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

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Presented at 4th INT. SYMPOSIUM ON HORMONE AND VETERINARY DRUG RESIDUE ANALYSIS, Antwerp, 4th-7th June, 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 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_5, 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

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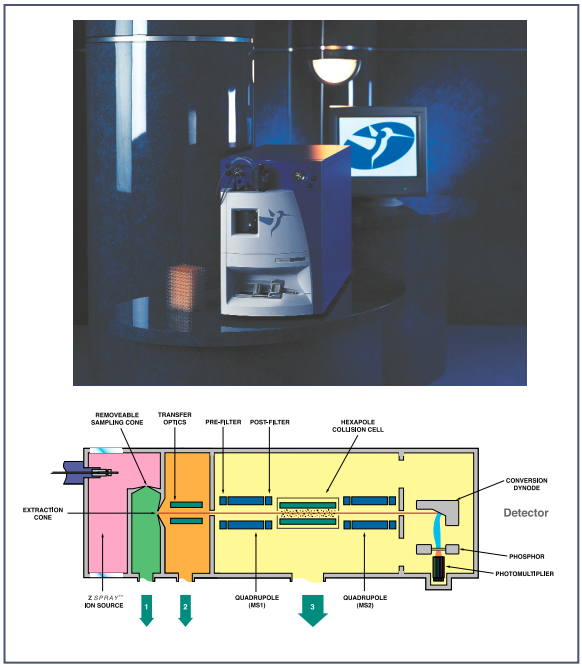

Figure 1.

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.

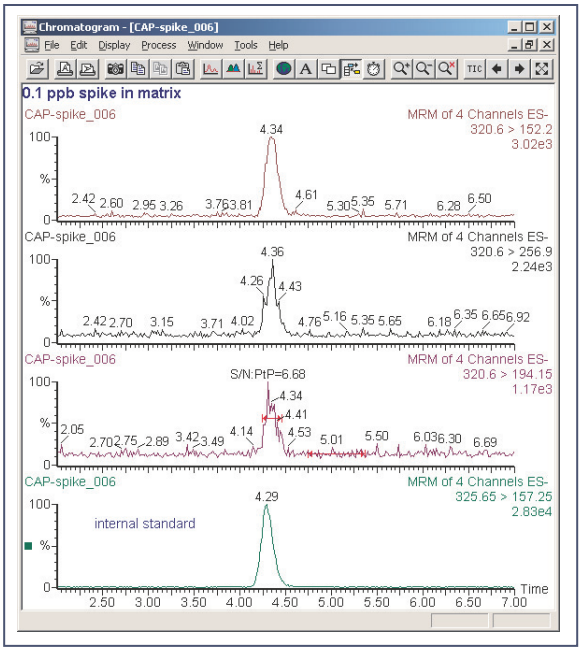

Figure 2.

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 reponse in the investigated concentration area and can be used for quantitation.

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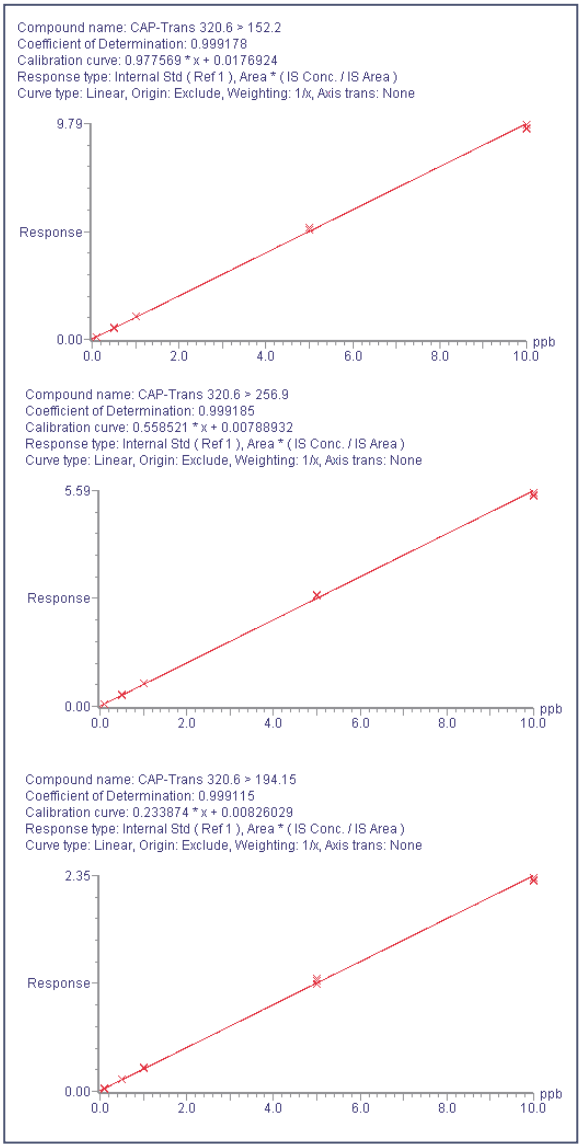

Figure 3.

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

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**.

0.5 ppb spike level

Injection	CAP - D5	CAP			CAP / CAP-D5		
	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
				AVG	0.281	0.119	0.495
				SD	0.015	0.005	0.011
				% RSD	5.187	3.854	2.273

0.1 ppb spike level

Injection	CAP - D5	CAP			CAP / CAP-D5		
	326 > 157	321 > 257	321 > 194	321 > 152	321 > 257	321 > 194	321 > 152
1	2977	214	71	327	0.0719	0.0238	0.1098
2	2859	172	81	313	0.0602	0.0283	0.1095
3	2786	197	69	325	0.0707	0.0248	0.1167
4	2811	162	67	301	0.0576	0.0238	0.1071
5	2802	176	76	308	0.0628	0.0271	0.1099
				AVG	0.065	0.026	0.111
				SD	0.006	0.002	0.004
				% RSD	9.841	7.977	3.240

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.

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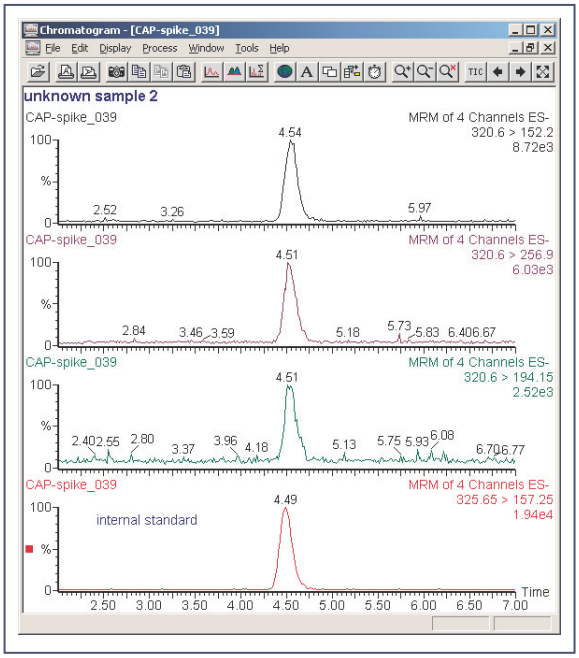

Figure 4.

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.

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