Determination of Tetracycline Antibiotics Residues in Chicken Muscle by Liquid Chromatography-Tandem Mass Spectrometry

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Abstract A sensitive and selective method is presented for simultaneous determination of tetracycline antibiotics TCs veterinary drugs. Oxytetracycline OTC tetracyclines TC and chlorotetracycline CTC in the chicken muscle were extracted and then solid-phase cleaned-up on a C18 reversed-phase column to obtain an extract suitable for analysis by liquid chromatography-tandem mass spectrometry LC-MS/MS. Electrospray ionization was applied and operated in the positive ion mode. The calibration curves were good linear between the peak areas and the mass concentrations of TCs from 25 to 500 μg/L with the correlation coefficient more than 0.99. The average recoveries from spiked chicken muscle at the three concentrations of 50 μg/kg 100 and 200 μg/kg were from 72.4% to 94.9% with relative standard deviation less than 11%. The detection limits of TCs were 10 μg/kg. The method was successfully validated for chicken muscle in compliance with the requirements set by Document No. 1 of 2003 dispatched by the Bureau of Animal Husbandary of Ministry of Agriculture. This method is suitable for the determination of OTC and CTC in chicken muscle.

Keywords liquid chromatography-tandem mass spectrometry LC-MS/MS oxytetracycline tetracyclines chlorotetracycline chicken muscle
1.1 Waters 2690 HPLC-micromass Quattro micro™

1.2 Waters Symmetry C_{18} 5 \mu m 2.1 mm i. d. \times 150 mm

1.4 TCSs \( \frac{1}{10} \) 10 000 r/min \( \frac{1}{10} \) 1 min

10.9 g 37.2 g 1 000 mL 12.9 g 10 mL

MCI \( \frac{1}{10} \) 10 000 r/min \( \frac{1}{10} \) 10 min \( \frac{1}{10} \) 5 mL

C_{18} 3 mL 2 mL 2 mL 2 mL

LC-MS/MS

1

2

2.1 1.4

OTC/TC/CTC

Fig. 1 Chromatograms of a blank sample spiked with 100 \mu g/kg of TCSs

1 100 \mu g/kg TCSs

N 7: 3 0.5 mL

2

2.3 1 mg/L 20 \mu L 2 g

10 \mu g/kg

2.5 mg/L 20 \mu L 2 g

10 \mu g/kg
2.4

The recovery of TCs in chicken muscle was assessed at concentrations of 50, 100, and 200 μg/kg. The results are shown in Table 1.

<table>
<thead>
<tr>
<th>Spiked/TCs/μg/kg</th>
<th>OTC recovery/ %</th>
<th>RSD/ %</th>
<th>TC recovery/ %</th>
<th>RSD/ %</th>
<th>CTC recovery/ %</th>
<th>RSD/ %</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>84.0</td>
<td>9.0</td>
<td>87.1</td>
<td>11</td>
<td>70.8</td>
<td>9.5</td>
</tr>
<tr>
<td>100</td>
<td>94.9</td>
<td>5.3</td>
<td>76.6</td>
<td>8.8</td>
<td>72.4</td>
<td>6.4</td>
</tr>
<tr>
<td>200</td>
<td>80.6</td>
<td>6.8</td>
<td>88.4</td>
<td>7.5</td>
<td>78.9</td>
<td>9.5</td>
</tr>
</tbody>
</table>

2.5 TCS

Table 1: Recoveries of TCs in chicken muscle (n = 6)

Table 2: ESI-MS/MS analysis conditions of tetracycline antibiotics

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Parent ion [m/z]</th>
<th>Daughter ion [m/z]</th>
<th>Cone voltage/V</th>
<th>Collision energy/eV</th>
</tr>
</thead>
<tbody>
<tr>
<td>OTC</td>
<td>461</td>
<td>426</td>
<td>25</td>
<td>20</td>
</tr>
<tr>
<td>TC</td>
<td>445</td>
<td>410</td>
<td>26</td>
<td>18</td>
</tr>
<tr>
<td>CTC</td>
<td>479</td>
<td>444</td>
<td>23</td>
<td>23</td>
</tr>
</tbody>
</table>

Fig. 2: Structures of tetracycline antibiotics


3.1 TCS

- M + H⁺.
- M + H – NH₃ – H₂O⁺.
- M + H – NH₃ – H₂O⁺.
- M + H – NH₃ – H₂O⁺.

References: