INTRODUCTION

One of the most widespread and lethal disease that is affecting honey bees is the American Foulbrood (AFB) caused by the spore-forming bacteria Paenibacillus larvae spp. larvae. Traditionally this disease has been treated with antibiotics as sodium sulfathiazole or oxytetracycline, but the possible presence of their residues in honey has conducted to the prohibition of their use in several countries. Nowadays, other antibacterial agents, mainly macrolides, are being tested to this purpose. One of them is tylosin.

The tylosin series of macrolides is composed of several related substances of which tylosin A (TA) is the major constituent. Other minor components include tylosin B or desmycosin (TB), tylosin C or macrocin (TC) and tylosin D or relomycin (Figure 1). The four of them contributes to the potency of tylosin, and all of them can be found in the technical product. The use of tylosin is only authorized under veterinary prescription in Europe, it can be used in EEUU since March of 2006.

In this work, a method to determine residues of antibacterial tylosins A, B, C y D in bee larvae by LC-ESI-MS is presented. The proposed method employs a simple solid-liquid extraction procedure for the isolation of tylosins from bee larvae. And finally, the method has been applied to the analysis of tylosin residues in bee larvae from veterinarian treated beehives, fed with the technical product which contains the four compounds.

TYLOSIN A, B, C AND D

The developed method was applied on a first trial to the analysis of TA, TB, TC and TD in bee larvae samples obtained from different beehives in the CAR of Marchamalo. In this purpose 15 beehives homogenous and with similar age were selected and divided into three groups:

- 5 were fed with placebo
- 6 were fed with a sugar mixture containing 200 mg Kg-1 of Tylosin TP (1 and 3)
- 6 were fed with a sugar mixture containing 400 mg Kg-1 of Tylosin TP (2 and 4)

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After the total consumption of the food and waiting for a month, bee larvae were collected from the treated beehives. The results are shown below (mg Kg-1):

<table>
<thead>
<tr>
<th>Compound</th>
<th>TA</th>
<th>TB</th>
<th>TC</th>
<th>TD</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>1</td>
<td>1.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>2</td>
<td>1.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>3</td>
<td>1.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>4</td>
<td>1.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

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LC CONDITIONS

- Column: Luna 5 μm, C18 100 A (150 x 4.60 mm I.d)
- Gradient elution programme:
  - Mobile phase flow-rate: 0.8 mL/min
  - Temperature: 25ºC
  - Injection volume: 90 μL
  - Wavelength: 280 nm
- Internal standard (I.S.): Roxithromycin

Extraction Procedure

- They were grinded in a mortar
- They were dried at 60ºC for 4 hours
- They were weighed 100 mg of bee larvae and the chitin was extracted with 2 mL MeOH
- They were centrifugated and the supernatant was collected after it was diluted in a ratio 1:2 with ultrapure water
- After filtration, the solution was injected

Analysis of real samples

As it has been proved, residues of TB, TC and TD can appear in bee larvae, so it will be necessary to measure and study all of them, not only TA, because it could lead to an error of 50% in the quantification of the presence of tylosin residues if only TA is considered.

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