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Development of a Direct Competitive Enzyme-Linked Immunosorbent Assay for Quantitation of Sodium Saccharin Residue in Food

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Abstract

Sodium saccharin is a common artificial sweetener. However, due to its possible carcinogenic effects and causing metabolic disorders, many countries have strictly regulated its use in food. In the study, we prepared a specific monoclonal antibody (mAb 2H11) using the new hapten (6-carboxylsaccharin) and developed a direct competitive enzyme-linked immunosorbent assay (dcELISA) for the screening of sodium saccharin residue in food. The half-maximum inhibition concentration (IC_{50}) and working range (IC_{20}–IC_{80}, the concentrations causing 20% and 80% inhibition by sodium saccharin) were 32.5 and 6.47–164 ng/mL, which was 6.5 times more sensitive than the previously reported immunoassay. The average recoveries of sodium saccharin in spiked food samples detected by dcELISA ranged from 82.1% to 117%. Among 70 food samples bought in the physical stores and online, sodium saccharin residues were only detected in 4 samples purchased online (1 canned pineapple, 2 winter jujube and 1 kimchi). The content measured by dcELISA agreed well with those determined by ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS). The developed dcELISA was proved to be a sensitive and accurate method for determining sodium saccharin in food.

Keywords

sodium saccharin; mAb; dcELISA; food additives

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Author Contributions

Zhao YJ and Wang M designed this study. Zhang M and Yang XL performed experiemntal analyses and discussed all data. Li ZF, Vasylieva N and Tan GY handled statistical analyses. Wang BM and Hammock BD wrote this manuscript.

Conflicts of Interest

All authors declare no conflicts of interest.

Practical Application: Quantitation of sodium saccharin residue in food is very necessary and important for consumers and regulatory agencies.

1 INTRODUCTION

Sodium saccharin was first synthesized in 1879. It is approximately 200–500 times sweeter than sucrose and helpful in controlling body weight and insulin level as it has no calories (Gong et al., 2016). However, because of its possible carcinogenic effects (Altschul, 1993; Khan, 1993), the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) stipulated that the concentration of saccharin in foods should not exceed 2.8 g/kg (Li & Fang et al., 2018). In the United States, European Union and China, the maximum residue level (MRL) of sodium saccharin was also set in different kind of foods (U.S. Food and Drug Administration, 2018; Nation Health Commission of the People's Republic of China, 2014; The European Parliament and the Council of the European Union, 2006). What's more, the frequent consumers of these sugar substitutes might be at increased risk of excessive weight gain (Fowler et al., 2008), metabolic syndrome (Duffey, Steffen, Van Horn, Jacobs, & Popkin, 2012), type 2 diabetes (Guy et al., 2013), and cardiovascular disease (Swithers, 2013). But the illegal and excessive use of sodium saccharin in foods was still reported from time to time. In China, some unscrupulous traders immersed immature winter jujubes in sodium saccharin solution to increase their sweetness and sold them in the market. In September 2015, sodium saccharin residue was detected in 2 winter jujube samples at levels of 0.05–0.22 g/kg in Hainan Province, China ("Sodium saccharin residue," 2015). In European Union, a study showed that one of the 48 soft drinks and nectars detected in Portugal exceeded the maximum permitted level (100 mg/L) for sodium saccharin according to the European Union and Portuguese legislation (Lino & Pena, 2010). In the United States, although artificial sweeteners were legally available, a survey showed that most parents (72%) disagreed with the statement "NNS are safe for my child to consume." (Sylvetsky, Greenberg, Zhao, & Rother, 2014).

In 2019, the study of Azeez et al. showed that long-term sodium saccharin consumption would increase the risk of obesity, diabetes, hepatic dysfunction, and renal impairment in rats (Azeez, Alkass, & Persike, 2019). Health effects and safety aspects of food are very important issues for consumers, and many people are concerned about safety of sodium saccharin (Grembecka, Baran, Błażewicz, Fijałek, & Szefer, 2014). So, it is necessary to develop detection methods for sodium saccharin. There are many instrumental detection methods for sodium saccharin in foods, such as high-pressure liquid chromatography (Holder & Bowman, 1980), liquid chromatography tandem mass spectrometry (Ens, Senner, Gygax, & Schlotterbeck, 2014; Ordóñez, Quintana, Rodil, & Cela, 2012), spectrophotometry (Ni, Xiao, & Kokot, 2009), ultra-performance liquid chromatography tandem mass spectrometry (Chen, Zhao, Shen, & Jin, 2012; Ma, Li, Wang, & Zhao, 2015), ambient flame ionization coupled with triple quadrupole tandem mass spectrometry (Li & Zhang et al., 2018). They all need expensive equipment and professional operators; and those methods are time consuming and not suitable for high-throughput detection (Wang et al., 2019). At present, ELISA plays an important role in food safety and environmental pollution monitoring (Zhang et al., 2016). In 2011, Wang et al. prepared a polyclonal antibody (pAb) against sodium saccharin and developed an indirect competitive enzyme-linked immunosorbent assay (icELISA) for the detection of sodium saccharin in foods. The 50% inhibition value (IC_{50}) and workable range (IC_{30}–IC_{70}) were 0.24 μg/mL and

0.05–12.8 μg/mL, respectively (Wang et al., 2011). However, the reported immunoassay only performed the sodium saccharin detection in the spiked samples and the sensitivity of the icELISA may not be sufficient for the analysis of low-content sodium saccharin residue.

In the research, we prepared a specific monoclonal antibody (2H11) with a novel hapten. Two different kinds of dcELISA formats, direct-coating mAb 2H11 and precoating goat anti-mouse antibody, were developed and compared, and finally a sensitive dcELISA for the high-throughput quantitation of sodium saccharin residue in food was established.

2 Materials and Methods

2.1 Reagents and Apparatus

Cell culture medium (Dulbecco's modified Eagle's medium, DMEM) and fetal bovine serum (FBS) were purchased from Gibco BRL (PaisLey, Scotland). The sodium saccharin and its analogues, goat anti-mouse IgG, 3,3',5,5'-Tetramethylbenzidine (TMB), goat anti-mouse IgG-horseradish peroxidase (IgG-HRP), and PEG2000 were purchased from Sigma (Shanghai, China). BALB/c mice were obtained from the Laboratory Animal Center of the Institute of Genetics (Beijing, China). SP2/0-Ag14 (HAT sensitive mouse myeloma cell line) was provided by College of Veterinary Medicine, China Agricultural University. The other reagents were purchased from J&K China Chemical Ltd (Beijing, China). The microplate reader and direct heat CO_2 incubator were purchased from Thermo (Franklin, MA, USA). 96-well polystyrene microtiter plates were purchased from Costar (Corning, NY, USA).

2.2 Preparation of Sodium Saccharin Hapten

The hapten (6-carboxylsaccharin) was synthesized from *p*-xylene according to the Scheme 1. Ten grams of *p*-xylene was added into 30 g $ClSO_3H$ by dropwise, then the mixture was stirred for 5 hours at room temperature and poured into 100 mL ice-water. The white solid appeared was collected and washed by water, then dried under vacuum to give compound A (White solid, yield: 98%). Five grams of compound A was dissolved in 50 mL tetrahydrofuran under stirring, while ammonia gas (prepared by adding 25% concentrated ammonia droplets to solid NaOH) was continuously introduced into the solution for 15 minutes. Then the mixture was poured into 200 mL water and extracted by ethyl acetate, the organic phase was dried and concentrated to give crude product, which was finally recrystallized by EtOH/Water (the volume ratio was 1:1) to give compound B (White crystals, yield: 80%). One gram of compound B was dissolved in 10 mL MeCN, then 9.8 g periodic acid and 81 mg CrO_3 were added into the solution, the mixture was stirred and refluxed for 0.5 h. After filtered with a sand core funnel, the filtrate was concentrated to give the crude product, which was recrystallized by EtOH to give compound 6-carboxylsaccharin (White powder, yield: 20%). ^{1}H NMR data of 6-carboxylsaccharin (300 MHz, MeOD): δ 8.59–8.49 (m, 2H), 8.14 (dd, J = 7.7, 0.9 Hz, 1H). ^{13}C NMR of 6-carboxylsaccharin (75 MHz, MeOD): δ 164.90, 159.58, 139.80, 137.01, 134.77, 130.52, 124.56, 121.27. HRMS calcd for 6-carboxylsaccharin $C_8H_5NO_5S$: [M - H^+] 225.9816, found 225.9814. The other sodium saccharin haptens (TJ-H2, TJ-H3 and TJ-H4) were synthesized according to the previous report (Ascenzio et al., 2014).

2.3 Preparation of Coating Antigen, Immunogen and Enzyme-labeled Conjugate

The protein conjugates were synthesized via active ester method as previously described (Cao et al., 2014). Five milligrams of the resulting hapten, 6.9 mg of N-hydroxysuccinimide and 12.4 mg of dicyclohexylcarbodiimide were dissolved in 0.6 mL of N,N-dimethylformamide and magnetically stirred at 25°C for 6 hours. The supernatant was divided into three parts and added to the protein solutions (10 mg of bovine serum albumin (BSA), ovalbumin (OVA) and horseradish peroxidase (HRP) in 5 mL of carbonate buffer, 50 mM, pH 9.6). The reactions were stirred overnight at 4°C, and then dialyzed 4 times with phosphate-buffered saline (PBS) (0.1 M phosphate buffer containing 0.9% NaCl, pH 7.5) and 2 times with ddH_2O at 4°C. The dialyzed solutions were lyophilized into powder and stored at −40°C. The conjugates of 6-carboxylsaccharin–OVA, 6-carboxylsaccharin–BSA and 6-carboxylsaccharin–HRP were used as coating antigen, immunogen and enzyme-labeled conjugate, respectively.

2.4 Monoclonal Antibody Production and Characterization

The immunization protocol and mAb production were the same as described previously (Zhang et al., 2016). The immunogen was mixed with an equal amount of complete (primary immunization) or incomplete Freund's adjuvant and emulsified, then the emulsified immunogen was injected into the abdominal cavity (0.1 mL) and back (0.05 mL at two points) of 6 female BALB/c mice (7 weeks old). One week after the third immunization, mouse antisera collected from the retrobulbar plexus were tested for anti-sodium saccharin antibody titer and sodium saccharin recognition properties by icELISA (supporting information 2). Seven days after the fourth immunization, the mouse with the best titer and inhibition was selected for booster immunization (intraperitoneal injection of 0.1 mL 6-carboxylsaccharin-BSA conjugate in PBS, 1 mg/mL). Three days after the booster immunization, PEG-2000 was used to fuse mouse spleen cells with SP2/0-Ag14 cell line at a ratio of 10:1. The hybridoma cells were spread in five 96-well plates (Corning, NY, USA) and cultured at cell incubator (37°C and 5% CO_2) for one week. After screening the supernatant by icELISA, the best performing hybridoma cell line was cloned by limiting dilution method. The resulting clones were further selected by icELISA. The selected hybridoma cell line was inoculated into the abdominal cavity of six 7-week-old BALB/c female mice. The collected ascites were purified by the saturated ammonium sulfate precipitation method, and then dialyzed 4 times with PBS and 2 times with ddH_2O at 4°C. The dialysis solution was lyophilized and stored at −40°C. The class and subclass of mAb were determined using a mouse antibody isotyping kit (Pierce, Rockford, IL, USA). The specificity of mAb was evaluated by cross-reactivity with sodium saccharin and other analytes in dcELISA. Cross-reactivity (%) = (IC_{50} of sodium saccharin / IC_{50} of other compound) ×100.

2.5 dcELISA

A comparison of two different dcELISA formats based on the immobilization of either anti-sodium saccharin antibodies or goat anti-mouse to the microplate was performed.

Format I (Precoating with Goat Anti-Mouse IgG). The microplate was coated with 200 μL of 1 μg/mL goat anti-mouse IgG in coating buffer (0.05 M carbonate buffer, pH 9.6) at 4°C

overnight. After washing the plate with PBST (Tween-20 diluted in PBS, 0.1% (v/v)) three times, 100 μL of sodium saccharin standard solution (250, 125, 62.5, 31.3, 15.6 7.8, 3.9 and 0 ng/mL) or analytes, 50 μL of sodium saccharin-HRP (0.25 μg/mL) and 50 μL of mAb 2H11 (1 μg/mL) in PBSTG (PBS with 0.1% (v/v) Tween-20 and 0.5% (w/v) gelatin) were added to each well.

Format II (Direct Coating). The microtiter plate was coated directly with 200 μL of 1 μg/mL mAb 2H11 in coating buffer at 4°C overnight. After washing the plate with PBST three times, 100 μL of sodium saccharin standard solution (250, 125, 62.5, 31.3, 15.6 7.8, 3.9 and 0 ng/mL) or sample analytes and 100 μL of sodium saccharin-HRP (0.25 μg/mL) in PBSTG were added to each well.

After incubating at 4°C for 1 h, the plates of two formats were washed again as before. Then 200 μL of substrate solution (4.0 mg of urea peroxide added to 10.0 mL of citrate-phosphate buffer (0.01 M citric acid and 0.03 M monosodium phosphate, pH 5.5) containing 0.1 mg/mL TMB) was added. Thirty minutes later, the reaction was terminated by adding 50 μL of the stop solution (1 M HCl) per well, and the absorbance at 450 nm was read with the microplate reader. The calibration curve data were imported into OriginPro 8.5 (OriginLab) and fit to a sigmoidal logistical equation for calculation.

2.6 Sample Collection and Fortification for Recovery Tests

The food samples were purchased at supermarkets around University of California, Davis (CA 95616, United States), China Agriculture University (Beijing, China) and Chinese e-commerce platforms. A total of 7 kinds of food samples (raspberry sauce, pepsi, orange juice, yogurt, canned pineapple, kimchi and winter jujube) were purchased, 10 items of each type, including 5 items purchased in physical stores and 5 items purchased online, the detail information of food samples was shown in Table S2. After crushing, 1 g (fresh weight) sodium saccharin-free food sample (determined by UPLC-MS/MS) was placed in 10 mL centrifuge tube. Fifty microliters of 2.5, 5, 10 μg/mL sodium saccharin standard solution in PBSTG were added and vortexed for 1 minute to obtain fortification levels of 125, 250 and 500 ng/g, respectively. The spiked samples were placed at 4°C overnight prior to extraction. Four millilitres of ethyl acetate, 1 mL of saturated saline and 0.2 mL of 1 M HCl were added and then vortexed for 2 min. After centrifugation for 5 min at 2898 *g*, 1 mL of the supernatant was collected into two 10 mL centrifuge tubes and dried under a gentle stream of nitrogen gas at 40°C. One was dissolved in 2.5 mL PBS for dcELISA, the other was dissolved in 2.5 mL 70% methanol and filtered with 0.22 μm membrane prior to UPLC-MS/MS analysis.

2.7 UPLC-MS/MS Analysis of Sodium Saccharin

Sodium saccharin was detected with ACQUITY UPLC system (Waters Corp., MA, USA) interfaced to a QTRAP6500 triple quadrupole-linear ion trap mass spectrometer (AB SCIEX Corp., MA, USA). The chromatographic column was ACQUITY UPLC BEH C18 column (2.1×100 mm, 1.7 μm). The mobile phases were 100% methanol (A) and 0.1% acetic acid-water (B), the elution condition was gradient elution, 0–3 min, the proportion of mobile phase A was increased from 40% to 50%, then kept at 50% in the next two minutes.

The flow rate of mobile phase was 0.4 mL/min, and the injection volume was 5 μL. The multiple reaction monitoring mode (MRM) chromatogram of sodium saccharin was shown in Figure S1. The ionization mode of the mass spectrometer was ESI, which adopted the negative ion MRM for quantitative analysis. The ionization temperature was 500°C. The optimized condition parameters were shown in Table S1. The linear correlations between the detection results of UPLC-MS/MS and dcELISA were run in Excel 2010, and the significance analyses of correlation were performed in IBM SPSS Statistics 19.

3 Results and Discussion

3.1 Characteristics of mAb 2H11

The best hybridoma cell line (2H11) was selected from 45 hybridoma cell lines by icELISA. After intraperitoneal injection, all six mice produced ascites. After purification, dialysis and lyophilization, a total of 300 mg of mAb 2H11 was obtained. The type of mAb 2H11 was IgG2a with κ light chain. The cross-reactivity of mAb 2H11 with 6-carboxylsaccharin, 6-nitrosaccharin, saccharin, 6-aminosaccharin and acesulfame was 260%, 163%, 124%, 81.7% and 0.53%, respectively. No competitive inhibition was observed up to 45000 ng/mL of other analytes (Table 1).

It has been discussed in numerous articles, that the structure of the immunizing hapten has a great influence on the affinity and specificity of the antibody, which in turn determines the performance of the immunoassay (Goodrow, Sanborn, Stoutamire, Gee, & Hammock, 1995). At present, there was only one report on immunoassay for sodium saccharin in foods with an IC_{50} of 243 ng/mL (Wang et al., 2011). The hapten used in the report was 6-Aminosaccharin (Scheme 2A), which was coupled with BSA by diazotization to obtain an immunogen. Since the formyl sulfonimide functional group has a strong electron-absorbing effect, the lone pair electrons of the nitrogen atom in the amino group will be transferred to the electron withdrawing group. Therefore, the amino group on 6-aminosaccharin is actually in an electron-deficient state, which is not conducive to the diazotization reaction (Dechristopher, Adamek, Lyon, Klein, & Baumgarten, 1974). So it is difficult to control the coupling ratio between the hapten and the carrier proteins and the quality of the immunogen. This may be the reason for the low sensitivity of the reported antibody. In this study, a carboxyl group was introduced to the benzene ring of saccharin to obtain the hapten (6-carboxylsaccharin) (Scheme 2B), which could be easy to couple with carrier proteins by activating the carboxyl group of the hapten. The 6-carboxylsaccharin avoided the deficiencies of the hapten reported in the previous work, and the IC_{50} value of dcELISA was 6.5 times lower than previously reported. In addition, different antibody types (monoclonal vs polyclonal) and ELISA formats (dcELISA vs icELISA) might also contribute to the difference in detection sensitivity.

The cross-reactivity results showed that mAb 2H11 could recognize sodium saccharin, saccharin, 6-carboxylsaccharin, 6-nitrosaccharin, 6-aminosaccharin and acesulfame with IC_{50} of 32.5, 26.2, 12.5, 19.9, 39.8 and 6138 ng/mL, respectively. Since 6-carboxylsaccharin, 6-nitrosaccharin and 6-aminosaccharin were neither metabolites of sodium saccharin (Bassoli & Merlini, 2003) nor used in foods (“The use of seven pesticides,” 2013), they would not affect the determination of sodium saccharin residue in

food. And the cross-reactivity of mAb 2H11 with acesulfame was only 0.5%, the probability of false positive results caused by acesulfame was low. The common feature of the above compounds is that they all contain a sulfonyl sulfonimide functional group. However, mAb 2H11 can't recognize another kind of saccharin derivative, such as TJ-H2, TJ-H3 and TJ-H4. A common feature of these compounds is that the hydrogen on the nitrogen atom was replaced by an alkyl group. Therefore, the formyl sulfonimide functional group on the saccharin may be the recognition site of mAb 2H11 (Scheme 2C). In addition the aromatic ring may also be important in their determination of the recognition site of the hapten. Acesulfame, without the aromatic ring shows only a low cross-reactivity although there are other structural differences in this chemical which could be responsible. In summary, the synthesis of the saccharin hapten should derive from the phenyl ring and retain the intact formyl sulfonimide functional group.

3.2 Optimization of dcELISA

Assay formats greatly influence important parameters such as sensitivity and absorbance (Schneider & Hammock, 1992). In the study, format I resulted in lower IC_{50} and about 2–3-fold increased absorbance at 450 nm compared to the format II (Figure 1). The possible reason was that the antigen binding site of mAb 2H11 was more fully exposed after captured by goat anti-mouse antibody, and the competitive reaction in format I was more sufficient. And in 2008, Zhu et al. thought that the reason might be that all the reactants of the competitive reaction were in the liquid phase for the second antibody coated format, while not for the directly antibody coated format (Zhu et al., 2008). Finally, format I was selected for the further analysis.

The standard sigmoidal inhibition curve of dcELISA for sodium saccharin was established using the optimum reagent dilutions based on monoclonal antibody 2H11 (Figure 2). The sigmodial curve regression equation was y = 0.078 + 0.924/(1 + (x/25.445)^1.018), with a correlation determination (R^2) of 0.9995. The inter-assay and intra-assay coefficient of variation was 1.44%–9.42% and 0.18%–3.33%. The IC_{50} and detection range (IC_{20}–IC_{80}) were 32.5 ng/mL and 6.47–164 ng/mL, respectively.

3.3 Analyses of Sodium Saccharin in Spiked Food Samples

Elaborated sample pretreatment and proper dilution were common method to minimize matrix interference in the immunoassay (Zhang et al., 2016). In this work, the sample solution was diluted 10-fold (0.25 g-2.5 mL), which effectively decreased the matrix effect on the dcELISA. The average recoveries of sodium saccharin in food samples detected by dcELISA and UPLC-MS/MS ranged from 82.1% to 117% and 74.9% to 121%, respectively (Table 2). The detection results of dcELISA were consistent well with those of UPLC-MS/MS, and the correlation was significant (p=1.98E-11). The correlation coefficient (R) was 0.954, showing that dcELISA was accurate for monitoring sodium saccharin residues in food samples (Figure 3).

3.4 Analyses of Sodium Saccharin in Different Food Samples

The food samples were serially numbered according to the purchased time and place. Sodium saccharin residue was detected by dcELISA and UPLC-MS/MS after sample

treatment (Table 3). All the samples bought in the physical stores were negative, but sodium saccharin residue was found in four samples bought online. The content in No.3 kimchi sample was 843 and 753 ng/g detected by dcELISA and UPLC-MS/MS, which didn't exceed sodium saccharin MRL in pickled vegetables of China (0.15 g/kg). Sodium saccharin was not allowed in canned pineapple and winter jujube in China, but 548, 122 and 51.4 ng/g were detected in No.6 canned pineapple, No.7 and No.10 winter jujube samples by dcELISA and 395, 90.5 and 50.5 ng/g by UPLC-MS/MS, respectively. Figure 3 shows that the UPLC-MS/MS data correlated well with those from dcELISA, (R, 0.989), and the correlation was significant. Nowadays, online shopping is becoming more and more popular, which brings a lot of convenience to people's life but does make the food safety supervision much more difficult. The dcELISA developed in the study was suitable for the rapid detection of sodium saccharin in food.

4 Conclusion

In the present work, we prepared a sodium saccharin monoclonal antibody (mAb 2H11) based on a novel hapten (6-carboxylsaccharin) and developed convenient and sensitive dcELISA for the analysis of sodium saccharin residue in food. The dcELISA showed an IC_{50} and working range of 32.5 and 6.47–164 ng/mL, respectively. And the fortification tests of sodium saccharin in blank food samples by dcELISA had average recoveries of 82.1% to 117%. In the detection of actual food samples, four of the 70 food samples were found to be positive, and the four food samples were all purchased online, while no sodium saccharin residue was detected in the samples purchased in the physical stores, indicating that the online food safety supervision must be improved and enhanced. The detection results of dcELISA were consistent with those of UPLC-MS/MS. Compared with UPLC-MS/MS, dcELISA was a more economical and convenient choice. The developed dcELISA was sufficient for high-throughput monitoring of residual sodium saccharin in food.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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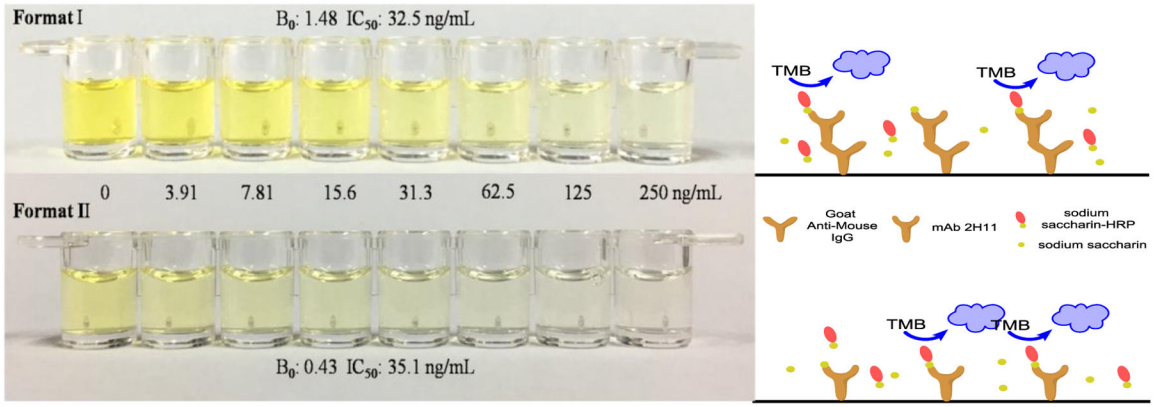


Figure 1-

Color development of standard inhibition curve for sodium saccharin and schematic diagram in two formats of dcELISA analysis. B_0 was the absorbance at 450 nm in the absence of sodium saccharin.

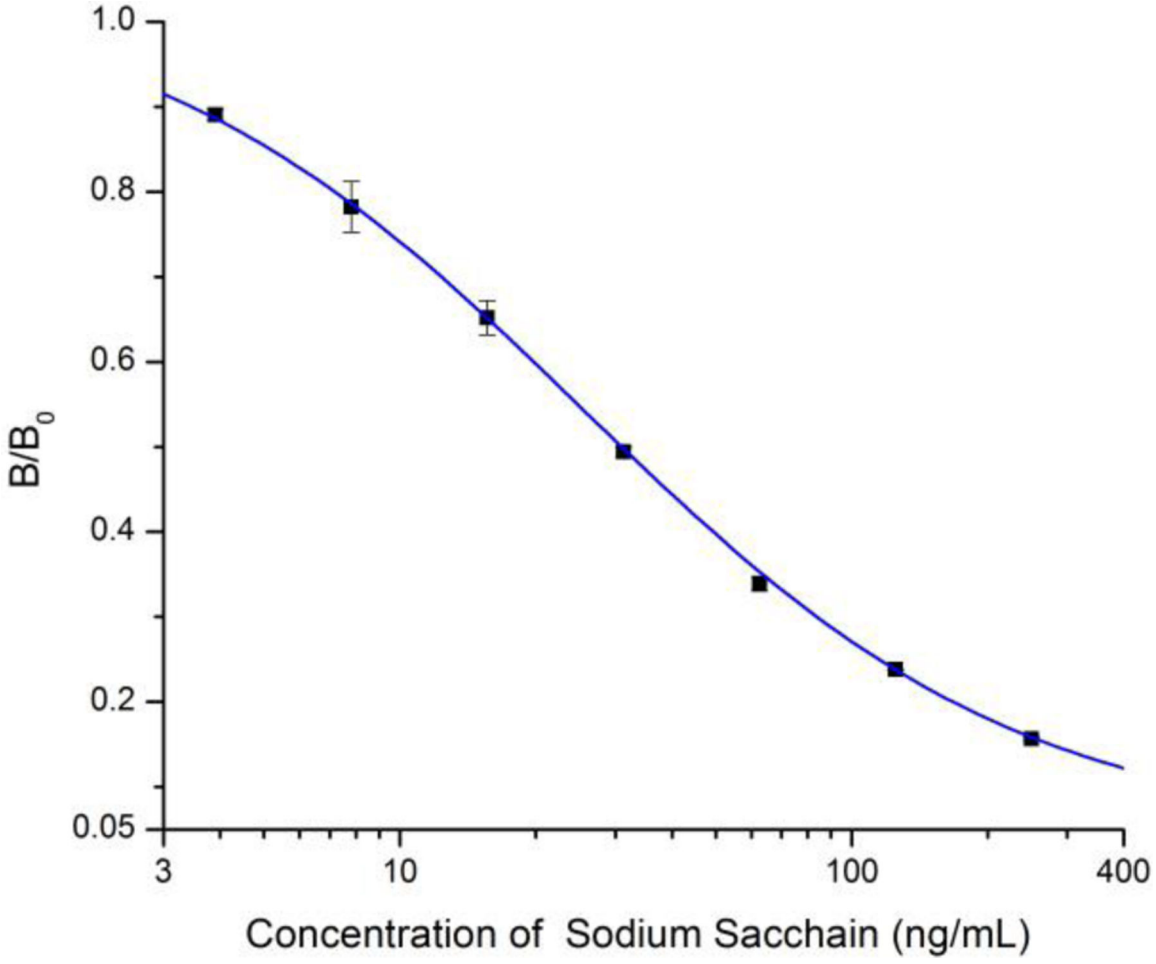


Figure 2-

Standard inhibition curve of sodium saccharin in dcELISA. Each value represented the mean of three replicates. B_0 and B were the absorbance at 450 nm in the absence and presence of sodium saccharin, respectively.

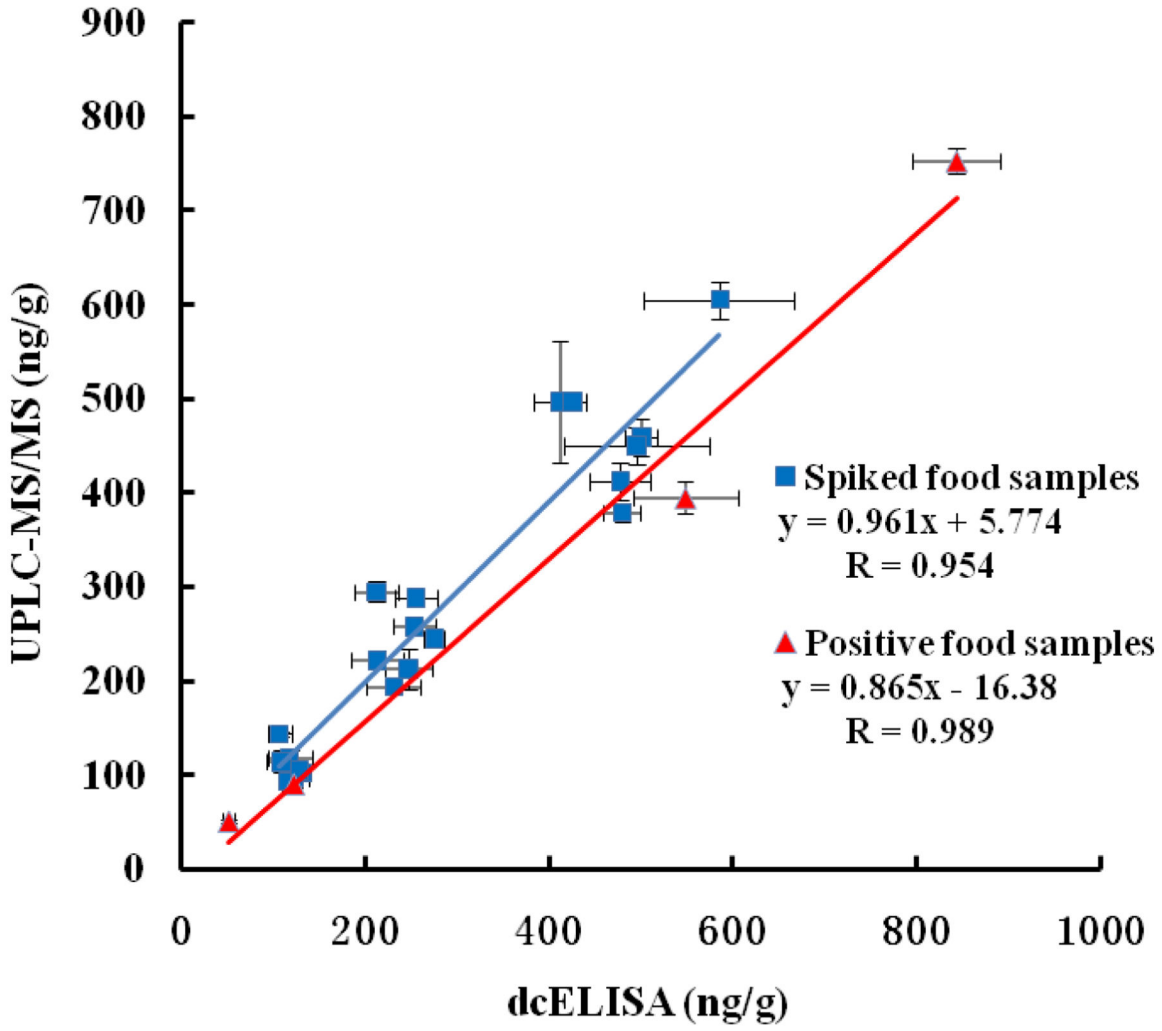
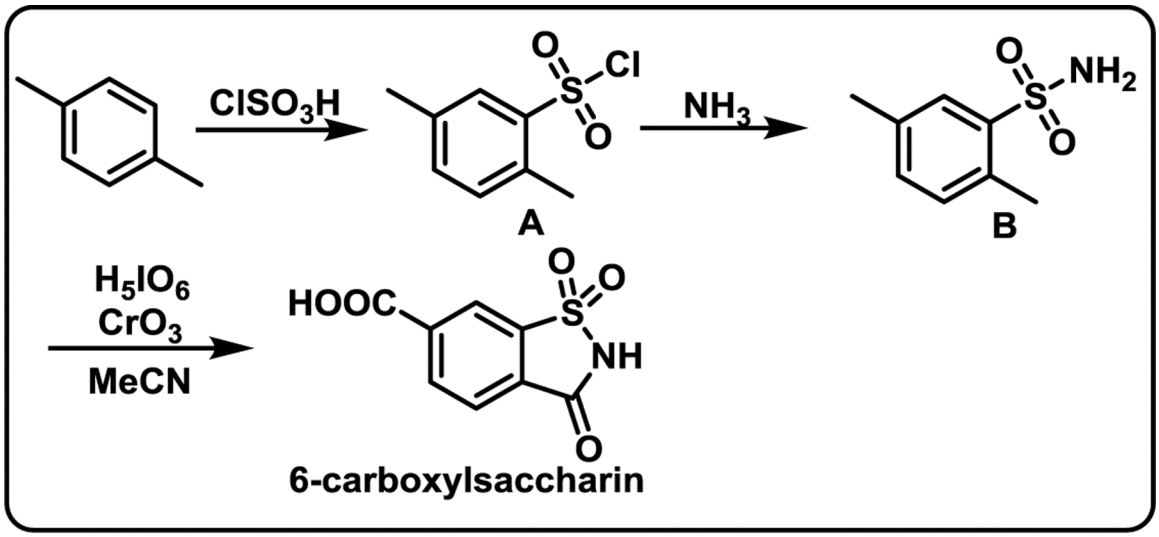
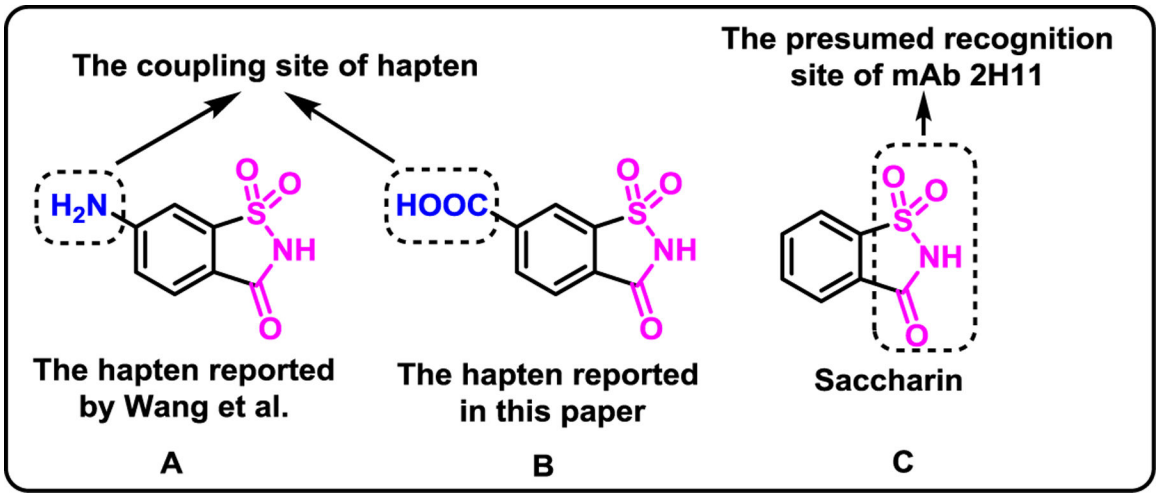


Figure 3-
Correlation analysises between dcELISA and UPLC-MS/MS for spiked food samples (data in Table 2) and positive food samples.



Scheme 1-
Synthetic route of the hapten (6-carboxylsaccharin).

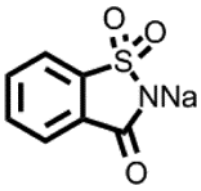
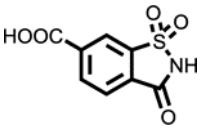
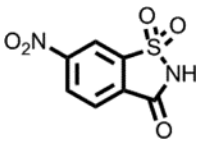
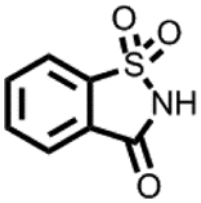
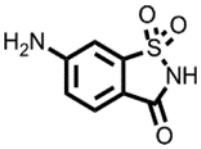
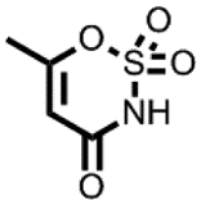
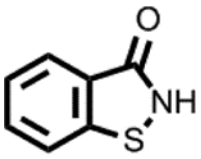


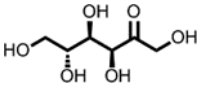
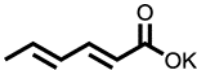
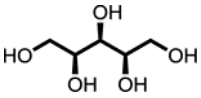
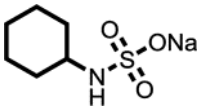
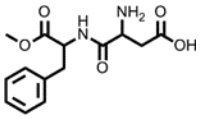
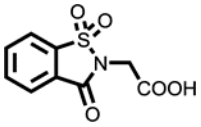
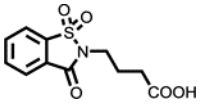
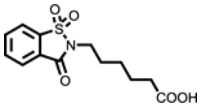
Scheme 2-

(A) The hapten of saccharin reported by Wang et al (B) The hapten of sodium saccharin reported in this paper (C) The presumed recognition site of mAb 2H11.

Table 1-

Cross-reactivity of sodium saccharin monoclonal antibody to sodium saccharin and other substances.

Analyte	Structure	IC_{50} (ng/mL)	Cross-reactivity[a] (%)
Sodium saccharin		32.5 ± 0.3[b]	100
6-Carboxylsaccharin		12.5 ± 0.4	260
6-Nitrosaccharin		19.9 ± 0.6	163
Saccharin		26.2 ± 0.9	124
6-Aminosaccharin		39.8 ± 1.0	81.7
Acesulfame		6138 ± 209	0.5
Benzo[d]isothiazol-3(2H)-one		NI[c]	0

Analyte	Structure	IC_{50} (ng/mL)	Cross-reactivity[a] (%)
D(−)-Fructose		NI	0
Potassium sorbate		NI	0
Xylitol		NI	0
Sodium cyclamate		NI	0
Aspartame		NI	0
TJ-H2		NI	0
TJ-H3		NI	0
TJ-H4		NI	0

[a]Relative cross-reactivity (%) = (IC_{50} of sodium saccharin / IC_{50} of other substances) ×100.

[b]Data represented means of triplicate samples ± standard deviations.

[c]No inhibition was observed up to 45000 ng/mL of the analytes.

Table 2-

Average recoveries of sodium saccharin in food samples by dcELISA and UPLC-MS/MS.

Category	Spiked concentration (ng/g)	Mean recovery (%, n = 3)	
		Detected by dcELISA	Detected by UPLC-MS/MS
Canned Pineapple	125	105±2.7	81.2±2.3
	250	84.9±9.7	118±4.4
	500	82.1±5.7	99.3±12.9
Raspberry Sauce	125	98.2±11.5	77.8±2.4
	250	85.3±11.2	88.9±3.9
	500	100±3.6	91.7±3.9
Kimchi	125	93.8±8.0	74.9±9.0
	250	92.4±11.7	77.7±1.3
	500	95.8±4.1	75.8±2.1
Pepsi	125	94.0±18.5	94.4±6.9
	250	101±9.3	103±1.8
	500	84.9±0.9	99.4±1.4
Yogurt	125	87.2±13.4	91.5±9.4
	250	98.7±10.0	85.0±8.7
	500	98.9±15.9	89.9±3.9
Orange Juice	125	85.8±10.9	115±2.1
	250	102±9.0	115±1.5
	500	117±16.4	121±4.1
Winter Jujube	125	103 ± 1.3	84.1±1.4
	250	110 ± 4.3	97.9 ±3.6
	500	95.3 ± 6.5	82.3 ±4.0

Table 3-

Sodium saccharin residue in food samples detected by dcELISA and UPLC-MS/MS.

Category	Number	Concs of sodium saccharin (ng/g)	
		dcELISA[a]	UPLC-MS/MS[a]
Raspberry Sauce	1–10	0	0
Pepsi	1–10	0	0
Orange Juice	1–10	0	0
Yogurt	1–10	0	0
Canned Pineapple	1–5,7–10	0	0
	6	548±56.8	395±16.7
Kimchi	1,2,4–10	0	0
	3	843±48.0	753±12.9
Winter Jujube	1, 2–6, 8, 9	0	0
	7	122±5.7	90.5±3.5
	10	51.4±6.7	50.5±1.4

[a]Data were the means of triplicate samples ± standard deviations.