# DETERMINATION OF NITROFURANS IN SHRIMP FEED USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY-UV DETECTION

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## Introduction

Nitrofurans are broad spectrum antimicrobials administered to food producing animals via feed for treatment, and prevention of bacterial and protozoal diseases. They are also used as growth promoters (Vass *et al.*, 2008). In veterinary practice, Furaltadone (Ft), Nitrofurazone (Nz), Nitrofurantoin (Nt) and Furazolidone (Fz) are the most commonly used nitrofurans (Barbosa *et al.*, 2007).

Consumption of food of animal origin, containing nitrofuran residues can elicit hazardous effects such as cancers on human consumers. Therefore, European Union (EU) has prohibited the use of nitrofurans on food producing animals.

Shrimp industry is one of the main foreign currency earning sectors among the aquaculture industries of Sri Lanka and has to maintain international standards such as regular monitoring for the presence of prohibited antibiotics etc.

A few HPLC methods have been developed to detect nitrofuran parent compounds in animal feed. Most of these methods use Solid Phase Extraction technique which requires expensive disposable cartridges. Therefore the objective of this paper was to develop an inexpensive and simple liquid-liquid extraction method coupled with High Performance Liquid Chromatography (HPLC)-Ultra Violet (UV) light to detect nitrofurans in shrimp feed.

## Materials and Methods

### *Materials*

All chemicals were of HPLC grade or analytical grade.

HPLC was performed using an Agilent 1100 series HPLC system connected to a DAD using a $C_{18}$ (Eclipse XDB , 250 mm × 4.6 mm, particle size 5 µm) analytical column. Data acquisition was controlled by ChemStation software, rev. A. 01.02 (Agilent Technologies, Waldbornn, Germany).

### *Standard solutions*

Nitrofuran (Ft, Nz, Nt and Fz) standards were obtained from Sigma-Aldrich, St. Louis, MO, USA and 1 mg/ml stock standard solutions were prepared by dissolving 5 mg of each nitrofuran in 5 ml of methanol. From the stock solution, a 10 µg/ml intermediate standard solution was prepared. A working mixed standard solution of 1 µg/ml was prepared by pooling 500 µl aliquots of individual intermediate standard solutions and making the final volume to 5 ml by adding 1 mM acetic acid: acetonitrile (70:30 v/v). All standard solutions

were stored in the dark at 4 °C and all preparation of solutions was carried out under shaded light.

***Samples***

Shrimp feed samples were directly obtained from reliable manufacturers and from each sample; 20 g was ground to obtain a fine powder. Then 1.0 ± 0.05 g from each powder was weighed and spiked with different volumes of working standard solutions to prepare feed samples with 100, 200, 300, 400, 500 and 1000 µg/kg nitrofurans and vortexed for 5 min . All samples were kept for 20 min before being subjected to extraction.

***Extraction***

A 3 ml of acetonitrile was added to 1 g sample of each powdered feed and mixed well in a glass tube and centrifuged at 1000 g for 15 min. The supernatant was collected to another glass tube and 1 ml of sodium chloride (10 %) and 5 ml of dichloromethane was added and each tube was vortexed for 2 min and allowed for phase separation. The organic layer was separated and 0.5 g of anhydrous sodium sulphate was added and the treated solution was extracted twice with 2 ml n-hexane and evaporated under mild nitrogen flow at 45 °C to dryness. The dry residue was dissolved in 1 ml of 1 mM acetic acid: acetonitrile (70:30 v/v) mixture and transferred to a HPLC vial. The extraction was carried-out under shaded light to prevent degradation of nitrofurans.

***HPLC-DAD analysis***

A 50 µl of the sample was injected into the HPLC system. The separation of nitrofurnas was performed with a 1 mM acetic acid and acetonitrile (70:30, v/v) mobile phase at a flow rate of 0.8 ml/min. The HPLC-UV analysis was performed at 375 nm and peak spectra were collected in the range of 90-400 nm.

The linearity of the detection method was checked using a seven point standard curve for Ft, Nz, Nt and Fz over the range 10, 20, 50, 100, 200, 500 and 1000 µg/kg on two separate occasions. The standard curves were obtained by plotting values of peak area against nitrofuran concentrations. The calibration curve parameters were calculated by ordinary linear regression. Recovery of the extraction method was assessed using triplicates of spiked feed samples at 100, 200, 300, 400,500 and 1000 µg/kg levels.

**Results**

The decision limit (3 x standard deviation + 2.33 x average matrix noise) of the each analyte was calculated and given in Table 1. Three times the decision limit of Ft (the highest decision limit) was taken as the lowest spiked level.

The analytes were found to be well separated in 20 min with sharp and symmetrical peaks when 1 mM acetic acid: acetonitrile (70:30, v/v) was used in isocratic mode.

The linear correlation coefficients ($r^2$) were above 0.999 for all nitrofurans tested. The mean recovery was in the range of 80 - 110 % for all the analytes except nitrofurantoin, for which the recovery was 58-65 % at 100, 200 and 300 µg/kg level (Table 1).

**Table 1. The decision limits, linear correlation coefficients ($r^2$) of the calibration curves drawn against different concentrations of nitrofurans and their recoveries at different concentrations**

| Analyte | Decision Limit (µg/kg) | Linear Correlation Coefficients ($r^2$) | Recovery | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 100 µg/kg | 200 µg/kg | 300 µg/kg | 400 µg/kg | 500 µg/kg | 1000 µg/kg |
| Ft | 34.04 ± 5.06 | 0.999 | 80 | 110 | 99 | 95 | 110 | 83 |
| Nz | 29.18 ± 7.31 | 0.999 | 80 | 105 | 110 | 91 | 110 | 81 |
| Nt | 17.44 ± 1.22 | 0.999 | 58 | 60 | 65 | 80 | 85 | 80 |
| Ft | 10.22 ± 0.78 | 0.999 | 80 | 100 | 95 | 106 | 91 | 80 |

## Discussion

Barbosa *et al.* (2003) have developed a method with solid phase extraction procedure using a gradient mobile phase to detect nitrofurans in animal feed. Further the percentage recovery for Barbosa's method (62 to 78 %) was found to be lesser than that found by the above method. According to the European Commission decision 2002/657/EC, if the assay concentration was ≥ 10 µg/kg the recovery should be within the range of – 20 % to + 10 %. In addition, decision limits of Barbosa *et al.* (2003) method ranged from 47 to 98 µg/kg and these limits were higher than the limits of the respective compounds in the present method.

## Conclusion

An inexpensive, simple, liquid-liquid extraction with isocratic elution mode for the rapid analysis of four nitrofurans in shrimp feed samples using HPLC-UV was developed.

## References

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