INTRODUCTION

For residue analysis, milk samples should be transported to a testing laboratory and stored at low temperature (frozen or refrigerated) pending analysis. Therefore, the evaluation of the stability of penicillin residues under different storage conditions and the knowledge of the optimal storage and processing conditions of the milk samples are key issues for successful laboratory analysis. A literature survey shows that the data regarding the effect of storage conditions on the stability of penicillin G in milk are limited [1, 2], whereas there are no studies concerning penicillin V.

Information about the effect of thermal processing on the stability of penicillin G and penicillin V residues in milk is also important from a technological point of view. Although pasteurized milk production consumes pasteurization processes, relatively few studies [3, 4] have been reported concerning the effect of heating on penicillin G residues. On the contrary, most information on penicillin G and penicillin V residues in milk and regulatory considerations are directed toward raw milk.

The aim of this study was to use an accurate and precise LC method to produce quantitative data about the change in concentration of penicillin G and penicillin V residues in fortified and incurred cow milk, and in aqueous solutions during cold storage and common heating processing methods.

MATERIALS AND METHODS

Animals, drug administration, and sampling

Five healthy Holstein Friesian cows of 4-5 years of age were used in this study. Each cow was administered three successive intramammary infusions with a penicillin G formulation at the dose of 300,000 IU/24 h. The animals were milked twice daily (12 h intervals) and the milk of the 2nd and 3rd milking, after medication, of all five cows was mixed and stored frozen at -20°C pending processing. Control milk was collected two days prior to the intramammary infusions.

Stability of penicillins in aqueous solutions

Effect of pH: Standard solutions containing 1.98 μg/ml penicillin G and 2.08 μg/ml penicillin V were prepared in 0.02 M phosphate buffer solution, pH 2, 3, 4, 5, 6, 7, 8, 9, and 10. These solutions were stored at 25°C for 60 min and the concentration of the analytes in each tube (n=6) was measured after 10, 20, 30, 40, 50 and 60 min of storage.

Effect of storage temperature: Standard solutions containing 1.98 μg/ml penicillin G and 2.08 μg/ml penicillin V were prepared in water and were stored for 90 days at 25°C in the dark and in the light, and at 4°C, 28°C and -20°C in the dark. The concentration of the analytes in each tube (n=6) was measured after 3, 6, 9, 12, 15, 20, 30, 45, 60 and 90 days of storage.

Effect of thermal processing: Standard solutions containing 495 mg/l penicillin G and 520 mg/l penicillin V were prepared in water. These solutions were dispensed into capped glass centrifuge tubes (n=6), 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 autoclaving (120°C for 10, 15, and 20 min).

Stability of penicillin residues in milk samples

Effect of freezing temperature: For fortified (penicillin G and penicillin V) and incurred (penicillin G) milk samples were frozen in capped polypropylene centrifuge tubes at -28°C and -80°C for 90 days. The concentration of the analytes in each tube was measured after 15, 30, 45, 60 and 90 days of storage.

Effect of thermal processing: For fortified and incurred milk samples were dispensed into capped glass centrifuge tubes, which were subjected to pasteurization (65°C and 72°C for 30, 45, 60, and 15 sec, 2, and 10 min, respectively), boiling (100°C for 2, 5, and 10 min) and autoclaving (120°C for 10, 15, and 20 min).

Sample preparation and LC analysis

Analysis of the milk samples was performed according to the method reported by Fletouris et al. [5]. In brief, milk is acidified at pH 3 and extracted with dichloromethane. The extracted penicillins are partitioned into a phosphate buffer, pH 7, and following addition of ammonium sulfate, the extracts are purified by treatment with diethyl ether and repartitioned into acetonitrile. The acetonitrile extracts are concentrated into phosphate buffer, pH 7, and after addition of tetrabutylammonium hydrogen sulfate the formed penicillin ion pairs are extracted into chloroform. The chloroform is evaporated to dryness and the residue is dissolved in mobile-phase to be further injected (100 μl) into a Nucleosil 100-5 C18, 5 μm column and analysed using a mobile phase consisting of acetonitrile and 0.02 M phosphate buffer (38:62 v/v), containing 5 mM tetrabutylammonium hydrogen succinate and adjusted at pH 6.

RESULTS

Stability of penicillins in aqueous solutions

Effect of pH: The data collected in this study show that both penicillin G and penicillin V were stable at pH values from 7 to 10 when stored for 60 min at 25°C. Contrary to neutral and alkaline pH, acidic pH significantly affects the stability of both penicillins. As regards penicillin G, the reduction of the concentration begins at pH 5 and becomes more pronounced with decreasing pH. When the pH of the aqueous solution of penicillin G was adjusted at 2, only 60 min were required for a 90% reduction of its concentration. Aqueous solutions of Penicillin V exhibit remarkable stability for 1 hour at pH 3 to 6, while at pH 2 a 20% reduction of its concentration was observed after storing the solution for 8 hours at 25°C.

Effect of storage temperature: Storage of aqueous solutions at 25 and 4°C significantly affects the stability of both penicillins (Table 1). As far as the stability of these penicillins to freezing is concerned, the results showed that 3 months of storage at -28 and -80°C did not produce any change on the concentration of both analytes in aqueous solutions.

Effect of thermal processing: The results concerning the stability of penicillin G and Penicillin V in aqueous solutions during common heating processing methods are presented in Table 1. The results show that both penicillins are resistant to heating treatments analogous to milk pasteurization, but boiling and sterilization significantly affects the concentration of penicillin G and penicillin V in aqueous solutions.

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>Concentration change (% ± SD) (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light (25°C)</td>
<td>Dark (25°C)</td>
</tr>
<tr>
<td>Penicillin G</td>
<td>Penicillin V</td>
</tr>
<tr>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>3</td>
<td>-14.8 ± 2.0</td>
</tr>
<tr>
<td>15</td>
<td>-98.1 ± 2.1</td>
</tr>
<tr>
<td>30</td>
<td>-100.0</td>
</tr>
<tr>
<td>60</td>
<td>-100.0</td>
</tr>
<tr>
<td>90</td>
<td>-100.0</td>
</tr>
</tbody>
</table>

Effect of freezing temperature: The results showed that penicillin G and penicillin V residues were stable for at least 90 days storage at -28°C and -80°C.

Effect of thermal processing: The results concerning the stability of penicillins residues in fortified cow milk samples during common heating processing methods are presented in Table 2. The results show that both penicillins are resistant to common pasteurization treatments of milk, but boiling and sterilization significantly affects the concentration of penicillin G and penicillin V in milk. Analogous reduction of the concentration of penicillin G residues in incurred cow milk samples was observed when these samples were subjected to boiling and sterilization conditions.

Stability of penicillin residues in milk samples

Effect of freezing temperature: The results showed that penicillin G and penicillin V residues were stable for at least 90 days storage at -28°C and -80°C.

Effect of thermal processing: The results concerning the stability of penicillin residues in fortified cow milk samples during common heating processing methods are presented in Table 2. The results show that both penicillins are resistant to common pasteurization treatments of milk, but boiling and sterilization significantly affects the concentration of penicillin G and penicillin V in milk. Analogous reduction of the concentration of penicillin G residues in incurred cow milk samples was observed when these samples were subjected to boiling and sterilization conditions.

CONCLUSIONS

The results of the present study clearly indicate that penicillin G, contrary to penicillin V, is very sensitive at acidic conditions. The aqueous solutions of both penicillins can be stored for at least 6 days at 4°C, whereas they can be stored with spliced and incurred cow milk samples for at least 3 months at -28°C and -80°C. Moreover, the pasteurisation of aqueous solutions and spliced and incurred cow milk samples does not affect penicillin concentration, but boiling and sterilization significantly reduce their concentration. The data presented in this study could help to improve laboratory analysis of penicillin G and penicillin V and protect public health.

REFERENCES