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Development and validation of an analytical method for the determination of semicarbazide in fresh egg and in egg powder based on the use of liquid chromatography tandem mass spectrometry

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Abstract

In this work the development of a method to determine semicarbazide (SEM) in fresh whole egg and in whole egg and egg white powders as those used in the food industry, by using LC–MS/MS is described. The method is based on a recently validated one for the determination of SEM in baby food. SEM is extracted from egg with 0.2 mol L^{-1} hydrochloric acid and derivatised with 2-nitrobenzaldehyde. The extract is neutralised and purified by passing through a solid phase extraction (SPE) cartridge. The 2-nitrobenzaldehyde derivative is eluted with ethyl acetate. The eluate is evaporated to dryness and the residue re-dissolved in methanol:water mobile phase. SEM is determined by reversed-phase LC–MS/MS. The "in-house" validation of this method has been performed taking into account the "*Harmonized guidelines for single-laboratory validation of methods of analysis*" (IUPAC Technical report) and the Commission Decision (2002/657/EC). The performance characteristics of the method were established by in-house validation procedures employing assays with standard solutions, sample blanks and spiked samples. Linearity, matrix effect, trueness, precision, selectivity, limits of detection and of quantification were determined. The fitness for purpose of this method was assessed based on its performance characteristics. LOD's of 0.15 and 0.4 μg kg^{-1} for whole fresh egg and industrial egg powders, respectively, were obtained. The LOQ for fresh whole egg was 0.2 μg kg^{-1} and for industrial egg powders 0.8 μg kg^{-1}. Linear calibration curves were obtained in the ranges 1–100 and 0.8–400 μg kg^{-1} for fresh whole egg and the egg powders, respectively.

Keywords: Semicarbazide; Nitrofurazone; Nitrofurans; Egg; Liquid chromatography tandem mass spectrometry

1. Introduction

Nitrofurazone (NFZ) is a banned antibiotic which belongs to the nitrofurans group. Nitrofurans have been used for the treatment of gastrointestinal and dermatological infections in cattle, swine, poultry, fish and shrimps, and also to treat bacterial diseases in bees. Since 1993 the use of nitrofurazone has been banned within the countries of the European Union (Commission Regulation 2901/93 (1993). Due to a very fast metabolism the "in vivo" half-life of the nitrofuran-type antibiotics is of only few hours [1,2]. For this reason some metabolites of the parent antibiotics, which accumulate in proteins are used as markers for the illegal use of nitrofurans. Semicarbazide was found to be a stable side chain metabolite of nitrofurazone and thus it is used as marker of nitrofurazone abuse in official residue controls [3]. Recently, Cooper and Kennedy reported that SEM has been found at parts per million concentrations in the retina of pigs fed therapeutic doses of nitrofurazone [4]. Nitrofuran derivatives have also been detected in eggs [5]. According to the literature, the presence of SEM in egg can be attributed to the illegal use of nitofurazone in poultry, but also to the use of hypochlorite for disinfection during egg braking operations. Hönicke et al. found up to 130–450 μg kg^{-1} SEM in egg white powder treated with 12% active chlorine [6].

Another source of SEM, also recently found, is by thermal decomposition of azodicarbonamide, which has been used as blowing agent in jar gaskets [7], and as additive in bread and bakery products [8,9]. The use of azodicarbonamide with the two mentioned purposes is nowadays banned within the countries of the European Union.

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Briefly, the method to analyse total SEM (free and bound to proteins) consists in four steps: hydrolysis of the protein-bound SEM to get it free in solution and simultaneous derivatisation of the free SEM with 2-nitrobenzaldehyde (2-NBA) to form the respective hydrazone derivative, clean-up by liquid–liquid or solid phase extraction (SPE), and final LC–MS/MS determination. Most of the published methods regarding the determination of SEM in food are based on the same schema with few modifications [10]. In this method the clean-up is performed by solid phase extraction on a polystyrene–divinylbenzene copolymer sorbent material, which enables a strong and quite selective retention of the nitroaromatic derivatives (owing to π–π interactions), whereas most of the matrix compounds are more weakly retained. In this way a tedious liquid–liquid extraction clean-up procedure is avoided.

The purpose of this work was to develop and validate a method for the determination of SEM in fresh egg and in egg powders as those used in food processing at industrial level. The method should deviate as little as possible from a method recently validated in a collaborative trial to determine SEM in baby food, in order to facilitate the work of food control laboratories [11,12].

2. Experimental

2.1. Apparatus

LC–MS/MS analyses were performed using an Agilent 1100 HPLC (Diegem, Belgium) coupled via an electrospray interface to a mass spectrometer Quattro Premier Micromass (Bruxelles, Belgium). The HPLC and MS/MS optimised analytical conditions are summarised in Table 1.

Table 1
Optimised conditions for the LC–MS/MS analysis of SEM in egg samples

HPLC conditions	
Sample temperature	$20 \pm 2\,^{\circ}C$
Injection volume	10 μL
Reversed-phase HPLC column	Atlantis C_{18} 150 × 2.1 mm, 3 μm (Waters, Milford, Massachusetts, USA)
Column temperature	$25 \pm 5\,^{\circ}C$
Flow	0.2 mL min^{-1}
Mobile phase solution	(A) Ammonium formate 5 mmol L^{-1}: (B) methanol (v/v) in a gradient condition

HPLC gradient

Time (min)	Ammonium formate 5 mmol L^{-1} (%)	Methanol (%)
0	80.0	20.0
5.0	5.0	95.0
9.0	5.0	95.0
10.0	80.0	20.0
16.0	80.0	20.0

MS/MS conditions

Source temperature	120 °C
Desolvation temperature	450 °C
Electrospray capillary voltage	3 kV
Collision cell entrance and exit energies	0 eV
Collision gas, argon, pressure into the cell	$\sim 10^{-4}$ mbar
Nitrogen as the dryer and cone gas at a flow rate ~700 and ~100 L h^{-1}, respectively	
Multiple reaction monitoring for the selected ions (Table 2)	
LC and MS conditions are programmed and controlled with MassLynx software	

2.2. Reagents and standards

All general reagents were of analytical reagent grade or higher. Semicarbazide hydrochloride (SEM) 99% pure and the isotopically labelled semicarbazide (1,2-[$^{15}N_2$, ^{13}C] SEM) 99% pure, used as internal standard, were supplied by Sigma–Aldrich (Bornem, Belgium) and Witega (Berlin, Germany), respectively. Stock solutions, 1 mg mL^{-1} of SEM and of 1,2-[$^{15}N_2$, ^{13}C] SEM free, were prepared independently in methanol. Intermediate solutions 10 μg mL^{-1} of SEM and of 1,2-[$^{15}N_2$, ^{13}C] SEM were prepared by dilution of the respective stock solutions with methanol. Spike solutions 20 and 200 ng mL^{-1} SEM and 100 ng mL^{-1} 1,2-[$^{15}N_2$, ^{13}C] SEM were prepared by dilution of the respective intermediate solutions in methanol. The stock, intermediate and spike solutions were stored at 2–8 °C and were stable at least for 1 year, 1 month and 1 week, respectively.

The derivatisation solution, 10 mg mL^{-1} 2-NBA, was prepared dissolving 100 mg of 2-NBA in 10 mL of methanol. This solution should be made fresh daily.

2.3. Samples

Two different types of samples were used to perform this study: fresh eggs purchased at local supermarkets, and whole egg powder and egg white powder which have been provided by the industry. The egg samples purchased at local supermarkets were stored at 2–8 °C and the egg powders were kept at room temperature the time that analyses have last.

2.4. Sample pre-treatment and analysis

Samples were allowed to warm up to room temperature and homogenised with a spatula prior to sub-sampling. Aliquots of 2.0 ± 0.03 g of fresh egg or 0.5 ± 0.03 g of egg powders were weighed into 50 mL centrifuge tubes. Extraction solution (10 mL of the 0.2 mol L^{-1} hydrochloric acid), internal standard (100 μL of the 100 ng mL^{-1} 1,2-[$^{15}N_2$, ^{13}C] SEM) and derivatisation solution (240 μL of 2-NBA 10 mg mL^{-1} in methanol) were added to the tubes. The centrifuge tubes were securely capped and vortex for ~30 s and then placed in a shaking water bath at $40 \pm 3\,^{\circ}C$ overnight. The samples were allowed to cool down to room temperature and 10 mL of a 0.2 mol L^{-1} dipotassium hydrogen orthophosphate solution and 800 μL of a 2 mol L^{-1} sodium hydroxide solution were added to neutralise the mixture. After vortexing the tubes for 30 s the samples were centrifuged at 4500 rpm for 15 min.

SPE cartridges were stored at room temperature. For conditioning of the cartridges 3 mL of ethyl acetate, 3 mL of methanol