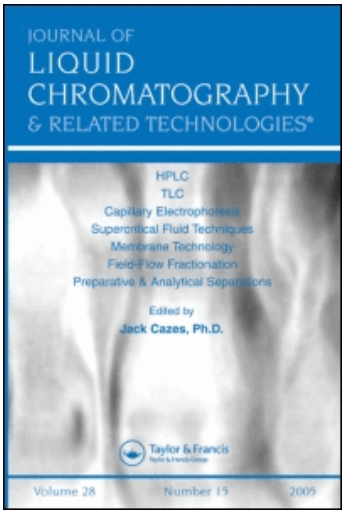

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HPLC/UV DETERMINATION OF SODIUM ACIFLUORFEN IN TROPICAL FISH

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ABSTRACT

This work reports the determination of sodium acifluorfen residue in fish species living in tropical rivers and lakes (*Piaractus mesopotamicus*). This determination was carried out through studies of LC_{50} - 96 hours and BCF- 192 hours (BCF = 1/100 LC_{50}). Controlled conditions in the static mode, with constant atmospheric air flow and dilution water at 25°C, were used in the laboratory tests. The fish samples were collected and submitted to appropriate analytical procedure. A method was developed that allows the residue quantitation of fish by HPLC/UV after soxhlet extraction and clean-up on silica gel and Florisil. The aquarium water samples were analyzed directly by HPLC/UV. The developed method proved to be adequate for LC_{50} and BCF determinations presenting recoveries above 75 %, with a relative standard deviation below 5 %.

INTRODUCTION

Pesticides have been widely used to eliminate forms of vegetable or animal life undesirable in agricultural cultivation and cattle raising, as well as in houses and gardens, and in public health projects, for combating the vectors which transmit illness.

These compounds, even when used correctly, can cause ecological and public health problems, favoring the appearance of new pests, eliminating pollinating insects and resulting in the slaughter of fish and birds. Another inconvenience caused by pesticides is the occurrence of toxic residues in foodstuffs, besides their persistence in the environment, especially in natural resources, so that they are transferred to other forms of life.[1]

The direct application of pesticides in lakes and rivers surfaces has been made to combat mosquitoes and other undesirable organisms, but their dispersion through the water bodies has been due especially, to the drainage water from soils contaminated by the systematic use of these pesticides in agriculture. Rain water transports these chemical compounds to the streams, rivers, lakes, estuaries and eventually to the oceans.[2]

Among the aquatic organisms, fish belong to the trophic levels in the aquatic food chain, which is used to evaluate water contamination. This evaluation of toxicity can be made by using such acute laboratory tests as Lethal Concentration (LC_{50}), as well as chronic tests called Bioconcentration Factor (BCF)[3]. BCF in aquatic organisms is primarily caused by passive partitioning of the chemicals between an aqueous (environment) and an organic (organism) compartment. BCF is also referred as the ratio of the concentration of a chemical in an organism to that in the environment, at steady-state equilibrium. It also correlates well with the chemical's octanol-water partition coefficient.[4-9]

Studies of the Bioconcentration Factor of these chemicals (accumulation directly from the aqueous environment), with representatives of various trophic levels such as algae, invertebrates and fishes, have demonstrated that increased bioconcentration occurs with increasing trophic level.[10]

Samples with high amounts of lipids, such as fish, are mainly extracted by soxhlet[11] or solid liquid extraction,[12, 13] and cleaned-up by adsorption columns containing adsorbents such as silica gel,[14] alumina[15] or Florisil,[11, 16-18] before being analyzed by techniques such as HRGC-ECD,[11] HRGC-MS,[13, 16] and HPLC-UV.[14]

This work determinated sodium acifluorfen residue in *Piaractus mesopotamicus*, a characteristic fish species in tropical rivers and lakes, through studies of LC_{50} - 96 hours and BCF - 192 hours (BCF=1/100 LC_{50}). These tests were carried out in laboratory controlled conditions in the static mode, with constant atmospheric air flow and dilution water (Standard Methods), at 25°C.[19] The fish samples were collected and submitted to an appropriate analytical procedure. A method was developed that allows the residue quantitation of fish by HPLC/UV after soxhlet extraction and clean-up on silica gel and Florisil. The aquarium water samples were analyzed directly by HPLC/UV.

EXPERIMENTAL

Fishes

Specimens of *Piaractus mesopotamicus*, in the juvenile period, were collected from the natural tank of CEPTA (Centro de Pesquisa e Treinamento em Aquicultura / Instituto Brasileiro do Meio Ambiente - IBAMA) Pirassununga - SP, Brasil.

The fish were acclimated in the laboratory for 30 days, in an asbestos box coated with epoxy resin layer. After this period of time, the fishes were transferred to a glass aquarium (100 L capacity) containing soft water, with concentrations of cations and anions controlled to be similar to the natural conditions. The population density was 1 g of fish per liter of water and the temperature was maintained at 25°C ± 1°C.

Acute Toxicity Test

The experimental procedure was based on EPA method 660/3-75-009.[20] The static system was selected as a model in a glass aquarium coated with a thin film of PVC to avoid contamination of the aquarium glass walls during 96 hours test.

Different concentrations of sodium acifluorfen, 69.28, 83.34, 90.71, 98.09 and 119.09 mg L^{-1} in water, were used during the experiment.

The minimum amount of sodium acifluorfen was determinated when the fish showed slight changes in their behavior and the maximum concentration, by 100 % death.

The LC_{50} calculation was carried out by computer using the JSPear Test method.[21] The dead organisms were collected and stored in a freezer (-18°C), until processed.

Bioconcentration Factor

BCF determination was realized in the same conditions as the acute toxicity tests (LC_{50}), with the organisms submitted to 1/100 of LC_{50} of sodium acifluorfen. The fish did not present a statistically measurable harmful effect during 192 hours of exposition.[22]

The experimental tests were performed in triplicate as the following:

- *blank*: soft water plus sodium acifluorfen (0.9184 mg L^{-1}); to evaluate chemicals and physical-chemical changes of the analyte during the test period.
- *control*: soft water plus organisms; to evaluate physiological conditions of organisms.
- *test*: soft water plus sodium acifluorfen (0.9184 mg L^{-1}) plus organisms.

Experimental protocol during tests: removing two fishes and 50 mL of aquarium water daily, which were stored in freezer (-18°C) or a refrigerator (10°C), respectively, for later analytical processing.

Sample

About 20 g of Pacu fish (*Piaractus mesopotamicus*) sample, taken as recommended by López et al.,[11] was cut and blended. Subsequently, the sample was freeze dried and submitted to extraction.

Extraction

A 1 g amount of sample was weighed and extracted in a Soxhlet extractor with 180 mL of methanol for 5 hours. The extract was then dried in a rotary evaporator under vacuum.

Clean-up

The residue was redissolved in 5 mL of methanol and transferred to a preparative column containing 10 g of silica, activated at 140°C for 4 hours and

conditioned with 30 mL of hexane. The column was sequentially eluted with 50 mL of hexane, 50 mL of dichloromethane, and 100 mL of methanol for the sodium acifluorfen. The methanol fraction was dried in a rotary evaporator and the residue was redissolved in 1 mL of methanol and then submitted to clean-up with Florisil, using a Florisil SPE cartridge conditioned with 10 mL of hexane. Elution involved 30 mL of hexane, that was discarded, 30 mL of dichloromethane and 40 mL of methanol. The fractions of dichloromethane and methanol were combined, dried under vacuum and dissolved in 1 mL of methanol, to be analyzed by HPLC/UV.

Analytical Conditions

The extracts obtained were submitted to HPLC analysis on a Shimadzu SPD-10 A liquid chromatograph, with a UV detector operated at 300 nm and a 20 μL injection loop. Analytical RP-HPLC separations were performed on a Supelco RP-18 (5 μm) column (250 mm x 4 mm I.D.) at 30°C. The initial elutions were performed with the mobile phase acetonitrile/water/acetic acid (60 : 40 : 15, v/v/v) at a flow rate of 1 $mL.min^{-1}$.

Recovery Study

This study was conducted through spiking an untreated sample of 1 g of fish or 20 mL of aquarium water with 1 mL of a standard solution of sodium acifluorfen in three different levels: 0.1, 0.5 and 1.0 mg L^{-1}. The fish sample was submitted to the extraction and clean-up processes. Water sample was analyzed directly without any preparative procedure. The tests were carried out five times to calculate standard deviation.

Quantitation

Sodium acifluorfen quantitation, in fish or water samples, was done by the external standard method. The sodium acifluorfen residue values for fish (R) in the samples were calculated according to the following equation:

$$R(mg/kg) = \frac{C \times Vf \times 100}{m \times r} \quad (1)$$

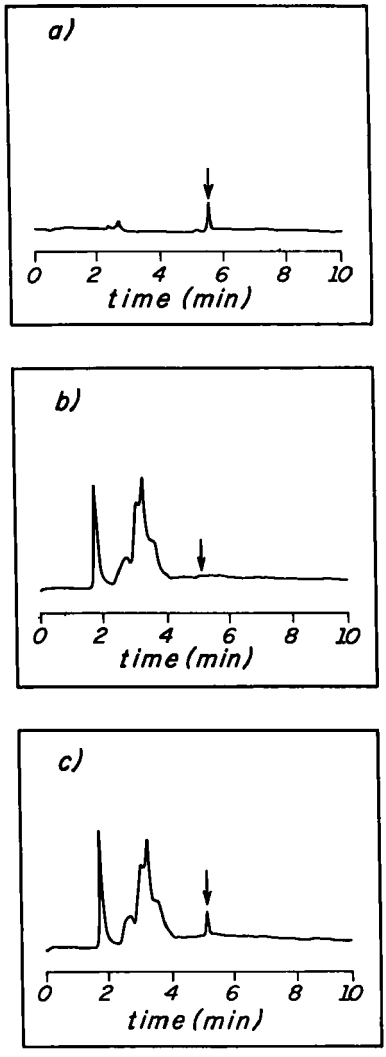

Figure 1. Chromatograms obtained by RP - HPLC with UV detection at 300 nm of: 1 mg L^{-1} of sodium acifluorfen (a), an untreated fish sample (b) and an untreated fish sample fortified with 1 mg L^{-1} of sodium acifluorfen. The arrows indicate the retention time of sodium acifluorfen.

Table 1

Recovery Study Using Fish and Aquarium Water Fortified with Sodium Acifluorfen in Three Levels and their Standard Deviations (S.D.) and Their Relative Deviations (R.S.D.)

Fortification Level (mg L^{-1})	Recovery (%)	
	Fish	**Water**
0.1	75.8 ± 5.2 (5.4)	94.1 ± 4.8 (5.0)
0.5	81.3 ± 4.9 (5.3)	97.2 ± 4.9 (5.5)
1.0	87.9 ± 5.2 (5.3)	99.3 ± 3.7 (4.5)

Recovery ± S.D. (R.S.D. %)

where:

C = concentration obtained from the analytical curve;
Vf = final dilution volume;
m = mass of fish;
r (%) = recovery.

RESULTS AND DISCUSSION

Figure 1, shows the chromatograms obtained with 1 mg L^{-1} of sodium acifluorfen standard (a), an untreated fish sample (b), and untreated fish sample fortified with 1 mg L^{-1} of sodium acifluorfen (c). A total absence of peaks at t_R = 5.7 minutes (retention time of sodium acifluorfen), in the chromatogram of an untreated fish sample (b), suggests no co-elution from matrix compounds.

Table 1, presents the results obtained in the recovery study of fish and aquarium water samples for three levels of sodium acifluorfen concentration.

The results obtained show that this method presented good recoveries for fish and water (higher than 75 %), presenting high efficiency, low standard deviation and good repeatability.

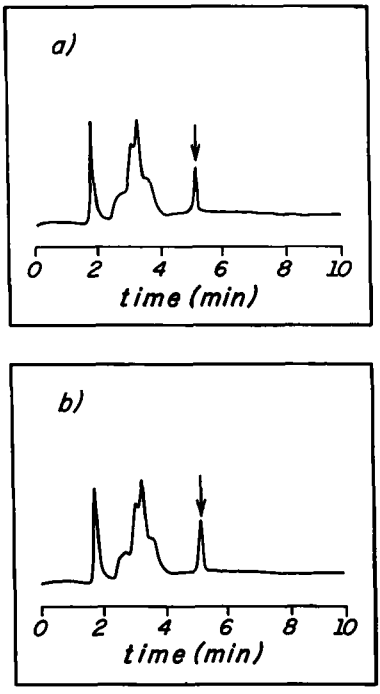

Figure 2. Chromatograms obtained by RP - HPLC with UV detection at 300 nm of: fish samples from the LC_{50}-96 hours test with sodium acifluorfen concentration in the aquarium of 69.28 mg L^{-1} (a) and 119.04 mg L^{-1} (b). The arrows indicate the retention time of sodium acifluorfen.

Table 2

Fish (*Piaractus Mesopotamicus*) Deaths Obtained in the LC_{50}-96 Hours Test Using 8 Test Organisms

Sodium Acifluorfen (mg L^{-1})	Mortality	LC_{50} (mg L^{-1})
69.28	0	
83.33	1	
90.71	5	91.84
98.09	7	
119.04	8	

Table 3

Residue (mg kg^{-1}) Determinated in Fish in the LC_{50}-96 Hours Test

Acifluorfen Sodium (mgL^{-1})	Residue (mg kg^{-1})
69.28	6.24
83.33	8.09
90.71	10.80
98.09	6.85
119.04	4.35

Acute Toxicity (LC_{50})

Figure 2, presents the chromatograms obtained with an extract of fish samples from the LC_{50} test at two sodium acifluorfen concentrations: 69.28 mg L^{-1} (a) and 119.04 mg L^{-1} (b).

Table 2 presents the deaths during the LC_{50}-96 hours test with different sodium acifluorfen concentrations with *Piaratus mesopotamicus* using 8 organisms in the juvenile period, with a 1 g of fish/L of water ratio. At the lowest concentration (69.28 mg L^{-1}) there were no deaths, but, in the subsequent experiments, the mortality increased proportionally with the increase in sodium acifluorfen concentration. The value obtained for LC_{50}-96 hours according to the JSPear program[21] was 91.84 mg L^{-1}, with a confidence range of 87.12-96.81 mg L^{-1}; where this concentration killed 50 % of the fish (*Piaractus mesopotamicus*).

In Table 3, the residue values (mg kg^{-1}) determinated for each sodium acifluorfen concentration in the LC_{50}-96 hours test can be observed.

The highest residue value determinated was 10.80 mg kg^{-1} when 90.71 mg L^{-1} of sodium acifluorfen was applied. This concentration approaches the determined LC_{50} value. After this point, increased sodium acifluorfen concentration decreased the residue value in fishes (Table 3).

Bioconcentration Factor

Figure 3 shows the chromatograms obtained with the aquarium water in the 8^{th} day of BCF test of water : *test* sample (a), *blank* sample (b) and *control* sample (c).

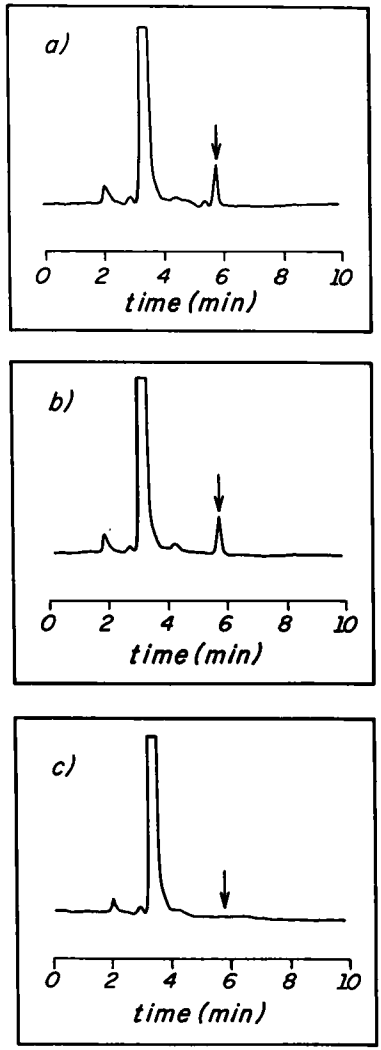

Figure 3. Chromatograms obtained by RP - HPLC with UV detection at 300 nm of water samples from the BCF test in the 8th day using 0.9184 mg L^{-1} of Sodium Acifluorfen (1/100 LC_{50}) in *test* water (a) *blank* water (b) and *control* water (c). The arrows indicate the retention time of Sodium Acifluorfen.

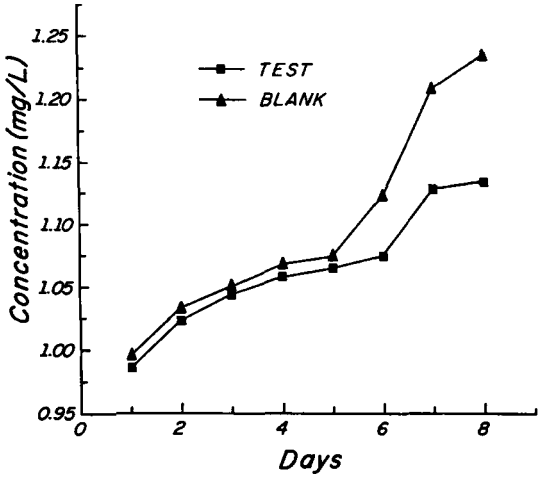

Figure 4. Study of the variation of the Sodium Acifluorfen concentration in the BCF test in aquarium water *test* and *blank*.

The chromatograms show the presence of sodiumacifluorfen at t_R = 5.7 minutes in the *blank* and *test* samples; note the absence of interfering peaks in the control sample. Figure 4 presents a study of sodium acifluorfen concentration during the BCF test. *Blank* aquarium water presented a slightly increased concentration during the time of test, which suggests evaporation of water. Concerning the test water aquarium, a decrease in the concentration of sodium acifluorfen occurred, which suggests the uptake of this compound by the fishes. No residue of sodium acifluorfen was detected present in fishes up to the limit of determination of the developed method (50 mg L^{-1}). It is likely that this compound is metabolized and eliminated by the organism, thus preventing the BCF determination.

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REFERENCES

1. C. Baird, **Environmental Chemistry**, W. H. Freeman and Company, USA, 1995.

2. A.S. Murty, **Toxicity of Pesticides to Fish**, Department of Zoology Nagarjuna - University Nagarjunanagar, India, Third Printing, 1988.

3. G. M. Rand, S. R. Petrocelli, **Fundamentals of Aquatic Toxicology Methods and Application**, Hemisphere Publishing Co, Washington USA, 1985.

4. C. T. Chiou, V. H. Freed, D. W. Schmedding, R. L. Kolnment, Environ. Sci. Technol., **11**, 475-478 (1977).

5. J. Kanazawa, Pestic. Sci., **12**, 417-424 (1981).

6. D. Mackay, Environ. Sci. Technol., **16**, 274-278 (1982).

7. C. T. Chiou, Environ. Sci. Technol., **19**, 57-62, (1985).

8. D. W. Hawker, D. W. Connell, Water Res., **6**, 701-707 (1988).

9. D. W. Hawker, D. W. Connell, Environ. Sci. Technol., **23**, 961-965 (1989).

10. G. A. Leblanc, Environ. Sci. Technol., **29**, 154-160 (1995).

11. J. E. Q. López, R. L. Aguilar, L. M. P. Diez, J. Chromatogr., **591**, 303-311 (1992).

12. P. W. Kohler, S. Y. Su, Chromatographia, **21**, 531-536 (1986).

13. H. R. Buser, M. D. Müller, C. Rappe, Environ. Sci. Technol., **26**, 1533-1540 (1992).

14. A. Olsson, A. Bergman, Ambio, **24(2)**, 119-123 (1995).

15. J. Satsmadjis, B. Iatridis, Centro, **2**, 57-61 (1985).

16. H. R. Buser, M. D. Müller, Anal. Chem., **64**, 3168-3175 (1992).

17. H. R. Buser, M. D. Müller, Environ. Sci. Technol., **27**, 1211-1220 (1993).

18. F. I. Onuska, M. E. Comba, J. A. Cobwrn, Anal. Chem., **52**, 2272-2278 (1980).

19. Standard Methods for the Examination of Water and Wastewater, Joint Editorial Board - Washington, DC, 12th ed. 1980.

20. Committe on Methods for Toxicity Tests with Aquatic Organisms - Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibian, Ecol. Res. Ser. EPA-660/3-75-009, 1975.

21. M. A. Hamilton, R. C. Russo, R. V. Thurston, Environ. Sci. Technol., **11**(7), 714-718 (1977),. Correction: 12(4), 417 (1978).

22. OECD Bioaccumulation Static Fish Test (Guidelines for Testing of Chemicals 305 D), 1981 a, 11.

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