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# CHLORAMPHENICOL CRISIS IN BENELUX: LC-MS/MS OFFERS THE ANALYTICAL SOLUTION !

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### Presented at Spring Meeting Dutch Society of Mass Spectrometry, Wageningen, Netherlands, 13th-17th May, 2002

### 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 have 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. Wyhowski 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-d<sub>5</sub>, 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<sup>-3</sup> mbar as collision gas



Figure 1.



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### **Results and Discussion**

Chloramphenicol- $d_5$  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.

Compound	Precursor ion (m/z)	Product ion (m/z)	Cone voltage (V)	Collision Energy (eV)
CAP	320.60	152.20	28	18
		256.90	28	11
		194.15	28	13
CAP-D5	325.65	157.25	28	18

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

**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.





Based on the response for the smallest ion at the 0.1 ppb level, an estimated LOD of about 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**.





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**.

Injection	Conc added (ppb)	Conc Found (ppb)	Injection	Conc added (ppb)	Conc Found (ppb)
1	0.1	0.098	1	0.5	0.471
2	0.1	0.091	2	0.5	0.474
3	0.1	0.095	3	0.5	0.475
4	0.1	0.091	4	0.5	0.502
5	0.1	0.092	5	0.5	0.490
	Avg	0.093		Avg	0.482
	St Dev	0.003		St Dev	0.013
	% RSD	3.265		% RSD	2.737
	% Accuracy	-7		% Accuracy	-4

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

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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**.

	040.05	1	040		1		
	CAP - D5		CAP			CAP/CAP-D5	
Injection	326 > 157	321 > 257	321 > 194	321 > 152	321 > 257	321 > 194	321 > 152
1	3021	795	373	1462	0.2632	0.1235	0.4839
2	2886	791	333	1395	0.2741	0.1154	0.4834
3	2802	822	342	1422	0.2934	0.1221	0.5075
4	2776	769	315	1380	0.2770	0.1135	0.4971
5	2587	773	317	1305	0.2988	0.1225	0.5044
	i.			AVG	0.281	0.119	0.495
				SD	0.015	0.005	0.011
				% RSD	5.187	3.854	2.273
			0.1 ppb s	pike level			
	CAP - D5	I	0.1 ppb s CAP	pike level	I	CAP / CAP-D5	
Injection	CAP - D5 326 > 157	321 > 257	0.1 ppb s CAP 321 > 194	pike level 321 > 152	321 > 257	CAP / CAP-D5 321 > 194	321 > 152
Injection	CAP - D5 326 > 157	321 > 257	0.1 ppb s CAP 321 > 194	pike level 321 > 152	321 > 257	CAP / CAP-D5 321 > 194	321 > 152
Injection	CAP - D5 326 > 157 2977	321 > 257 214	0.1 ppb s CAP 321 > 194 71	pike level 321 > 152 327	321 > 257 0.0719	CAP / CAP-D5 321 > 194 0.0238	321 > 152 0.1098
Injection 1 2	CAP - D5 326 > 157 2977 2859	321 > 257 214 172	0.1 pph s CAP 321 > 194 71 81	<u>321 &gt; 152</u> 327 313	321 > 257 0.0719 0.0602	CAP / CAP-D5 321 > 194 0.0238 0.0283	321 > 152 0.1098 0.1095
Injection 1 2 3	CAP - D5 326 > 157 2977 2859 2786	321 > 257 214 172 197	0.1 ppb.s CAP 321 > 194 71 81 69	pike level 321 > 152 327 313 325	321 > 257 0.0719 0.0602 0.0707	CAP / CAP-D5 321 > 194 0.0238 0.0283 0.0248	321 > 152 0.1098 0.1095 0.1167
Injection 1 2 3 4	CAP - D5 326 > 157 2977 2859 2786 2811	321 > 257 214 172 197 162	0.1 ppb s CAP 321 > 194 71 81 69 67	pike level 321 > 152 327 313 325 301	321 > 257 0.0719 0.0602 0.0707 0.0576	CAP / CAP-D5 321 > 194 0.0238 0.0283 0.0248 0.0238	321 > 152 0.1098 0.1095 0.1167 0.1071
Injection 1 2 3 4 5	CAP - D5 326 > 157 2977 2859 2786 2811 2802	321 > 257 214 172 197 162 176	0.1 pph s CAP 321 > 194 71 81 69 67 76	pike level 321 > 152 327 313 325 301 308	321 > 257 0.0719 0.0602 0.0707 0.0576 0.0628	CAP / CAP-D5 321 > 194 0.0238 0.0248 0.0248 0.0238 0.0271	321 > 152 0.1098 0.1095 0.1167 0.1071 0.1099
Injection 1 2 3 4 5	CAP - D5 326 > 157 2977 2859 2786 2811 2802	321 > 257 214 172 197 162 176	0.1 ppb s CAP 321 > 194 71 81 69 67 76	121 > 152 321 > 152 327 313 325 301 308 AVG	321 > 257 0.0719 0.0602 0.0707 0.0576 0.0628 0.065	CAP / CAP-D5 321 > 194 0.0238 0.0283 0.0248 0.0238 0.0271 0.026	321 > 152 0.1098 0.1095 0.1167 0.1071 0.1099 0.111
Injection 1 2 3 4 5	CAP - D5 326 > 157 2977 2859 2786 2811 2802	321 > 257 214 172 197 162 176	0.1 ppb s CAP 321 > 194 71 81 69 67 76	pike level 321 > 152 327 313 325 301 308 AVG SD	321 > 257 0.0719 0.0602 0.0707 0.0576 0.0628 0.065 0.006	CAP / CAP-D5 321 > 194 0.0238 0.0248 0.0238 0.0248 0.0238 0.0271 0.026 0.002	321 > 152 0.1098 0.1095 0.1167 0.1071 0.1099 0.111 0.004

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 according to the EU recommendations. **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.



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