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# Determination of nitrofurans in animal feeds by liquid chromatography-UV photodiode array detection and liquid chromatography-ionspray tandem mass spectrometry

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**Abstract**

Within the EU, the use of nitrofurans is prohibited in food production animals. For this reason detection of these compounds in feedingstuffs, at whatever limit, constitutes an offence under EU legislation. This detection generally involves the use of analytical methods with limits of quantification lowers than 1 mg kg$^{-1}$. These procedures are unsuitable for the detection and confirmation of trace amounts of nitrofurans in feedingstuffs due to contamination. It is well known that very low concentrations of these compounds can be the source of residues of nitrofuran metabolites in meat and other edible products obtained from animals consuming the contaminated feed. The present multi-compound method was capable of measuring very low concentrations of nitrofurantoin (NFT), nitrofurazone (NFZ), furazolidone (FZD) and furaltadone (FTD) in animal feed using nifuroxazide (NXZ) as internal standard. Following ethyl acetate extraction at mild alkaline conditions and purification on $NH_2$ column, the nitrofurans are determined using liquid chromatography with photodiode-array detection (LC-DAD). It was observed a CCα ranged from 50 to 100 μg kg$^{-1}$. The liquid chromatography-tandem mass spectrometric (LC-MS/MS) procedure was used to confirm the identity of the suspected presence of any of the nitrofuran compounds.

*Keywords:* Nitrofurans; Feeds; Liquid-chromatography; Photo-diode array detection; Tandem mass spectrometry

## 1. Introduction

Nitrofurans are Schiff's base derivatives of nitrofuraldehyde known to have a broad-spectrum of antimicrobial activity. They were widely used as feed additives in food-producing animals like poultry, swine, cultured fish and shrimps, for treatment and prevention of various gastrointestinal infections caused by bacteria or protozoa and as growth promoters [1]. A great advantage of these compounds in comparison with other antimicrobial agents, is the slowly development and only to a limited extent of the in vivo bacterial resistance. The most common nitrofurans are furazolidone (FZD), nitrofurantoin (NFT), nitrofurazone (NFZ) and furaltadone (FTD) (Fig. 1). These compounds are rapidly metabolized in vivo, leading to a significant decrease of their parent compounds levels in plasma. The elimination half-lives of nitrofurans are very short, and some hours after administration it is almost impossible to detect any residues of parent compounds in edible tissues. A concomitant accumulation of their protein-bound residues is observed, and their detection is possible over large periods of time [2–4]. Based on the evidence of carcinogenic and genotoxic effects of these bound metabolites [5], European Union (EU) has forbidden, for more than a decade, the use of nitrofuran drugs in food producing animals [6].

The detection of nitrofuran metabolite residues in large amount of samples from poultry and aquaculture products imported to Europe from some Southeast Asian and South American countries, and also within the EU in pork and poultry meat, provoked the so called nitrofuran crisis during 2002–2003 [7]. This circumstance had demonstrated the necessity of an effective control of the illegal use of these compounds. The EU Commission Decision of 13 March, 2003 [8] was established the minimum required performance limit (MRPL) of 1 μg kg$^{-1}$ for

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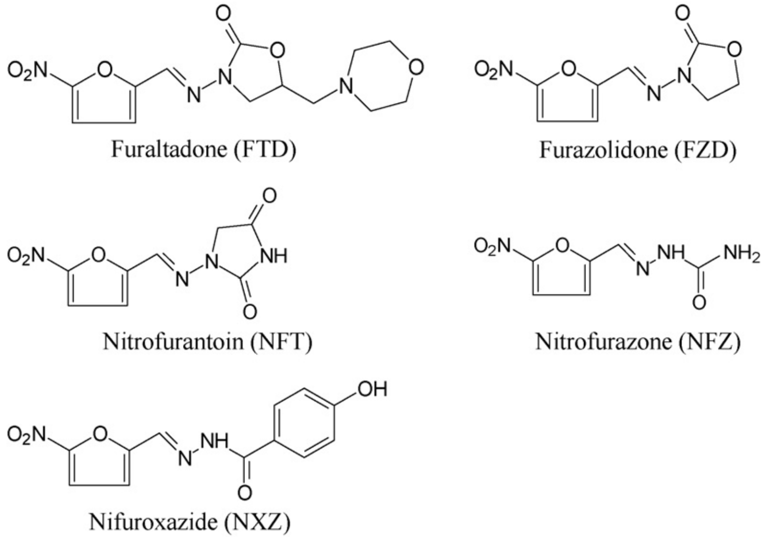

Furaltadone (FTD)

Furazolidone (FZD)

Nitrofurantoin (NFT)

Nitrofurazone (NFZ)

Nifuroxazide (NXZ)

Fig. 1. Chemical structure of furaltadone, furazolidone, nitrofutantoin, nitrofurazone and nifuroxazide (IS).

each nitrofuran metabolite based on the great efficiency of liquid chromatography tandem mass spectrometry (LC-MS/MS) methodologies for detecting bound nitrofuran metabolites in edible tissues.

Nitrofuran compounds were usually administered to animals by means of medicated feeds or at drinking water. Concentrations in feeds ranging from 8 to 400 mg kg$^{-1}$ were considered appropriate depending of the intended use. However, McCracken et al. [9] have confirmed the possibility for the detection of very low concentrations of nitrofuran metabolites in animal tissues exposed to a diet of contaminated feeds with furazolidone and furaltadone at levels as low as 30 μg kg$^{-1}$.

This means that low concentrations of these compounds can be the source of nitrofuran residue metabolites in meat and other edible products obtained from animals consuming the contaminated feed. So, animal feedingstuffs must be analyzed with analytical procedures capable of measuring very low concentrations of nitrofurans to assure its "fit-for-purpose". Nevertheless, the majority procedures of liquid chromatography with ultraviolet detection (LC-UV) available for nitrofuran feed control, shown limits of quantification around 1 mg kg$^{-1}$, were usually applied for a single nitrofuran compound and were not suitable for nitrofuran multicomponent screening and confirmation procedures [10–12]. The aim of this paper was to develop a methodology for screening and confirmation the presence of furazolidone (FZD), nitrofurantoin (NFT), furaltadone (FTD) and nitrofurazone (NFZ) at low concentrations in animal feed, in order to satisfy the above referred needs on feed control.

## 2. Experimental

### 2.1. Reagents and materials

All chemicals and solvents used were of analytical reagent grade except solvents used in mobile phase that were HPLC grade. Deionized water was obtained from a Mill-Q System (Millipore, Bedford, USA). Ethyl acetate, glacial acetic acid, ammonium hydroxide solution containing NH3 >30%, acetone, methanol, acetonitrile, *N*,*N*–dimethylformamide and ammonium acetate were supplied by VWR (Darmstadt, Germany). HPLC solvents were filtered through a 0.45 μm nylon membrane (Whatman, Maidstone, USA) and Sep-Pack NH2 Cartridges (6 mL, 1 g) were purchase from Waters (Milford, USA). PVDF Mini-uniprep$^{TM}$ vial were purchased from Whatman.

A Retsch ZM 200 miller (Haan, Germany), Mettler Toledo PC2000 and AE100 balances (Greifensee, Switzerland), a Heraeus Megafuge 1.0 centrifuge (Hanau, Germany), a rotary vacuum evaporator (Büchi, Flawil, Switzerland) and a speed-Vac concentrator (Thermo electron corporation, Milford, USA) were used to prepare samples, to perform extraction and clean-up procedures.

Liquid chromatography with diode array detection (LC-DAD) determinations were performed in an HP/Agilent 1100 Series HPLC system with a diode array detector (HP/Agilent Techonologies, Waldbronn, Germany), using a Lichrospher 60, RP-select B, 5 μm, 250 mm × 4 mm analytical column with a Lichrospher 60, RP-select B, 5 μm, 4 mm × 4 mm preguard column (Merck, Darmstadt, Germany). Data acquisition was controlled by a ChemStation for LC 3D® software, rev. A.10.01 (Agilent Techonologies, Waldbronn, Germany).LC-MS/MS was performed in an Agilent 1100 Series HPLC system (Agilent Techonologies, Waldbronn, Germany) coupled to a Triple Quadrupole System Sciex API 3000 (Applied Biosystem, Foster City, USA) tandem mass detector, with a Zorbax Eclipse XDB–C18, 5 μm, 150 mm × 2.1 mm column, with a guard column Zorbax Eclipse XDB, C8, 5 μm, 12.5 mm × 2.1 mm (Agilent Technology, Palo Alto, USA). Data acquisition was controlled by a Sciex Analyst® software, Version 1.4.1 (Applied Biosystems, Foster City, CA, USA).

### 2.2. Standard solutions

Furazolidone, furaltadone, nitrofurantoin, nitrofurazone and nifuroxazide, which were used as internal standard