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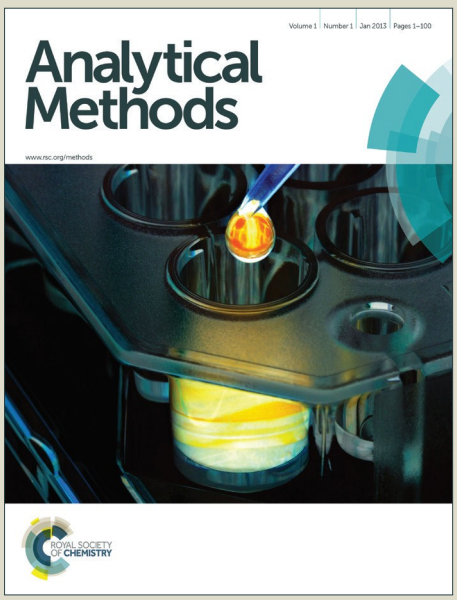

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ARTICLE TYPE

Extraction and determination of sulfadiazine and sulfathiazole in milk using magnetic solid phase extraction-HPLC-UV

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Abstract

Sulfonamides are heat stable antibacterial drugs and residues of them in milk, increase the risk of human exposure and may cause food-borne illness. In this study, Fe3O4 magnetic nanoparticles (Fe3O4 MNPs) have been successfully used for extraction of sulfadiazine (SDZ) and sulfathiazole (STZ), from milk samples. The effect of magnetic adsorbents amount, volume of extracted milk and sample pH were investigated and optimized. The experimental results showed that suggested method possessed suitable analytical performance. Linearity was obtained over a concentration range of 0.25, 0.5, 1.0, 2.0 and 4 MRL, with regression coefficients ranging from 0.9985 to 0.9978 for SDZ and STZ. The limits of detection (LOD) and quantification (LOQ), for two analytes, were 10 and 30 ng mL^{-1}, respectively. The proposed method was used to the analysis of different milk samples, and excellent recoveries in the range of 92.9 – 102.4% were obtained. These results indicated that the proposed method can be broadly used in monitoring of low concentrations of drugs in milk samples and open fascinating perspectives in future studies.

1. Introduction

Sulfonamides (SAs) are a large group of synthetic antibiotics which routinely used in veterinary medicine and have proven effective antimicrobial agents since their discovery after 1930s. SAs competitively inhibit conversion of p-aminobenzoic acid (PABA) to dihydropteroate, which bacteria need for folic acid synthesis[1]. Sulfadiazine (SDZ) and sulfathiazole (STZ) belong to the sulfonamide family and are daily consumed worldwide to control of animal illness. The widely used of SAs related to their Low cost, effectiveness as growth promoters of livestock, broad antibacterial spectrum, but an insufficient withdrawal period and unnecessary administration of SAs may result in their residues in milk. The presence of sulfonamide residues in milk, because of high consumption of milk and stability of SAs, is a great concern[2], also some of them induce adverse effects, such as allergic reactions or antibiotic resistance in humans[3-5]. Several reports have indicated the presence of some of these compounds in milk[6-8]. To ensure milk safety for the consumers, the European Union (EU) commission, Drug Administration (FDA) and Codex Alimentarius has established a Maximum Reside Limit (MRL) of 100 µg L^{-1} for the sum of all SAs in food of animal origin, such as milk [9, 10]. Use of a sensitive and reliable pre-concentration procedure to eliminate the matrix interference for determination of SAs in milk samples is vital. Liquid-liquid extraction, solid phase extraction (SPE) and recently, new methodologies, so-called Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) and dispersive liquid- liquid micro extraction, have been proposed for the extraction of veterinary drug residues[11]. Several methodologies have been reported for pre-concentration of SAs in milk samples[12, 13]. Traditional pre-treatment methods have some disadvantages such as require large amounts of toxic organic solvents and so relatively expensive, be tedious and time consuming[14]. To date, the new area of research in pre-treatment methods is focused on the novel adsorbents in solid phase extraction techniques. Nano-meter-sized adsorbents have attracted considerable interest owing to their special properties[15-17]. Magnetic solid-phase extraction (MSPE) is a new mode of SPE, that the packing of the adsorbent into the SPE cartridge is not require, but the adsorbent dispersed in sample solution and removed by appropriate magnetic field, thus the phase separation could conveniently realized and shows great advantages in separation science[12]. The adsorbent could be magnetic or magnetizable adsorbents. Magnetic nanoparticles (MNPs) as the adsorbents have been effectively used in pre-concentration and removal of some organic and inorganic compounds[18-26]. The MNPs are magnetic and are attracted to a magnetic field and enriched nanoparticles removed from a matrix by using a magnetic field. Magnetic solid phase extraction (MSPE) can be accomplished without the need of centrifuging or filtration, which causes separation simpler and faster [27]. In this study we measured the efficiency of the MNPs for extraction of SDZ and STZ from milk samples.

2. Experimental

2.1 Chemicals

Analytical standards (pestanal quality) of SDZ and STZ were purchased from Sigma–Aldrich (Germany). Iron oxide (II,III) magnetic nanoparticles was purchased from Sigma–Aldrich (Germany). The HPLC-grade acetonitrile, methanol, and all analytical grade extraction solvents were purchased from Merck (Darmstadt, Germany). Deionized water was prepared from a Milli-Q water purification system at 18.2 MΩ cm (Bedford, MA, USA). Individual 100.0 µg/mL stock standards of SDZ and STZ were prepared by dissolving of each SAs in Methanol. These solutions were stored at – 20 ° C. A 1.0 µg mL $^{-1}$ mixture of SDZ and STZ was prepared by appropriate dilution of stock standards solutions with deionized water.

2.2 Instrumentation

The chromatographic analyses were carried out using a Waters Breeze (Waters, Milford, MA, USA), HPLC system equipped with a binary HPLC pump (Waters 1525), Waters 717 plus auto-sampler and a Waters 2487 dual λ absorbance UV detector (Waters, Milford, MA, USA). The reverse phase column was a Waters Nova-pak® C-18, 150 mm ×3.9 mm ID, 4 µm particle size (Waters Milford, MA, USA). The mobile phase consisted of water/ acetonitrile (90:10 v/v) at a flow rate of 1.0 mL min^{-1}. The column was maintained at 40º C and the eluent was monitored at 270 nm[28].

2.4. Sample preparation

The milk samples obtained from retail stores on July 2014. Five millilitres of milk sample was placed into a 50 mL centrifuge tube, and 10.0 millilitres acetonitrile was added to the tube, the mixture was vigorously shaken for 3 min, and then centrifuged at 3500 rpm for 2 min. Two millilitres of supernatant was transferred to another tube and diluted with 8.0 mL deionized water. The diluted extract was used for MSPE procedure.

2.5. MSPE procedure

The 0.2 g Fe3O4 MNPs was added into 10.0 mL diluted extract and the mixture was ultrasonicated for 5 min. Then, the enriched MNPs were separated from solution by using a strong magnet and the supernatant was discarded. The target analytes were desorbed by 2.0 mL acetonitrile after shaking for 1 min (repeated for two times). The eluate was dried under nitrogen stream at 30 ºC. The residue was dissolved in 1.0 mL mobile phase, and 100 µL of this solution was injected to HPLC.

3. Results and discussion

3.1 Optimization of MSPE conditions

In order to obtain a high MSPE efficiency, the effect of different parameters such as amounts of adsorbent, volume of the diluted extract and pH of sample was investigated by spiking the blank milk sample with 100 µg of each SDZ and STZ per Kg of milk.

3.1.1 Amounts of adsorbent

MNPs offer a noticeable higher surface ratio as compared with the common sorbents (micro-sized). Thus, fewer amounts of MNPs are needed to obtain acceptable results. MNPs amounts were added in the range of 0.05-0.3 g. The results are shown in Fig. 1. When the amount of adsorbent was 0.2 g, the efficiency of extraction reached the maximum and after that it decreased. Based on the obtained results, 0.2 g MNPs was used as optimum amount.

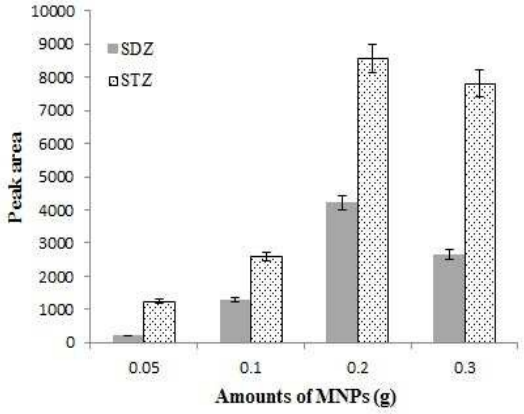

Fig.1 Effect of amounts of MNPs on the adsorption of SDZ and STZ.

3.1.2 Volume of the diluted extract

Milk is a complex biological matrix due to its high protein and fat contents, in which proteins are supposed to avoid analysis of target analytes. So, in this study we diluted the supernatant (milk extracted obtained with acetonitrile) to reduce the matrix effect. Different volumes of supernatant (1-3 mL) were diluted with deionized water to final volume of 10 ml. According to the obtained results (Fig. 2.) the best recovery was achieved by 2 mL of the milk extracted. When volume of the extracted was more than 2 mL, the adsorption of the target analytes decreased, which may be attributed to negative effects of matrix on MNPs.

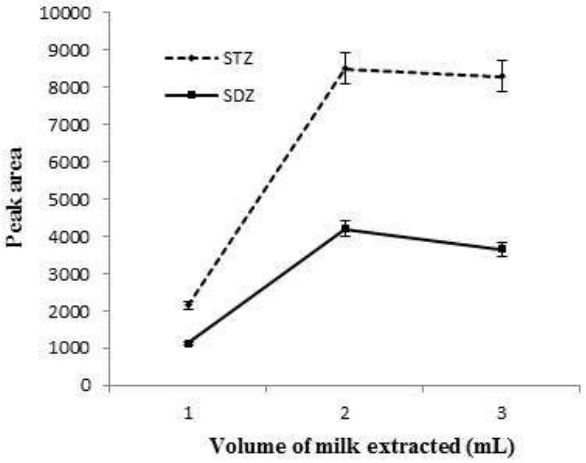

Fig. 2 Effect of volume of milk extracted on the adsorption of SDZ and STZ.

3.1.3 pH of the diluted extract

pH of the solution is one of the most important factors which can influence the extraction efficiency because of its effects on surface binding-sites of the adsorbent and aqueous chemistry. The pH values of the solutions were adjusted at 3.5, 7.0 and 10.5 by adding diluted hydrochloric acid and sodium hydroxide solution, respectively. The experimental data showed that there was a decrease in recovery at alkaline and acidic conditions; its

may be related to isoelectric point (pI) of Fe_3O_4 MNPs (pH 6.8)[29], Low extractions of analytes at pH of 3.5, may be due to the competition of proton with analytes ions for sorption on the MNPs surface and in the higher pH (pH 10.5), same phenomena could be happened for anionic compounds . So, further study was done at pH 7.0.

4. Method validation

4.1 Precision and accuracy

Relative standard deviations (RSDs %) for analysis of the target analytes was based on three-replicate. Precision was determined by with in day and between-day repeatability. The accuracy of the method was determined by spiking a blank milk sample at three levels (0.5, 1 and 2 MRL) and extracted by described MSPD method (Tab.1). The recoveries were achieved between 92.9% and 102.4%, which confirm the accuracy of the suggested method. The limits of detection (LOD) and quantification (LOQ), for two analytes, were obtained by progressively diluted standard solution to the peak height of analytes was 3 and 10 times of the back ground noise. Based on the signal-to-noise ratio of 3 and 10, the LODs and LOQs were 10 and 30 ng mL-1, respectively.

Table 1 relative recovery of SDZ and STZ in milk samples [a]

			Intra-day (n=3)	Inter-day (n=3)
Analyte	R^2	Spiking levels (ng mL^{-1})	Mean recovery ± RSD (%)	Mean recovery ± RSD (%)
SDZ	0.9989	50.0	102.4±1.61	93.4 ± 5.51
		100.0	98.3±2.55	92.4 ± 3.67
		200.0	92.9±8.06	88.2 ± 4.83
STZ	0.9979	50.0	98.7±2.65	91.4 ± 7.32
		100.0	99.2±3.04	97.2 ± 4.24
		200.0	97.3±1.11	95.8 ± 6.11

[a] Extraction conditions: the amount of MNPs: 0.02 g; the volume of milk extracted; 2.0 mL; extraction time; 5 min; desorption solvent: 4.0 mL acetonitrile.

4.2 Calibration curve

Calibration curves were obtained by spiking deionized water over a concentration range of 0.25 to 4 MRL, and following the procedure previously explained under the optimized conditions. Correlation coefficients were 0.9985 to 0.9978 for SDZ and STZ, respectively.

4.3 Analysis of cow milk samples

The validated method was applied for the quantitative determination of SAs residues in 10 milk samples obtained from retail store in July 2014. The whole milk samples were analyzed by MSPD. None of samples were contaminated to SAs.

5. Conclusions

In this study, the efficiency of SDZ and STZ extraction from milk samples by using Fe_3O_4 MNPs as adsorbent was evaluated. The results established that the method is suitable for the rapid extraction of target analytes from milk samples at ppb levels. Due to very high surface areas of these MNPs, suitable results can be obtained by using fewer amounts of MNPs adsorbents than micron-size sorbents. Also, magnetically assisted recoverably of MNPs resulted in a reduction of analysis time and easier practical applications. Thus, the method described denotes an appropriate procedure for controlling the quality of food and opens new ideas to monitor low concentrations of drugs in different matrix. The RSDs% indicated that this method have good repeatability and same as other research the RSDs% was lower than 15% [28]; the obtained recoveries confirm the accuracy of the validated method. In another report, two SPE cartridges were employed for cleanup and pre-concentration of SAs in meat extract before capillary electrophoresis detection[30], but in our research we used lower amounts of Fe_3O_4 MNPs (0.2 g) for cleanup and pre-concentration of SAs. Cavaliere et al , used SPE as a clean up method for SAs residues in milk, after loading, washing and eluting steps the recovery of their method was between 76 to 112%, but here we reports the better recoveries at 3 different levels of spiked sample with fast extraction step [31]. Gao et al , reported the good efficiency of magnetic particles for extraction of SAs in milk and in our research we find same results[12]. The validated method was successfully applied for the determination of SDZ and STZ, in milk samples, and contamination over the EU MRL was not detected.

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Notes and references

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