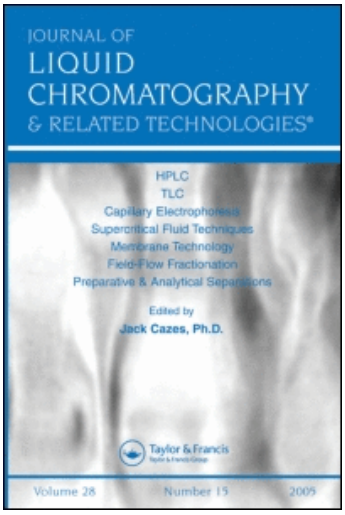

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Determination of Diflubenzuron and Teflubenzuron in Fish Feed By High Performance Liquid Chromatography

Victor Hormazabal[a]; Magne Yndestad[a]
[a] Division of Food Hygiene, Norwegian College of Veterinary Medicine, Oslo 1, Norway

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DETERMINATION OF DIFLUBENZURON AND TEFLUBENZURON IN FISH FEED BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Víctor Hormazábal, Magne Yndestad

Division of Food Hygiene
Norwegian College of Veterinary Medicine
P.O. Box 8146-Dep.
N-0033 Oslo 1, Norway

ABSTRACT

A simple method for the determination of diflubenzuron and teflubenzuron in fish feed by HPLC is presented. The samples were extracted with acetone - tetrahydrofurane and diluted with tetrahydrofurane, prior to the HPLC. The lower limit of quantification was 0.25 and 0.4 g/kg. for diflubenzuron and teflubenzuron in fish feed, respectively.

INTRODUCTION

Diflubenzuron (DFB) and teflubenzuron (TFB) are insecticides belonging to a group of compounds that act by inhibiting chitin biosynthesis and deposition.[1] DFB and TFB are effective in the control of a variety of insects.[2] TFB is applied at regular intervals throughout the growing season of some fruits to control insect pests. It can also be used to reduce the incidence of "contaminants" such as mites on harvested fruit.[3,4]

Infestation with sea lice, *Lepeophtheirus salmonis* and *Caligus elongatus*, is a growing problem in fish farming. The lice damage the skin, causing unthriftiness, and in severe cases the lice may even result in death of the fish. The parasite may also transmit microbial pathogens, and infestations may impact upon wild salmonids. A comparison of infestations between wild and farmed fish shows an usually higher prevalence and abundance on the latter, indicating enhanced transmission of sea lice under farm conditions.[5,6,7]

A range of methods (chemical, physical and biological) have been introduced for controlling sea lice. Treatment with, chemotherapeutics has included the use of dichlorvos, trichlorfon, azamethiphos, carbaryl, ivermectin, pyrethrum and hydrogenperoxide.[6]

The pesticides DFB from Solvay Duphar and TFB from Cyanamid which can be given orally (in feed) to salmon, are two of the newest drugs introduced in the treatment of sea lice in salmon. Methods for the determination of DFB in fish tissues,[8] in environmental samples,[9] foresty substrates,[10] and residues in cabbage under sub-tropical field conditions,[11] have been described. Only one method for the determination of TFB in fish tissues has been described.[8] However, none of the published methods appear to be applicable to medicated fish feed.

The purpose of the present study was thus to develop a simple HPLC method for the routine analysis of DFB and TFB in fish feed.

MATERIALS AND METHODS

Materials and Reagents

Samples of commercial fish feed free of DFB and TFB were used. For testing of the method, medicated (commercial) fish feeds containing DFB and TFB were used.

All chemicals and solvents were of analytical or HPLC grade. The fish feeds were produced by Ewos and Skretting. Diflubenzuron (Solvay Duphar), was donated by Ewos Aqua A.S. (Skårer, Norway) and teflubenzuron (Cyanamid) was donated by Skretting (Stavanger, Norway). Stock solutions (1mg/mL) of DFB and TFB were prepared by dissolving the compounds in tetrahydrofurane. The solutions were stored in the refrigerator.

Chromatographic Conditions

The analyses were performed on a Perkin-Elmer HPLC system, consisting of a Series 410 Bio solvent delivery system, an ISS 100 sampling system (with acetonitrile as flushing liquid) equipped with a Lauda RMT6 cooler (12°C) from Messgeräte Werk Lauda (Lauda Königshafen, Germany), and a LC 235C diode array detector (Perkin-Elmer, Norwalk, CT, USA). The detector was operated at 250 nm (fixed wavelength). The integration was carried out using the software programme Turbochrom 4.0 (Perkin-Elmer), which was operated on a Brick personal computer connected to a BJ-330 printer (Canon).

The analytical column (stainless steel, 15 cm x 4.6 mm. ID) and guard column (stainless steel , 2 cm x 4.6 mm. ID), were packed with 5 μm particles of Supelcosil LC - ABZ (Supelco, Bellefonte, PA, USA). The guard column was connected to an A.318 precolumn filter with an A-102X frits (Upchurch Scientific, USA).

The mobile phase was a mixture of acetonitrile-water-tetrahydrofurane (50 : 37 : 13). The flow rate was 1 mL/min.

Sample Pretreatment

Exactly 0.5 g ground feed was weighed into a 50 mL graduated centrifuge tube with screw cap (Nunc, Roskilde, Denmark), and made up to 50 mL volume with acetone-tetrahydrofurane (6 : 4). The sample was blended, placed in an ultrasonic bath for 5 min (at room temperature) and then left in the extraction fluid for 10 min before again being placed in an ultrasonic bath for 5 min. The sample was mixed and centrifuged for 3 min (3000 rpm). A 1 mL volume of tetrahydrofurane was added to 100 μl of the supernatant and the mixture then blended. The samples (10 μl) were injected into the HPLC system at intervals of 12 min for the determination of DFB and TFB.

Calibration Curves And Recovery Studies

The calibration curves for DFB and TFB were obtained by spiking feed samples with standard solutions, to yield 0.3, 0.5, 0.75, 1.0, and 2.0 g/kg and 0.5, 0.75, 1.0, 2.0 and 3.0 g/kg, of DFB and TFB in fish feed, respectively. Duplicate samples were used. The recovery rates were determined by comparing results of analysis of the spiked samples with those of standard solution. The linearity of the standard curves for DFB and TFB in fish feed was tested using peak-height measurements.

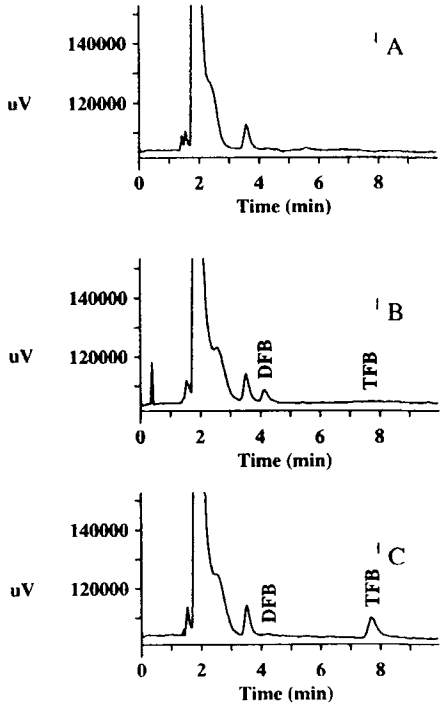

Figure 1. Chromatograms of extracts from 0.5g fish feed. A) drug-free fish feed, B) "real" sample of fish feed contains 0.6g/Kg DFB, C) "real" sample of fish feed contains 2.2 g/Kg TFB.

RESULTS AND DISCUSSION

Chromatograms of commercial samples of clean feed and of medicated fish feed containing DFB and TFB are shown in Figures 1. The standard curves were linear in the investigated areas; 0.3 to 2.0 g/kg for DFB and 0.5 to 3.0 g/kg for TFB.

The linearity of the standard curves for DFB and TFB in fish feed was 0.999 and 0.997, respectively, when using the external standard method of calculation. The precision and recovery for DFB and TFB from fish feed were also calculated, and are shown in Table 1.

Table 1

Material	No. of Samples	Amount in Spiked Samples (g/kg)	Recovery % DFB Mean	DFB S.D.	TFB Mean	TFB S.D.
Feed	8	0.30	91.43	1.84		
(1g)	8	0.75	92.97	2.44		
	8	0.75			90.79	0.86
	8	3.00			90.27	1.61

S.D. = standard deviation.

The extraction procedures were validated, and showed good recovery of DFB and TFB. The recovery varied from 91.4 to 93 % and from 90.8 to 90.3% for DFB and TFB , respectively. The precision of these recovery studies varied from 1.8 to 2.4 % and from 0.9 to 1.6 % for DFB and TFB in fish feed, respectively.

For DFB, the limit of quantification was 0.25 g/kg and the limit of detection 0.15 g/kg. The limit of quantification for TFB was 0.4 g/kg and the limit of detection 0.3 g/kg.

In Norway, commercial medicated fish feed usually contains about 0.6 and 2 g/kg of DFB and TFB, respectively. The potential number of samples that can be dealt with per day is limited only by the duration of the HPLC procedure. The assay shows good precision when using the external standard method.

The method described is rapid, simple, robust and sufficiently sensitive,with good recovery. The quantification is linear over a wide concentration range.

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