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A Broad-Spectrum Antibody-Based Immunochromatographic Assay for Rapid Screening of BADGE and Its Derivatives in Canned Foods

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Abstract: Bisphenol A diglycidyl ether (BADGE) is widely existed in the inner coating of canned foods. It migrates into food and generate various derivatives in the process of storage, such as Bisphenol A (2, 3-dihydroxypropyl) glycidyl ether (BADGE·H_2O), Bisphenol A (3-chloro-2-hydroxypropyl) glycidyl ether (BADGE·HCl) and Bisphenol A (3-chloro-2-hydroxypropyl) (2, 3-dihydroxypropyl) glycidyl ether (BADGE·HCl·H_2O), which have negative effects on human health. A gold nanoparticle-based immunochromatographic assay for simultaneous detection of BADGE and its derivatives was developed by using a broad-spectrum polyclonal antibody, and the detection can be finished in 15 min. The visualization of results was processed by Adobe Photoshop CC software to achieve quantitative analysis and the detection limit (IC_{15}) is 0.97ng/mL. The recoveries of BADGE and its derivatives at various spiking levels in canned food samples ranged from 79.86% to 93.81%. The detection results of the proposed immunochromatographic assay were also validated by HPLC analysis, and got good consistency (R^2=0.9580).

Keywords: Bisphenol A diglycidyl ether; derivatives; broad-spectrum polyclonal antibodies; AuNPs; immunochromatographic; canned food

1. Introduction

Bisphenol A diglycidyl ether (2, 2-Bis (4-glycidyloxyphenyl) propane, BADGE) is a condensation product of BPA and epichlorohydrin. It can be used as additive of polyester fiber and scrubber of hydrochloric acid. It is often used to remove hydrochloric acid in organosol resin, and widely exists in the inner wall of metal cans [1-3]. If the chemical reaction is not complete during the coating manufacturing process, BADGE may remain in food cans [4, 5]. In addition, it may migrate to food contents during processing and storage [6, 7]. Due to the complexity of food matrix, when BADGE contact with acidic or greasy food, hydrolysis or chlorination reaction will occur to generate various derivatives, such as Bisphenol A (2, 3-dihydroxypropyl) glycidyl ether (BADGE·H_2O), Bisphenol A (3-chloro-2-hydroxypropyl) glycidyl ether (BADGE·HCl) and Bisphenol A (3-chloro-2-hydroxypropyl) (2,3-dihydroxypropyl) glycidyl ether (BADGE·HCl·H_2O) [8, 9]. BADGE may lead to abnormalities of human endocrine system, immune system or nervous system, and affect normal reproductive and genetic functions [10-12]. In fact, BADGE and its derivatives residues had been detected in canned foods such as canned seafood, canned meat products, and energy drinks from markets [13, 14]. Based on the toxicological research of BADGE and its derivatives and the pollution status in food, European legislation requires that the specific migration limits (SMLs) of BADGE and its hydrolysis derivatives (BADGE·H_2O) were set at 9 mg/kg in foodstuffs or in food simulants, and hydrochloric derivatives (BADGE·HCl and BADGE·HCl·H_2O) should not exceed 1 mg/kg [15].

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There are many analytical methods for bisphenol-dihydrate glycerol ether compound, which mainly concentrated on liquid chromatography-ultraviolet detection (HPLC-UV) [16], high performance liquid chromatography-fluorescence (HPLC-FLD) [17-19], high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) [20-22] and gas chromatography-mass spectrometry (GC-MS) [23]. Although LC-MS / MS and GC-MS / MS have good accuracy and high sensitivity, they are expensive, complex, and require trained operators, which mean they may not be suitable for rapid primary on-site detection. Compared with the instrumental analysis method, the immunoassay method can be used for detection a variety of samples at the same time, and can meet the needs of mass sample detection in the market. The immunochromatographic strip assay eliminates the culture and washing step in the enzyme-linked immunoassay method (ELISA), and the results are given within 15-20 minutes, which means it provides an effective screening method for rapid detection of small molecular contaminants [24-26]. At present, gold nanoparticle-based immunochromatographic assay has been widely used in rapid detection in the fields of diagnosis and small molecular detection [27-30].

To our best knowledge, at present, there have studies on the production of monoclonal antibodies that can recognize BADGE and the establishment of ELISA method for detecting BADGE in lake water [31]. The preparation of broad-spectrum polyclonal antibodies that can recognize BADGE and its derivatives and the establishment of immunochromatographic assay have not been reported. Actually, during the migration of BADGE to food contents, hydrolysis or chlorination products may also appear in food matrix. As a result, it is of great significance to establish a rapid and simple immunochromatographic assay to realize the quantitative and qualitative detection of multiple residues on site.

In this work, we developed an immunochromatographic assay using Au nanoparticles (AuNPs) and broad-spectrum polyclonal antibody according to the competitive principle, and the visualization results were processed by Adobe Photoshop CC software to achieve quantitative analysis. Canned food samples were selected for the reliability compliance test and the immunochromatographic strip method can meet the rapid screening of a large number of samples in the market.

2. Results

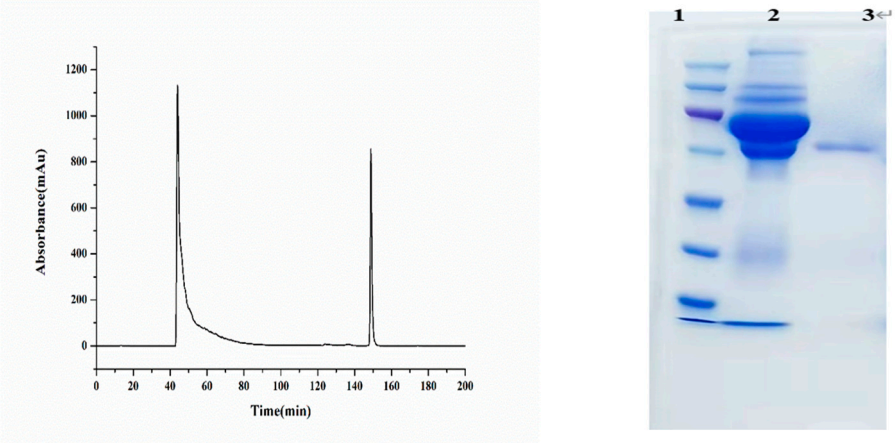
2.1. Screening of broad-spectrum antibodies

The standard solution of BADGE derivatives was prepared, the IC_{50} values were determined and the cross-reactivities were calculated by ic-ELISA. The recognition capability of the selected antiserum to BADGE derivatives was investigated. As shown in Table 1, the cross-reactivities of antiserum PAb-1 to BADGE·HCl, BADGE·H_2O and BADGE·HCl·H_2O were 79.6%, 175.8% and 110.8%, respectively. Compared with other antisera, the antiserum PAb-1 had better recognition capability to BADGE and its derivatives and could be considered as broad-spectrum toward BADGE and its derivatives. Then, the antibody was purified by protein A-Sepharose 4B affinity chromatography, and characterized by SDS-PAGE. Figure 1(a) was the antibody purification obtained by AKTA, and the second peak represented the target antibody. Figure 1(b) showed the SDS-PAGE of the purified antibody, compared with the antiserum, there only has one protein band, indicating that the impurity protein of the antiserum has been eluted completely.

Table 1. Cross-reactivity of BADGE with analogues of four antisera.

Target	Antisera							
	PAb-1		PAb-2		PAb-3		PAb-4	
	IC_{50} (ng/mL)	CR (%)	IC_{50} (ng/mL)	CR (%)	IC_{50} (ng/mL)	CR (%)	IC_{50} (ng/mL)	CR (%)
BADGE	51	100	65	100	26	100	29	100
BADGE·HCl	64	79.6	114	57.0	57	45.6	60	48.3
BADGE·H_2O	29	175.8	51	127.4	76	34.2	47	61.7

BADGE·HCl·H₂O	46	110.8	59	110.1	82	31.7	70	41.4



(a) (b)

Figure 1. (a) The antibody purification of AKTA. (b) The SDS-PAGE of antibody purification. 1: Marker; 2: antiserum; 3: Target antibody.

2.2. Characterization of AuNPs and labelling with the antibody

The characterizations of AuNPs and the antibody conjugates were confirmed by UV-vis, as shown in Figure 2. The prepared AuNPs had an absorption peak at 521 nm and a strong signal under UV. By the conjugation of antibody and AuNPs, the surface plasmon band was red shifted to 532 nm which indicated that AuNPs were successfully coupled with antibody.

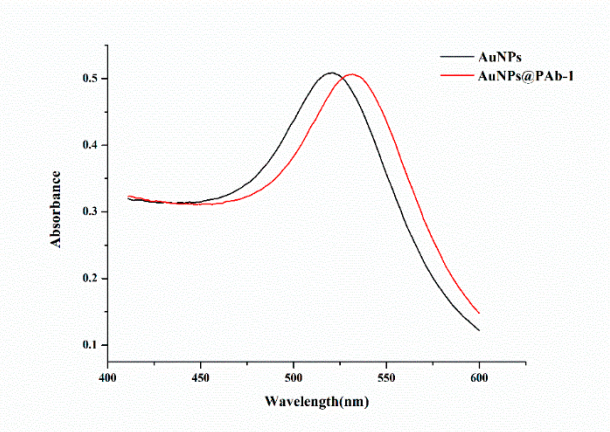


***Figure* 2.** Wavelength scanning of AuNPs and AuNPs-labelled antibody.

2.3. The optimization of AuNPs-labelled antibody

Preparation of AuNPs labeled protein was essentially the process of antibody being adsorbed on the surface of AuNPs. The combination of AuNPs and antibody mainly depended on pH and the amounts of antibody. When the pH of the system was close to the isoelectric point of the antibody, the binding was stable. The pH value of AuNPs solution was adjusted by adding different volumes of K_2CO_3 solution, and excessive antibodies was added for labeling. As shown in Figure 3, the absorbance value first increased and then decreased with the increase of the amount of K_2CO_3 solution, and when the volume was 15 μL, the absorbance was the largest. Therefore, to obtain the maximum absorption of the AuNPs-labelled antibody, 15 μL of K_2CO_3 solution was selected for adjusting the pH.

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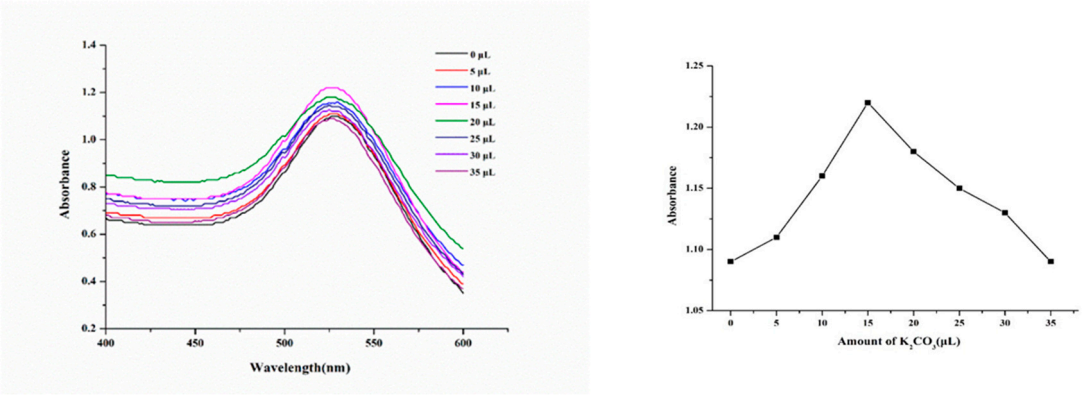
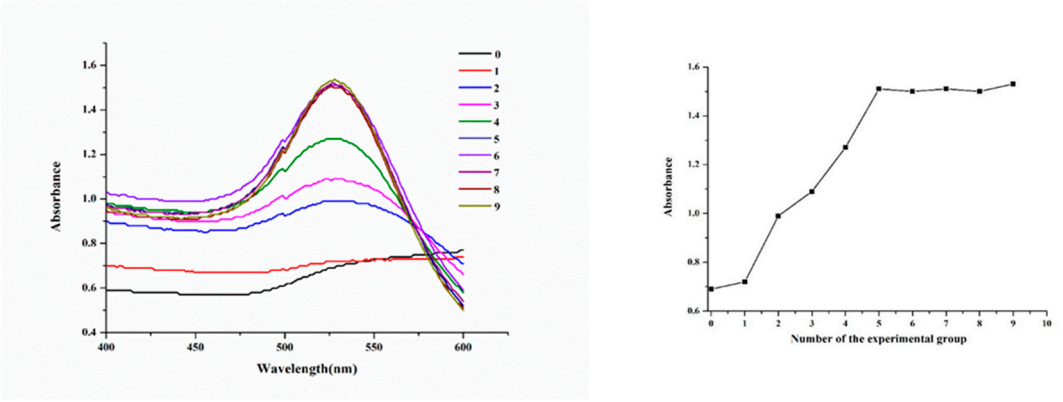


(**a**) (**b**)

Figure 3. (a) Wavelength scanning of AuNPs-labelled antibody at different pH. (b) OD_{521} of AuNPs-labelled antibody at different pH.

The amount of the antibody for labelling was also a key factor. The lack of antibody would result in surface instability of AuNPs, while the overdose would lead to a waste of antibody. According to Figure 4, when the dosage of antibody was 9.35 μg, the amount of antibody labeling had reached saturation, 20% more than this dose was the actual amount required for labeling 1 mL AuNPs solution.



(**a**) (**b**)

Figure 4. (a) Wavelength scanning of AuNPs-labelled antibody at different amount. (b) OD_{521} of AuNPs-labelled antibody at different amount.

2.4. Establishment of AuNPs immunochromatographic method

The immunochromatographic strip assay was established based on an antibody–antigen reaction and competitive immunoreactions. The process was shown in Figure 5. The reaction principle was that if BADGE was absent from the sample, the AuNPs labelled antibody would bind to the coating antigen at the T zone, making the T zone become red, and it was determined to be negative results. When the samples contained BADGE, it would compete with coating antigen on T zone to combine with AuNPs labelled antibody. The higher the concentration of BADGE in the samples, the weaker the color of the T zone. The concentration of coating antigen and AuNPs labelled antibody in the lateral flow immunoassay were optimized. As shown in Figure 6 (a), the color of the T zone gradually deepened with the increase of the concentration of the coating antigen. When reaching 20 μg/mL, the color was no longer significantly deepened. Therefore, 20 μg/mL was selected for the next experiment. As shown in Figure 6 (b), when the volume of AuNPs labelled antibody reached 20 μL, the color did not significantly deepen, so 20 μL was selected as the optimal amount.

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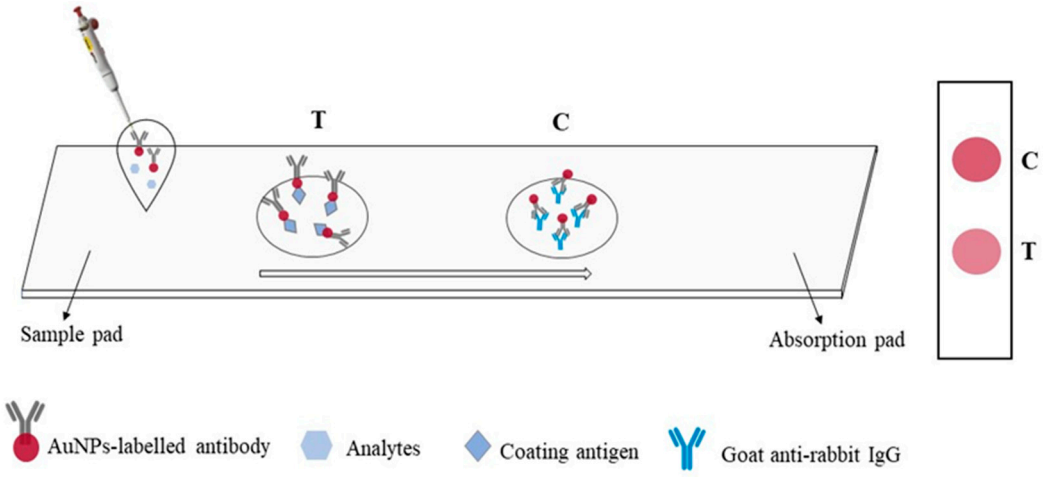

Figure 5. Schematic diagram of AuNPs lateral-flow immunoassay.

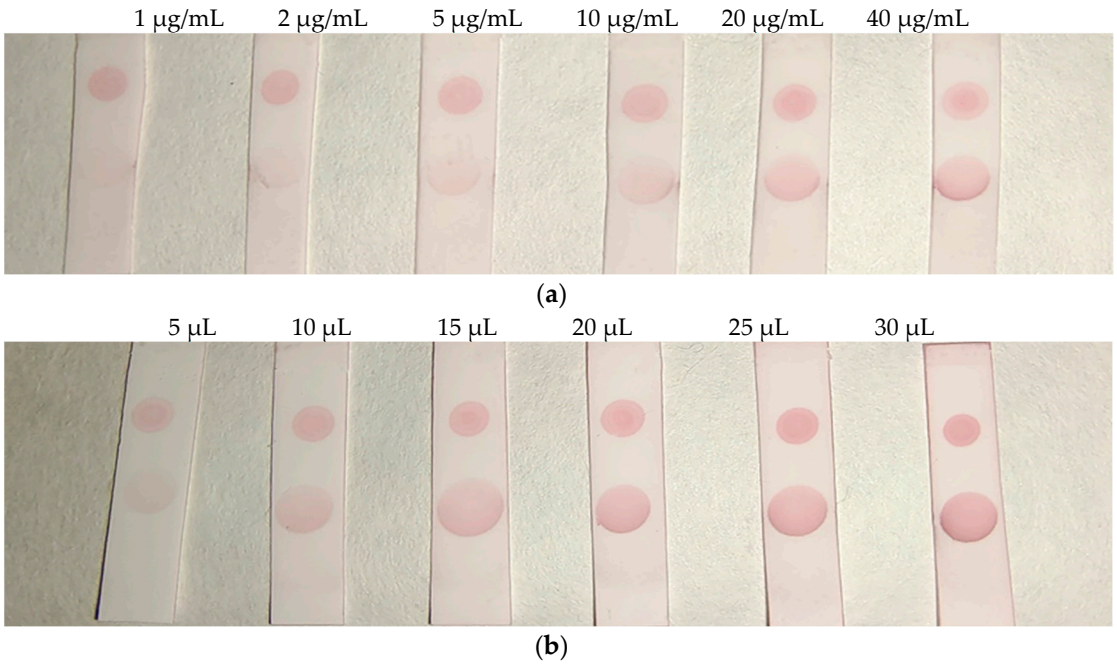

Figure 6. (a) Optimization of concentration of coating antigen. (b) Optimization of amount of AuNPs-labelled antibody.

Under the optimal conditions, the AuNPs labelled antibody was mixed with different concentrations of BADGE and added to the strip, as shown in Figure 7 (a), the higher the concentration of BADGE, the lighter the color of T zone. When the concentration of BADGE reached 1 ng/mL, the color of T zone can be distinguished from the negative sample, so the visual detection limit (vLOD) was 1 ng/mL.

Color intensities were quantified by Adobe Photoshop CC software, the grayscale variation of T zone was shown in Figure 7 (b) and the standard curve was drawn by calculating inhibition ratio Figure 7 (c). The calculated limit of detection (cLOD) was 0.97 ng/mL (IC_{15}, concentration at inhibition rate of 15%), which was in general agreement with the visual results.

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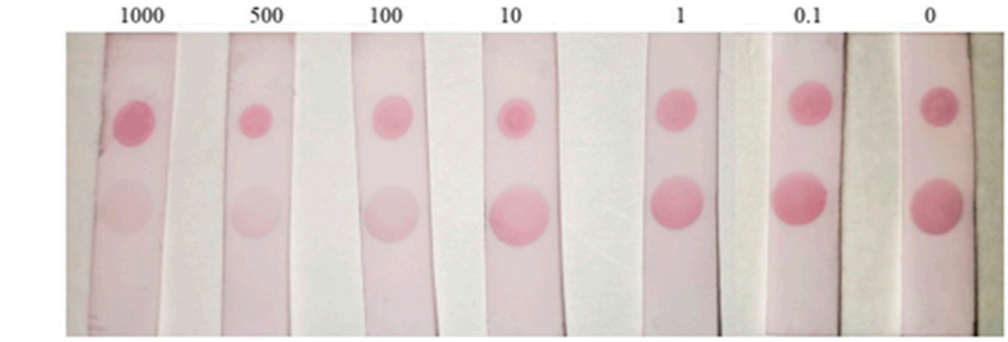
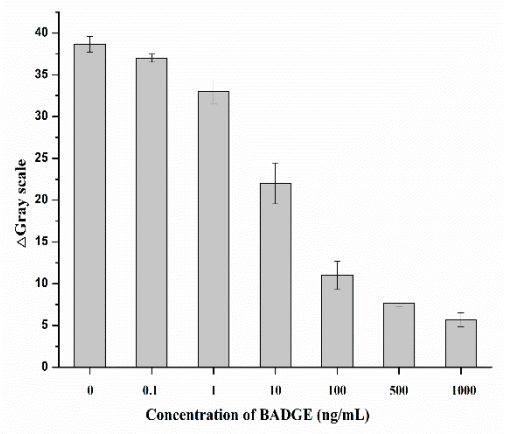
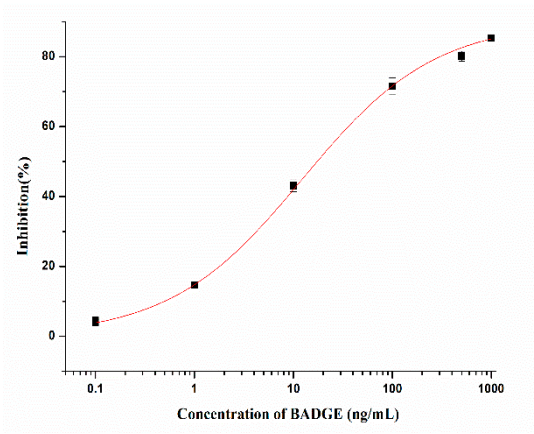


(a)



(b)



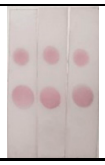
(c)

Figure 7. (a) Visual results of AuNPs lateral-flow immunoassay. (b)Grayscale variation of T zones with different concentrations. (c) Quantitative analysis of the visualization results for BADGE detection.

2.5. Stability analysis of AuNPs-PAb immunochromatographic strip

The test strips were stored at 4℃ for 1, 3, 5 and 7 days, and the color was observed by immunochromatographic reaction with 1 ng/mL of BADGE standard solution, and the results are shown in Table 2. There was no significant change in the color of the T region, which indicates that the test strips have good stability.

Table 2. Stability analysis of AuNPs-PAb immunochromatographic strip.

Storage time（Day）			
1	3	5	7
			

2.6. Matrix effect

In the analysis of the actual sample, the matrix may interfere with the accuracy of detection. Therefore, in order to eliminate the influence of matrix on the detection performance, the samples were diluted at different multiples (5-fold, 10-fold, 20-fold, 40-fold) and then analyzed by the ic-ELISA. As shown in Figure 8, the sample curve almost overlapped with the standard curve by 40-fold dilution with 10% methanol-PBS (V/V). Therefore, samples could be diluted 40-fold and analyzed by the immunochromatographic method.

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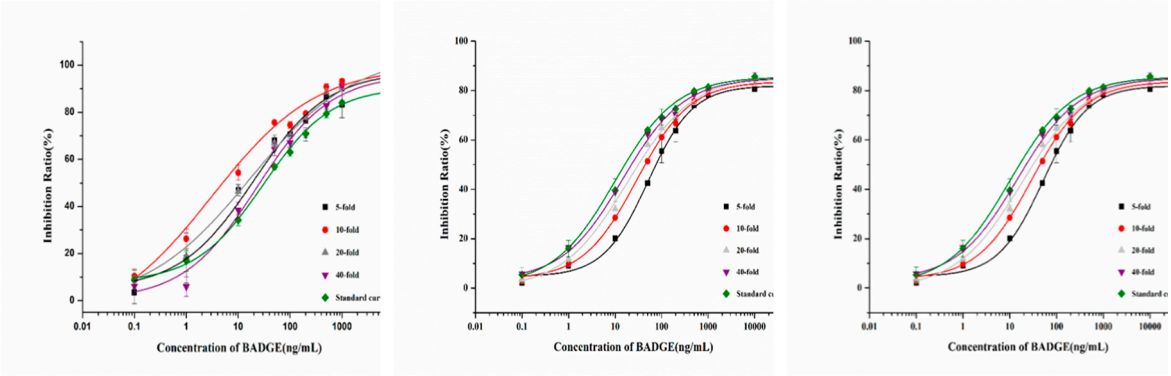

Figure 8. The optimization of dilution factor of the sample extracting solution. (a) Canned luncheon meat. (b) Canned yellow peach. (c) Red bull drink.

2.7. Recovery Experiments

Spiking and recovery tests with BADGE were performed by the immunochromatographic method and HPLC (Table 3). The recovery rate of BADGE in canned luncheon meat, canned yellow peach and red bull drink samples ranged from 79.86% to 93.81%. These results were basically the same as the HPLC results (Figure 9, R^2=0.9580), showing that the immunochromatographic method had high accuracy and reliability.

Table 3. Recoveries of BADGE and its derivatives in spiked samples by AuNPs lateral-flow immunoassay and HPLC (n=3).

Sample	Spiked conc.(ng/g)	HPLC		AuNPs lateral-flow immunoassay	
		mean ± SD (ng/g)	Recovery (%)	mean ± SD (ng/g)	Recovery (%)
Canned luncheon meat	50	45.28±0.02	90.56	45.67±1.66	91.35
	100	95.71±0.10	95.71	79.86±3.51	79.86
	250	247.56±0.11	99.02	209.10±5.59	83.64
Canned yellow peach	50	43.30±0.03	86.60	44.10±2.60	88.20
	100	96.93±0.09	96.93	91.69±6.94	91.69

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	250	243.95±0.74	97.58		213.76±5.27	85.50
	50	46.97±0.03	93.94		43.17±3.78	86.35
Red bull drink	100	94.82±0.11	94.82		87.35±3.97	87.35
	250	231.41±0.50	92.56		234.54±6.48	93.81

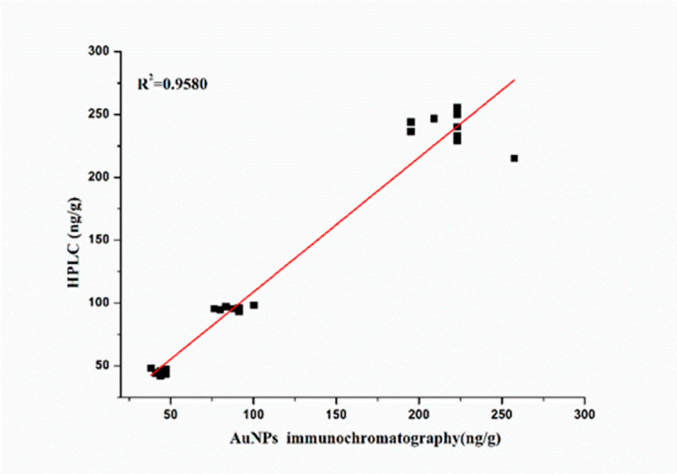

Figure 9. Correlation analysis between AuNPs lateral-flow immunoassay and HPLC.

2.8. Sample analysis using the immunochromatographic strip assay

The immunochromatographic strip assay and HPLC were used to detect the concentration of BADGE and its derivatives in different kinds of canned samples to assess the practicability of the established method. Because of BADGE and its derivatives may simultaneously exist in actual samples, we have detected the total amount of BADGE and its derivatives by the proposed immunochromatographic strip. As shown in Table 4, the total amount of BADGE compounds in samples determined by immunochromatographic strip was consistent with that by HPLC. It is proved that the immunochromatographic strip assay can be used to detect real samples.

Table 4. Detection of BADGE and its derivatives in samples by AuNPs lateral-flow immunoassay and HPLC (n=3).

Sample	BADGE and its derivatives	HPLC		AuNPs lateral-flow immunoassay	
		mean ± SD (ng/g)	Total amount	Detection of concentration mean ± SD (ng/g)	Actual concentration

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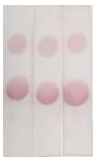
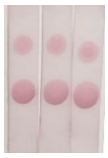
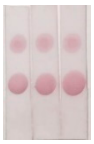
							mean ± SD (ng/g)
Canned luncheon meat	1	BADGE	45.03±0.83	130.06		3.14±5.44	125.72
		BADGE·HCl	85.03±0.36				
		BADGE·H_2O	ND				
		BADGE·HCl·H_2O	ND				
	2	BADGE	39.00±0.80	110.76		2.57±1.09	103.69
		BADGE·HCl	71.76±0.19				
		BADGE·H_2O	ND				
		BADGE·HCl·H_2O	ND				
Canned yellow peach	3	BADGE	47.85±0.39	102.11		2.48±0.84	99.42
		BADGE·HCl	ND				
		BADGE·H_2O	54.25±0.31				
		BADGE·HCl·H_2O	ND				
	4	BADGE	61.39±0.25	144.78		3.41±1.72	136.50
		BADGE·HCl	ND				
		BADGE·H_2O	83.42±0.07				
		BADGE·HCl·H_2O	ND				
Red bull drink	5	BADGE	50.54±0.77	176.92		4.27±2.64	170.81
		BADGE·HCl	ND				
		BADGE·H_2O	126.37±0.82				
		BADGE·HCl·H_2O	ND				
	6	BADGE	56.49±2.16	193.68		4.67±5.44	187.14
		BADGE·HCl	ND				
		BADGE·H_2O	137.18±0.62				
		BADGE·HCl·H_2O	ND				

3. Discussion

BADGE is used as an additive or starting agent in coatings for cans. It is commonly used to avoid direct contact between food matrix and metals in packaging materials. During the processing, storage and transportation, BADGE is prone to migrate to the food matrix and generate hydrated derivatives or chlorinated derivatives, which can accumulate in the human body through the food chain, causing abnormalities in the endocrine system and nervous system. Anna et al. [32] studied the toxicity and potential lipid destruction of BADGE in human placental JEG-3 cells, and found that it can interfere with lipid metabolism and alter the cellular lipidome, ultimately causing disease. Instrumental analysis is applied for the detection of BADGE and its derivatives. Gallo et al. [33] simultaneously determined eight kinds of bisphenol substances in soft drinks by liquid chromatography-fluorescence (LC-FD), including BADGE and BFDGE residues.

Due to the wide polarities of BADGE and its derivatives, conventional liquid chromatography requires complex sample pretreatment to complete the separation, which cannot meet the requirements of rapid detection. The traditional instrumental analysis method cannot realize the

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simple and rapid analysis of large numbers of samples, and it is difficult to real-time feedback the real migration residual pollution status.

Immunoassay based on antibodies or other biological molecules with recognition function have the advantages of rapid analysis process with simple pretreatments, which can be an effective supplementary method for conventional instrument analysis. In fact, immunoassay has been widely for the analysis of various pollutants in the field of food safety detection because of its reliability, efficiency and cost-saving. It has also been recommended by authoritative testing departments for rapid screening of large quantities of samples. Canned food has the advantages of convenience and nutrition, which is widely accepted and consumed in large quantities. Therefore, the health hazards caused by bisphenol diglycidyl ether compounds in packaging materials cannot be ignored. Based on the requirements of consumers for the safety monitoring of food in daily contact, long-term, multi-frequency, high-throughput sample analysis is needed to draw regular conclusions from a large number of analysis results, and immunoassay methods fully meet the requirements of such research. Guan [34] developed a fluorescence polarization (FP) assay for simultaneous monitoring BPA, BPF, BADGE, and BFDGE in canned tuna, and the detection limits were 0.35, 0.08, 0.10 and 0.49 mg/L, respectively. The results are not sensitive for the detection of BADGE, which may be the fact that the receptor cannot completely replace the biological antibody to achieve high affinity recognition to the target object. Due to the coexistence of BADGE and its derivatives, we developed broad-spectrum polyclonal antibodies that can recognize BADGE and its derivatives. In this study, a rapid immunochromatography method based on gold nanoparticles was established to detect BADGE and its hydrolyzed and chlorinated derivatives in canned food. The assay can be finished in 15 min and the visualization of results are processed by Adobe Photoshop CC software to achieve quantitative analysis and the detection limit (IC_{15}) is 0.97ng/mL. It can meet the requirements of real-time screening and detection of large quantities of samples.

4. Materials and Methods

4.1. Chemicals and Reagents

$HAuC_{l4}·4H_2O$, trisodium citrate dihydrate ($C_6H_5Na_3O_7$), sodium chloride (NaCl), potassium carbonate (K_2CO_3), disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4·12H_2O$), sodium phosphate dibasic dihydrate ($Na_2HPO_4·2H_2O$), H_2SO_4, potassium dichromate ($K_2Cr_2O_7$), Tween-20, ethyl acetate, hexane, acetonitrile, methanol were purchased from Sinopharm Chemical Reagent (Shanghai, China). The chemical standards of BADGE, BADGE·H_2O, BADGE·HCl and BADGE·HCl·H_2O were purchased from Macklin (Shanghai, China). Bovine serum albumin (BSA), non-fat milk powder and goat anti-rabbit IgG-HRP were purchased from Sangon Biotech (Shanghai, China). The canned luncheon meat, canned yellow peach and red bull drinks were purchased from local supermarket (Zhenjiang, China).

4.2. Instruments

HH-A magnetic stirrer was purchased from Zhongda instrument factory (Changzhou, China), UV-1801 ultra-violet spectrophotometer was purchased from Ruili Company (Beijing, China). HPLC was performed on a LC-20AD module (Shimadzu, Japan) coupled to ultraviolet detector.

4.3. Screening of broad-spectrum antibodies

Our research group has preliminarily screened four antisera which have certain recognition ability to BADGE and its derivatives in the preliminary experiment. The cross reactivity of four antisera to BADGE derivatives was investigated to determine the broad-spectrum recognition specificity of antisera. The cross-reactivity (%) was calculated as follows:

$$\text{Cross reactivity (\%)} = \text{IC50 (BADGE)} / \text{IC50 (other analogues)} \times 100$$

4.4. Preparation of AuNPs

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The AuNPs were produced by the sodium citrate method. Briefly, 100 mL 0.01 % HAuCl4 solution was heated and kept boiling. Then, 4 mL of 1% sodium citrate was quickly added under stirring, the solution was heated and stirred for 5~10 min until the color was stable. The obtained AuNPs solution was cooled to room temperature and stored at 4°C.

4.5. Preparation of AuNPs-labelled antibody

4.5.1. Optimization of pH

AuNPs with different pH were adjusted by adding different volumes of K_2CO_3 solution. Excess amounts of antibodies were added under each condition and stood for 10 min at room temperature, then 10% NaCl was added and vortex mixing. After standing for 10 min, the color was observed and scanned by UV-vis spectroscopy.

4.5.2. Optimization of the amounts of antibody

10 tubes of AuNPs solution were taken in glass tube, the pH were adjusted with 0.2M K2CO3 solution. According to Table 5, different amounts of antibody was added to AuNPs solution and stood for 10 min at room temperature, then 10% NaCl was added and vortex mixing. After standing for 10 min, the color was observed and scanned by UV-vis spectroscopy.

10 mL AuNPs with the optimal pH were adjusted by 0.2M K_2CO_3 solution, the antibody was added to the following solution, and then mixed. The mixture reacted at 4℃ for 3 h, and then 10% BSA was added to block the excess binding sites on AuNPs for 1 h. After the reaction, the solution was centrifuged at 12000 rpm for 20 min, the precipitation was re-dissolved in 1 mL PB solution containing Tween-20 and BSA and stored at 4℃ for use.

Table 5. Optimization of the amounts of antibody.

Number	0	1	2	3	4	5	6	7	8	9
Antibody（μg）	0	0.37	1.49	3.36	5.98	9.35	13.51	18.33	23.93	30.32
AuNPs（mL）	1	1	1	1	1	1	1	1	1	1
10%NaCl（μL）	100	100	100	100	100	100	100	100	100	100

4.6. Establishment of AuNPs immunochromatographic method

In this work, NCM film with smooth backing was used to prepare immunochromatographic strip, including sample zone, test zone (T), control zone (C) and absorbent zone. The NCM films were cut to certain size and the test zone was coated with coating antigen, the control zone was coated with goat anti-rabbit IgG. Then, the strip was dried at room temperature and stored at 4℃. A certain volume of AuNPs labelled antibody was mixed with 100 μL BADGE standard solution at different concentrations and incubated for 10 min. The mixture was then added to the sample zone, after 15 minutes, the liquid flows completely through T and C zones. The visualization results were recorded by taking photos with smart phone, and the results of T zone were processed by Adobe Photoshop CC software to realize quantitative analysis.

$$\text{Inhibition (\%)} = (\triangle G - \triangle G1)/(\triangle G) \times 100\%$$

G: grayscale variation without BADGE; G_1: grayscale variation of BADGE with different concentration.

4.7. Sample analysis

The canned luncheon meat, canned yellow peach and red bull drinks were selected for sample analysis, which obtained from a local supermarket of Zhenjiang. Canned luncheon meat samples were treated as follows: 2.0 g of samples were homogenized with 10 mL hexane and treated with ultrasonic assisted extraction for 30 min. Then the mixture centrifuged at 4500 rpm for 10 min. The

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supernatant was extracted with 5 mL acetonitrile twice. The acetonitrile extracts were evaporated to dryness at 40°C under nitrogen. After that, the residues were re-dissolved in 2 mL methanol. Canned yellow peach samples were treated as this method: the sample of 2 g was weighed and 5 mL of ethyl acetate was added as extraction solvent. The mixture was shaken for 20 min in the shaker and 30 min in an ultrasonic bath. Then the mixture centrifuged at 4500 rpm for 15 min. The supernatant was evaporated to dryness under nitrogen stream. Then, the extract was re-dissolved in 2 mL methanol. 5 mL red bull drinks were centrifuged at 4500 rpm for 10 min, the supernatant was filtered by 0.22 μm membrane before analysis. The samples were separately spiked with BADGE at different concentrations (2, 10, 20 ng/mL) to evaluate the accuracy of the AuNPs immunochromatographic method, and the results were confirmed by HPLC simultaneously.

The Hypersil GOLD C_{18} column (4.6 mm×250 mm, 5 μm) was employed for chromatographic separation in HPLC analysis. The mobile phase was composed of 40% ultrapure water and 60% acetonitrile. The eluent flow rate was 0.5 mL/min, the injection volume was 10 μL and the temperature of the column oven was maintained at 30°C.

5. Conclusions

To summarize, in this study, we screened a broad-spectrum polyclonal antibody that can recognize BADGE and its derivatives including BADGE·HCl, BADGE·H_2O and BADGE·HCl·H_2O. A lateral flow immunochromatographic assay for detecting BADGE and its derivatives in canned food was developed by coupling the antibody with AuNPs, and the visualization results were processed by Adobe Photoshop CC software to achieve quantitative analysis. The method was applied to the actual sample detection and compared with the results of HPLC, it was proved that the method was reliable and accurate. In addition, the immunochromatographic strip assay could meet the requirements of rapid screening of large quantities of samples in the market because of its advantages of simple, rapid operation, cost-effectiveness and non-instrumental.

Author Contributions: Q.Y. and C.Z. constructed the project; C.Y., Q.Y. and C.Z. designed the experiments; C.Y., J.H. and Y.Z. performed the experiments; C.Y., C.Z. and W.W. analyzed the data; C.Y. wrote the paper. All authors have read and agreed to the published version of the manuscript.

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