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The determination of biurea: A novel method to discriminate between nitrofurazone and azodicarbonamide use in food products

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Abstract

Recently doubts have arisen on the usefulness of semicarbazide as marker residue for the illegal use of the antibiotic nitrofurazone (NFZ) in aquaculture and poultry production. Most notably azodicarbonamide (ADC) has been implicated as an alternative source of semicarbazide. ADC is used in some countries as a dough conditioner at concentrations up to 45 mg kg^{-1}. The use of ADC-treated flour or dough in coated or breaded food products may generate false non-compliant results in the analytical method for nitrofurazone metabolites, which is currently in use. During the dough preparation process ADC is largely reduced to biurea, which can be considered as an appropriate marker residue of ADC. Thus far no methods have been published for the determination of biurea in food commodities. Due to its polar nature it is very difficult to generate sufficient retention on conventional C_{18} HPLC columns. With a TSK amide HILIC type column good retention was obtained. A straightforward extraction-dilution protocol was developed. Using a mixture of dimethyl formamide and water biurea was nearly quantitatively extracted from a variety of fresh, coated and processed products. Mass spectrometric detection was performed with positive electrospray ionisation. The sensitivity and selectivity of the mass spectrometer for biurea was very good, allowing detection at concentrations as low as 10 μg kg^{-1}. However, in some extracts severe ion suppression effects was observed. To overcome the implications of ion suppression on the quantitative performance of the method an isotopically-labelled biurea internal standard was synthesized and incorporated in the method. The method developed can be used effectively in nitrofurazone analysis to eliminate the risk of false non-compliant results due to the presence of azodicarbonamide-treated components in the food product.

Keywords: Azodicarbonamide; Biurea; Semicarbazide; Nitrofurazone; Breaded products

1. Introduction

Nitrofurans (furazolidone (FZD), furaltadone (FTD), nitrofurazone (NFZ), nitrofurantoin (NFT)) comprise a group of antibiotic substances that are cheap and highly effective drugs for the treatment of bacterial diseases in farm animals and in aquaculture. They have been extensively used in intensive farming for many years, but due to concerns raised regarding the human health risks associated with the use of these drugs and the lack of sufficient safety data, nitrofurans as a group have been banned in the European Union (EU) in the 1990s [1,2]. Methods developed for the detection of abuse originally aimed at the parent drugs themselves. But as these are very quickly metabolized in the living organism the usefulness of these methods was rather limited. However, during metabolism tissue-bound nitrofuran adducts are formed in substantial amounts and as these adducts are only very slowly released, they remain in the animal for weeks or even months after medication. Feeding experiments have shown that of the nitrofurans NFZ produces the highest concentrations tissue-bound adducts in pig muscle tissue, with an estimated half-life of 15 days [3]. Although little is known about the identity of these tissue-bound adducts, it has been known for many years that upon acid hydrolysis the hydrazone bond is cleaved releasing the hydrazine side chain which, after derivatisation with 2-nitrobenzaldehyde, can be isolated and analysed by LC-UV or LC–MS/MS (Fig. 1) [4–6].

Much of the early work focused on FZD, but it is now well established that all four nitrofurans are metabolized in a similar fashion and for each of the nitrofurans a specific side chain is released: FZD: 3-amino-2-oxazolidinone (AOZ); NFZ: semicarbazide (SEM); FTD: 5-morpholino-3-amino-2-

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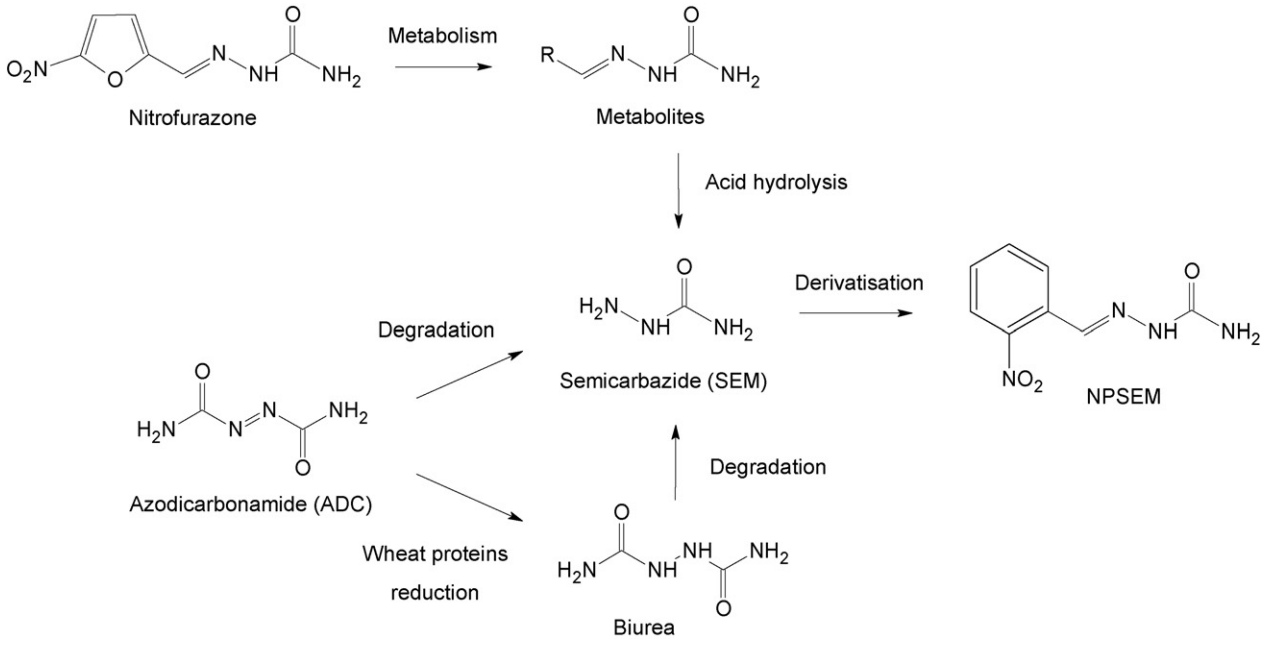

Fig. 1. The formation of semicarbazide by acid hydrolysis of nitrofurazone metabolites and as a degradation product from azodicarbonamide treatment of flour.

oxazolidinone (AMOZ) and NFT: 1-aminohydantoin (AHD). Methods directed at the detection of these marker metabolites have developed quickly in recent years and are now routinely used by laboratories around the world. The implementation of sensitive LC–MS/MS methods in 2002 revealed a large-scale abuse of nitrofuran drugs, most notably in aquaculture products originating from South-East Asia and in poultry products from certain countries in South-East Asia and South America. The EU responded by issuing protective measures [7,8]. The result was a large increase of numbers of consignments tested for nitrofuran metabolite residues and many non-compliant results above the MRPL of 1 $\mu g\,kg^{-1}$ [9] were reported, mainly on residues originating from FZD, FTD and NFZ. In the months following, there was a marked decrease in the number of incidences reported for FZD and FTD, but for NFZ the number of non-compliant results dropped less significantly.

Moreover, SEM was found in products for which it was considered highly unlikely that NFZ had been used in the production process. Most notably a high incidence of (high concentrations) of SEM in breaded and processed food commodities triggered the search for alternative sources of SEM. This resulted early 2003 in the identification of azodicarbonamide (ADC) as a likely candidate to produce SEM in the standard nitrofuran protocol [10,11]. ADC is used as a flour improving (bleaching) agent and as a dough conditioner at concentrations up to 45 $mg\,kg^{-1}$ [12]. During dough preparation ADC is almost quantitatively converted to biurea (hydrazodicarbonamide) by the reaction with the wet flour [11,13,14]. It has been postulated that acid hydrolysis of biurea can form SEM with an efficiency of approx. 0.1% [11]. ADC has been removed from the list of permitted dough treatment agents in the mid 1990s, but in the USA the FDA still permits the use of ADC [12]. ADC is used in Brazil [11] and Canada [14,15] as well.

ADC has also been widely used as a blowing agent for the production of gasket seals in food jars, and it has been connected to the elevated levels of SEM encountered in baby food products [16,17]. It was shown that SEM is a minor decomposition product formed from ADC during heat treatment needed for the sealing of the jars [18]. The European Union has prohibited the use of ADC as a blowing agent for cap seals that may come into contact with foodstuffs in 2005 [19]

The findings of non-compliant results on SEM for breaded and coated products has prompted AFSSA in their position of Community Reference Laboratory on nitrofurans to advice the National Reference Laboratories to physically remove the coating from the meat part in breaded samples. Furthermore, in case of a non-compliant sample for the total residue content of SEM, the analysis should be repeated for the bound residues of SEM only [20]. Although the proposed procedure may be considered as the best approach under the given circumstances, it is not a very satisfactory situation. Moreover, there are strong indications that SEM formed from ADC may bind covalently to proteins or polycarbohydrates during the bread baking process [21]. Therefore, the presence of tissue-bound adducts alone does not provide sufficient evidence of NFZ abuse. It is important that a method is made available that can discriminate between NFZ and ADC as the source of SEM residues in food products.

As ADC is typically added to the flour in the 10–40 $mg\,kg^{-1}$ range, substantial amounts of biurea will be present in the ADC-treated batter or bread [11,13]. Even if the bread coating comprises only one percent of the total food product (e.g. after physical removal of the breaded parts) it will still contain biurea in concentrations of 100 $\mu g\,kg^{-1}$ or more. Considering the molecular structures of NFZ and biurea, it is highly unlikely that biurea will be produced metabolically from NFZ. Furthermore, no other biological sources of biurea or commercial uses other than as an intermediate for the production of ADC, are