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Determination of nitrofuran metabolites in shrimp muscle by liquid chromatography-photo diode array detection

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3-Amino-2-oxazolidinone (PubChem CID: 65725)
3-Amino-5-morpholino-methyl-1,3-oxazolidinone (PubChem CID: 3016406)
Semicarbazide (PubChem CID: 5196)
1-Aminohydantoin (PubChem CID: 72823)

ABSTRACT

The use of nitrofurans on any animal in the European Union (EU) and any animal or animal food products intended for export to the EU was banned in 1993 (except furazolidone which was banned in 1995) due to the carcinogenicity of the parent drugs and their metabolites. Thereafter, the screening of food of animal origin for nitrofurans and their metabolites became mandatory for all exports to the EU. This paper describes a High Performance Liquid Chromatography – Diode Array Detection (HPLC-DAD) method to detect tissue bound nitrofuran metabolites, namely 3-amino-2-oxazolidinone (AOZ), 3-amino-5-morpholino-methyl-1,3-oxazolidinone (AMOZ), semicarbazide (SEM) and 1-aminohydantoin (AHD). The bound residues were hydrolysed and derivatized into the corresponding nitrophenyl derivatives (NPAOZ, NPAMOZ, NPSEM and NPAHD) with 2-nitrobenzaldehyde and analysed by HPLC-UV at 275 nm. The linearity of the photometric detector response to the four derivatized nitrofuran metabolites was verified using matrix-matched calibration standards in the range of 1–20 μg/kg and the average correlation coefficients for NPAOZ, NPAMOZ, NPSEM and NPAHD were 0.9960, 0.9951, 0.9984 and 0.9993, respectively. The decision limit of the method was below the Minimum Required Performance Limit (MRPL) of 1 μg/kg, for all four nitrofuran metabolites, which makes it compatible with the EU requirements. The average recoveries for (fortified samples between 1 and 5 μg/kg) AOZ, AMOZ, SEM and AHD were 107%, 107%, 115% and 114%, respectively. This is the first report of a HPLC-DAD method detecting nitrofuran metabolites below the MRPL according to our understanding. This method has been successfully used with aquaculture and poultry products; however, currently it is validated according to EU guidelines only for shrimp muscle tissue.

1. Introduction

The nitrofurans are a group of broad-spectrum antimicrobials active against Salmonella sp., coliforms, Mycoplasma sp., Coccidia sp. and certain protozoa (Delatour et al., 2003). Furazolidone, furaltadone, nitrofurantoin and nitrofurazone are the four main members of this group (Kennedy, Young, & McCracken, 2003). They are rapidly metabolized in the body within hours after administration (Leitner, Zöllner, & Lindner, 2001). However, their metabolites reside in animal body for weeks, possibly months, as protein bound residues (Conneely, Nugent, & O'keeffe, 2002) (Fig. 1). The use of nitrofurans was banned in the European Union (EU) member states in 1993, with the exception of furazolidone due to the carcinogenicity of the parent drugs and their metabolites (EC., 1993). The ban was extended to cover furazolidone in 1995 due to the carcinogenicity of the parent drug and the unavailability of data concerning the safety of its metabolites (EC., 1995). Thereafter, it is prohibited to use nitrofurans on any animal in the EU and any animal or animal food products intended for export to the EU.

In the early 1990's the Laboratory of the Veterinary Science Division (VSD) in the Department of Agriculture and Rural Development, Belfast, developed an LC-MS method to detect furazolidone with a detection limit of 2 μg/kg (Kennedy et al., 2003). However, it was later found that this method has a limited value, because the tissue concentrations of furazolidone were very low due to rapid excretion of the drug and instability of the residues. In 1980s RIKILT (State Institute for Quality Control of Agricultural Products, Wageningen, Netherlands) showed that mild acidic

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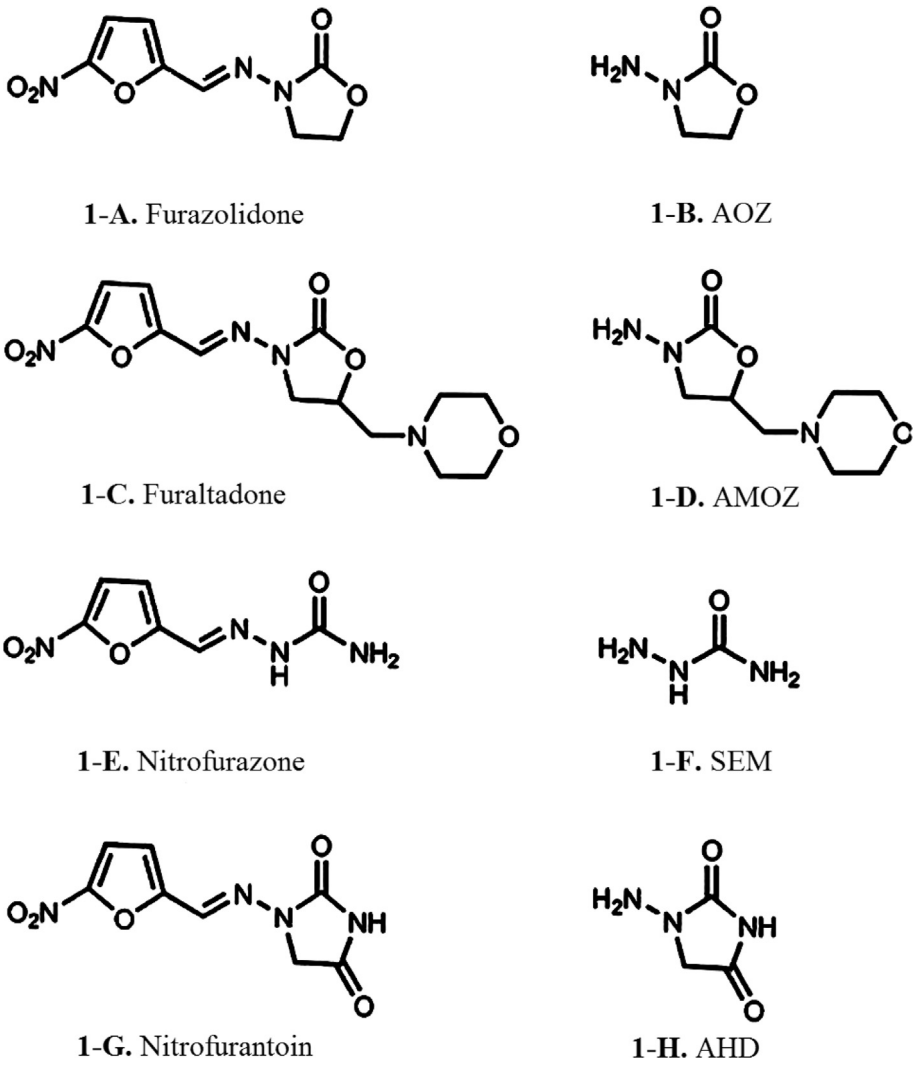

Fig. 1. A-H. Structures of four nitrofuran antibiotics (on the left side) and their respective free metabolites (on the right side).

treatment could release a compound called 3-amino-2-oxazolidinone (AOZ) from the stable tissue-bound furazolidone residues (Fig. 2) (Hoogenboom, Berghmans, Polman, Parker, & Shaw, 1992; Hoogenboom, Van Kammen, Berghmans, Koeman, & Kuiper, 1991). Later, RIKILT developed an HPLC UV method for detection of AOZ and showed that it is a more reliable analytical method for screening tissues of animals treated with furazolidone than the parent compound. After the European Commission has launched the FoodBRAND project to overcome the limitations associated with nitrofuran issue, the project team developed a sensitive method to detect all four metabolites of the nitrofuran drugs. The method utilizes a LC-MS/MS and meets the criteria set out by the EU for unequivocal confirmation of the presence of these metabolites (EC., 2002; Kennedy et al., 2003). At present a Minimum Required Performance Limit (MRPL) for the nitrofuran metabolites, in poultry and aquaculture products, has been set at 1.0 μg/kg (EC., 2003).

The shrimp farming industry has been one of the most important economic activities in the aquaculture sector of many developing countries including Sri Lanka due to the high demand in the EU member states, Japan and USA. However, due to fundamental deficits in disease control, management and biosecurity practices the disease prevalence is high in shrimp farms (Munasinghe, Stephen, Abeynayake, & Abeygunawardena, 2010). High disease prevalence, lack of resources and facilities to test food of animal origin for such banned antimicrobials and inadequate legislative frame work may increase the risk of abusing nitrofurans by the shrimp farmers. Therefore, it is essential to screen shrimp (and other food of animal origin) for banned antimicrobial compounds including nitrofurans to safeguard the consumers and export industry. The purpose of this study was to establish a financially sustainable method to screen nitrofuran metabolites namely 3-amino-2-oxazolidinone (AOZ), 3-amino-5-morpholino-methyl-1,3-oxazolidinone (AMOZ), semicarbazide (SEM) and 1-aminohydantoin (AHD) in shrimp muscle tissue conforming to the EU criteria.

2. Material and methods

2.1. Reagents and chemicals

The solvents used were of HPLC grade or above, unless otherwise stated. Double distilled and deionized water, filtered through a 0.45 μm nylon filter was used. The standard substances (AOZ,