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Establishment of a method to detect sulfonamide residues in chicken meat and eggs by high-performance liquid chromatography

J.M.K.J.K. Premarathne [a, b, *], D.A. Satharasinghe [a], A.R.C. Gunasena [a], D.M.S. Munasinghe [a], P. Abeynayake [a]

[a] Faculty of Veterinary Medicine and Animal Science, University of Peradeniya, Peradeniya, 20400, Sri Lanka
[b] Faculty of Livestock, Fisheries and Nutrition, Wayamba University of Sri Lanka, Makandura, 60170, Gonawila, Sri Lanka

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ABSTRACT

Sulfonamides are a group of antimicrobials used for treatment and prevention of infectious diseases in humans and animals. In veterinary practice, sulfonamides are extensively used due to its broad spectrum of activity and low cost. A multi-residue analysis of seven sulfonamides (sulfadiazine, sulfathiazole, sulfapyridine, sulfamerazine, sulfamethazine, sulfamethizole, and sulfamethoxypyridazine) in chicken and eggs using HPLC- DAD detection method has been proposed. Chicken and egg samples were homogenized and extracted with distilled water:ethyl acetate (1:3, v/v) liquid-liquid extraction. The extracts were defatted with n-heptane and dried under nitrogen flow at 55 °C. The dry residue was dissolved in 500 μL methanol:acetic acid:water mixture with a ratio of 10:4:36 (v/v/v) and 10 μL of the sample was subjected to HPLC determination under the following conditions: column, Luna 5 μ C18; particle size, 5 μm; mobile phase, 17 mM acetic acid: methanol: acetonitrile (83:10:7, v/v/v); flow rate, 1.0 mL/min; and detection, 270 nm. The specificity was evaluated by analyzing 30 different blank samples of chicken and eggs in order to verify the absence of potential interfering compounds. No interfering peaks were found around the retention time of analytes in the matrices under investigation. The linear correlation coefficients (r^2) for 50–250 ppb range were above 0.99 for all sulfonamides tested. The mean recoveries for chicken and eggs spiked at 50, 100, and 150 ppb were in the range of 86–108% for all analytes. Repeatability and within laboratory reproducibility of the developed method was determined at 100 ppb and quantified as the relative standard deviation was lower than 15%. The decision limits were between 108 and 116 ppb for all analytes whilst the detection capability of all analytes ranged from 129 to 140 ppb. An inexpensive and simple liquid-liquid extraction with isocratic elution mode for rapid analysis of residues of seven sulfonamides in chicken and egg samples using HPLC-DAD detection was established in this study.

1. Introduction

Sulfonamides are a group of antimicrobials used for treatment and prevention of infections in humans and in animals (Bishop, 2005). In veterinary practice, sulfonamides (SAs) are extensively used due to their broad spectrum of activity and low cost (Saif et al., 2003). In addition, SAs are used as feed additives to promote growth in animals (Bishop, 2005).

Improper use of sulfonamides can lead to the development of resistant microorganisms and the occurrence of residues in animal origin food products (Giguere et al., 2006). Sulfadiazine (SDZ) has been shown to have toxic effects especially on the thyroid (Tsai, Kondo, Ueyama, & Azama, 1995). Therefore to ensure consumer safety, the European Union (EU) has set the maximum residue limits (MRLs) of 100 ppb for sulfonamides in food of animal origin.

Various physiochemical and immunological methods have been developed to detect and quantify sulfonamide residues in animal origin food products. Among them are single or simultaneous analysis of sulfonamides by immunoassay methods (De Keizera, Bienenmann-Plouma, Bergwerff, & Haasnoota, 2008; Zhang et al., 2007) or biosensor immunoassay (Haasnoot, Bienenmann-Ploum, & Kohen, 2003; Situ, Crooks, Baxter, Ferguson, & Elliottet, 2002), multi-residue analysis of sulfonamides using liquid

* Corresponding author. Faculty of Livestock, Fisheries and Nutrition, Wayamba University of Sri Lanka, Makandura, 60170, Gonawila, Sri Lanka.
E-mail address: krissjayaruk@yahoo.com (J.M.K.J.K. Premarathne).

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chromatography with UV detection (Biswas, Rao, Kondaiah, Anjaneyulu, & Malik, 2007a, 2007b; Di Sabatino, Di Petra, & Benfenati, 2007; Furusawa, 2002; Furusawa & Kishida, 2001; Kao, Chang, Cheng, & Chou, 2001; Perez et al., 2002), liquid chromatography with fluorescence detection with post or pre-column derivatization (Gehring et al., 2006; Stoev & Michailova, 2000; Zotou & Christina, 2010), and liquid chromatography-mass spectrometry (Sheridan, Policastro, Thomas, & Rice, 2008; Zheng, Zhang, Peng, & Feng, 2008). The method developed by Kao et al., (2001) can detect eight sulfonamides (SAs) namely sulfadiazine (SDZ), sulfathiazole (STZ), sulfamerazine (SMR), sulfamethazine, sulfamonomethoxine, sulfamethoxazole, sulfaquinoxaline, and sulfadimethoxine in chicken and swine muscles. Six SAs including STZ, SMR, SMZ, sulfachlorpyridazine, sulfamethoxazole, and sulfamonomethoxine could be detected in pasteurized milk (Perez et al., 2002). Some methods are capable of detecting only two SAs, SDZ and sulfadimethoxine (SDM) in milk samples (Furusawa, 2000). Furusawa (2002) has developed a method for the analysis of SMZ, sulfamonomethoxine (SMM), sulfadimethoxine (SDM), and sulfaquinoxaline (SQ) in eggs.

In Sri Lanka poultry industry contributes 70% of the total livestock production in Sri Lanka and generally there is an increasing trend towards the consumption of chicken meat and eggs among Sri Lankans. Furthermore, Sri Lanka exports surplus poultry products to neighboring countries (Ministry of Livestock and Rural Community Development, 2011). Exporting food of animal origin requires compliance with the specific animal health, public health, and hygienic standards together with the verification of compliance with importing country requirements on residues of veterinary therapeutics, pesticides, and contaminants. In order to comply with the ISO 22000 standard and hazard analysis critical control point (HACCP) system, it is essential to maintain the animal origin food products free of antimicrobial residues.

It is evident that there are a huge number of methods which could be used to analyze SA residues in different matrices. Still there are methods being developed in order to cater the specific needs of animal husbandry practices and importing country. Considering the above issues, a method to detect SAs in the food of animal origin is of great concern. Most of the HPLC methods adopted solid-phase extraction (SPE) (Di Sabatino et al., 2007; Kim, Choi, Kim, & Lee, 2002) with post or pre-column derivatization which is expensive and laborious. When considering the current state of food safety and quality assurance issues in Sri Lanka, there is a growing demand for availability methods capable of detecting antimicrobial residues. Therefore, it is necessary to develop an affordable method according to the requirements of Sri Lanka which is capable of detecting SAs in the matrices and has a great consumer demand with the potential to be exported to other countries. Therefore, the objective of this study is to develop a simple and economical liquid-liquid extraction method coupled with HPLC–DAD to detect seven commonly used SAs in chicken meat and eggs in routine residue monitoring.

2. Materials and method

2.1. Chemicals and reagents

Acetonitrile, *n*-heptane, methanol, and ethyl acetate were of HPLC grade obtained from BDH HiperSolv, VWR International, UK; while acetic acid and HCl were of analytical grade obtained from Ajax Finechem, Australia and BDH, VWR International, UK respectively. De-ionized double distilled water was obtained by a Milli-Qm purification system (Millipore, USA).

2.2. Standard solutions

Sulfonamide standards; sulfadiazine (SDZ), sulfathiazole (STZ), sulfapyridine (SPD), sulfamerazine (SMR), sulfamethazine (SMT), sulfamethizole (SMZ), and sulfamethoxypyridazine (SMP) were obtained from Sigma-Aldrich, USA (Fig 1). A stock standard solution of each sulfonamide was prepared in methanol to obtain a final concentration of 1000 ppb. Stock standard solutions were stored in amber colored glass bottles at −20 °C and were stable for 3 months. An intermediate standard solution of 100 ppb was prepared by diluting the stock solution in methanol and was stable for 4 weeks when stored at 4 °C. The working solutions of 10 ppb were freshly prepared by diluting intermediate stock solutions with a mixture of 17 mM acetic acid: methanol: acetonitrile (83:10:7, v/v/v) for each analysis.

2.3. Sample preparation

Known negative chicken muscle and whole egg samples were stored at −20 °C prior to use as blank matrices. Chicken muscle samples were cut into small pieces and homogenized using an Ultra Turrax homogenizer (IKA, Germany) at 24,000 rpm for 3 min. Eggs were broken and both yolk and albumen were homogenized using a homogenizer at 24,000 rpm for 1 min.

2.4. Fortification of samples

A portion of 3 g ± 0.05 of homogenized chicken muscle and egg samples were spiked with working SAs standard mixture at three levels to obtain 50, 100, and 150 ppb for each compound in chicken or egg which was equivalent to 0.5, 1.0, and 1.5 times the European Union MRLs. Fortified samples were allowed to stand at room temperature for 30 min before further analysis.

2.5. Sample extraction

A 3g ± 0.05 of homogenized chicken muscle or egg sample was added with 0.5 mL HCl (0.1 M). Then, 3 mL distilled water and 4.5 mL ethyl acetate were added to the same sample and mixed thoroughly by vortexing. Next sample was centrifuged at 3000 rpm for 10 min. The supernatant was pipetted into a test tube. The obtained pellet was extracted again with 4.5 mL ethyl acetate. The extracts were combined and evaporated under a mild nitrogen flow at 55 °C using a heating block (Techne, UK). Finally, the dry residue was dissolved in 500 μL methanol:acetic acid:water (10:4:36, v/v/v) mixture. Thus, the prepared solution was extracted twice with 2 mL *n*-heptane to remove any excess fat. The resulting solution was filtered through a 0.45 μm disposable syringe filter and transferred to a HPLC vial.

2.6. HPLC analysis

Liquid chromatography was performed using the Agilent 1100 series HPLC system connected to DAD (Agilent Technologies, Germany) using a Luna C_{18}, 5 μm, 250 mm × 4 mm analytical column. Data acquisition was controlled by ChemStation software, rev. A. 01.02 (Agilent Technologies, Germany). 10 μL of the sample were injected into the HPLC system by an automatic sample injector. The separation of SAs was performed with 17 mM acetic acid:-methanol:acetonitrile (83:10:7, v/v/v) mobile phase at a flow rate of 1 mL/min. The HPLC-DAD analysis was performed at 270 nm and the peak spectra were collected in the range of 190–400 nm.