ABSTRACT

A liquid chromatography/tandem mass spectrometry (LC-MS/MS) multivariate method for the simultaneous quantification of tetracycline derivatives in pork and chicken muscle is described. The method was validated according to the European requirements for veterinary drug residues and all analytical parameters were found to be in conformance. The selected method is also validated according to the European requirements for veterinary drug residues and all analytical parameters were found to be in conformance. The selected method is also validated according to the European requirements for veterinary drug residues and all analytical parameters were found to be in conformance.

Methods

1. Chemicals and Materials

Tetracycline (Tc), oxytetracycline (OTC), and chlortetracycline (CTC) were obtained from Sigma-Aldrich (United States). LC grade acetonitrile and methanol, reagent grade ammonium acetate, disodium hydrogen phosphate, and orthophosphoric acid were purchased from Acros Organics (Geel, Belgium). Hydrogen Phosphate Solution.

2. Standards and Solutions

Tetracycline hydrochloride standard solution (1000 μg/mL) was prepared in methanol and was stored at -20 °C. Oxytetracycline disodium salt (Na2EDTA) in 1625 mL McIlvaine Buffer, and sonicated 5 min in an ultrasonication bath. The tubes were centrifuged 5 min at 1000 rpm and supernatants were transferred to a clean centrifuge tube. Two more extractions were effectuated with 20 mL and 10 mL of pH 4.0 Na2EDTA McIlvaine buffer solution, and then they were sonicated 5 min in an ultrasonication bath. The tubes were centrifuged 5 min at 1000 rpm and supernatants were transferred to a clean centrifuge tube.

3. Sample Cleanup with Manual and Automatic SPE method

The HPLC system was Agilent 1100 system with Agela Durashell C18 (5 µm, 100 Å, 2.1x150 mm) to carry out gradient elution analysis. The mobile phases used for mass spectrometric analysis were a mixture of 0.3% formic acid in water, and the phase B-0.3% formic acid in acetonitrile. The gradient elution condition was as follows: Phase-A: 0.3% formic acid in water; and the phase B: 0.3% formic acid in acetonitrile. The gradient elution condition was as follows: Phase-A: 0.3% formic acid in water; and the phase B: 0.3% formic acid in acetonitrile.

4. Analytical Procedure

The eluates were evaporated to dryness under nitrogen stream on a 45 °C and were reconstituted with methanol-water solution (3:7, v/v). The final eluates were filtered using 0.45 µm nylon membrane for tested by LC-MS/MS.

5. Apparatus and Operation Conditions

AB Sciex PXQ® 4500 tandem mass spectrometer with electrospray ionization in positive ion mode was used. The ion source was set by tuning multiple ions. The method was intended to be used in multiple laboratories. The eluates were evaporated to dryness under nitrogen stream on a 45 °C and were reconstituted with methanol-water solution (3:7, v/v). The final eluates were filtered using 0.45 µm nylon membrane for tested by LC-MS/MS.

6. Analytical Procedure

The eluates were evaporated to dryness under nitrogen stream on a 45 °C and were reconstituted with methanol-water solution (3:7, v/v). The final eluates were filtered using 0.45 µm nylon membrane for tested by LC-MS/MS.

7. Sample Cleanup with Manual and Automatic SPE method

The HPLC system was Agilent 1100 system with Agela Durashell C18 (5 µm, 100 Å, 2.1x150 mm) to carry out gradient elution analysis. The mobile phases used for mass spectrometric analysis were a mixture of 0.3% formic acid in water, and the phase B-0.3% formic acid in acetonitrile. The gradient elution condition was as follows: Phase-A: 0.3% formic acid in water; and the phase B: 0.3% formic acid in acetonitrile.

8. Results and Discussion

The HPLC system was Agilent 1100 system with Agela Durashell C18 (5 µm, 100 Å, 2.1x150 mm) to carry out gradient elution analysis. The mobile phases used for mass spectrometric analysis were a mixture of 0.3% formic acid in water, and the phase B-0.3% formic acid in acetonitrile. The gradient elution condition was as follows: Phase-A: 0.3% formic acid in water; and the phase B: 0.3% formic acid in acetonitrile. The gradient elution condition was as follows: Phase-A: 0.3% formic acid in water; and the phase B: 0.3% formic acid in acetonitrile.

9. CONCLUSIONS

In conclusion, compared to the two liquid-phase extraction techniques, the automated SPE cleanup methods outlined above with Cleanup RfP 7-column and Durashell C18 chromatographic column are convenient to obtain better recovery for the determination of tetracycline antibiotics residues in pork and chicken meat. Quantification at 10 μg/kg for samples by LC-MS/MS detection was readily achieved. The method by SPE-LC-MS/MS has been successfully applied to the detection of Tc, OTC, and CTC in various samples, including pork and chicken meat, and its applications in the field of food safety and environmental monitoring, clinical testing and forensic analysis.

Figure 1. LC-MS/MS chromatogram of blank sample

Figure 2. LC-MS/MS chromatogram of spiked (10 μg/kg) sample

Table 1. Extractable tetracycline derivatives in meat

<table>
<thead>
<tr>
<th>Tetracycline Derivative</th>
<th>Concentration (μg/kg)</th>
<th>Recovery (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetracycline (Tc)</td>
<td>7.95</td>
<td>81.64</td>
<td>97.23</td>
</tr>
<tr>
<td>Oxytetracycline (OTC)</td>
<td>11.86</td>
<td>80.8</td>
<td>97.27</td>
</tr>
<tr>
<td>Chlortetracycline (CTC)</td>
<td>76</td>
<td>76.0</td>
<td>81.26</td>
</tr>
</tbody>
</table>

Table 2. Detection of tetracycline antibiotics in meat samples by SPE-LC/MS/MS

<table>
<thead>
<tr>
<th>Tetracycline Derivative</th>
<th>Concentration (μg/kg)</th>
<th>Recovery (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetracycline (Tc)</td>
<td>7.95</td>
<td>81.64</td>
<td>97.23</td>
</tr>
<tr>
<td>Oxytetracycline (OTC)</td>
<td>11.86</td>
<td>80.8</td>
<td>97.27</td>
</tr>
<tr>
<td>Chlortetracycline (CTC)</td>
<td>76</td>
<td>76.0</td>
<td>81.26</td>
</tr>
</tbody>
</table>

Notes: M=micellar SPE method. A=automatic SPE method. R=manual SPE method. 1=All the samples spiked with standards solutions (150 μg/kg) of the Tcs. 2=The samples spiked with standards solutions (50 μg/kg) of the Tcs. 3=The samples spiked with standards solutions (10 μg/kg) of the Tcs. 4=The samples spiked with standards solutions (5 μg/kg) of the Tcs.