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Analysis of methyl parathion in tilapia filets using a simple solid phase extraction clean-up and GC-NPD

Análise de paration metílico em filés de tilápia utilizando clean-up com cartucho de extração em fase sólida e CG-NPD

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Summary

A simple and efficient procedure is presented for the analysis of methyl parathion in tilapia (*Oreochromis niloticus*) filet. A C18 solid phase extraction clean-up and a gas-chromatography with NPD detection was used. The methods indicated no significant matrix effect as verified by the recovery efficiency. The limits of detection and quantification were 0.024 and 0.080 $\mu g.g^{-1}$, respectively. The method was successfully applied for the analyses of methyl parathion in fishes subjected to Folisuper™ administration.

Key words: *Aquaculture; Bioaccumulation; Parathion methyl; Tilapia.*

Resumo

Um método simples e eficiente é apresentado para a análise do paration metílico em filés de tilápia (*Oreochromis niloticus*). Foram empregados um *clean-up* com cartucho de extração em fase sólida C18 e cromatografia gasosa com detector de nitrogênio e fósforo. O método não exibiu efeito matriz significante, como pode ser verificado pela eficiência na recuperação. Os limites de detecção e quantificação foram 0,024 e 0,080 $\mu g.g^{-1}$, respectivamente. O método foi satisfatoriamente aplicado para análises de paration metílico em peixes submetidos ao tratamento com Folissuper™.

Palavras-chave: *Aquicultura; Bioacumulação; Paration metílico; Tilápia.*

1 Introduction

The use of non-regulated pesticides to control fish ectoparasites and fish predators is becoming a usual practice in Brazilian aquaculture activities (LUVIZOTTO-SANTOS, 2007). Some organophosphates (OF) such as methyl parathion (MP) have been used during lerneosis and argulosis infestations. In addition, OF are also used to prevent piscivorous insects such as Odonata and Hemiptera. Some works provide information about the use of methyl parathion during aquaculture practices (FIGUEIREDO and SENHORINI, 1990; SENHORINI et al., 1991; SILVA et al., 1993; LESTER and ROUBAL, 1995; ANÔNIMO, 2000; AGUIAR et al., 2004; CRUZ et al., 2004; CRUZ, 2005). The accumulation of MP in fish tissues has been accessed in different species (PAN, 2008), however, the applied methods, in many cases, are laborious and time consuming.

It is common sense that agrochemical, veterinary drug residues and heavy metals may accumulate in aquaculture products at unsafe levels that are potentially harmful to human health. Analysis for routine assessment of chemotherapeutic residues in the aquaculture products presupposes a sensitive, simple, efficient, and preferably low-cost method. The objective of the present study was to develop a rapid and simple cleanup step to detect and quantify MP in fish filets.

Tilapia is among the most cultivated fish in Brazil (around 37.8% of total inland aquaculture), mainly in the Southeastern region (ROUBACH et al., 2003; IBAMA, 2007) and for this reason *Oreochromis niloticus* was chosen for this study.

2 Material and methods

2.1 Chemicals

Methyl parathion (O,O-dimethyl O-4-nitrophenyl phosphorothioate) were supplied by Chem Service (West Chester, USA). Ethyl acetate HPLC grade (J.T. Baker, USA) was used without further purification. Stock solution of MP (100 $\mu g.mL^{-1}$) was prepared by dissolving MP in ethyl acetate and then stored in freezer (–20 °C). Sodium sulfate anhydrous of analytical grade was obtained from F. Maia Indústria e Comércio Ltda. (Cotia, SP, Brazil). Commercial insecticide Folisuper™ was obtained from Nufarm (São Paulo, SP, Brazil). The anesthetic solution was prepared by diluting an appropriate amount of commercial benzocaine gel (DFL Indústria e Comércio SA, Rio de Janeiro, RJ, Brazil) in water.

2.2 Fish sampling

Tilapia *O. niloticus* (105.7 ± 31.9 g) contaminated with MP was obtained through a treatment simulation experiment against ectoparasites using the commercial insecticide Folisuper™, in accordance with the procedure usually conducted by Brazilian fish farmers (LUVIZOTTO-SANTOS, 2007). The fishes were kept in 10,000 L concrete ponds located at the Center for Water Resources and Applied Ecology, School of Engineering of São Carlos (Universidade de São Paulo, Brazil) with the following characteristics: static system, sediment layer of 10 cm, density of 8 $fishes.m^{-3}$, and feeding regime of 2 times per day with commercial pelletized food. Immediately before the pesticide administration (0.25 $g.m^{-3}$ of active ingredient) and 5 h after the pesticide administration, the fishes were caught and anesthetized with benzocaine (2%) prior to decapitation. Sampling procedures were performed as approved by the Animal Care Committee from the Brazilian College of Animal Experimentation (COBEA, 2008).

2.3 Sample pretreatment

Exactly 5.0 g of filet (free of bones and scales) were homogenized with a mixer (food processor) in a glass beaker with 30 mL of cold ethyl acetate and 15 g of sodium sulfate anhydrous for approximately one minute. All contents were transferred to a centrifuge tube and centrifuged at 5,000 rpm for 10 min at 4 °C. An aliquot of 6 mL of the supernatant was collected and filtered using an unconditioned C18 cartridge (Bakerbond – 500 mg). The eluate was injected (1 µL) into the GC system.

The recovery rates were determined by comparing the analysis results of the MP spiked *O. niloticus* filets with those of the standard solution (calibration curve). Exactly 5.0 g of tilapia filet from the experimental pond (before pesticide administration) were spiked with an appropriate amount of a MP standard stock solution to provide a nominal concentration of 0.68, 3.40 and 6.80 $\mu g.g^{-1}$. The samples were homogenized, centrifuged and filtered as described above. All experiments were carried out in quintuplicate.

2.4 Chromatographic conditions

The analyses were performed using a Shimadzu GC 17A gas chromatograph with a nitrogen-phosphorus detector (NPD). A capillary column HP35 (25 m x 0.25 mm) coated with a 0.25 µm thick film of methyl silicon was used. 1 µL of the sample at splitless mode was injected, and the carrier gas (N) flow-rate was 0.8 $mL.min^{-1}$. Hydrogen and air were used as auxiliary gases for NPD. The oven temperature was programmed for an initial hold of 2 min at 150 °C, then increased to 10 $°C.min^{-1}$ until a temperature of 250 °C and kept for 5 min. The detector and injector temperature were 250 °C.

3 Results and discussion

3.1 Calibration curve

The calibration curve for MP was obtained by spiking ethyl acetate with stock solution. These were prepared in triplicate by dilution to yield 0.05; 0.1; 0.25; 0.5; 1.0; 1.5 and 2.0 $\mu g.mL^{-1}$. The standard curve was linear in the investigated range. The equation obtained by regression analysis was: Y = –1,524.85 + 39,764.18 X, (R = 0.9995).

3.2 Limits of detection and quantification

The limits of detection (LOD = 0.024 $\mu g.g^{-1}$) and quantification (LOQ = 0.080 $\mu g.g^{-1}$) were estimated at 3 and 10 s, respectively, where *s* is a standard deviation of measured noise signal (n = 20) in retention time window after a blank injection. A blank solution was prepared using fish filets (n = 5) from the experimental pond before pesticide administration.

3.3 Recovery studies

Good recovery averages and precision (standard deviation) were obtained in the analysis of spiked filets (Table 1). Apparently, the methods exhibit no significant matrix effect as verified by the recovery efficiency. This efficiency is similar to that obtained by other authors analyzing MP residues in fish tissues (SABHARWAL and BELSARE, 1986; OHAYO-MITOKO and DENEER, 1993; SOUMIS et al., 2003).

3.4 MP waterborne exposition

A real situation of tilapia exposure to MP was performed in an experimental pond where fishes were exposed to Folisuper™ for 5 h. Typical chromatograms obtained after injection of fish filet extracts from uncontaminated and MP exposed tilapias are shown in Figures 1a and b, respectively.

The amount of MP in *O. niloticus* filets accumulated after this period was 4.15 ± 0.22 $mg.kg^{-1}$. A bioaccumulation ratio calculated as MP concentration in tissue divided by the MP concentration in water was estimated in approximately 17 fold. Similar values of bioaccumulation ratio in fishes were determined by other authors in different experimental conditions (SABHARWAL and BELSARE, 1986; FAVARI et al., 2002).

4 Conclusions

The simple cleanup step proposed to detect and quantify MP using GC-NPD analysis demonstrates to be accurate and rapid. This method could be considered sufficiently sensitive and reliable for routine analysis of *O. niloticus* filet following waterborne exposure to MP commercial formulation at the concentration levels usually administered by Brazilian fish farmers.

Table 1. Mean recovery (R) and standard deviation (s) of MP from spiked muscles from *O. niloticus*.

MP ($\mu g.g^{-1}$)	Replicas	(R ± s) %
0.68	N = 5	93.93 ± 6.57
3.40	N = 5	97.67 ± 5.60
6.80	N = 5	102.91 ± 3.83

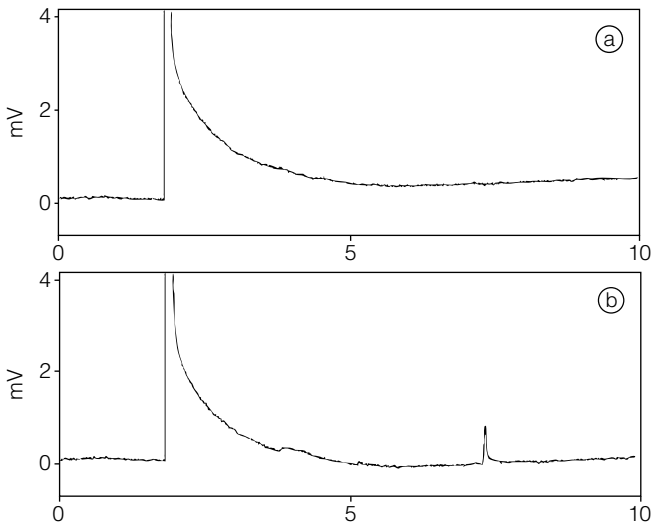

Figure 1. Chromatograms (GC-NPD) of filet extracts from fishes before a) and after b) MP exposition.

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