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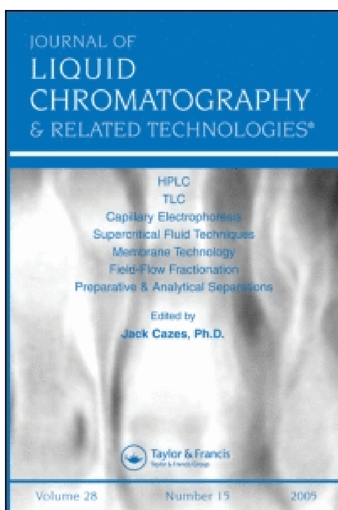


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# Simple and Rapid Method of Analysis for Furazolidone in Meat Tissues by High-Performance Liquid Chromatography

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JOURNAL OF LIQUID CHROMATOGRAPHY, 18(9), 1871-1877 (1995)

# SIMPLE AND RAPID METHOD OF ANALYSIS FOR FURAZOLIDONE IN MEAT TISSUES BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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## ABSTRACT

A simple method for the determination of furazolidone in meat tissues by HPLC using nitrofurazone as internal standard is presented. The samples were extracted with acetonitrile, the organic layer then being separated and evaporated to dryness. The lower limit of quantification was 3 µg/kg with an injection volume of 20 µl, the recovery of furazolidone varying from 97 to 100 %.

## INTRODUCTION

Nitrofuran derivatives (e.g. furazolidone, furaltadone, nitrofurazone) are widely used in Europe in the prevention and treatment of gastro-intestinal infections in pigs, calves and poultry caused by E. coli and Salmonella spp.(1). Severe side-effects (e.g. neurotoxicity,growth depression, haemorrhagic



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diathesis) have been reported especially in association with furazolidone in food producing animals (2).
Nitrofurans are metabolised to a great extent in food-producing animals, and since the demonstration of their mutagenic and (pro) carcinogenic properties, the use of nitrofurans has been strictly regulated in most countries (1-3).
Several analytical methods for the determination of nitrofurans in biological materials based on high-performance liquid chromatography have been published (3-9). The methods are however time-consuming and require the use of large quantities of chemical reagents.
The purpose of the present study was to develop a rapid, simple, and sufficiently sensitive method, for the determination of furazolidone, which required minimal sample manipulation and only small quantities of chemical reagents.

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## MATERIALS AND METHODS

### Materials and Reagents

Samples of cows meat were used.
All chemicals and solvents were of analytical or HPLC grade. Furazolidone and nitrofurazone were purchased from Sigma Co. (St. Louis, MO, USA). Stock solutions (1mg/ml) of furazolidone were prepared by dissolving the compounds in acetonitrile. Stock solutions (1mg/ml) of nitrofurazone were prepared in acetone and ultrasonicated for 1 min. Working standards were prepared by dilution with water. The solutions were stored in the refrigerator.
Spin-X centrifuge filter units from Costar (Cambridge, MA, USA) were 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 Köningshafen, Germany), and a LC 235C Diode Array detector (Perkin-Elmer, Norwalk, CT, USA). The detector was operated at 365 nm. The integration was carried out using the software programme Omega-2 (Perkin-Elmer), wich was operated on an Olivetti M300 personal computer connected to a Bj-330 printer (Canon).

The analytical column (stainless steel, 25 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 two solutions, A and B (75:25). Solution A was 0.02 M heptane sulphonate-0.025 M trisodium phosphate, made by dissolving 4.45 g/l 1-heptane sulphonic acid sodium salt (Supelco) and 9.5 g/l trisodium phosphate 12-hydrate (Merck) in ca. 750 ml of water when preparing 1 litre of solution. The pH was then adjusted to 2.5 with 5 M phosphoric acid and the solution made up to volume with water. Solution B was acetonitrile. The mobile phase was degassed with helium before use.
The flow-rate was 1 ml/min. The samples were injected at intervals of 10 min. Aliquots of 20 μl were injected into the column.

## Sample pretreatment

The stepwise procedure for pretreatment of tissue samples is shown in Fig. 1.
The tissue sample, 3 g of ground muscle, was weighed into a 50-ml centrifuge tube with a screw cap (NUNC, Roskilde, Denmark). Volumes of 200 μl of internal standard solution (nitrofurazone 1 μg/ml) and 6.8 ml acetonitrile were added. The mixture was homogenized for approx. 6 sec. in an Ultra-Turrax TP 18/2 (Janke & Kunkel KG, Ika Werk, Staufen,F.R.G.), and then centrifuged for 5 min. (5000 rpm.). 6.5ml of the supernatant (corresponding to 1.95 g meat), were transferred into a glass-stoppered centrifuge tube, and 2 ml 5 M NaCl added. The sample was shaken vigorously for 10 s., and centrifuged for 2 min. at 3000 rpm. The upper layer was transferred to another glass-stoppered tube. The organic layer was evaporated to dryness at 43°c under a stream of nitrogen. The dry residue was dissolved in 250 μl 0.02M 1-heptane sulfonic acid sodium salt-0.01M di-sodium hydrogenphosphate-2-hydrate (Ferax, Germany), (the pH was then adjusted to 6.0 with phosphoric acid) and acetonitrile (80:20). After 1 ml of hexane had been added, the sample was again whirlimixed. After centrifugation for 4 min, the hexane layer was discharged. The water-based phase was filtered through a Costar Spin-X centrifuge filter unit (low type) with 0.22 μm cellulose acetate binding by centrifugation for 4 min. at 10000 rpm. (5600g). Aliquots of the filtrate were injected into the HPLC system.

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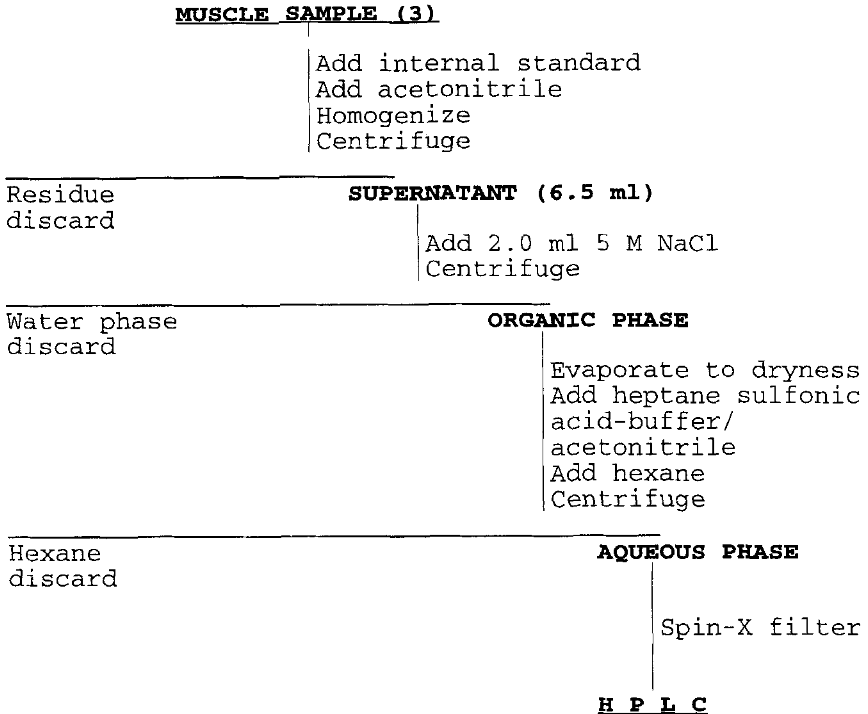


**FIGURE 1**
Extraction and clean-up procedure for furazolidone and nitrofurazone from meat.

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Calibration curves and recovery studies

The calibration curves for furazolidone were obtained by spiking muscle tissue samples with standard solutions and internal standard to yield 2, 3, 5, 10, 20, 50, 100, 200 ng/g of furazolidone and 0.2 µg of nitrofurazone (IS). Duplicate samples were used. The recovery rates were determined by comparing results of analysis of the spiked muscle samples with those of standard solution. The linearity of the standard curves for furazolidone in muscle was tested using peak-height measurements and the internal standard.

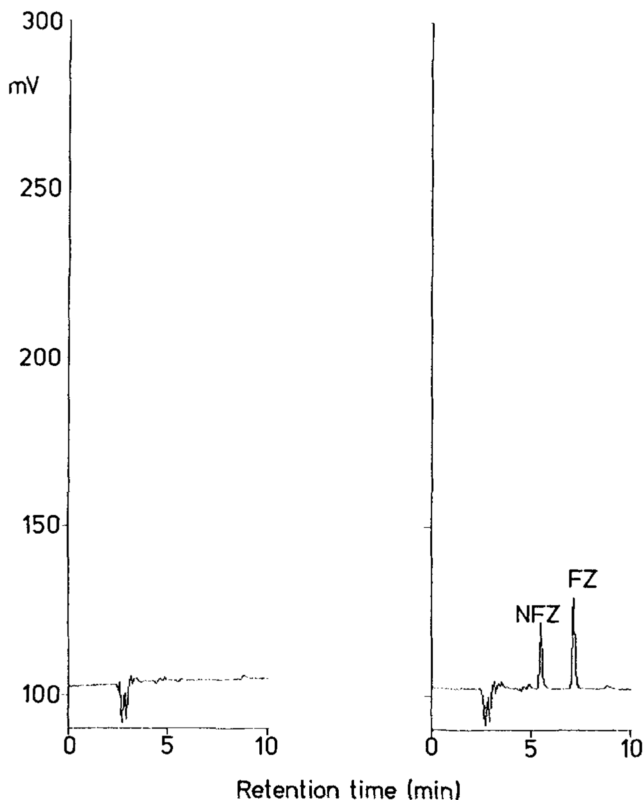


**FIGURE 2**

Chromatograms of extracts from meat.

A - drug-free meat; B - meat spiked with furazolidone and nitrofurazone.

## RESULTS AND DISCUSSION

Chromatograms of clean muscle, and spiked samples are shown in Figure 2. The standard curves were linear in the investigated areas; 2 - 200 ng/g for furazolidone. The linearity of the standard curve was 0.9997 when using the internal standard method. The external standard method of calculation gave a linearity

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**TABLE 1.**

Recovery and repeatability for furazolidone and nitrofurazone from spiked samples of muscle.

| Tissue | No. of samples | Amount in spiked samples (µg/g) | Recovery % NTZ. Mean | NTZ. S.D. | FZ. Mean | FZ. S.D. |
|---|---|---|---|---|---|---|
| Meat | 8 | 0.01 | | | 100 | 1.2 |
| (3g) | 8 | 0.1 | | | 97 | 1.7 |
| | 8 | 0.066 | 90 | 2.4 | | |
| | 8 | 0.066 | 88 | 2.5 | | |

NTZ. = nitrofurazone
FZ. = furazolidone
S.D. = standard deviation

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coefficient of 0.9995. The precision and recovery for furazolidone and nitrofurazone (internal standard) from muscle were also calculated and are shown in Table 1. The extraction procedures were validated, and showing good recovery of both furazolidone and nitrofurazone. The recovery of furazolidone from muscle tissue varied from 97 to 100 % and that of the internal standard from 88 to 90 %, respectively. The precision of these recovery studies varied from 1.2 to 1.7% for furazolidone and from 2.4 to 2.5% for nitrofurazone. The calculations were also performed without internal standard.

The method was tested under practical conditions by analysing about 60 samples from different animals (cows and pigs). No interference was seen. During analysis, when calibrating the curves, and when performing recovery studies, no special attention was paid to light intensity, a factor considered to be of great significance by other authors.

This study has shown that residues of the antibacterial compound furazolidone in meat may be determined using minimal sample manipulation. The cost of chemicals and the manual work-up procedures is also reduced compared to previously published methods. An experienced technician can carry out sample clean-up of about 18-24 samples per day. The assay shows good precision both when using internal and external standard method. The

limit of quantification was 3 ng/g for furazolidone. The method is robust, sensitive and specific, with good recovery of both substances. The method is demonstrated to be efficient for quantification of residues of drug of furazolidone. Only small amounts of solvents are necessary.

## ACKNOWLEDGEMENT

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