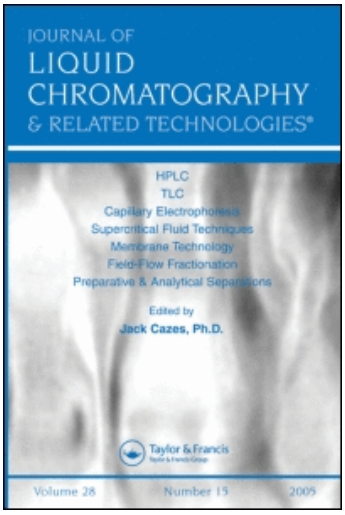

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Determination of Praziquantel in Medicated Fish Feed and Sediment by HPLC

V. Hormazábal[a]; M. Yndestad[a]
[a] Divison of Food Hygiene Norwegian College of Veterinary Medicine, Norway

To cite this Article Hormazábal, V. and Yndestad, M.(1995) 'Determination of Praziquantel in Medicated Fish Feed and Sediment by HPLC', Journal of Liquid Chromatography & Related Technologies, 18: 6, 1231 — 1238
To link to this Article: DOI: 10.1080/10826079508009287
URL: http://dx.doi.org/10.1080/10826079508009287

JOURNAL OF LIQUID CHROMATOGRAPHY, 18(6), 1231-1238 (1995)

DETERMINATION OF PRAZIQUANTEL IN MEDICATED FISH FEED AND SEDIMENT BY HPLC

VÍCTOR HORMAZÁBAL AND MAGNE YNDESTAD

Divison 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 praziquantel (2-cyclohexylcarbonyl-4-oxo-1,2,3,6,7,11b-hexahydro-4H-pyrazino [2,1-a]isoquinoline-4-one) in medicated fish feed, and sediment by HPLC, is presented. The calibration curves were linear in the investigated areas, 0.5 - 5 mg/g praziquantel for fish feed and 30 500 ng/g for sediment, and the recovery rates were 99 to 100%, respectively.

INTRODUCTION

Praziquantel (PQ) is a drug known for its broad-spectrum activity against trematodes and cestodes (1, 2, 3, 4). The presence of tapeworm in farmed Atlantic salmon (Salmo salar) and rainbow trout (Oncorhynchus mykiss), has become an increasing problem in Norwegian fish farming (5) and presumably also in other countries. PQ is often used in treatment against this parasite, being administered as medicated feed. After

administration, some of the drug will enter the sediment under the net pens, as reported for various other drugs (6 - 9).
Various assay procedures for studying PQ levels in serum and other body fluids have been reported, namely: radiometry (10), fluorimetry (11), gas chromatography (12), and biological assay (13). A HPLC method for the determination of PQ in serum (14) and tissues (15) has also been published.
However, none of the published methods appeared to be applicable to medicated fish feed and sediments.
The purpose of the present study was thus to develop a rapid and efficient HPLC method for routine analysis of PQ in fish feed and sediments.

MATERIALS AND METHODS

Materials and Reagents

Samples of fish feed and sediments free of PQ were used. The fish feed was produced by Skretting (Stavanger, Norway). The sediment was taken from an area with no fish farming activity or known effluents possibly containing antibiotics or chemotherapeutical substances.
All chemicals and solvents were of analytical or HPLC grade. PQ (Droncit vet."Bayer") was donated by Bayer Kjemi A.S. (Oslo, Norway). Stock solutions (1 mg/ml) of PQ were prepared by dissolving the compound in a small amount of acetone (6 ml), and diluting to volume with water. Working standards were prepared by dilution with water. The working standard solutions, when stored in the refrigerator are stable for five days.
Solution A was 0.02 M 1-heptane sulfonic acid sodium salt (Supelco, USA) - 0.01 M di-sodium hydrogen phosphate-2-hydrate (Ferax, Germany), made by dissolving 4.45 g/l heptane sulphonate and 1.779 g/l di-sodium hydrogen phosphate-2-hydrate in c. 750 ml of water when preparing 1 litre of solution. The pH was then adjusted to 6 with 2 M phosphoric acid and the solution made up to volume with water.
A Spin-X centrifuge filter unit with a 0.2 µm nylon membrane from Costar (Cambridge, MA, USA) was also employed.

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 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 205 nm. The integration was carried out using the software programme Omega-2 (Perkin-Elmer), which was operated on an Olivetti M300 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 mobile phase was a mixture of water-acetonitrile (60:40 for fish feed and 61:39 for sediments). The flow rate was 1.0 ml/min. for fish feed and 1.0 ml/min. for 3 min. followed by 0.8 ml/min. for 6 min. for sediments. The samples (10 µl for fish feed and 20 µl for sediments) were injected at intervals of 10 and 12 min., respectively.

Sample pretreatment

Fish feed. The stepwise procedure for the pretreatment of fish feed is shown in Fig. 1.
0.5 g ground feed was weighed into a 50 ml graduated centrifuge tube with screw cap (Nunc, Roskilde, Denmark), and 6 ml of acetone added. The sample was mixed for 5 s., and then left to stand with the extraction fluid for 5 min. before again being whirlimixed for 5 s. The homogenate was then made up to 50 ml volume with water-acetonitrile (60:40). The sample was blended, and then centrifuged for approximately 3 min. (3000 rpm). To 0.5 ml of the supernatant was added a 4.5 ml volume of water-acetonitrile (60:40) and the mixture blended. Approximately 0.5 ml of the water-based phase was filtered through a Spin-X centrifuge filter, by centrifugation for 3 min. at 10000 rpm. (5600 g). Aliquots of the filtrate (10 µl) were injected into the HPLC system.

Sediment. The sediment sample (2 g) was weighed into a 50 ml centrifuge tube with screw cap (Nunc). Volumes of 200 µl water (or standard) and 6 ml acetone were added.

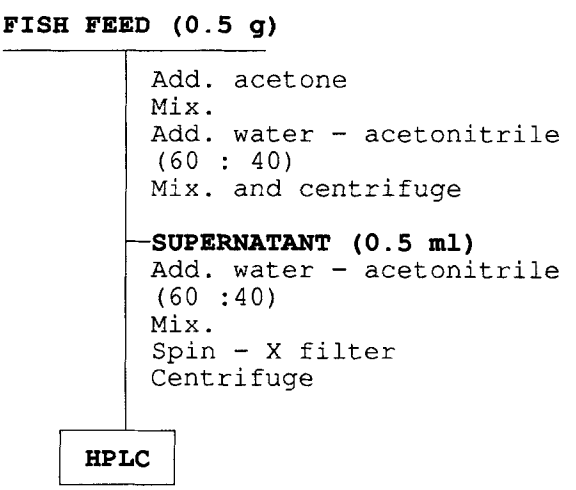

FIGURE 1

Extraction and clean-up procedure for PQ from fish feed.

The sample was mixed for 5 s., and then left to stand with the extraction fluid for 5 min. before again being whirlimixed for 5 s., and then centrifuged for 3 min. (5000 rpm).
4.1 ml of the supernatant (corresponding to 1 g sediment) were transferred into a glass-stoppered centrifuge tube, and 50 µl 1 M NaOH and 5ml diethylether-hexane (3:2) added. The sample was shaken vigorously for 5 s., and centrifuged for 3 min. at 3000 rpm. The upper layer (acetone, diethylether, hexane) was transferred to another glass-stoppered tube. The organic layer was evaporated to dryness at 60 °C under a stream of nitrogen. After adding 1 ml methanol-solution A (70:30) and 1 ml hexane to the dry residue, the sample was again whirlimixed. After centrifugation for 3 min., the hexane layer was discharged, and 2 ml of 0.01 M phosphoric acid-acetonitrile (60:40) added. The sample was again mixed and c. 0.5 ml filtered through a Spin-X centrifuge filter.
Aliquots of the filtrate (20 µl) were injected into the HPLC system.

Calibration curves and recovery studies

The calibration curves for PQ were obtained by spiking fish feed and sediment samples with standard solutions, to yield 0.5, 1.0, 1.5, 3.0, and 5.0 mg/g, and 30, 50, 75, 100, 200 and 500 ng/g of PQ for fish feed and sediment, respectively. Duplicate samples were used. The recovery rates were determined by comparing results of analysis of the spiked fish feed and sediment samples with those of standard solution. The linearity of the standard curves for PQ in fish feed and sediment was tested using peak-height measurements.

RESULTS AND DISCUSSION

Chromatograms of clean and spiked sediment samples, clean samples of fish feed, and a commercial sample of medicated fish feed containing PQ, are shown in Figure 2.

The standard curves were linear in the investigated areas; 0.5 - 5.0 mg/ml, and 30 - 500 ng/g, in fish feed and sediment, respectively. The linearity of the standard curves was 0.9998 and 0.9999 for fish feed and sediment, respectively, when using the external standard method of calculation. The precision and recovery rates for PQ from fish feed and sediment were also calculated, and are shown in Table 1.

The extraction procedures were validated, and showed good recovery of PQ. The recovery of PQ varied from 99 to 100 % for fish feed and sediment, respectively. The precision of these recovery studies varied from 0.37 to 1.07 % and from 0.32 to 2.04 %, for PQ in fish feed and sediment, respectively.

The limit of quantification of PQ was 30 ng/g for sediment, and 0.1 mg/g for fish feed, respectively. No interference was seen during analysis, when calibrating the curves, or when performing recovery studies.

The fish feed clean-up procedure was tried out on sediment. However the limit of quantification in sediment was only 200 ng/g, when aliquots of the filtrated extract (25 µl) were injected into the HPLC system. Unfortunately, when 30 µl or more were injected, minor residues of endogenous compounds in the sediment extracts interfered with PQ in the chromatograms.

The method was tested under practical conditions by analysing about 40 different sediment samples from a

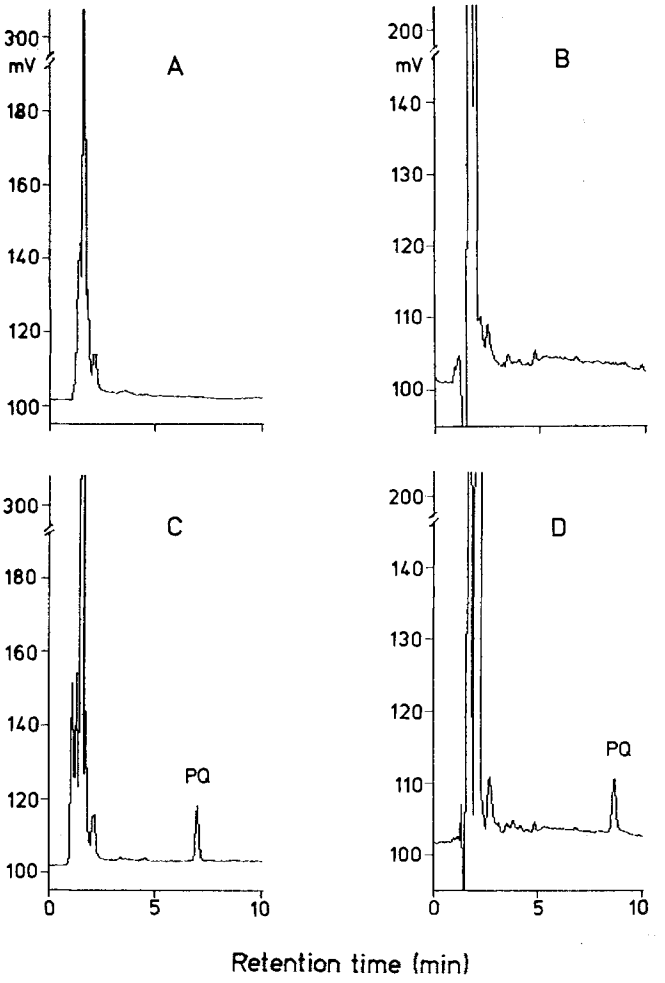

FIGURE 2

Chromatograms of extracts from fish feed and sediment. **A**: Drug-free fish feed, **B**: drug-free sediment, **C**: "real" sample of fish feed contains 1.5 mg/g PQ, **D**: Sediment spiked with PQ (500ng/g).

T A B L E 1

Recovery and repeatability for PQ from spiked samples of fish feed and sediment.

Material	No. of samples	Amount in spiked samples (mg-µg/g)	Recovery % PQ Mean	S.D.
Fish feed	8	0.50	99	1.07
(0.5 g)	8	3.00	100	0.37
Sediment	8	0.05	99	2.04
(2g)	8	0.50	100	0.32

S.D.= standard deviation

field study, as well as six "real" samples of medicated fish feed produced by Skretting (Stavanger, Norway). No interfering peaks were observed in the chromatograms

CONCLUSION

This study showed that the content of the antiparasitic compound, PQ, in medicated fish feed, and residue levels in sediment, can be determined by very simple procedures. The assay shows good precision when using the external standard method. The method is robust, simple, and sufficiently sensitive, with good recovery. The quantification is linear over a wide concentration range. The amount of solvents required is minimized. The pretreatment of samples by liquid-liquid extraction combined with centrifugation filters, is preferable to solid-phase extraction columns when performing the pretreatment manually. The chromatographic system was specific with regard to PQ.

A C K N O W L E D G E M E N T S

We are grateful to the Norwegian Research Council for financial support.

REFERENCES

1 Gönnert R. and Andrews P. (1977). Z. Parasitenkd. 52; 129 - 150.

2 Andrews P., Thomas H., Pohlke R. and Seubert J. (1983). Med. Res. Reviews 3; 147 - 200.

3 Groll E. (1984). Adv. Pharmacol. Chemoth. 20; 219-238.

4 San Martín Durán M. L., Caamano-García F., Fernádez Casal J., Leiro J. and Ubeira F. M. (1989). Aquaculture 76; 199 - 201.

5 Engelstad M., Berland B. and Bristown G. A. (1990). Norsk Fiskeoppdrett, 15; 50.

6 Bjørklund, H., Bondestam, J. and Bylund, G., (1990). Aquaculture, 89 : 359 - 367.

7 Jacobsen, P. and Berglind, L., (1988). Aquaculture, 70 : 365 - 370.

8 Samuelsen, O. B., (1989). Aquaculture, 83 : 7 - 16.

9 Nygaard K., Lunestad B. T., Hektoen H., Berge J. A. and Hormazábal V. Aquaculture, 104 (1992) 31 - 36.

10 Steiner K., Garbe A., Diekmann H. W. and Nowak H.; Eur. J. Drug Metab. Pharmacokinet., 1 (1976) 85.

11 Pütter J.; Eur. J. Drug Metab. Pharmacokinet., 4 (1979) 143.

12 Diekmann H. W,; Eur. J. Drug Metab. Pharmacokinet., 4 (1979) 139.

13 Andrews P.; Vet. Med. Nachr. (Engl. Ed.). (1976) 154.

14 Xiao S. H., Catto B. A. and Webster L. T.; J. of Chromatogr., 275 (1983) 127.

15 Rogstad A., Hormazábal V. and Yndestad M.; J. of Chromatogr. 391 (1987) 328 - 333.

Received: September 7, 1994
Accepted: September 20, 1994