Food Chemistry 134 (2012) 2526–2531

Contents lists available at SciVerse ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Establishment of magnetic beads-based enzyme immunoassay for detection of chloramphenicol in milk

Jing Xu [b], Weiwei Yin [b], Yuanyang Zhang [b], Jian Yi [b], Meng Meng [a,*], Yabin Wang [b], Huyin Xue [a], Taichang Zhang [a], Rimo Xi [a,*]

[a] College of Pharmacy, State Key Laboratory of Medicinal Chemical Biology and Tianjin Key Laboratory of Molecular Drug Research, Nankai University, Tianjin 300071, PR China
[b] The School of Chemistry and Chemical Engineering, Shandong University, Jinan, Shandong 250100, PR China

ARTICLE INFO

Article history:
Received 3 August 2010
Received in revised form 8 January 2012
Accepted 15 April 2012
Available online 21 April 2012

Keywords:
Chloramphenicol
Monoclonal antibody
Immunomagnetic assay
Drug residue

ABSTRACT

In this research, magnetic beads-based enzyme immunoassays were investigated for rapid analysis of chloramphenicol (CAP) in milk. To improve sensitivity of CAP determination, two kinds of immunomagnetic separation methods were designed and compared. Magnetic polystyrene microspheres were conjugated with anti-CAP antibody (Method I) or goat-anti-mouse IgG (Method II). The whole determination could be finished in 1.25 h. Both methods showed high sensitivity to CAP in buffer, and obtained an IC_{50} value of 0.05 ng mL^{-1} for Method I and 0.4 ng mL^{-1} for Method II. The methods showed high specificity, only showing a little cross-reaction towards CAP succinate. The two methods were applied to detect CAP in milk. The recovery rates were 80–106% and the coefficients of variation (CVs) were 4.7–15%. The immunomagnetic assay showed promising potential in rapid screening field for CAP analysis. Between the two methods, Method I is more sensitive, and Method II is more suitable for producing a general assay by changing a primary antibody for another analyte.

© 2012 Elsevier Ltd. All rights reserved.

1. Introduction

Chloramphenicol (CAP, Fig. 1), a broad-spectrum antibiotic, has been widely used in animal husbandry as a low-price and effective veterinary drug. Recent research showed that CAP administration is associated with haematological toxicity in humans. It may induce bone marrow depression and aplastic anaemia (Kasten, 1999). Its use in food and animal feeds has been banned in many countries (Gaudin, Cadieu, & Maris, 2003). In 1994, the European Community banned the use of CAP in food producing animals (European Community Regulations, 1990, 1994). In 2002, the European Union (EU) introduced the concept of minimum required performance limit (MRPL) and revised the technical criteria applied in the screening and confirmatory analysis of veterinary drug residues in food of animal origin (Commission Decision, 2002). The MRPL for CAP residues in food of animal origin was set to be 0.3 μg kg^{-1} (Commission Decision, 2003). To meet the requirement of lower detection limit of 0.1 μg kg^{-1}, the U.S. Food and Drug Administration (FDA) has been developing sensitive analytical methods for CAP. In 2007, the Ministry of Agriculture in People's Republic of China announced that the use of CAP and CAP succinate as veterinary drugs was prohibited (No. 824 Bulletin, 2007). However, CAP is still abused and its residues have been found in various food samples, such as muscle, shrimp, milk and honey. To effectively monitor illegal use of CAP, a simple, sensitive and specific analytical method is urgently required to rapidly detect CAP in foods for screening purpose.

Traditional methods for detecting CAP involve planar chromatography (Santos & Ramos, 2006), gas chromatography (GC) (Cerkvenik-Fajs, 2006), high-performance liquid chromatography (HPLC) (Shen & Jiang, 2005), gas chromatography-mass spectrometry (GC–MS) (Xie, Liu, Qiu, Han, & Liu, 2005), liquid chromatography– mass spectrometry (LC/MS) (Fujita, Ito, Nakamura, Watai, & Taniguchi, 2008; Turnipseed, Roybal, Pfenning, & Kijak, 2003) and LC/MS/MS (Jani, Shukla, & Bhagwat, 2006; Sheridan, Policastro, Thomas, & Rice, 2008). These methods are expensive, complicated, and not suitable for analysis of a large number of samples for screening purpose. With the advantages of simplicity, cost-effectiveness and high sample throughput, immunoassays (Wang, Zhang, Gao, Duan, & Wang, 2010; Samsonova, Fedorova, Andreeva, Rubtsova, & Egorov, 2010) have lately gained increasing interest as an alternative methods for large monitoring programs, such as chemiluminescent-ELISA (Zhang, Zhang, Shi, Eremin, & Shen, 2006), fluorescence polarisation immunoassay (FPIA) (Gasilova & Eremin, 2010) and time-resolved fluoroimmunoassay (TRFIA) (Li, Hu, Huo, & Xu, 2006). The reported rapid immunochemical assays for CAP determination are listed in Table 1. Among these methods, TRFIA is the most sensitive assay, but the analysis protocol is not

* Corresponding authors. Tel.: +86 22 23499986; fax: +86 22 23507760.
E-mail addresses: mengmeng@nankai.edu.cn (M. Meng), xirimo2000@yahoo.com (R. Xi).

0308-8146/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodchem.2012.04.083

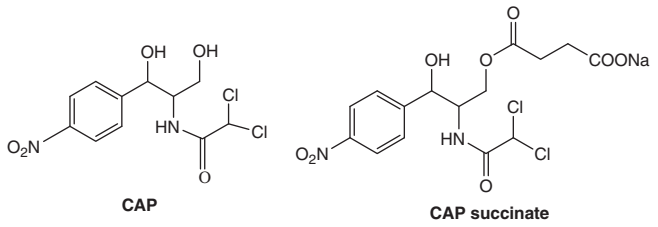

Fig. 1. Structures of CAP and CAP succinate.

fast (>2 h), usually needs expensive instrument for signal measurement. Immunochromatographic assay is the most rapid, but it is not so sensitive (LOD = 10 ng g^{-1}).

Immunomagnetic assay, raised by Merck Serono (Geneva, Switzerland) in 1980s, has been increasingly and widely applied to test environmental contaminants in recent years (Shelver, Kamp, Church, & Rubio, 2007; Dequaire, Degrand, & Limoges, 1999; Tanaka et al., 2004). This method integrates magnetic separation and mostly enzyme-linked immunosorbent technology to increase sensitivity and specificity. Being associated with the beads, the biological function of the antibody is not influenced. As a result, the antibody coupled with the magnetic particles could be separated and enriched in a magnetic field, and the sensitivity of the antibody is greatly improved. Until now, there is no immunomagnetic ELISA reported for CAP rapid analysis. In this study, the sensitivity of magnetic beads-based ELISA method was systemically investigated. The effect of magnetic separation on assay sensitivity was studied by attaching primary antibody (anti-CAP, Method I) or secondary antibody (goat-anti-mouse IgG, Method II) onto the surface of magnetic microspheres. The sensitivity (IC_{50} values) was 0.05 ng mL^{-1} (Method I) and 0.4 ng mL^{-1} (Method II), and the whole determination could be completed in 1.25 h.

2. Experimental

2.1. Chemicals and instruments

Chloramphenicol (CAP), CAP succinate and 1-ethyl-3-(dimethylaminopropyl) carbodiimide hydrochloride (EDC) were purchased from Sigma–Aldrich (St. Louis, MO). Goat anti-mouse IgG, CAP–HRP (horseradish peroxidase) and anti-CAP monoclonal antibody were provided from Xiniya Biotechnology Co., Ltd. (Jinan, China). 3,3′,5,5′-Tetramethylbenzidine (TMB) was purchased from Shanghai Sangon Biological Engineering Technology (Shanghai, China). Monodisperse magnetic polystyrene microspheres with carboxyl as the surface functional group [0.5–1 μm, 1% (w/v)] and magnetic separator were provided by Tianjin Baseline Chromtech Research Centre (Tianjin, China). Ethanol, hydrogen peroxide (30%) and other reagents were of chemical grade and supplied by Guangmang Chemical Co. (Jinan, China). Yili skimmed milk (fat content = 1.3 g/100 g) was purchased from local supermarket (Tianjin, China). The immunomagnetic assay was carried out in tubes by magnetic separation and spectrophotometrically read with an automatic microplate reader Model 680 of Bio-Rad (California, USA).

2.2. Buffers and solutions

The following buffers were used: phosphate-buffered saline (PBS, pH7.4) containing 138 mM NaCl, 1.5 mM KH_2PO_4, 8 mM $Na_2HPO_4{\cdot}H_2O$ and 2.7 mM KCl; washing buffer (PBST): PBS with addition of 0.05% (v/v) Tween20; citrate buffer (pH5.0): 19 mM citric acid, 33.5 mM $Na_2HPO_4{\cdot}H_2O$; substrate solution: 400 μL of 0.6% TMB-DMSO mixed with 100 μL of 1% H_2O_2 in citrate–acetate buffer (pH5.5); stopping solution: 2 M H_2SO_4.

2.3. Development of magnetic beads-based immunoassays

2.3.1. Modification of magnetic beads

For CAP separation, magnetic beads were modified by different antibodies. Method I: 100 μL of magnetic beads [0.5–1 μm, 1% (w/v)] were mixed with 100 μL of anti-CAP antibody and 1 mg EDC in tubes. The reaction was allowed to proceed at 37 °C for 2 h, or room temperature overnight. Then, the mixture was washed for five times with washing buffer in a magnetic field. Finally, the immunomagnetic microspheres were dispersed in PBS and stored at 4 °C before use. Method II: 100 μL of magnetic beads [0.5–1 μm, 1% (w/v)] reacted with 100 μL of goat-anti-mouse IgG at 37 °C for 2 h via 1 mg EDC. The modified magnetic particles were then washed and stored as described for Method I.

2.3.2. Procedure of the two magnetic beads-based immunoassays

The flow chart of the two methods is displayed in Fig. 2.

Method I: After magnetic beads were modified as described above, they were mixed with 50 μL of CAP-HRP and 50 μL of analyte samples (CAP standard solution or CAP samples), and the mixture was incubated for 1 h at 37 °C with gentle shaking. Then,

Table 1
Comparison of rapid immunochemical assays for CAP determination.

Immunoassays	Label	Sensitivity	Matrix	Assay duration	References
This work	HRP	IC_{50} = 0.05 ng/mL (Method I) IC_{50} = 0.4 ng/mL (Method II)	Milk	1.25 h	This work
ELISA	HRP	LOD = 1 ppb	Water	<24 h	Campbell, Mageau, Schwab, and Johnston (1984)
ELISA	HRP	IC_{50} = 10.5 ppb	Shrimp	7 h	Sai et al. (2010)
Chemiluminescence immunoassay	HRP-SuperSignal® substrate	IC_{50} = 0.47 ng/mL	Water	>1.5 h	Xu et al. (2006)
Chemiluminescence immunoassay	HRP-luminol	LOD = 6 ng/g	Chicken	About 3 h	Zhang et al. (2006)
Chemiluminescence immunoassay	HRP-luminol	LOD = 0.05 ppb	Water	2 h	Lin, Han, Liu, Xu, and Guan (2005)
Fluorescence polarisation immunoassay (FPIA)	Fluorescein	LOD = 10 ng/mL (Buffer) LOD = 20 μg/kg (Milk)	Milk	10 min	Gasilova and Eremin (2010)
Time-resolved fluoroimmunoassay (TR-FIA)	Europium (Eu)	LOD = 0.5 g/g	Shrimp Chicken	>2 h	Shen et al. (2006)
Test strips	Colloidal gold nanoparticles	LOD = 10 ng/mL	Milk	10 min	Byzova, Zvereva, Zherdev, Eremin, and Dzantiev (2010)
Test strips	Colloidal gold nanoparticles	LOD = 10 ng/g	Aquaculture tissues	5 min	Li, Liu, Xu, and Chu (2007)

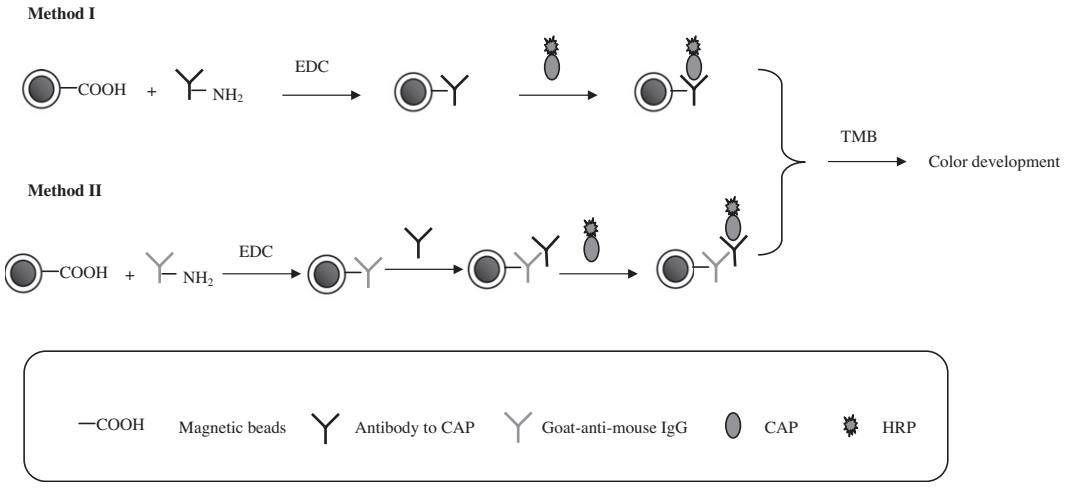

Fig. 2. The flow chart of the two immunomagnetic assays to detect CAP.

the tubes were washed in magnetic field, and the substrate solution (TMB, 50 μL) was added to incubate for 15 min. Then, the mixture was transferred to a 96-well microtiter plate. Finally, the enzyme reaction was stopped with 2 M H_2SO_4 (100 μL/well) and the absorbance values at 450 nm were measured.

Method II: The goat-anti-mouse IgG modified magnetic beads were connected with CAP antibody (100 μL) at 37 °C for 1 h. Then 50 μL of CAP-HRP and 50 μL of analyte samples (CAP standard solution or CAP samples) were added, and the mixture was incubated for 1 h at 37 °C. The tubes were then washed with PBST for three times in a magnetic field. 50 μL of TMB substrate solution was added and the tubes were incubated for 15 min. Then, the mixture was transferred to a 96-well microtiter plate. The enzyme reaction was stopped with 2 M H_2SO_4 (100 μL/well) and the absorbance values of the plate were measured at 450 nm.

2.4. Checkerboard procedure

Checkerboard procedure was performed to select the optimal concentrations of immunomagnetic beads, CAP-HRP and antibody for the competitive immunomagnetic assay.

Method I: Immunomagnetic beads were diluted to 1/200, 1/400, 1/800, 1/1600 and 1/3200. A series of CAP-HRP solution (1/1250–1/20,000) were used. Method II: Beads were diluted to 1/20, 1/40, 1/60 and 1/80. A series of anti-CAP antibody (1/200–1/2000) and CAP-HRP (1/1000–1/10,000) were used.

2.5. Sensitivity determination

The optimal immunomagnetic beads and CAP-HRP (50 μL/tube) concentrations were acquired according to the checkerboard procedure above. The sensitivity of CAP antibody was determined according to inhibition curve. The tested level of CAP (50 μL/tube) was 0.0001–100 ng mL^{-1} in Method I, and 0.001–100 ng mL^{-1} in Method II. The standard calibration curve with final CAP concentrations of 0.005, 0.01, 0.1, 1, 5 ng mL^{-1} in Method I and 0.01, 0.1, 0.5, 1, 5 ng mL^{-1} in Method II were run in PBS. The linear relationship between logarithmic concentration of CAP and the B/B_0 values was evaluated. B_0 is the absorbance value without addition of CAP, and B is the absorbance value with CAP.

2.6. Comparison with conventional ELISA method

The sensitivity of the proposed immunoassay was compared with that of conventional immunoassay. A competitive ELISA method was developed as the ordinary immunoassay. A 96-well plate was coated with 100 μL of goat-anti-mouse IgG (1:1000) at 37 °C for 2 h. The plate was washed for three times, and anti-CAP antibody (1:4000) was added (50 μL/well) and incubated at 37 °C for 1 h. After washing, CAP standard solution (50 μL/well) and CAP-HRP (1:3000, 50 μL/well) was added at 37 °C. After the plates were incubated for another 30 min, TMB substrate solution was added (100 μL/well). After 15 min, chromogenic reaction was inhibited by adding stopping solution (100 μL/well), and absorbances were measured at 450 nm.

2.7. Specificity of the assay

CAP succinate in different concentrations was used as the competitor and determined as the immunomagnetic assay described above. The IC_{50} value (competitor concentration that leads to a 50% decrease of the maximum signal) was obtained. The cross-reactivity was calculated according to the equation: cross-reactivity = IC_{50} (CAP)/IC_{50} (analogues) × 100%.

2.8. Matrix effects

The skimmed milk [fat content = 1.3% (w/w)] was centrifuged and diluted to 1/5, 1/10, and 1/20 with PBS. The inhibition curves were drawn using 0.0001–100 ng mL^{-1} (Method I) and 0.001–100 ng mL^{-1} (Method II) CAP solution as the competitors.

2.9. Recovery study in milk samples

Milk samples spiked with 0.5 and 1 ng mL^{-1} CAP was analysed by the methods. Afterwards, recoveries were calculated from standard calibration curve. The procedure was repeated for four times on the same day and three different days to calculate the inter-assay and intra-assay coefficients of variation (CVs).

3. Results and discussion

3.1. Sensitivity

Immunoassay is advantageous in simple procedure, rapid analysis and cost-effectiveness. Now, there are many ELISA test systems for analysis of veterinary drugs in solution, food and biological samples. In the process of immunomagnetic assay, the immunomagnetic beads complex was separated and enriched in a magnetic field. Therefore, the tested compounds would be effec-

tively enriched from a large volume on a surface of the magnetic carriers. As a result, the sensitivity of the antibody was significantly improved. The optimum dilution of magnetic beads, CAP-HRP and antibody concentration were selected according to the checkerboard experiment. For Method I, the dilution factor of the immunomagnetic particles CAP-HRP solution was 1/400 and 1/10,000, respectively. For Method II, the immunomagnetic beads were diluted to 1/40. Antibody to CAP antibody and CAP-HRP were diluted to 1/500 and 1:4000, respectively.

Based on these optimal conditions, the sensitivity of antibody could be determined from the inhibition curve (Fig. 3-A). The IC_{50} value obtained by immunomagnetic assay was 0.05 ng mL^{-1} for Method I and 0.4 ng mL^{-1} for Method II. To compare with classic immunoassay, a competitive ELISA method was developed and tested. The IC_{50} value of the ELISA method was about 0.72 ng mL^{-1} (Fig. 3-A), demonstrating that the immunomagnetic assay established in this research is more sensitive. The standard calibration curve was generated in PBS. 0.005–5 ng mL^{-1} and 0.01–5 ng mL^{-1} of CAP solution were applied as competitors for Method I and Method II, respectively. Both methods showed excellent linear tendency in the measured range. The linear relationship of B/B_0 values versus CAP concentration was $y = -0.1503x + 0.3056$, (Method I; $R^2 = 0.9996$); $y = -0.2294x + 0.4047$ (Method II, $R^2 = 0.9826$), where y is the B/B_0 values, and x is the logarithmic concentration of CAP.

In comparison of these two methods, Method I is advantageous in better sensitivity and simple operation. The higher sensitivity for Method I might be caused by that the antibody was directly connected with the beads, which would extremely increase the binding ratio of the antibody with CAP. Compared with the reported rapid immunochemical assays (Table 1), Method I is even more sensitive than chemiluminescence immunoassay (IC_{50} of 0.47 ng mL^{-1} in water, Xu, Peng, Hao, Jin, & Wang, 2006), and time-resolved fluoroimmunoassay (TRFIA) (LOD of 0.5 g g^{-1} in shrimp and chicken, Shen et al., 2006). For Method II, CAP antibody was connected with beads after goat-anti-mouse IgG conjugation, so, the immobilized antibody molecule on the surface of magnetic beads was less than that for Method I, and the sensitivity might be lower too. However, Method II possesses wider application in detection analytes by binding of the antibody for another analyte with goat-anti-mouse IgG, and could be developed as a general detection agent.

3.2. Specificity

The assay specificity was studied by determining cross-reactivity of CAP analogues, using CAP succinate (0.0001–1000 ng mL^{-1})

Table 2
Cross-reactivity (CR) values of anti-CAP antibodies with CAP analogue.

Compound	Method I		Method II	
	IC_{50}[a]	CR[b] (%)	IC_{50}	CR (%)
CAP	0.05	100	0.4	100
CAP succinate	0.88	5.7	4.1	9.8

[a] IC_{50} was the competitor concentration where the absorbance value was a half as that of no competitor.
[b] CR% was the percentage ratio of IC_{50} for CAP to that for the test compounds.

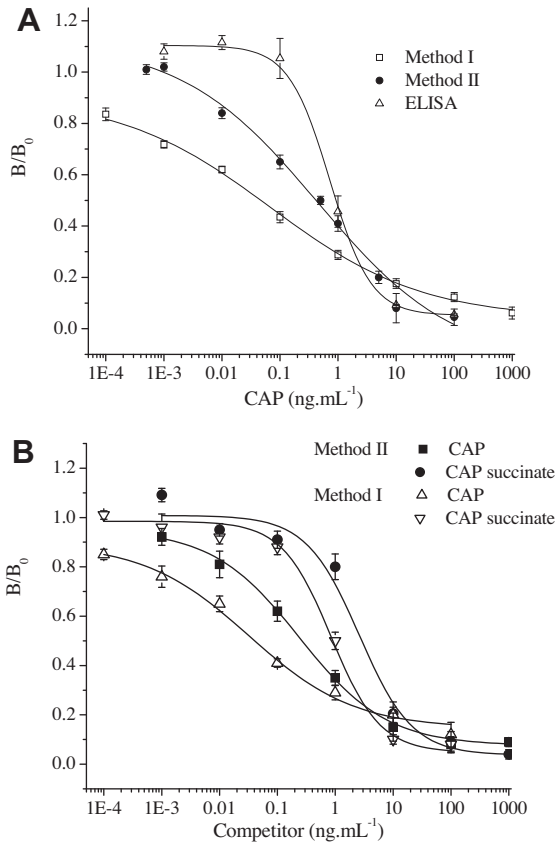

Fig. 3. Inhibition curve of anti-CAP antibody with CAP (A) and CAP succinate (B) as a competitor ($n = 5$).

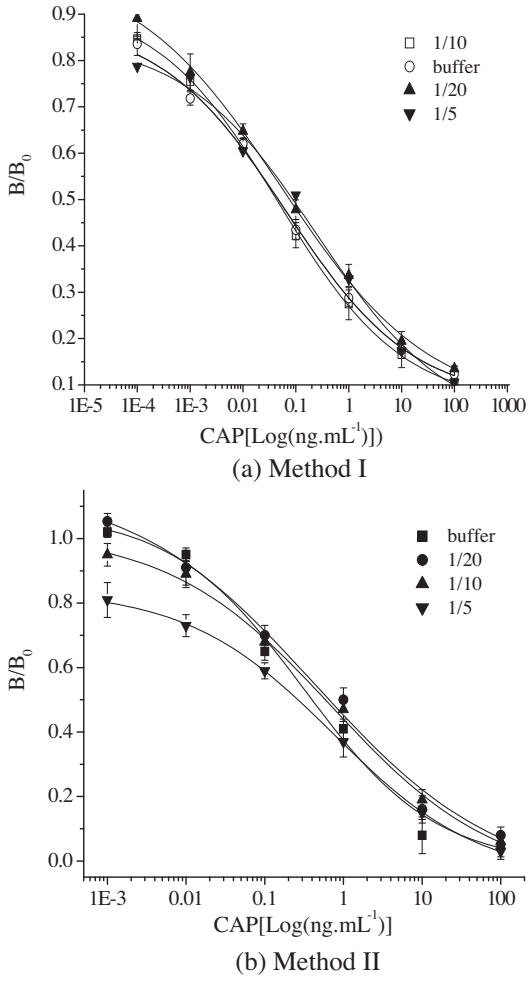

Fig. 4. Inhibition curves of anti-CAP antibody with CAP in buffer and milk of different dilutions.

Table 3
Recoveries for detection of CAP in milk sample by the immunomagnetic methods.

Method	Method I		Method II	
Spiked level ($ng\,mL^{-1}$)	0.5	1	0.5	1
Intra-assay (n = 4)[a]				
Measured ($ng\,mL^{-1}$)	0.45 ± 0.04	1.06 ± 0.05	0.46 ± 0.05	0.92 ± 0.06
Recovery (%)	90	106	92	92
CV (%)	8.9	4.7	10.8	6.5
Inter-assay (n = 3)[b]				
Measured ($ng\,mL^{-1}$)	0.43 ± 0.05	1.01 ± 0.09	0.40 ± 0.06	0.89 ± 0.07
Recovery (%)	86	92	80	89
CV (%)	11.6	8.9	15.0	7.9

[a] Intra-assay variation was determined by four replicates on a single day.
[b] Inter-assay variation was determined by three replicates on three different days.

as competitor (Fig. 3-B). As is indicated in Table 2, the methods showed good specificity, only displaying a low cross-reactivity towards CAP succinate (<10%).

3.3. Recovery of the assay in milk samples

As a veterinary drug, CAP is still used to treat animal diseases; therefore, CAP residue usually appears in muscle, shrimp, milk and honey. In our research, milk was chosen as an actual system to study the stability and recovery of the assay. To reduce the matrix effect, milk samples without CAP addition were diluted (1/5, 1/10, 1/20) and detected by the immunomagnetic assay. The results of the two methods were shown in Fig. 4a and b. The result indicated that the matrix effect could be ignored at a dilution of 1/10 (Method I) and 1/20 (Method II), since the responding IC_{50} was close to that in buffer. Therefore, 1/10 and 1/20 dilutions of milk was used to evaluate recoveries of Method I and Method II, respectively. 0.5 and 1 $ng\,mL^{-1}$ of CAP were spiked in milk samples and analysed. The recoveries determined according to the standard curve were summarised in Table 3, which shows that the recoveries were 80–106% and the CVs were in the range of 4.7–15.0%, indicating the good stability of the two methods.

4. Conclusions

In conclusion, two competitive ELISA methods using magnetic particles as a solid-phase carrier were established to detect CAP in milk sample. An average IC_{50} value of 0.05 and 0.4 $ng\,mL^{-1}$ was obtained for Method I and Method II, respectively. The linear range was 0.005–5 $ng\,mL^{-1}$ (Method I, $R^2 = 0.9996$) and 0.01–5 $ng\,mL^{-1}$ (Method II, $R^2 = 0.9826$). The sensitivity for both immunomagnetic assays are more sensitive than classic ELISA method ($IC_{50} = 0.72\ ng\,mL^{-1}$). The antibody showed good specificity, only displaying a low cross-reactivity towards CAP succinate. The recovery rates and the CVs were satisfactory in milk system, therefore, the immunomagnetic assay could be used as an alternative screening method to detect CAP residue in milk and diary foods. Between the two methods, Method I is more sensitive and suitable to detect CAP in food samples, and Method II possesses wider application as a general agent by using the antibodies of different analytes for binding with goat-anti-mouse IgG on the surface of magnetic beads.

Acknowledgements

We would like to thank the financial support of National Natural Science Foundation (Nos. 81173017 and 31101277), the National High-Tech Research and Development Program of China (863 Program, Nos. 2010AA10Z402 and 2007AA06A407), Tianjin Science and Technology Program (No. 09ZCKFSH07500), the Scientists-Company Cooperation Project of the Ministry of Science and Technology of China (SQ2009GJA0002591) and the Fundamental Research Funds for the Central Universities (No. 65011121).

References

Byzova, N. A., Zvereva, E. A., Zherdev, A. V., Eremin, S. A., & Dzantiev, B. B. (2010). Rapid pretreatment-free immunochromatographic assay of chloramphenicol in milk. *Talanta, 81*(3), 843–848.
Campbell, G. S., Mageau, R. P., Schwab, B., & Johnston, R. W. (1984). Detection and quantitation of chloramphenicol by competitive enzyme-linked immunoassay. *Antimicrobial Agents and Chemotherapy, 25*(2), 205–211.
Cerkvenik-Fajs, V. (2006). Performance characteristics of an analytical procedure for determining chloramphenicol residues in muscle tissue by gas chromatography-electron capture detection. *Biomedical Chromatography, 20*(10), 985–992.
Commission Decision 2002/657/EC. Official Journal of European Communities, L221, 8.
Commission Decision 2003/181/EC. Official Journal of European Communities, L71, 17.
Dequaire, M., Degrand, C., & Limoges, B. (1999). An immunomagnetic electrochemical sensor based an a perfluorosulfonate-coated screen-printed electrode for the determination of 2,4-dichlorophenoxyacetic acid. *Analytical Chemistry, 71*(13), 2571–2577.
European Community Regulations, 2377/90.
European Community Regulations, 1430/94.
Fujita, K., Ito, H., Nakamura, M., Watai, M., & Taniguchi, M. (2008). Determination of chloramphenicol residues in bee pollen by liquid chromatography/tandem mass spectrometry. *Journal of AOAC International, 91*(5), 1103–1109.
Gasilova, N. V., & Eremin, S. A. (2010). Determination of chloramphenicol in milk by a fluorescence polarization immunoassay. *Journal of Analytical Chemistry, 65*(3), 255–259.
Gaudin, V., Cadieu, N., & Maris, P. (2003). Inter-laboratory studies for the evaluation of ELISA kits for the detection of chloramphenicol residues in milk and muscle. *Food and Agricultural Immunology, 15*(3–4), 143–157.
Jani, G., Shukla, J., & Bhagwat, S. (2006). Determination of chloramphenicol residues in milk and milk-products using LC/MS/MS. *Asian Journal of Chemistry, 18*(4), 3040–3048.
Kasten, M. J. (1999). Clindamycin, metronidazole, and chloramphenicol. *Mayo Clinic Proceedings, 74*(8), 825–833.
Li, X. Q., Hu, Y. M., Huo, T. M., & Xu, C. L. (2006). Comparison of the determination of chloramphenicol residues in aquaculture tissues by time-resolved fluoroimmunoassay and with liquid chromatography and tandem mass spectrometry. *Food and Agricultural Immunology, 17*(3–4), 191–199.
Li, K., Liu, L. Q., Xu, C. L., & Chu, X. G. (2007). Rapid determination of chloramphenicol residues in aquaculture tissues by immunochromatographic assay. *Analytical Sciences, 23*(11), 1281–1284.
Lin, S., Han, S. Q., Liu, Y. B., Xu, W. G., & Guan, G. Y. (2005). Chemiluminescence immunoassay for chloramphenicol. *Analytical and Bioanalytical Chemistry, 382*(5), 1250–1255.
No. 824 Bulletin, Ministry of Agriculture of the People's Republic of China (2007).
Samsonova, J. V., Fedorova, M. D., Andreeva, I. P., Rubtsova, M. Y., & Egorov, A. M. (2010). Characterization of anti-chloramphenicol antibodies by enzyme-linked immunosorbent assay. *Analytical Letters, 43*(1), 133–141.
Sai, N., Chen, Y. P., Liu, N., Yu, G. G., Su, P., Feng, Y., Zhou, Z. J., Liu, X. Y., Zhou, H. Y., Gao, Z. X., & Ning, B. A. (2010). A sensitive immunoassay based on direct hapten coated format and biotin–streptavidin system for the detection of chloramphenicol. *Talanta, 82*(4), 1113–1121.
Santos, U., & Ramos, F. (2006). Analytical methodologies for chloramphenicol residues determination in food matrixes: A brief review. *Current Pharmaceutical Analysis, 2*(1), 53–57.
Shelver, W. L., Kamp, L. M., Church, J. L., & Rubio, F. M. (2007). Measurement of triclosan in water using a magnetic particle enzyme immunoassay. *Journal of Agricultural and Food Chemistry, 55*(10), 3758–3763.
Shen, H. Y., & Jiang, H. L. (2005). Screening, determination and confirmation of chloramphenicol in seafood, meat and honey using ELISA, HPLC–UVD, GC–ECD, GC–MS-EI-SIM and GC–MS-NCI-SIM methods. *Analytica Chimica Acta, 535*(1–2), 33–41.
Shen, J. Z., Zhang, Z., Yao, Y., Shi, W. M., Liu, Y. B., & Zhang, S. X. (2006). A monoclonal antibody-based time-resolved fluoroimmunoassay for chloramphenicol in shrimp and chicken muscle. *Analytica Chimica Acta, 575*(2), 262–266.
Sheridan, R., Policastro, B., Thomas, S., & Rice, D. (2008). Analysis and occurrence of 14 sulfonamide antibacterials and chloramphenicol in honey by solid-phase extraction followed by LC/MS/MS analysis. *Journal of Agricultural and Food Chemistry, 56*(10), 3509–3516.
Tanaka, T., Takeda, H., Ueki, F., Obata, K., Tajima, H., Takeyama, H., Goda, Y., Fujimoto, S., & Matsunaga, T. (2004). Rapid and sensitive detection of 17 beta-estradiol in environmental water using automated immunoassay system with bacterial magnetic particles. *Journal of Biotechnology, 108*(2), 153–159.
Turnipseed, S. B., Roybal, J. E., Pfenning, A. P., & Kijak, P. J. (2003). Use of ion-trap liquid chromatography–mass spectrometry to screen and confirm drug residues in aquacultured products. *Analytica Chimica Acta, 483*(1–2), 373–386.

Wang, L., Zhang, Y., Gao, X., Duan, Z. J., & Wang, S. (2010). Determination of chloramphenicol residues in milk by enzyme-linked immunosorbent assay: Improvement by biotin–streptavidin-amplified system. *Journal of Agricultural and Food Chemistry, 58*(6), 3265–3270.

Xie, M. X., Liu, Y., Qiu, Y. M., Han, J., & Liu, Y. Z. (2005). Determination of chloramphenicol residue in animal tissues by solid phase extraction and gas chromatography–mass spectrometry. *Chinese Journal of Analytical Chemistry, 33*(1), 1–4.

Xu, C. L., Peng, C. F., Hao, K., Jin, Z. Y., & Wang, W. K. (2006). Chemiluminescence enzyme immunoassay (CLEIA) for the determination of chloramphenicol residues in aquatic tissues. *Luminescence, 21*, 126–128.

Zhang, S. X., Zhang, Z., Shi, W. M., Eremin, S. A., & Shen, J. Z. (2006). Development of a chemiluminescent ELISA for determining chloramphenicol in chicken muscle. *Journal of Agricultural and Food Chemistry, 54*(16), 5718–5722.