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## RESEARCH ARTICLE

# VALIDATION OF A METHOD FOR DETERMINATION OF ORGANOPHOSPHATES IN BLACK-BEAN SAMPLES

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## ABSTRACT

The presence of pesticides residues above the Acceptable Daily Intake values and the Maximum Residue Limits is worrying in the point of view of food safety. In order to identify and determine the presence of organ phosphorus pesticides in bean samples, a multiresidue technique of extraction method was applied, and the analyses were conducted by gas chromatography coupled to a thermoionic flame detector (GC-FTD). This study aimed to validate the methodology of determination of organophosphates by studies of selectivity, linearity, intermediate precision, limit of detection, limit of quantification, and accuracy study by addition and recovery. The method was selective for all analytes, because there was no interference higher than 30% of the limit of quantification. The limits of detection were between 1.60 and 52.96 ng.kg$^{-1}$ and the quantification limits were between 3.30 and 176.0 ng.kg$^{-1}$. Regarding intermediate precision, the differences of area values were ≤ 15%, in different concentrations, except for phorate. The correlation coefficients (*r*) of linearity curves were higher than 0.99 for all active ingredients, within the working range applied. The recovery percentage was between 70 and 120%, except for phorate. Thus, the methodology was satisfactory for identification and determination of ten from the eleven organophosphates analyzed in beans.

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## INTRODUCTION

The extensive use of pesticides in agriculture is a major cause of contamination of surface and ground waters, especially due to the action of rain and irrigation waters that cause leaching, drainage and spreading. The active ingredients belonging to the group of organophosphates (OP) are widely used as insecticides, miticides, nematicides and fungicides, and poisoning by these products is similar to other acetylcholinesterase inhibitors (Liu *et al.*, 2008). High performance liquid chromatography (HPLC) and gas chromatography (GC) techniques are traditionally used in pesticide residue analysis. These procedures allow the separation and quantification of various compounds with high resolution, and include the possibility of multiresidues analysis (Collins, 1997; Li *et al.*, 2007).

In order to identify and determine the presence of eleven organophosphorus pesticides, in black-bean samples, a multiresidue technique of extraction was applied, and the analyses were carried out by gas chromatography coupled to a thermoionic flame detector (GC-FTD). This study aimed to validate a methodology of identification and quantification of OP multiresidue added to beans samples, applying studies of selectivity, linearity, intermediate precision, limit of detection, limit of quantification, addition and recovery.

## MATERIALS AND METHODS

Standards were purchased from Dr. EhrenstorferGmBH® (Augsburg, Germany) with the following purities: chlorpyrifos (98.5%), etione (98.0%), malathion (99.0%), methamidophos (98.0%), parathion-methyl (98.5%), phenthoate (96.5%), phorate (99.0%), pirimiphos-methyl (99.5%), terbuphos (93.0%), pyrazophos (98.5%), and triazophos (81.0%).

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The standards solutions were prepared in ethyl acetate pesticide grade, from Tedia® (Fairfield, USA), purity: 99.9%. The multiresidue extraction employed acetic acid HPLC grade, purity: 97.9%; acetonitrile HPLC grade, purity: 99.9%; all from Tedia® (Fairfield, USA); magnesium sulfate and sodium acetate ACS grade, purity: 99.0%, from Sigma-Aldrich® (St. Gallen, Switzerland); and Bondesil® PSA 40 UM, from Agilent Technologies® (Saint Clara, USA). Purified water was from Advantage A10Millipore® system, resistivity 18,2 MΩ.cm a 25º C. Chromatographic analyses were performed in a gas chromatography GC-2010 system from Shimadzu, equipped with a flame thermionic detector (FTD).

For chromatographic separation a capillary column DB5 (30m x 0.25 mm x 0.25 µm) was employed, using helium as carrier gas, nitrogen gas as make up, with splitless injection at 250° C. The temperature program was started at 80° C, followed by a 25° C $\text{min}^{-1}$ increase to 130° C, 15° C $\text{min}^{-1}$ to 210° C, and 4° C $\text{min}^{-1}$ to 270° C (held for 5 min). The FTD was maintained at 280° C and make-up gas at 15 mL $\text{min}^{-1}$ flow. The air and hydrogen gas flows were set at 140 and 4 mL $\text{min}^{-1}$, respectively. An analytical curve was constructed with triplicate injections of the standard solutions of the eleven OP (chlorpyrifos, etione, malathion, methamidophos, parathion-methyl, phenthoate, phorate, pirimiphos-methyl, terbuphos, pyrazophos, and triazophos) in eight different concentrations: 0.006 $\text{mg.kg}^{-1}$, 0.008 $\text{mg.kg}^{-1}$, 0.018 $\text{mg.kg}^{-1}$, 0.027 $\text{mg.kg}^{-1}$, 0.036 $\text{mg.kg}^{-1}$, 0.045 $\text{mg.kg}^{-1}$, 0,06 $\text{mg.kg}^{-1}$, 0,07 $\text{mg.kg}^{-1}$.

A multiresidue method of pesticides extraction was adapted from QuEChERS method (Quick, Easy, Cheap, Effective, and Safe Ruged) described by Lehotay, Maštovská and Lightfield (2005). This work also includes studies of selectivity, intermediate precision, limit of detection (LD), limit of quantification (LQ), and recovery. LD was obtained considering the signal-to-noise ratio of 3 and 10. LQ was calculated by multiplying LD by 3.33 (Ribani *et al*., 2004). Intermediate precision was determined from the estimated relative standard deviation (RSD) of triplicate injections of three dilutions. Accuracy was evaluated by recovery studies, performed by adding standard solutions containing a pool of OP in three different dilutions a = 0.016 $\text{mg.kg}^{-1}$, b = 0,022 $\text{mg.kg}^{-1}$ and c = 0,44 $\text{mg.kg}^{-1}$. A fourth aliquot was unfortified, and recovery of added standards was determined using the latter as a reference. Recovery percentages were obtained by subtracting the concentrations found in doped aliquots of samples minus the concentrations of the unfortified.

## RESULTS AND DISCUSSION

Regarding sensitivity, there were no intrinsic interferences from black bean samples in retention times close to those of the analytes. Chromatograms of standards solutions at eight different concentrations and the linear analytical curves can be seen in Figure 1. The analytical curve of methamidophos just had *r* values close to 1.0 standards in five different concentrations, and just in six concentrations in phorate and parathion-methyl.

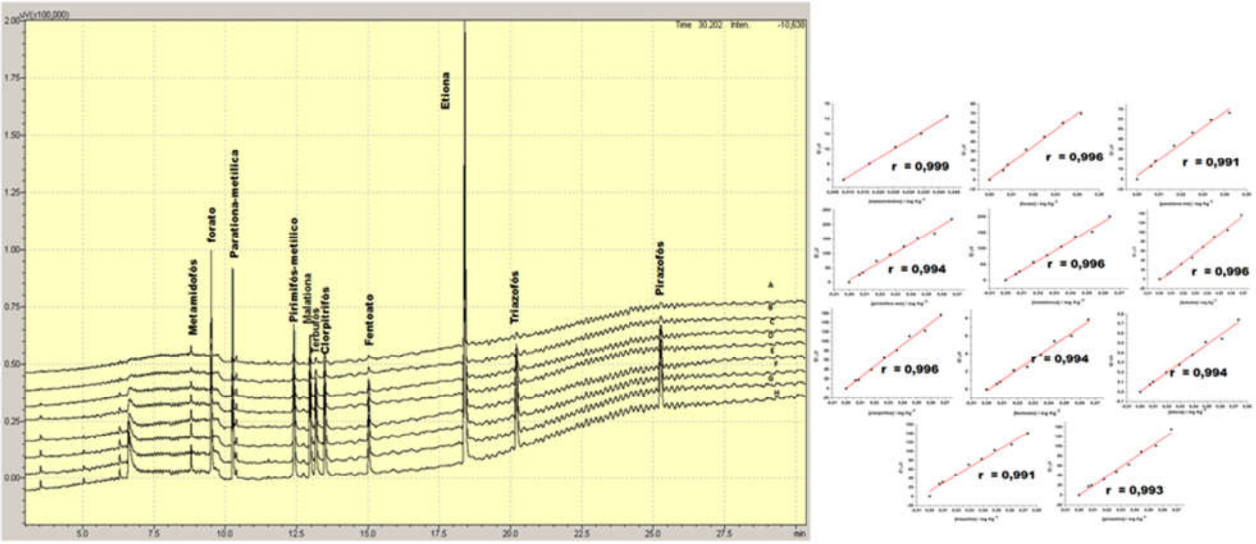

Figure 1. Chromatograms of OP standard solutions at eight different concentrations and the resultant analytical curves

Table 1. Intermediate precision in three different concentrations and RSD

| | Concentration (mg $\text{Kg}^{-1}$) | | | RSD (%) | | |
|---|---|---|---|---|---|---|
| Organofosforados | a | b | c | a | b | C |
| Metamidophos | 0.017 | 0.026 | 0.043 | 15.47 | 16.73 | 7.65 |
| Phorate | 0.017 | 0.025 | 0.042 | 7.51 | 15.42 | 10.47 |
| Parathion-me | 0.017 | 0.025 | 0.042 | 7.35 | 15.16 | 11.43 |
| Pyrimiphos-me | 0.017 | 0.026 | 0.044 | 9.90 | 13.94 | 7.38 |
| Malathion | 0.017 | 0.026 | 0.043 | 8.64 | 11.15 | 4.71 |
| Chlorpyriphos | 0.018 | 0.027 | 0.046 | 11.52 | 12.67 | 7.96 |
| Terbuphos | 0.018 | 0.027 | 0.045 | 4.25 | 13.01 | 13.33 |
| Phenthoate | 0.017 | 0.026 | 0.044 | 19.29 | 11.09 | 21.56 |
| Etione | 0.020 | 0.029 | 0.049 | 9.07 | 8.20 | 4.50 |
| Triazophos | 0.019 | 0.029 | 0.049 | 11.67 | 8.26 | 10.82 |
| Pyrazophos | 0.018 | 0.027 | 0.045 | 20.50 | 14.08 | 10.05 |

**Table 2. Percentage of recovery of OP**

| OP | a (%) | b (%) | c (%) |
|---|---|---|---|
| Metamidophos | 92.42 | 102.55 | 119.62 |
| Phorate | 130.93 | 188.45 | 126.98 |
| Parathion-me | 115.76 | 212.7 | 160.53 |
| Pyrimiphos-me | 75.49 | 90.02 | 76.78 |
| Malathion | 64.35 | 103.5 | 61.72 |
| Chlorpyriphos | 96.96 | 126.55 | 99.7 |
| Terbuphos | 90.12 | 98.45 | 87.14 |
| Phenthoate | 76.87 | 119.21 | 81.34 |
| Etione | 83.84 | 100.09 | 80.97 |
| Triazophos | 118.74 | 32.72 | 122.96 |
| Pyrazophos | 120.05 | 119.67 | 116.03 |

Bastos *et al.* (2012) observed a regression varying from 0.01 to 0.05 µg.mL$^{-1}$ in 40 of the 53 organophosphates evaluated. The LD values varied from 1.6 to 62.93 ng.L$^{-1}$, and the LQ values ranged from 3.31 to 176.26 ng.L$^{-1}$, and regarding the selectivity studies, the samples are appropriate for the method evaluation. The results of intermediate precision for the eleven OP are presented according to relative standard deviation percentage (RSD) in Table 1. The results are within the accepted limits by the validation script chromatographic methods to area values ≤ 15%, with the exception of phenthoate at 0.017 and 0.044 mg.kg-1, pyrazophos at 0.018 mg.kg-1 and methamidophos at 0.026 mg.kg-1. Gobo *et al.* (2004) using GC-FTD obtained satisfactory results for intermediate precision of organophosphates, with the exception of methamidophos. The percentages of OP recovery are expressed on Table 2. In column a, the concentration of OP which was added to the common bean aliquot was 0.016 mg.kg$^{-1}$, in column **b** the concentration of OP added was 0.022 mg.kg$^{-1}$ and in **c** it was 0,44 mg.kg$^{-1}$.

As observed in Table 2, the recovery percentage of methamidophos, chlorpyrifos, etione and pyrazophos is inside the limits recognized by the Brazilian laws that varied from 80 to 120% with all three dilution of standard solutions of OP added to the common beans samples. Parathion-methyl, phenthoate and triazophos had good recoveries only with the addition of 0.016 mg.kg$^{-1}$. Pirimiphos-methyl and malathion had good recoveries for addition of 0.022 mg.kg$^{-1}$. Terbuphos had acceptable recovery at 0.016 and 0.044 mg.kg$^{-1}$. Gobo *et al.* (2004) had satisfactory recoveries for all concentrations in study of OP residues added to tomatoes. Bastos *et al.* (2012) obtained satisfactory recovery values for all pesticides added to milk samples with exception of dichlorvos.

## Conclusion

The methodology was satisfactory for identification and determination of ten from the eleven analyzed organophosphates added to beans samples.

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## REFERENCES

Bastos, L. H. P., Gouvêa, A. V., Málaga, F., Cardoso, M. H. W. M., do Couto, J. S., da Nóbrega, A. W. 2012. Analytical method implementation for determination of organophosphate residues in milk by gas chromatography with flame photometric detector. *Química Nova*, 35(8), 1657-1663.

Collins, C. H. 1997. *Introduction to Chromatographic Methods*. Ed. UNICAMP, Campinas.

Gobo, A. B, Kurz, M. H., Pizzutti, I. R., Adaime, M. B., Zanella, R. 2004. Development and validation of methodology for the determination of residues of organophosphorus pesticides in tomatoes. *Journal of the Brazilian Chemical Society*, 15(6), 945-950.

Lehotay, S. J., Maštovská, K., Lightfield, A. R. (2005). Use of buffering and other means to improve results of problematic pesticides in a fast and easy method for residue analysis of fruits and vegetables. *Journal of AOAC International*. 88, 615.

Li, L., Zhou, Z., Pan, C., Qian, C., Jiang, S., Liu, F. 2007. Determination of organophosphorus pesticides in soybean oil, peanut oil and sesame oil by low-temperature extraction and GC-FPD. *Chromatographia*. 66(7-8), 625-629.

Liu, J. H., Chou, C. Y., Liu, Y. L., Liao, P. Y., Lin, P. W., Lin, H. H., Yang, Y. F. 2008. Acid-base interpretation can be the predictor of outcome among patients with acute organophosphate poisoning before hospitalization. *American Journal of Emergency Medicine*. Jan. 26(1):24-30.

Ribani, M., Bottoli, C. B. G., Collins, C. H., Jardim, I. C. S. F., Melo, L. F. C. 2004. Validation of chromatographic and electrophoretic methods. *Química Nova*, 27(5), 771- 78.

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