Gold immunochromatographic assay for simultaneous detection of sibutramine and sildenafil in slimming tea and coffee

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Weight gain and obesity are worldwide problems prompting a search for many health supplements that provide benefits such as revitalization, skin-beautification, visual improvement, and slimming effects, mainly based on herbal formulations as an alternative treatment. Obesity may be defined as decreased physical activity, energy intake greater than energy expenditure, poor eating habits, neurogenic abnormalities, and genetic factors that have negative impact on the quality of life [1–3]. Sibutramine (SB) is one of the most widely used herbal formulations or dietary supplements used for weight loss. It has also been used to treat polycystic ovary syndrome, and usually found in anorexic drugs used in herbal slimming [4–6]. Sildenafil (SID) is a phosphodiesterase inhibitor and commonly used for erectile dysfunction treatment. It acts by blocking phosphodiesterase 5 (an enzyme that promotes the breakdown of cyclic guanosine monophosphate), which increases blood flow. Common side effects of SID include headaches, heartburn and flushed skin [7–9]. The use of SB and SID as additives in commercial foods and their unregulated intake are illegal and can affect human health and even threaten the lives of consumers [10,11]. Therefore, an effective and rapid method for detection of them is needed to guarantee the quality of functional foods and to protect consumers effectively.

Several techniques have been used to detect SB and SID such as gas chromatography [12–15], high performance liquid chromatography [16–18], and liquid chromatography-tandem mass chromatography [19–22]. Critically, these methods need time and expert personnel to run the analyses, require cleaning procedures, and they are of low

sensitivity and expensive. Furthermore, these methods do not meet current legislative requirements [23–26]. Alternatively, immunoassays are popularly used because they have high sensitivity and specificity, and are less time-consuming and inexpensive. The determination of SID in functional food using an enzyme-linked immunosorbent assay (ELISA) has been developed [27], which is useful for the analysis of multiple samples. Even though ELISAs have high sensitivity, specificity, ease of use and low cost, they require long incubation times and multiple steps for the enzyme reactions, which are unsuitable for on-site detection [28–31].

The recently developed immunochromatographic or lateral flow immunoassay (LFIA) test strips are popular for detecting various analytes, including drugs, pesticides, toxins, and antibiotics [32–34]. LFIA tests are performed by spraying all reagents onto the strips, and thus they are easy to use, sensitive, specific and rapid, which represent a major advantage over other methods (results can be seen within 5–10 min) [35,36]. To our knowledge, no LFIA has been developed to determine SB, though it is currently used for rapid screening of SID in functional foods [37]. In this study, we aim to develop an LFIA to simultaneously determine SB and SID in slimming tea and coffee.

For the LFIA, gold nanoparticles (AuNPs) were firstly prepared, and then combined with monoclonal antibody (mAb). Briefly, a solution of HAuCl_4 (10 mmol L^{-1} , 12.5 mL) made with ultrapure water (487.5 mL, filtered through a membrane $0.22 \mu\text{m}$) was boiled and constantly stirred for 15 min. Then, 1 mL of sodium citrate tribasic dihydrate (10%, w/v) was added and stirred until a red-

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wine color developed (after 30 min). After this, the solution was cooled to room temperature, and the obtained AuNPs (Fig. 1) were stored at 4°C before antibody labeling. Purified anti-SB and anti-SID mAbs were produced by our laboratory, and used for the preparation of the colloidal gold (CG)-labelled mAbs. Briefly, a certain amount of K_2CO_3 (0.1 mol L^{-1}) was added into the CG solution (10 mL) to adjust the pH to 7.0. Then, 0.4 mL of purified mAb, adjusted to 0.2 mg mL^{-1} with borate buffer (0.02 mol L^{-1}), was added to the CG, and the mixture left for 35 min at room temperature. Free CG was blocked with 1 mL of 10% (w/v) bovine serum albumin (BSA) for 2 h at room temperature. After the blocking step, the solution was centrifuged for 45 min at $6266 \times g$ at 4°C to remove the unconjugated antibody. After centrifugation, the supernatant was removed, and 1 mL of resuspension buffer (0.02 mol L^{-1} Tris-HCl, 0.1% Tween-20, 0.1% poly(ethylene glycol) (PEG), 1% polyvinylpyrrolidone (PVP), 5% sucrose, 4% trehalose, 2% sorbitol, 1% mannitol, 0.04% NaN_3 , and 0.2% BSA) was added. The suspension of CG-mAb conjugate was stored at 4°C.

A schematic representation of the assay is shown in Fig. 2, including the capture and control reagents used to spray onto the nitrocellulose (NC) membrane, plastic backing plate (polyvinyl chloride, PVC), conjugate pad with CG-mAb, sample pad, and absorbent pad (all pasted onto a plate). The coating antigen hapten-SID-BSA (test line 1, T1), hapten-SB-OVA (test line 2, T2) and the

Figure 1 Characterization of colloidal AuNPs (15 nm) by using transmission electron microscopy.

control line (C, goat anti-mouse IgG antibody) were sprayed onto the NC membrane ($0.9 \mu\text{L cm}^{-1}$), representing the two test lines and the control. After being sprayed onto the NC membrane, the capture and control reagents were dried at 37°C overnight. Then the plate was cut into narrow strips (2.9 mm wide) by using a strip cutter.

Due to the stability and the charge of the conjugation,

Figure 2 Schematic and illustration of typical strip test results.

the pH may affect its performance. In this study, BSA (10%, 1 mL) was used to stabilize the CG-antibody conjugate and reduce the nonspecific background. The experimental condition, including concentration of each antibody (8 and 10 $\mu\text{g mL}^{-1}$) and amount of K_2CO_3 (4 and 8 $\mu\text{L mL}^{-1}$ added in the AuNP solution), was optimized. After optimization, K_2CO_3 (0.1 mol L^{-1} , 4 $\mu\text{L mL}^{-1}$ added) and 8 $\mu\text{g mL}^{-1}$ of the concentrated antibody were used for further experiments. While developing this assay, choosing the appropriate surfactant was very important because of incompatibility issues between the surfactant and protein. In this study, the mAb-AuNPs were diluted five times with the suspension buffer (0.02 mol L^{-1} Tris-HCl, 500 mL) containing 5% sucrose, 0.1% PEG, 0.1% Tween-20, 5% trehalose, and 0.2% BSA, mixed with six different types of reagents: Tween-20, PVP, PEG, BSA, Brij-30, and ON-870 (each concentration was 5%), respectively, and sprayed onto the conjugate pad. After optimization, based on the color intensity at 0 ng mL^{-1} (control) and cut-off (500 ng mL^{-1}) using phosphate buffer saline (PBS, 0.01 mol L^{-1} , pH 7.4) (Fig. 3a), the most stable and intense deep red color on the test line was seen by using the surfactant ON-870. Different concentrations of the two analytes were mixed with PBS (0.01 mol L^{-1} , pH 7.4). Fig. 3b shows that a negative sample (0 ng mL^{-1}) had a deep color and the color be-

came weaker when a higher concentration of analyte was added, and completely disappeared at 500 ng mL^{-1} of SB and SID.

The sensitivity and specificity of the LFIA were evaluated by determining each analyte separately, to ensure no interaction between the two. The results, shown in Fig. 4, demonstrate that there is no interference between the anti-SB and anti-SD, suggesting that the coating antigen is specific. The difference in structure between the two analytes is the reason for the absence of interference. This LFIA could also be used as a multi-component strip because of its efficiency and capability of determining two different kinds of analytes.

Three types of slimming tea and three types of coffee (free from SB and SID) were obtained from a local market. Approximately 1 g of the sample was added into a 15-mL polypropylene centrifuge tube containing 5 mL of ethanol, and vortexed for 10 min. Then, the sample was treated by ultrasound in an ultrasonic bath for 20 min. It was then centrifuged at 6226 $\times g$ for 10 min at room temperature, and the supernatant was filtered through a 0.22- μm polytetrafluoroethylene membrane. Finally, the supernatant (1 mL) was added to 1 mL of PBS containing 0.5% Tween-20 (PBST) and the samples were spiked with a solution of SB and SID (100 $\mu\text{g mL}^{-1}$, diluted in methanol). Each concentration of the spiked sample was analyzed six times. The spiked SB and SID concentrations were 0 (control), 10, 25, 50, 100, 250, and 500 ng mL^{-1} . We optimized the extraction method by using 10%–50% ethanol. As shown in Fig. 5a, extraction using 50% ethanol demonstrates the best color intensity

Figure 3 (a) Optimization of the experimental condition: results using six kinds of reagents for SB and SID. 1, Tween-20; 2, PVP; 3, PEG; 4, BSA; 5, Brij-30; and 6, ON-870, detected by using 0.01 mol L^{-1} PBS (pH 7.4) with 0 ng mL^{-1} (A) and 500 ng mL^{-1} (B). (b) LFIA for detection of the two analytes in 0.01 mol L^{-1} PBS (pH 7.4). (1–8) represent concentrations of 0, 10, 25, 50, 100, 250, 500 and 1000 ng mL^{-1} . C, control line; T1, SID; and T2, SB.

Figure 4 Typical photo images for detection of the single analyte, SB or SID. C, control line; T1, SID; and T2, SB.

Figure 5 (a) Optimization of ethanol concentration in the extraction samples. A, 10%; B, 20%; C, 30%; D, 40%; and E, 50%. SB and SID concentration: 1, 0 ng mL^{-1} ; 2, 500 ng mL^{-1} . (b) Results for determination of analytes in the samples. (1–7) represent concentrations of 0, 10, 25, 50, 100, 250 and 500 ng mL^{-1} . C, control line; T1, SID; and T2, SB.

in the control and the two test lines. A standard solution of SB and SID was spiked for each sample ($100 \mu\text{g mL}^{-1}$, prepared with methanol) and the final SB and SID concentrations were 10, 25, 50, 100, 250, and 500 ng mL^{-1} (six replicates). Fig. 5b shows that the color becomes weaker with increasing SB and SID concentration. Vigorous color intensity could be seen with naked eye on the test line when the concentration of analyte was 0 ng mL^{-1} , and the color became weak when a higher concentration of analyte was added, and finally it completely dis-

appeared at 500 ng mL^{-1} of SB and SID for each sample.

In summary, this study aimed to develop a sensitive LFIA for the rapid detection of two different analytes (SB and SID) that may exist simultaneously in slimming tea and coffee. Three types of slimming tea and three types of coffee were tested, and all the results were seen within 5 min, by using semi-quantitative detection with naked eye. The cut-off value after optimization was 500 ng mL^{-1} for SB and SID in PBS (0.01 mol L^{-1} ; pH 7.4). These results demonstrate the potential of LIFA as an effective

and rapid method for simultaneous detection of multiple components in functional foods.

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Author contributions Xu C and Kuang H designed the experiments. Suryoprabowo S and Liu L performed the experiments. Cui G and Kuang H participated in data analysis. Xu C drafted the manuscript, and all authors read and approved the manuscript prior to submission.

Conflict of interest The authors declare that they have no conflict of interest.

Steven Suryoprabowo is a PhD candidate under the supervision of Prof. Chuanlai Xu at Jiangnan University. His research interests focus on developing rapid and sensitive methods to determine analytes using immunochromatography strip test.

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胶体金免疫层析分析方法用于同时检出减肥茶和咖啡中西布曲明和西地那非

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摘要 本文建立了一种方便、快速、高特异性的胶体金免疫层析检测方法, 用于同时检测减肥茶和咖啡中的西布曲明(SB)和西地那非(SID). 我们首先制备了胶体金纳米粒子(CG), 然后使之与单抗结合形成CG-mAb, 将CG-mAb与抗原分别喷涂到基板上形成结合垫和检测条带, 其中所需的抗原和抗体均由本实验室制备. 经过条件优化, 该方法在PBS缓冲溶液(0.01 mol L⁻¹, pH 7.4), 以及三种减肥茶和三种咖啡样本中, 对SB和SID的消线值均为500 ng mL⁻¹; 检测时间仅需5 min. 该方法对减肥茶和咖啡样本中SB和SID的快速检出具有实际应用价值.