DEVELOPMENT OF A RAPID AND SENSITIVE LC-MS/MS METHOD FOR THE IDENTIFICATION AND QUANTITATION OF CHLORAMPHENICOL IN SEAFOOD SAMPLES

Yasmine Govaert1, Amaya Janosi1, Peter Batjoens1, Jean-Marie Degroodt1, Nico Van Eeckhout2, Bart de Craene2, Karine Clauwaert2 and Jan Claereboudt2

1IPH-LP, Juliette Wytsmansstraat 14, B-1050 Brussels, Belgium. 2Micromass BV, Bedrijvencentrum Vilvoorde, Mechelsesteenweg 277 Box 9, B-1800 Vilvoorde, Belgium

INTRODUCTION

The application of veterinary drugs in aquaculture can lead to residue problems, which require the development of suitable and fast analytical methods. This particular problem was demonstrated in 2001 in the Netherlands and Belgium where shrimps contaminated with chloramphenicol were imported from the Far East and became part of a larger consignment of animal feed delivered to firms in Germany, Austria, Denmark, Poland and Romania.

In order to allow a faster control of these seafood samples we developed in this study a rapid and sensitive LC-MS/MS method for the identification and quantitation of chloramphenicol in shrimps using liquid chromatography coupled to a new compact triple quadrupole MS/MS system.

EXPERIMENTAL CONDITIONS

Sample preparation

The extraction of chloramphenicol from seafood products was performed according to a previously described extraction procedure (J.M.Degroodt, B. Wykowski de Bukanski, J. De Groof, H. Beernaert and S. Srebrnik, J. Liquid Chrom., 15,13, 2355-2371, 1992). Briefly 10 gr of shrimp tissue (to which the internal standard, chloramphenicol-d5, was added at a concentration of 1 ppb) was vortexed with 12 ml of ethyl acetate and centrifuged. The organic phase was evaporated to dryness and redissolved in petroleum ether: ammonium acetate (7:1, v/v). The mixture was vortexed and centrifuged again. Three ml of pentane was added to the aqueous phase. After vortex and centrifugation, 2 ml of ethyl acetate was added to the aqueous phase. The ethyl acetate phase was evaporated to dryness and dissolved in mobile phase.

LC conditions

- HPLC system: Waters Alliance 2695
- Column: Alltech ALTIma C18 (3.2 x 150 mm, 5 μm)
- Mobile phase: water:methanol (45:55, v/v)
- Flow rate: 400 μl/min
- Injection volume: 10 μl
- AutoDivert Valve: The Rheodyne valve on the front panel of the mass spectrometer was programmed to divert the HPLC effluent during the first 2 minutes and the last minute of the run to the waste.

MS conditions

- Mass spectrometer: Micromass Quattro Micro (Figure 1)
- Ionisation mode: ES negative ion
- Capillary voltage: 3.5 kV
- MS/MS: Argon at 3.3 x 10^{-3} mbar as collision gas
RESULTS AND DISCUSSION

Chloramphenicol-d₅ was used as internal standard for quantification purposes. Table 1 summarises the multiple reaction monitoring (MRM) transitions and conditions used in the analysis of chloramphenicol and its deuterated analogue.

Table 1  Precursor and product ions of chloramphenicol obtained under optimal ESI (-) MS/MS conditions

<table>
<thead>
<tr>
<th>Compound</th>
<th>Precursor ion (m/z)</th>
<th>Product ion 1 (m/z)</th>
<th>Cone voltage (V)</th>
<th>Collision Energy (eV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAP</td>
<td>320.60</td>
<td>152.20</td>
<td>28</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>256.90</td>
<td>28</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>194.15</td>
<td>24</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>CAP-D₅</td>
<td>325.65</td>
<td>157.25</td>
<td>28</td>
<td>18</td>
</tr>
</tbody>
</table>

Based on the response for the smallest ion at the 0.1 ppb level, an estimated LOD of less than 0.05 ppb could be realistic.

For the evaluation of the linearity, blank shrimp extracts were fortified with known concentrations (0 - 0.1 - 0.5 - 1 - 5 and 10 ppb) of chloramphenicol. The internal standard was added to each sample in a concentration of 1 ppb. Each concentration level was injected three times. The calibration curve was generated by means of QuanLynx software. The typical linearity of response is demonstrated in Figure 3 (the three different MRM transitions were used to produce a separate calibration curve, each of the individual MRM transitions showed a linear response in the investigated concentration area and can be used for quantitation.

Figure 2 shows the LC-MS-MS chromatograms obtained for the analysis of shrimp sample fortified at 0.1 ppb with chloramphenicol. The internal standard was in a concentration of 1 ppb.
Both fortified shrimp samples at 0.1 and 0.5 ppb were injected five times. The accuracy of the method was evaluated by comparing the mean of the measured concentrations with the theoretical concentration added to the samples. The results are presented in Table 2.

### Table 2  Accuracy of the developed LC-MS/MS method for the determination of chloramphenicol in shrimps

The precision of the injection was also evaluated by calculating the relative standard deviation (%RSD) of the ratio (area chloramphenicol / area internal standard) for all the three transitions. The results are presented in Table 3.

### Table 3  Precision of the injection

The method was then tested on some real life shrimp samples issued from the chloramphenicol crisis. One sample was found negative while two samples were found contaminated with chloramphenicol at 0.43 and 0.80 ppb. The chloramphenicol was clearly identified in these samples (three different MRM transitions were monitored, the intensity ratios of the different transitions were calculated) according to the EU recommendations (only two MRM transitions need to be monitored). Figure 4 shows the LC-MS-MS chromatograms obtained for the real sample at 0.43 ppb.
CONCLUSION

A rapid and sensitive method for the identification and quantitation of chloramphenicol using liquid chromatography with a new compact mass spectrometry (LC-MS/MS) system was developed and was successfully applied on real life shrimp samples.

The authors thank H. Kayirebe for her technical assistance in the preparation of the samples.

ACKNOWLEDGEMENTS

The Belgian Veterinary Inspection is acknowledged for the financial support.
Author to whom all correspondence should be addressed:

KARINE CLAUWAERT
MICROMASS BV,
BEDRIJVENCENTRUM VILVOORDE,
MECHELESESTEENWEG 277 BOX 9, B-1800 VILVOORDE, BELGIUM
TEL: + 32 (0)2 253 45 50
FAX: + 32 (0)2 253 45 55
e-mail: karine.clauwaert@micromass.net