INTRODUCTION
The combination of sulfonamides and diaminopyrimidines (pentamidine sulfonamides) are widely used antibiotics in the veterinary practice in a number of animal species, including cows and goats. The existing EU Maximum Residue Limits (MRLs) for all substances belonging to the sulfonamide group and for TMP in milk are 100 μg/kg and 50 μg/kg, respectively (Commission Regulation (EU) No 37/2010).

Various analytical methods that can be employed in the analysis of sulfonamides have been published in a review (Wang et al., 2006). Papapanagiotou et al. (2005) reported the effect of heating treatments on sulfanilamide residues in cow milk. Another study (Roca et al., 2013) investigated the thermal stability of eight sulfonamides spiked in skimmed milk powder, by means of kinetic models and thermodynamic parameters, using a LC-MS/MS method and examining degradation percentages. Other studies (Davodi et al., 2011; Xu et al., 1996) have reported on the effect of cooking methods on sulfadiazine and trimethoprim residues in edible tissues of broiler chicken using microbiological methods and ormetoprim and sulfadimethoxine cooking effects in muscle of channel catfish, respectively. Rose et al. (1995) have reported on the effect of cooking on sulfanilamide.

The aims of the study were to develop a reliable and robust, fully in-house validated LC-UV method for the simultaneous quantification of SDZ and TMP in cow milk which could be used in heat treatment stability studies in cow and goat milk.

MATERIALS AND METHODS

Sample extraction and cleanup
One and a half (1½) % cow milk sample was accurately weighed in a 20 mL centrifuge tube. The sample was extracted with 10 mL of dichloromethane using a vortex mixer for 2 min at high speed. The sample was centrifuged at 4000 g for 3 min. Eight (8) mL of the organic phase were transferred to a 20 mL centrifuge tube and 3 mL of n-hexane and 1 mL of phosphoric acid (0.1M) were added. The mixture was vortexed for 1 min and following centrifugation at 4000 g for 3 minutes, the upper aqueous layer was transferred to another tube and finally 100 μL were used for high-performance liquid chromatography.

LC-UV analysis
The mobile phase contained 20% acetonitrile and 80% 0.005 M ortho-phosphoric acid (v/v). Following its preparation, the mobile phase was passed through 0.2 μm Nylon-66 filter (Anachem, Luton, UK) and degassed using helium. The mobile phase was delivered in the system at a rate of 1 mL/min. The stationary phase was Nucleosil 100-5 C18, 5-μm material in a Mackeray Nagel LC column 250 x 4.6 mm id. The stationary phase was kept equilibrated at 40°C. The injection volume was 100 μL. The UV detector was set at 275 nm with a sensitivity of 0.01 a.u.f.s. Under the established conditions, SDZ eluted at 5.6 min and TMP at 7.6 min.

Effect of heat treatments on SDZ and TMP
The concentrations of SDZ and TMP in standard samples in water were quite stable in all heat treatments examined in this study. This is important when viewed vis a vis the stability of both analytes after heating treatments of fortified milk samples (see below). Results concerning SDZ are in agreement with those by Rose et al. (1995) for sulfadimidine.

RESULTS AND DISCUSSION

Effect of heat treatments on water solutions of SDZ and TMP
Fortified milk samples with 412.8 ng/g SDZ and 1028.0 ng/g TMP were dispensed into capped glass centrifuge tubes, which were subjected to pasteurization (65°C and 72°C for 30, 45 and 60 min, and for 15 sec, 2 and 10 min, respectively), boiling (100°C for 2, 5 and 10 min) and sterilization (121°C for 2, 5 and 10 min) and spiking (121°C for 10, 15 and 20 min). Six replicates were carried out for all the mentioned treatments.

Effect of heat treatments on cow and goat milk samples fortified with SDZ and TMP
Fortified milk samples with 412.8 ng/g SDZ and 1028.0 ng/g TMP were dispensed into capped glass centrifuge tubes, which were subjected to pasteurization (65°C and 72°C for 30, 45 and 60 min, and for 15 sec, 2 and 10 min, respectively), boiling (100°C for 2, 5 and 10 min) and sterilization (121°C for 10, 15 and 20 min). Six replicates were performed for all the above-mentioned treatments.

Table 1. Effect of heating treatments on the stability of sulfadiazine (412.8 ng/g) and trimethoprim (1028.0 ng/g) in cow and goat milk

<table>
<thead>
<tr>
<th>Heating treatment</th>
<th>Temperature/time</th>
<th>Cow milk</th>
<th>Goat milk</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Concentration (%)</td>
<td>SDZ (ng/g)</td>
<td>SDZ</td>
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<td></td>
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<td>65°C/30 min</td>
<td>65°C/45 min</td>
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</table>

CONCLUSIONS
The results of the present study clearly indicate that SDZ residues in both types of fortified milk (cow and goat) samples are stable when exposed to temperature/time combinations equivalent to both Low Temperature Long Time (LTLT) and High Temperature Short Time (HTST) pasteurization.

REFERENCES


