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Semicarbazide: Natural occurrence and uncertain evidence of its formation from food processing

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ABSTRACT

The use of nitrofuran antibiotics in food-producing species has been banned within the European Union (EU) since 1993. Analysis of their residues in food has focused on using LC-MS/MS for the determination of their metabolites using stable isotopes as internal standards. Since AB SCIEX launched the first commercial LC-MS/MS instrument in 1989, most of these have used Selective Reaction Monitoring (SRM) technique. Triple quadrupole LC-MS/MS systems suffer from cross-talk, which can lead to incorrect identification and quantitation. Concern over residual nitrofuran has persisted for a long time, with most of the nitrofuran alerts originating from semicarbazide (SEM) in shrimp. The aim of this study is to review evidence from the literature and experimental studies concerning the formation of SEM from food processing and to discuss the natural occurrence of SEM in shrimp and aquatic organisms. Although SEM can form from azodicarbonamide, biurea, and homologous compounds, biurea readily reacts with 2-nitrobenzaldehyde (2-NB) to form a derivative that LC-MS/MS has incorrectly identified as SEM. Concerning the natural occurrence of SEM in crustaceans, cross-talk and misleading data have been frequently reported in the literature. Cross-talk symptoms should be carefully considered because they can affect the reliable detection and determination of SEM using a stable isotope as the internal standard. Twelve cases of non-compliance in shrimp from China and Korea were investigated: 7 cases of amino-2-oxazolidinone (AOZ) in the steamed meat of Pacific white shrimp, 1 case of AOZ in freshwater prawns, and 4 cases of SEM in freshwater prawns. The ease of purchasing medical-grade nitrofurans on the web and monitoring data suggest that the natural occurrence of SEM in prawns and shrimp is questionable, and that adulteration is a more likely scenario.

1. Introduction

Nitrofurazone (5-nitro-2-furaldehyde semicarbazone), a nitrofuran, is a veterinary antibacterial drug widely used for fish (Fig. 1). It is often used in shrimp farming and poultry production because of its growth-promoting properties and to prevent and control bacterial infections (Pereira, Pampana, Donato, & De Nucci, 2004). Because of the potential carcinogenicity and mutagenicity of nitrofurans and their metabolites, the use of any nitrofuran in food-producing animals within the European Union (EU), or in any animal destined for export into the EU, has been banned since the mid-1990s (Commission Regulation, 1995, Samsonova, Douglas, Cooper, Kennedy, & Elliott, 2008). Rapid metabolism, with an *in vivo* half-life of a few hours, has been reported as evidence for the instability of residues of the parent compound (McCracken, Blanchflower, Rowan, McCoy, & Kennedy, 1995). After administering nitrofurazone to chickens or pigs, the compound is rapidly metabolized to semicarbazide (SEM), a hydrazine derivative (Fig. 1) that can easily bind to tissue proteins (Cooper et al., 2005; McCracken, Rhijn, & Kennedy, 2005). Therefore, the parent compound is unsuitable for monitoring purposes. The most commonly used analytical methods for nitrofurans are based on acid hydrolysis of protein-bound residues followed by their conversion to nitrobenzaldehyde (NBA) derivatives and analysis by LC-MS/MS (Cooper & Kennedy, 2005; Hoogenboom, Van Kammen, Berghmans, Koeman, & Kuiper, 1991; Leitner, Zöllner, & Lindner, 2001). The International Agency for Research on Cancer (IARC) classified SEM as 'unclassifiable as to carcinogenicity to humans' (Group 3) in 1987 (International Agency for Research on Cancer (IARC), 1987) but the toxicities of nitrofurazone and SEM remain controversial. The European Food Safety Authority (EFSA) reported

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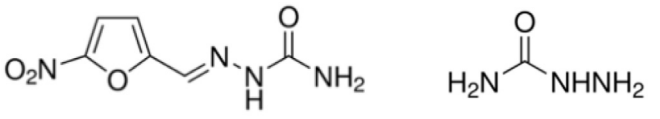

Nitrofurazone Semicarbazide (SEM)

Fig. 1. The structure of 5-nitro-2-furaldehyde semicarbazone (nitrofurazone) and its metabolite semicarbazide (SEM).

four chemical substances that combine with SEM (EFSA, 2005): (1) a metabolite of the veterinary antibiotic nitrofurazone, (2) a thermal break-down product of azodicarbonamide (ADC), (3) a reaction product of hypochlorite action on food additives and (4) a decomposition product of ADC when added to improve flour, a procedure that has been forbidden within European countries (European Food Safety Authority (EFSA), 2003).

The natural presence of SEM has been reported in the shells of crustaceans such as crayfish, shrimp, prawn, and soft-shell crab (McCracken et al., 2013; Saari & Peltonen, 2004; Van Poucke et al., 2011). Crews suggested that a potential natural source of SEM in honey is the sudden increase in arginine levels in heather pollen shortly before and during honey production. Other possible sources of SEM are unidentified precursors and environmental contaminants, including urine from sheep or wild animals (Crews, 2014). Despite the controversy over the toxicological effects and the natural occurrence of SEM, it will need to be detected as a bound residue biomarker of nitrofurazone in order to control the illegal use of nitrofurazone in the food processing of animals. The aim of this work was to elucidate and identify evidence for the naturally occurring formation of SEM, and its occurrence as the result of food processing, through a review of the literature and experimental results.

2. Materials and methods

2.1. Literature study

A literature search was carried out using more than 150 science databases. The search included the synthesis of nitrofurans, use and pharmacokinetics, toxicological data, methods of analysis and monitoring results. The literature search mainly aimed at finding information on: 1. The occurrence of SEM in food and other matrices; 2. Methods of analysis that were used for identification, monitoring, and investigation; and 3. The relationship between occurrence, theories, and methods of analysis (Table 1).

2.2. Analysis of SEM

Several chemicals are used in the analysis of SEM in shrimp meat and shell, e.g., SEM hydrochloride (≥99%), 2-nitrobenzaldehyde (2-NB, ≥99%), 3-amino-2-oxazolidinone-d4 (AOZ-d4, analytical standard grade), 3-amino-5-morpholino-methyl-1,3-oxazolidinone-d5 (AMOZ-d5, analytical standard grade), amino-2-oxazolidinone (AOZ, analytical standard grade), 3-amino-5-morpholino-methyl-1,3-oxazolidinone (AMOZ, certified reference grade) and 1-aminohydantoin (AHD, analytical standard grade). All of these chemicals were obtained from Sigma–Aldrich (St. Louis, MO, USA). Formic acid, hydrochloric acid, acetonitrile, and methanol for HPLC analyses were obtained from J.T Baker (Phillispsburg, NJ, USA). Tissue-bound nitrofuran metabolite residues were quantitated using a modification of a previous method for the determination of nitrofuran metabolites (Cooper et al., 2005). Briefly, 5 g of minced shrimp meat or 5 g of ground shell, and 5 g of minced whole shrimp were taken for analysis. Analytes were released by hydrolysis with dilute hydrochloric acid and simultaneously derivatised with 2- nitrobenzaldehyde with or without the addition of stable isotope internal standards. An Acquity ultra-performance liquid chromatography (UPLC) system coupled to a Waters XEVO triple quadrupole mass spectrometer equipped with an electrospray ion source was used for LC-MS/MS analyses. Separations were performed on a C18 UPLC BEH Acquity column (50 mm × 2.1 mm; 1.7 μm particle diameter) from Waters. Data processing was carried out using Masslynx 4.1 software from Waters.

The reaction products of the chemical reaction of biurea with 2-NB were studied using biurea purchased from TCI (>99.0%, Tokyo, Japan). A biurea solution in methanol was added to 5 g of a negative shrimp meat blank to make 20 ng/g and 200 ng/g, which were then analysed according to the same derivatisation and LC-MS/MS procedure as used for the nitrofuran metabolites.

3. Results

3.1. Uncertain evidence for natural occurrence from the literature

Nitrofuran antimicrobials were first synthesized in the 1950's for human use. The first drug in this class to be introduced into clinical practice was a simple derivative of 5-nitrofurfural and semicarbazone, called nitrofurazone (Lednicer & Mitscher, 1977). Its use was prohibited in Europe ('zero tolerance') at any stage in the raising of animals for food. Europe introduced a 'minimum required performance limit' (MRPL) of 1 μg/kg for four nitrofurans (furazolidone, furaltadone, nitrofurantoin, and nitrofurazone, measured as their respective tissue-bound metabolites) in poultry meat and aquaculture (CD, 2003). As marker metabolites, AOZ (3-amino-oxazolidone), AMOZ (3-amino-5-morpholinomethyl-1,3-oxazolidone) and AHD (1-aminohydantoin) are considered sufficient evidence to infer the use of the parent drugs. SEM, however, is a fairly ubiquitous small organic molecule which has many sources other than nitrofurazone (Points, Burns, & Walker, 2015).

3.1.1. Lack of analytical evidence

SEM is a decomposition product of azodicarbonamide (ADC), which is used as a blowing agent in the production of gaskets for the lids of food jars (Stadler et al., 2004). SEM is plausible, in terms of its chemical structure, as a minor thermal decomposition product of ADC. To identify SEM from ADC, co-workers of this article previously developed an LC-MS method for SEM without derivatisation or acid hydrolysis (Read & Castle, 2003). The Food Standards Agency in the United Kingdom (Food Standards Agency, 2003/2004) reported that this study used an independent method of testing for free SEM. The presence of SEM in the gaskets was thought to result from the use of the azodicarbonamide (ADC) blowing agent that was used during the manufacture of the plastic. At the same time as the presence of SEM was reported in foods associated with plastic gaskets, there were also reports, using other analytical methods, of the presence of SEM in a range of foods where it would not be expected. In all cases, the method of analysis that was used involved an acid hydrolysis and derivatisation step. It was hypothesized that the presence of SEM could be an artefact of this particular method. For example, SEM may have resulted from the hydrolysis of ADC or some of its impurities during the analytical work-up. An alternative analytical method was required, initially to look for the presence of free SEM in ADC, and also at the impurities in biurea (BU) and urazole (UR). In work commissioned by the Agency, liquid chromatography mass spectrometry (LC-MS) and LC-MS/MS methods were developed and reported for the determination of underivatised SEM in ADC, BU, and UR in free solution. This experimental results from this report did not provide clear