

Liquid chromatography coupled to high-resolution-Orbitrap mass spectrometry for the determination of nitrofuran metabolites in fish samples

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Abstract

Nitrofurans (NFs) are synthetic antibiotic drugs employed for the treatment of bacterial diseases in livestock production or as food additives in industrial farming of food-producing animals. The most widely used NFs in veterinary medicine are nitrofurantoin (NFT), furazolidone (FZD), nitrofurazone (NFZ) and furaltadone (FTD). After intake, NFs are extensively metabolized into their corresponding metabolites (NFM), identified as 1-amino-hydantoin (AHD), 3-amino-2-oxazolidone (AOZ), semicarbazide (SEM) and 3-amino-5-methyl-morpholino-2-oxazolidinone (AMOZ), for nitrofurantoin, furazolidone, nitrofurazone and furaltadone, respectively. NFs and their metabolites are suspected to possess carcinogenic and mutagenic potency, therefore their application in food and animal production was banned in the EU in 1995 and in the USA in 2002. Since parent NFs are extensively metabolized to tissue-bond metabolites, recent analytical methods have been focused on the determination of the NFMs instead of the parent compounds. In the present study a modified extraction technique including at first a hydrolysis-derivatization step [O'Keeffe et al., 2004] was validated and applied for the simultaneous determination of four of NFMs residues (AHD, AOZ, SEM, AMOZ) in fish samples from aquacultures located in North Western Greece.

Keywords: Nitrofuran metabolites, fish, UHPLC-LTQ-Orbitrap-MS

1. Introduction

Nitrofurans (NFs) are synthetic antibiotic drugs used as veterinary medicines or as feed additives in industrial farming of food-producing animals. Aquaculturists also use NFs to treat diseases found in aquatic animals, such as fish, shrimp, and crabs. The four most widely used NFs in veterinary medicine are nitrofurantoin (NFT), furazolidone (FZD), nitrofurazone (NFZ) and furaltadone (FTD) which all contain a characteristic 5-nitrofurans ring [Kim et al., 2015]. After intake, NFs are extensively metabolized into their corresponding metabolites (NFMs), which are highly stable and identified as 1-amino-hydantoin (AHD), 3-amino-2-

oxazolidone (AOZ), semicarbazide (SEM) and 3-amino-5-methyl-morpholino-2-oxazolidinone (AMOZ), for nitrofurantoin, furazolidone, nitrofurazone and furaltadone, respectively. Their application in food and animal production was banned in the EU in 1995 and in the USA in 2002, because several studies indicated their ability to possess carcinogenic and mutagenic potency. A minimum required performance level (MRPL) of 1 µg kg⁻¹, for each nitrofurans metabolite in meat product has been established by the EU. Nevertheless, NFs are still being used to treat animal diseases in some countries because of their efficiency, availability and relatively low cost [Zhang et al., 2016]. Since parent NFs are extensively metabolized to tissue-bond metabolites, recent analytical methods have been focused on the determination of the NFMs instead of the parent compounds.

2. Methodology

Fish samples (sea bream and sea bass) were obtained from an aquaculture in North Western Greece. The samples were blended using a food processor and mixed thoroughly. A well homogenized sample (1 g) was weighed and placed into a 50 mL centrifuge tube and Internal standard working solution was added. Hydrochloric acid, water and 2-NBA were added and the sample was vortexed. The sample was then derivatised at 37 °C for 18 hours. Following derivatization, the sample was neutralized by the addition of di-potassium hydrogen orthophosphate. The pH value was adjusted to pH 7 ± 0,5 using sodium hydroxide. For the liquid-liquid extraction, ethyl acetate was added to the tube. The sample was vortexed, shaken for 20 minutes and centrifuged for 10 minutes at 4000 rpm. The procedure was repeated one more time. The organic fractions were combined and evaporated till dryness. The residue was reconstituted in 400 µL H₂O with 0.01% CH₃COOH and injected into the chromatographic system. Detection of the target analytes was performed using a UHPLC-LTQ-Orbitrap-MS system in positive ionization mode in a total run of 10 min.

3. Findings

The validation scheme followed was based on the US Food and Drug Administration FDA . Good linearity was obtained in all cases exhibiting excellent coefficients of determination (R^2). The method precision achieved in terms of repeatability and within-lab reproducibility was low enough, expressed as relative standard deviation (R.S.D.). Recoveries obtained were satisfactory for all metabolites (above 75%) while limits of detection found at the low ppb level (0.2 ppb – 0.5 ppb).

4. Conclusions

An analytical methodology combining derivatization for pretreatment, neutralisation and LLE for extraction, was developed and evaluated exhibiting excellent performance for the all the target metabolites. The detection and quantification of the target compounds in fish samples were carried out using the innovative Hybrid LTQ Orbitrap mass spectrometry technology. The ultimate purpose was the successful application in the field of fish sample analysis from aquacultures in North Western Greece.

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