Supporting information:

Development of a monoclonal antibody based-ELISA for the detection of chloramphenicol in shrimp, feed and milk samples and validated by LC-MS/MS coupled with immunoaffinity clean-up

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Table S1. The primarily cross-reactivity values of the ELISAs based on the supernatants of 2D2 and 1B1 with Chloramphenicol, Thiamphenicol and Florfenicol

Compound	2D2[a]		1B1[b]	
	IC_{50} (ng mL^{-1})	CR (%)	IC_{50} (ng mL^{-1})	CR (%)
Chloramphenicol	0.59	100	1.65	100
Thiamphenicol	>10,000	<0.01	32.2	5.13
Florfenicol	>10,000	<0.01	136.4	1.21

[a] The cross-reactivity values of the ELISA based on the supernatants of 2D2 with Thiamphenicol and Florfenicol were less than 0.01%.

[b] The cross-reactivity values of the ELISA based on the supernatants of 1B1 with Thiamphenicol and Florfenicol were 5.13 % and 1.21%, respectively. Clearly, the cross-reactivity values of the ELISA based on the supernatants of 1B1 with Thiamphenicol and Florfenicol were higher than that of 2D2, indicating lower specificity.

Table S2. Optimized ELISA parameters including coating antigen, mAb and GaMIgG-HRP and the corresponding IC_{50} values

Coating antigen		mAb	GaMIgG-HRP	IC_{50} [a]
name	dilution	dilution	dilution	(ng mL^{-1})
CAP-BSA_1	1:2000	1:14000	1:5000	0.81-1.4
	1:5000	1:7000	1:10000	1.03-1.62
CAP-BSA_2	1:2000	1:14000	1:5000	0.36-0.51
	1:5000	1:7000	1:10000	0.78-1.21
CAP-OVA_1	1:2000	1:14000	1:5000	3.52-5.23
	1:5000	1:7000	1:10000	3.41-7.57
CAP-OVA_2	1:2000	1:14000	1:5000	2.83-4.65
	1:5000	1:7000	1:10000	2.32-6.67

[a] Values were calculated from standard curves run on six consecutive days.

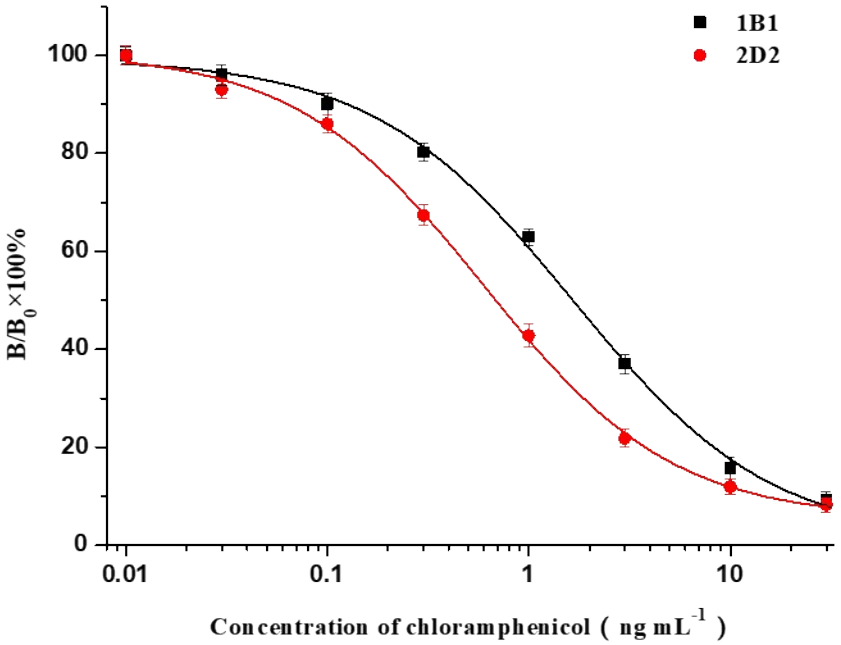

Fig. S1. The primarily standard curves of the ELISA for CAP based on the supernatants of 1B1 (black) and 2D2 (red). The IC_{50} values were 1.65 ng/mL and 0.59 ng/mL, respectively. Clearly, the sensitivity of the ELISA using supernatant of 2D2 (red) is higher than that of 1B1 (black).

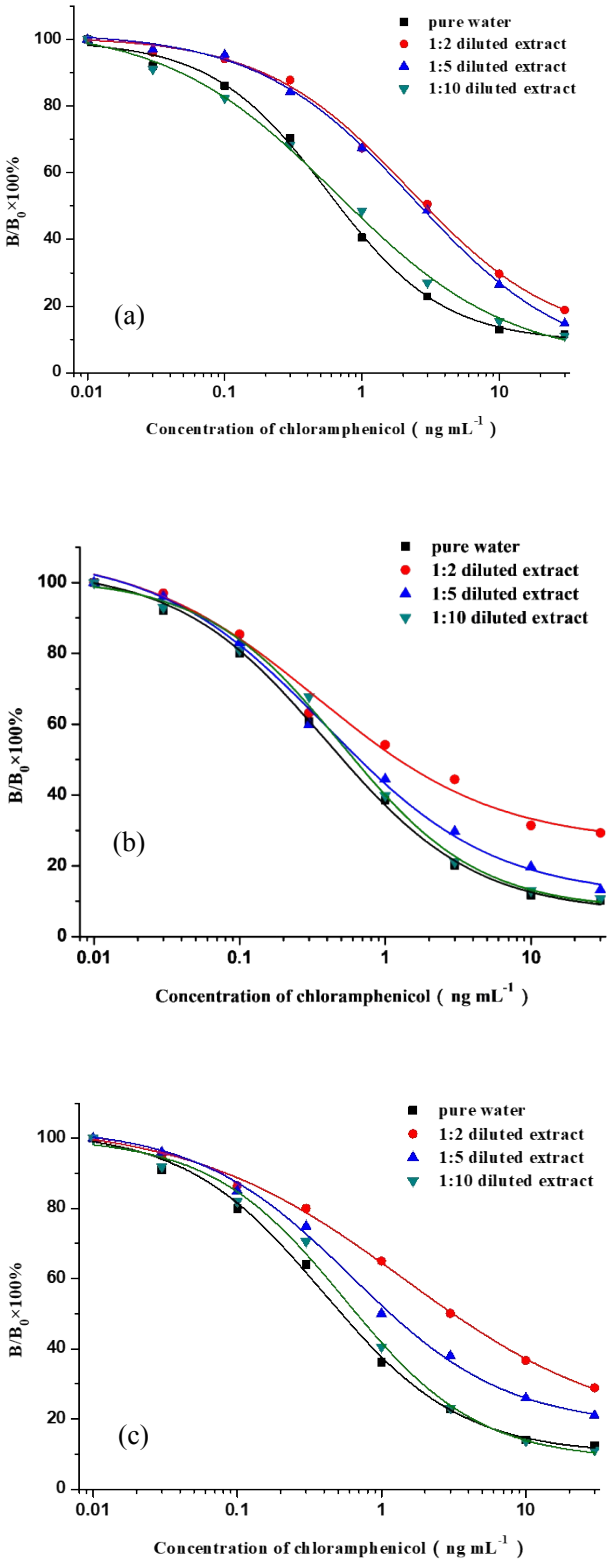

Fig. S2. Matrix effect of shrimp, feed and milk samples on the standard curves of the ELISA for CAP detection. (a) Standard curves of the ELISA for CAP performed in pure water and 1 : 2, 1 : 5, 1 : 10 extracts from shrimp sample, (b) standard curves of the ELISA for CAP performed in pure water and 1 : 2, 1 : 5, 1 : 10 extracts from feed sample, (c) standard curves of the ELISA for CAP performed in pure water and 1 : 2, 1 : 5, 1 : 10 extracts from milk sample

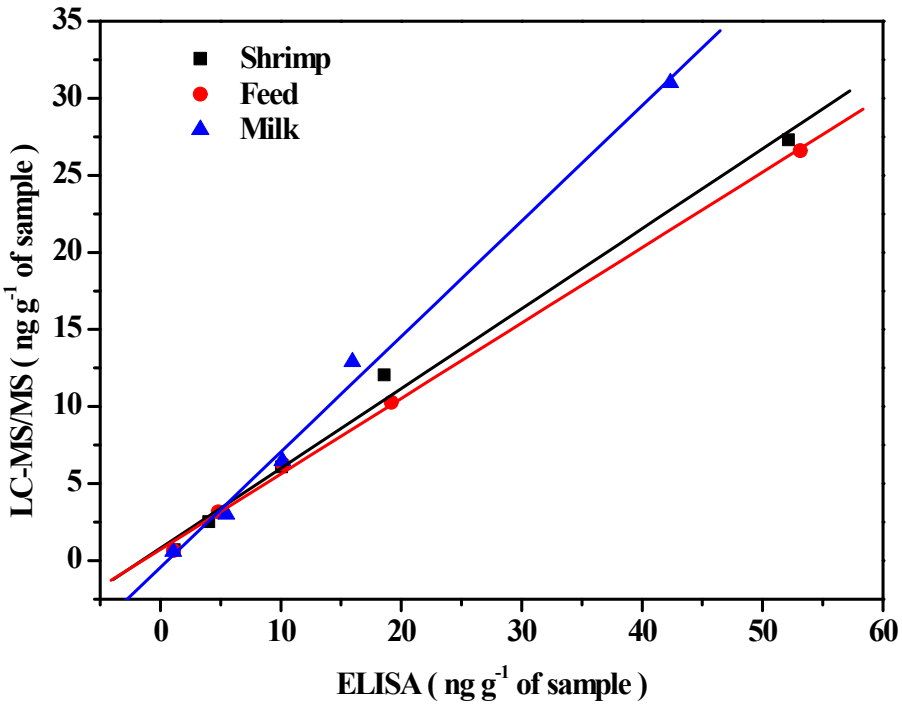

Fig. S3. The correlations between the ELISA and LC-MS/MS coupled with the convenient SPE clean-up for the detection of CAP in three spiked samples

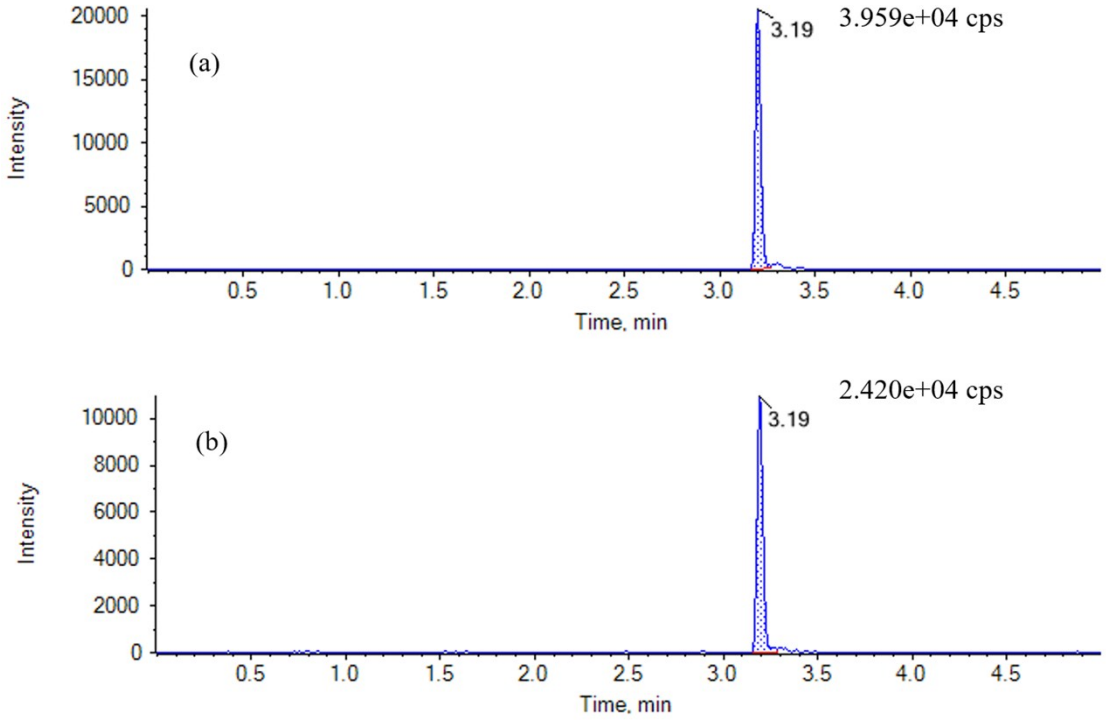

Fig. S4. The MRM chromatograms of the feed samples spiked at the 50 ng g^{-1} level of CAP and measured by LC-MS/MS-IAC and LC-MS/MS-SPE (The peak area signals are shown on the graphs)

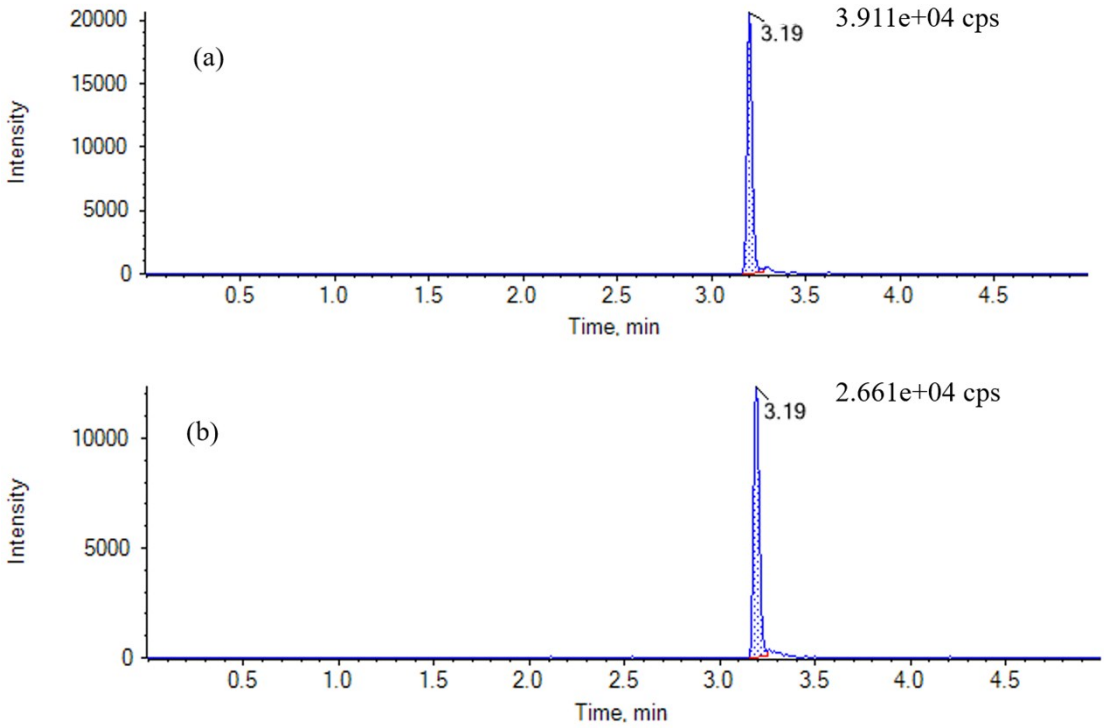

Fig. S5. The MRM chromatograms of the milk samples spiked at the 50 ng g^{-1} level of CAP and measured by LC-MS/MS-IAC and LC-MS/MS-SPE (The peak area signals are shown on the graphs)