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Monitoring of sulfonamide antibacterial residues in milk and egg by polymer monolith microextraction coupled to hydrophilic interaction chromatography/mass spectrometry

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ARTICLE INFO

Article history:

Received 15 May 2008

Received in revised form

15 July 2008

Accepted 17 July 2008

Published on line 29 July 2008

Keywords:

Polymer monolith microextraction

Hydrophilic interaction

chromatography–electrospray

ionization mass spectrometry

Sulfonamide antibacterial

Milk

Egg

ABSTRACT

A simple, rapid, and sensitive method for the determination of traces of thirteen sulfonamide antibacterials in milk and eggs is presented. This method is based on the combination of polymer monolith microextraction (PMME) technique with hydrophilic interaction chromatography/mass spectrometry (HILIC/MS). The extraction was performed with a poly(methacrylic acid–ethylene glycol dimethacrylate) monolithic capillary column while the subsequent separation was carried out on a Luna NH₂ column by HILIC. To obtain optimum results, several parameters relating to HILIC and PMME were investigated. After optimization, acetonitrile (contain 0.05% formic acid, v/v) was used as the elution solution, which was well compatible with the mobile phase in HILIC. Good linearities were obtained for thirteen SAs with the correlation coefficients (R^2) above 0.997. The limits of detection ($S/N=3$) of the method were found to be 0.4–5.7 ng mL⁻¹ of SAs in whole milk and 0.9–9.8 ng g⁻¹ of SAs in eggs. The recoveries of thirteen SAs in two matrices ranged from 80.4 to 119.8%, with relative standard deviations less than 11.8%.

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1. Introduction

Sulfonamides (SAs) comprise a large number of synthetic antimicrobial agents. More than ten SAs are routinely used in food-producing animals for therapeutic, prophylactic, and growth-promoting purpose [1]. Improper use of them, such as excessive administration and inappropriate withdrawal period, may result in SA residues in animal-derived food products. This is of great concern because there is a possibility that exposure to these drugs could reduce the effectiveness of human therapeutic drugs and some of the compounds such as sulfamethazine are carcinogen [2]. To protect consumers from risks related to SA residues, the European Union (EU) set a safe maximum residue limits (MRLs) of 100 ng g⁻¹ of SAs

as a total in milk (regulation EC/281/96). China established a MRLs of 100 ng g⁻¹ for the sum of SAs and a MRL of 25 ng g⁻¹ for sulfamethazine only [3]. Although several SAs have been approved for medicinal purposes in chickens, their use is forbidden in laying hens to avoid possible residues in eggs [4]. Consequently, the development of simple, rapid and efficient methods for monitoring sulfonamides residue in food is highly desired for the sake of food safety.

In order to inspect the SA residues in foodstuff, high-performance liquid chromatography (HPLC) method has been frequently used because it tends to be efficient in separating closely related compounds with low volatility [5]. HPLC with UV detection has been successfully applied to determine several SA residues in milk and eggs [6–13]. In recent

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doi:10.1016/j.aca.2008.07.033

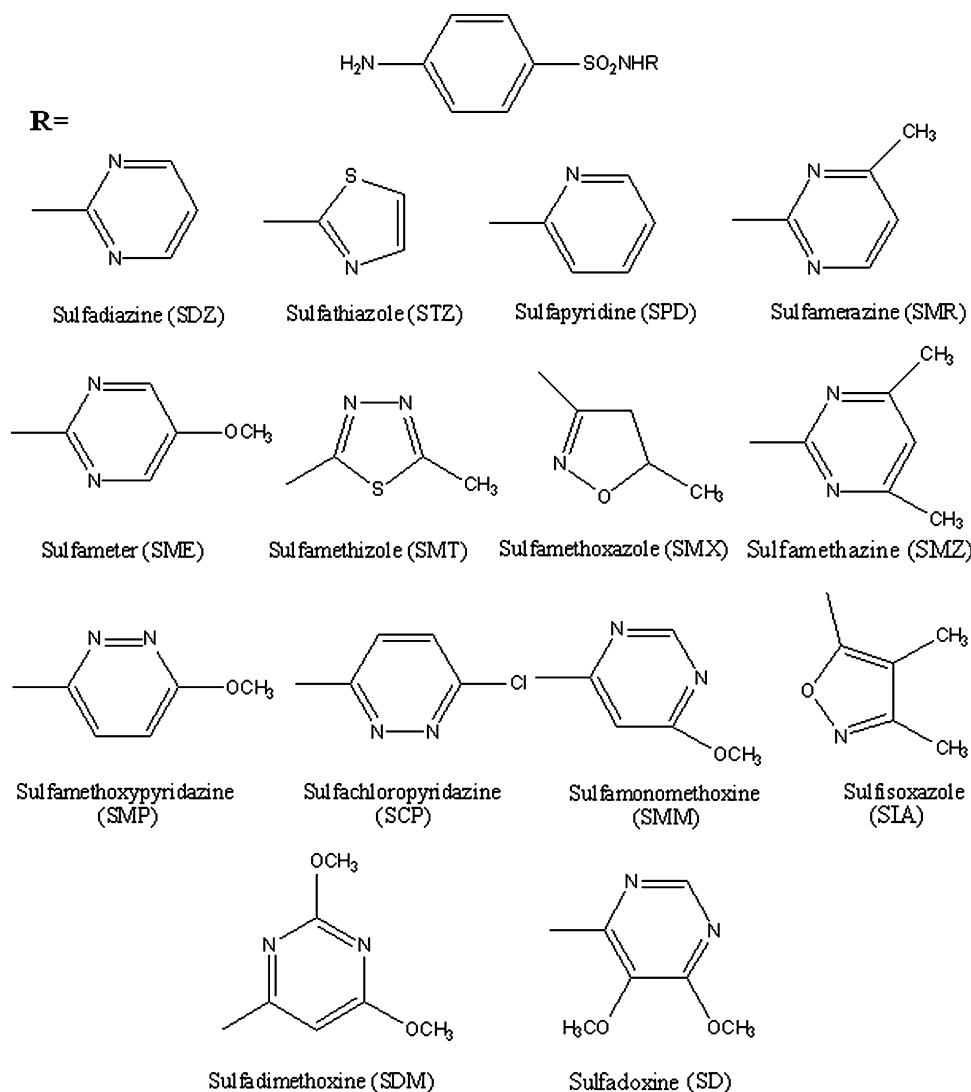


Fig. 1 – Molecular structures of selected sulfonamide antimicrobials.

years, HPLC with mass spectrometry (MS) detection [4,14–20] has been favored by many analysts due to their higher sensitivity and their ability to provide compound confirmation. Due to the complexity of biological samples, several pretreatment methods, such as solid-phase extraction (SPE) [4,12,20], liquid–liquid extraction (LLE) [7,8,15], and matrix solid-phase dispersion (MSPD) [6], have been applied for the extraction of SAs from milk and eggs samples before HPLC separations. However, these methods demand complex extraction and cleanup procedures, or large volumes of sample and organic solvent. To overcome those problems, some techniques such as ultrafiltration [10,11], MSPD using hot water as extractant [20] and on-line microdialysis [9] have been reported for extraction of SAs in biological matrixes.

Polymer monolith microextraction (PMME) is one kind of solid-phase microextraction, in which polymer monolith was used as sorbents [21–23]. The synthesis of PMME materials requires only one-step polymerization and a simple post-treatment procedure, and the porous structure and surface properties of the polymer are usually tunable [24]. Since this format of the extraction material could be regarded

as a multi-channel separation media that combined to a whole and will provide sufficient extraction phase to contact with sample solution, the extraction efficiency is expected to improve greatly compare with coated capillaries [22]. Another advantage is that convective mass transfer procedure in the monolithic column [25] is preferable during extraction procedure than the diffusive mass transfer in the conventional liquid phase coated capillaries, which will help to complete the extraction procedure within a shorter time. Based on poly(methacrylic acid-ethylene glycol dimethacrylate) (poly(MAA-EGDMA)) monolith, this technique has been coupled on-line or off-line with reversed-phase HPLC (RPLC) for the determination of several analytes in biological samples such as serum, urine, milk, egg, honey and pork [26–29]. The polymer monolith showed stability within the entire range of pH and exhibited excellent biocompatibility in dealing with biological samples [21]. Recently, Wen has developed a method for the monitoring of five SA residues in milk by on-line coupling PMME to RPLC with a UV detector [27]. The mobile phase was used as the desorption solvent of PMME. Due to the low concentration of organic solvent in mobile phase (30%

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