 integrator (Dani Strumentazione Analitica, Monza, Italy) and IQ3 software for data acquisition were used. The simultaneous resolution of organophosphorous pesticides under analysis was performed on a Supelco-SPB 35 column (30 m x 0.53 mm id, 0.5 µm film thickness; Supelco, Bellefonte, PA, USA) with detector temperature at 245°C, injector temperature at 275°C, and initial temperature of the oven at 160°C with ramp of 2.5°C $min.^{-1}$ to 205°C, maintained for 2.5 min., followed by ramp 20°C $min.^{-1}$ up to 275°C, maintained for 2.5 min. and carrier gas flow rate of N_2 (99.999%) at 8 mL $min.^{-1}$; injector operation in splitless mode; 1 µL of the sample was injected.

Bean samples

Bean samples were purchased in the southern region of Minas Gerais (real samples). Beans samples without any kind of OPPs (organic production, blank samples) were used for the method´s optimization and validation. Real samples, collected in five towns from the southern region of Minas Gerais (Figure 2), were frozen and stored at –20°C, not exceeding 72h before analysis.

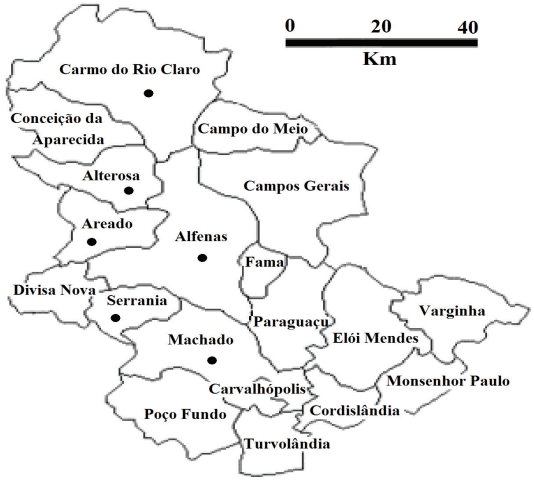

Figure 2. Map and location of the five towns in southern of Minas Gerais where bean samples were purchased.

Extraction procedure – Liquid-Liquid Extraction (LLE)

Beans were ground in a blender and 50 g were weighed in a 200-mL beaker. Sample was then placed in a 500-mL beaker to which were added 50 mL distilled water, 30 g sodium chloride and 200 mL acetone. The mixture was strongly stirred for 3-5 minutes and filtered with a vacuum Buchner funnel. The filtrate was transferred to a separator funnel, 150 mL dichloromethane were added and strongly stirred for 3-5 minutes. The aqueous phase was discarded and the organic phase was filtrated with anhydrous sodium sulfate. The filtrate was collected in a 250-mL beaker to determine a volume of 175 mL which, in turn, was transferred to a round bottom flask and evaporated in vacuum to a final volume 3-5 mL. This volume was completed to 10 mL and the solution was used to determine the organophophorous pesticides.

Method validation

The validation strategy method included the verification and determination of parameters such as selectivity, recovery, linearity, limit of detection (LOD), limit of quantification (LOQ), precision and accuracy in spiked bean samples (FDA, 2001).

Selectivity was evaluated by analyzing bean sample (blank samples) to ensure that no compound of the bean interfered with the target analytes and corresponding internal standard. Blank samples were extracted by LLE and the extracts submitted to GC-FPD analysis.

To determine the extraction recovery, bean samples (50 g) were spiked in triplicate at three levels of concentration each and submitted to extracting procedures. The recovery was calculated after extraction and expressed as percentage of the amount extracted.

For linearity study, bean samples were spiked with standard solutions of the analytes: diazinon (0.24 – 8.56 μg g^{-1}), ethion (0.86 – 13.67 μg g^{-1}), fenitrothion (0.56 μg g^{-1}, internal standard), malathion (0.52 – 18.87 μg g^{-1}), methyl parathion (0.23 – 8.14 μg g^{-1}) and methyl pirimiphos (0.28 – 10.25 μg g^{-1}). Curves of analytes (5 calibrators) were constructed using peak area ratio of OPPs to the internal standard versus nominal concentrations of the analytes. Method linearity was investigated by the evaluation of the linear regression and was expressed by the coefficient of correlation (r).

The method´s detection limit (LOD), defined as the lowest concentration that could be verified in the presence of analyte, was obtained by using bean samples (n = 2) spiked with different concentrations of analytes.

The method´s quantification limit (LOQ), defined as the lowest concentration that could be determined with accuracy and precision below 20% over five analytical runs (FDA, 2001), was obtained by bean samples (n = 5) spiked at different concentrations of analytes.

Quality control samples were used for precision and accuracy assays: whereas precision was calculated as coefficient of variation (CV, %) of within-day (n = 3) and between-day (n = 3) analysis, accuracy was determined as the percentage of deviation between nominal and measured concentration (relative error, RE%). The method´s precision and accuracy were evaluated using bean samples spiked with analytes at the three different concentrations.

Results and discussion

Method development and validation

A simple gas chromatographic flame photometric detection method for the separation of diazinon, ethion, fenitrothion (I.S), malathion, methyl parathion and methyl pirimiphos was performed to determine OPPs in bean samples. The analytical conditions were selected based on the literature (SANTOS-NETO; SIQUEIRA, 2005; IAL, 1995) and after testing the different parameters, such as column types, injector, detector and column temperatures, and other chromatographic and sample preparation conditions. The optimized chromatographic conditions are Supelco-SPB 35 column (30 m x 0.53 mm id, 0.5 μm film thickness; Supelco, Bellefonte, PA, USA) with detector temperature at 245°C, injector temperature at 275°C, and initial temperature of the oven at 160°C with ramp of 2.5°C $min.^{-1}$ to 205°C, maintained for 2.5 min., followed by ramp 20°C $min.^{-1}$ up to 275°C, maintained for 2.5 min. and carrier gas flow rate of N_2 (99.999%) at 8 mL $min.^{-1}$; injector operation in splitless mode and 1 μL of the sample was injected.

Some compounds were evaluated as internal standard (I.S.), among which fenitrothion was chosen. However, this compound is also used as pesticide in bean crop. The presence of the analyte may be observed in this method. However, some modifications on method should be performed to quantify the analyte. Figure 3 presents a typical chromatogram of blank samples with IS (fenitrothion).

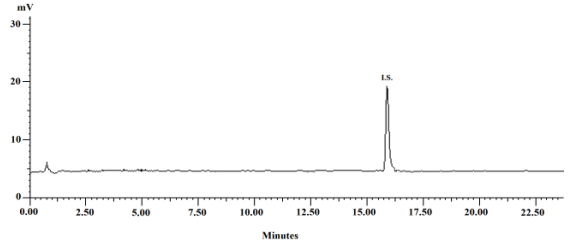

Figure 3. Typical chromatogram of blank sample of bean (IS – fenitrothion).

Figure 4 shows a typical chromatogram of OPPs under analysis: diazinon, ethion, fenitrothion (I.S), malathion, methyl parathion and methyl pirimiphos in spiked sample beans.

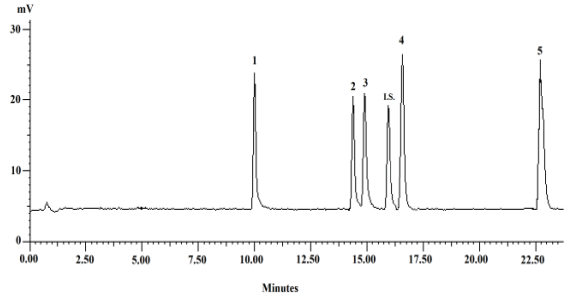

Figure 4. Typical chromatogram of spiked bean sample referring to the separation of diazinon (1), methyl parathion (2), methyl pirimiphos (3), fenitrothion (I.S) (4), malathion (5) and ethion (6).

Chromatograms show that the developed method did not present interfering elements in the blank bean sample. Recoveries of diazinon using LLE ranged between 97.02 and 99.21%, with % CV lower than 5.5 %. Recoveries of ethion ranged between 88.03 and 92.02%, with % CV lower than 3.2%. In the case of malathion, the recoveries using LLE ranged between 94.56 and 97.59%, with % CV lower than 4.5%. Recoveries ranged between 98.02 and 99.98% and between 93.47 and 95.42%, respectively, for methyl parathion and methyl pirimiphos, with both presenting % CV lower than 3.1%.

Calibration curves were prepared by plotting the relative area of each OPPs against the analyte concentration in the bean sample. Linear equations, correlation coefficient and CV (%) are shown in Table 1. All *r* were > 0.99, showing excellent linearity.

The quantification limits for all analytes were the same as the limits of detection (Table 1). Further, % CV and % RE employing LLE were lower than 15%.

The method´s precision and accuracy were evaluated by calculating the coefficient of variation (CV, %) for three determinations of each OPPs at three different concentrations (low, medium and high) on three consecutive days performed under the same experimental conditions. Data indicate agreement with recommendations of literature; neither CV nor RE exceeded the 15% rate, which proved that the assay method is reproducible on different days (Table 2).

Application of method in the analysis of real samples of beans

It is very important to evaluate the presence of OPPs in foods. Beans constitute a product consumed daily by most of the population and the identification and monitoring of possible residues of OPPs is mandatory. Intoxication risks are real because the misuse of OPPs is a common event and may affect directly or indirectly the environment, animals and humans.

The samples were collected in five towns from the southern region of Minas Gerais (Table 3).

Table 3. Origin and varieties of bean samples.

Brazilian City	Variety of studied beans			
	Carioca	Rosinha	Preto	Canário
Alfenas	4	2	1	2
Carmo do Rio Claro	1	1	1	2
Areado	2	1	2	1
Alterosa	1	0	2	1
Serrania	0	2	1	0
Sub-total	8	6	7	7
Total	28			

*The samples were purchased on the local market of each town.

Table 1. Linearity, LOD and LOQ of method for OPPs under analysis.

OPPs	Linear equation[a]	Correlation coefficient (*r*)	Range ($\mu g\ g^{-1}$)	CV (%)[b]	LOD = LOQ[c] ($\mu g\ g^{-1}$)	Conc. Analyzed ($\mu g\ g^{-1}$)	CV (%)[d]	RE(%)[c]
diazinon	y = 3.1854x – 0.6087	0.9985	0.24 – 8.56	4.4	0.24	0.25	13.4	4.04
ethion	y = 2.5347x + 2.2828	0.9919	0.86 – 13.67	6.7	0.86	0.89	7.8	3.49
malathion	y = 2.5054x – 1.9756	0.9955	0.52 – 18.87	6.9	0.52	0.57	11.8	8.77
methyl parathion	y = 3.1765x – 0.6945	0.9959	0.23 – 8.14	9.3	0.23	0.27	14.1	14.81
mehtyl pirimiphos	y = 3.0881x – 0.6010	0.9987	0.28 – 10.25	8.6	0.28	0.30	10.5	6.67

Table 2. Precision and accuracy of the method for analysis of OPPs in spiked bean samples.

OPPs	diazinon			ethion			malathion			methyl parathion		methyl pirimiphos			
Within-day (n = 3)[a]															
Nominal concentration ($\mu g\ g^{-1}$)	0.58	1.43	4.28	1.54	5.16	9.67	0.81	3.15	9.44	0.51	1.36	4.07	0.57	1.71	5.13
Analyzed concentration ($\mu g\ g^{-1}$)	0.58	1.41	4.36	1.57	5.52	9.97	0.87	3.02	9.64	0.55	1.27	4.17	0.53	1.66	4.96
Precision[b] (CV, %)	1.86	12.46	4.84	5.37	3.48	14.18	4.76	14.97	0.25	1.68	12.82	2.82	1.04	13.01	0.51
Accuracy[c] (RE, %)	-0.65	-1.29	1.79	3.06	6.43	3.01	7.52	-4.28	2.15	6.47	-6.92	2.31	-7.14	-2.75	-3.37
Between-day (n = 3)[d]															
Nominal concentration ($\mu g\ g^{-1}$)	0.58	1.43	4.28	1.54	5.16	9.67	0.81	3.15	9.44	0.51	1.36	4.07	0.57	1.71	5.13
Analyzed concentration ($\mu g\ g^{-1}$)	0.60	1.47	4.34	1.54	5.22	9.57	0.75	3.01	9.64	0.56	1.29	3.82	0.56	1.66	5.03
Precision (CV, %)[e]	6.79	4.29	9.67	2.26	2.57	5.88	4.56	9.03	11.46	5.77	11.47	12.34	10.68	4.56	3.34
Accuracy (RE, %)[fg]	3.37	3.02	1.43	0.13	1.08	-1.07	-8.36	-4.40	2.13	8.25	-4.89	-6.49	-1.18	-3.23	-1.86

Although the sample collected did not present any OPPs (Figure 5), current method was able to analyze five OPPs in bean samples. More samples may be collected from areas where bean crops could be polluted.

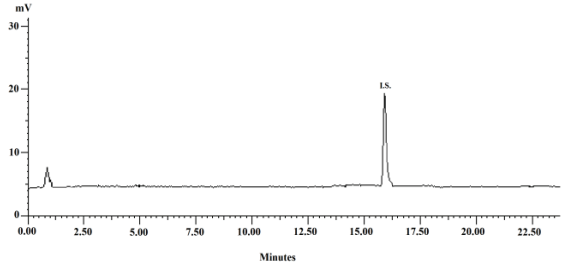

Figure 5. Typical chromatogram of real sample of bean (IS – fenitrothion).

Conclusion

The optimized method was adequate to analyze the OPPs under analysis in real bean samples. The validated method presented all parameters in accordance with the confidence criteria established in the literature. The method employs relatively inexpensive equipment and sample preparation techniques employed in routine analyses. The absence of OPPs in the samples could be attributed to the degradation that occurred between the use of OPPs and their commercialization, the presence of levels below LOD/LOQ and the non-use of OPPs in their cultivation.

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