THE FABRICATION OF TEST STRIP FOR SIBUTRAMINE HCl DETECTION IN SLIMMING TRADITIONAL HERBAL MEDICINE

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Received 30 November 2022
Accepted 30 January 2023

Abstract

The development of an optical sensor-based test strip for the detection sibutramine HCl adulteration in traditional herbal products has been studied. The medium of the test strip was cellulosic paper immobilized with Dragendorff's reagent and tetraethyl orthosilicate (TEOS) as the precursor using the sol-gel method. The presence of sibutramine HCl changed the color of the test strip from yellow to orange-red. The change in color was scanned and converted to RGB values using the ImageJ software. The intensity of the RGB value correlates with the concentration of the analyte. The performance of the test strip had good linearity in the range of 0,1-1,5 mM of sibutramine HCl, and the correlation coefficient (R) was 0,9872. The limit of detection, limit of quantification, precision (% RSD), and percentage of accuracy were 0,2 mM, 0,8 mM, less than 5%, and approximately 80-90%, respectively. The strip test is low cost and simple. Thus, it can be used as an alternative to detect sibutramine HCl in traditional herbal products.

Keywords*: optical sensor, sibutramine HCl, slimming herbal product, sol-gel method*

Introduction

Sibutramine hydrochloride is an anti-obesity agent widely used for weight loss. It can stimulate thermogenesis by producing norepinephrine and serotonin reuptake inhibitors that cause the body to produce heat and cause the tissue to be fat (Clapham *et al.*, 2002; Suneetha *et al*., 2011). Serotonin and dopamine inhibition make consumers feel full, which helps in the dietary process. Sibutramine HCl can be consumed at doses of 10-15 mg/day. However, it has adverse effects such as palpitations, insomnia, and cardiovascular disease, which are undoubtedly harmful to human health (Ouyang *et al*., 2017; Arterburn *et al*., 2003). Consequently, Badan Pengawas Obat dan Makanan (BPOM) withdrew sibutramine HCl from the Indonesian market in October 2010 (BPOM, 2010).

Herbal medicines contain active ingredients and phytochemical compounds from plants used for medicinal purposes. Because it is considered to have few side effects, it is widely consumed by the public in short time intervals. BPOM declared that, as synthetic chemical, sibutramine HCl should not be added to herbal medicine products. There are many reports of other synthetic chemical discoveries in herbal medicines, such as paracetamol in herbal pains, sildenafil citrate in herbal dietary products, mefenamic acid, and ibuprofen in analgesic traditional medicines (Rahmadani *et al*., 2021; Dural *et al*., 2020; Hayun *et al*., 2016; Sukmawati *et al*., 2021; Simaremare *et al.*, 2018). Thus, the detection of sibutramine HCl in herbal medicines is necessary to prevent the dangers of excessive drug use.

Previous methods for sibutramine HCl detection include UV spectroscopy (Chandokar *et al.*, 2008), HPLC (Diefenbach *et al.*, 2009), HPLC-MS

(Huang *et al*., 2008; Bhatt *et al*., 2007), potentiometry (Gohari *et al*, 2019), GC-MS (Sardela *et al*., 2009), thin layer chromatography (Salma *et al.*, 2022), RP-HPLC (Suthar *et al.*, 2009) and TLC-imaging (Phattanawasin *et al.*, 2012). These methods are sensitive and accurate but require complicated preparation and trained operators, and are quite expensive and, time-consuming for sample preparation and analysis. The other method is distance-based determination using a paper analytical device (Karamahito *et al.,* 2021). This method is low cost and easy to use for analyte sensing. However, measurement under large changes in ambient conditions can affect the velocity of the solution and alter the results (Cate *et al.*, 2013). Therefore, an accurate, efficient, and simple method for sibutramine HCl is required for the preparation of herbal products.

One alternative for sibutramine HCl detection is a colorimetric chemical sensor. Principal sensing is based on the chemical reaction between the analyte and reagent, which can form a color change in the presence of an analyte. The intensity of the color was related to the concentration of the analyte. Because colorimetric sensors are fast and easy to use in society, many researchers have applied them in any application (Liang *et al.*, 2021). Several researchers have reported β-lactam detection using colorimetric techniques using different reagents (Abedalwafa *et al.*, 2019), narcotics (Smith *et al.*, 201), pesticides (Qian and Lin *et al.*, 2015), bacteria (Carey *et al*., 2011), proteins (Xu *et al.*, 201), aminophylline (El-shabouri *et al.*, 1989), pH in aqueous solutions (Shojaefard *et al.*, 2022) and other analytes.

The colorimetric method was developed by dropping or immersing the reagent and analyte solution onto filter paper. The pores of the filter paper may cause reaction sensing, and color formation can be detected by the naked eye or other instruments. However, filter paper is less sensitive to the immobilization of volatile reagents and solutions such as Dragendroff's reagent; thus, the color formation is less clear. Furthermore, other techniques for good reagent immobilization on the test strip are needed to make the sensor more sensitive to detection without reducing reagent activity (Thohir *et al.*, 2022).

The sol-gel method has been successfully used in the manufacture of paper-based optical sensors. The most popular precursors for sol-gel synthesis are metal alkoxides such as tetraethoxysilane (TEOS) and tetramethoxysilane (TMOS). Porous silica gel allows the entrapment or immobilization of organic and inorganic reagents and acts as a transducer where the reaction and sensing analyte occur. In previous studies, sol gel-based test strips have been applied to different analytes, such as mercury in analgesic herbal medicine (Amaliyah *et al.*, 2021), tyrosine in human saliva (Vyas *et al.*, 2020), tyramine in vinegar (Qiao *et al.*, 2021), hydrazine (Beduk *et al.*, 2019), pH in cementitious materials (Inserra *et al.*, 2020; Othman *et al.*, 2016; Mohammad *et al.*, 2019) and volatile organic compounds (Duffy *et al.*, 2021).

Based on the facts mentioned above, this study was conducted to investigate sol-gel-based test strips for sibutramine HCl analysis. This test strip can be a fast, inexpensive, simple, and accurate alternative for consumers to determine sibutramine HCl. The recognition agent used was Dragendorff's reagent, which reacts with sibutramine HCl to form an orange-red color change. The performance of the sensor was evaluated based on the validation parameters and applied to slimming herbal products.

Research Methods

Materials

All chemicals were of analytical grade. Sibutramine HCl standard (BPOM),

Dragendorff's reagent p. a. (Merck), tetraethyl orthosilicate (TEOS) (Merck), methanol 96% p. a. (Merck), HCl 37% p. a. (Merck), distilled water, and Whatman paper no. 1 (GE Healthcare). Two brands of slimming traditional herbal medicines were used as samples, purchased from the local market (sample A from Madura and sample B from Semarang). Based on the product labels, the ingredients of the samples consisted of several types of herbs and did not contain sibutramine HCl.

Instrumentation

The instruments used in this research were FTS1000 Varian Fourier Transform Infrared Spectroscopy (FTIR), Flexsem 1000 type SU 1000 Scanning Electron Microscopy (SEM), EPSON L3250 scanner, hotplate Faithful, micropipette Dlab, and glassware.

Sol-Gel and Strip Test preparation

This method was similar to that reported by Thohir *et a.l* (2022). A mixture of 2 mL TEOS as the precursor and 2 mL methanol was stirred at room temperature for 30 min. Then, the mixture was added by 2 mL of Dragendorff's reagent, 683 µL of distillated water and 0,5 mL of HCl 3 M dropwise. The mixture was stirred for 5 h. Next, to prepare the medium for the immobilization sol-gel, 1 cm × 1 cm Whatman papers were immersed into the sol mixture and left for 24 h for hydrolysis and condensation. Later, the Whatman papers were dried at room temperature and left for two days to maximize wet gel aging before use.

Sibutramine HCl detection procedure

The bottom fabricated strip test was coated with an isolative to prevent reagents and analytes from entering the test zone and leakage through the device. The detection mechanism is illustrated in Figure 1. A volume of 10 µL of sibutramine HCl 0,1 to 1,5 mM was dropped onto the strip test. The sensing process was performed for 2 min until an orange color was formed. These color changes were scanned using an Epson Scanner, and the images were converted to RGB intensity using ImageJ software. The RGB color used to determine the concentration of sibutramine HCl was green because it was complementary to orange, which is a sign of the presence of sibutramine HCl. Later, the green intensity was subtracted from the blank intensity to obtain the Δintensity or absolute value (Nurrahmah *et al.*, 2022). The same procedure was used to measure sibutramine HCl in artificially slimmed herbal products. The concentration of the sample was calculated using the calibration curve of the sibutramine HCl standard. All analyses were performed in triplicate.

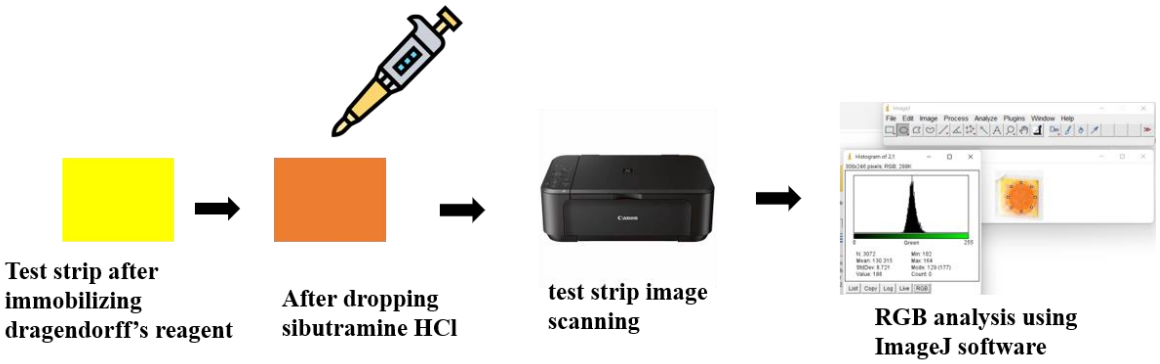

Figure 1. Mechanism of sibutramine HCl detection

Results and Discussion

Fabrication and characterization of test strip

Test strips were prepared using the sol-gel method. The precursors used were TEOS and methanol as solvent. In this method, hydrolysis and condensation occurred. Hydrolysis converts the Si-OR on TEOS into Si-OH in the presence of water and subsequently undergoes a polymerization process to form Si-O-Si. The reactions that occur are as follows (Chaudury *et al.*, 2007):

$$\text{-Si-OR} + H_2O \rightarrow \text{-Si-OH} + \text{ROH} \quad \text{(hydrolysis)} \qquad (1)$$

$$\text{-Si-OR} + \text{HO-Si-} \rightarrow \text{-Si-O-Si-} + \text{ROH} \quad \text{(alcohol condensation),} \qquad (2)$$

$$\text{-Si-OR} + \text{HO-Si} \rightarrow \text{-Si-O-Si-} + H_2O \quad \text{(water condensation)} \qquad (3)$$

In cellulosic media, gels that form Si-O-Si fill the cavities of cellulosic paper without forming chemical bonds or interactions (Thohir *et al.*, 2022). The aging time aims to separate the sol into wet gel and reduce cracks during the drying process. To produce the xerogel type, water and methanol are evaporated from the glass cavities, and the volume of the xerogel shrinks gradually (Lev *et al.*, 1995).

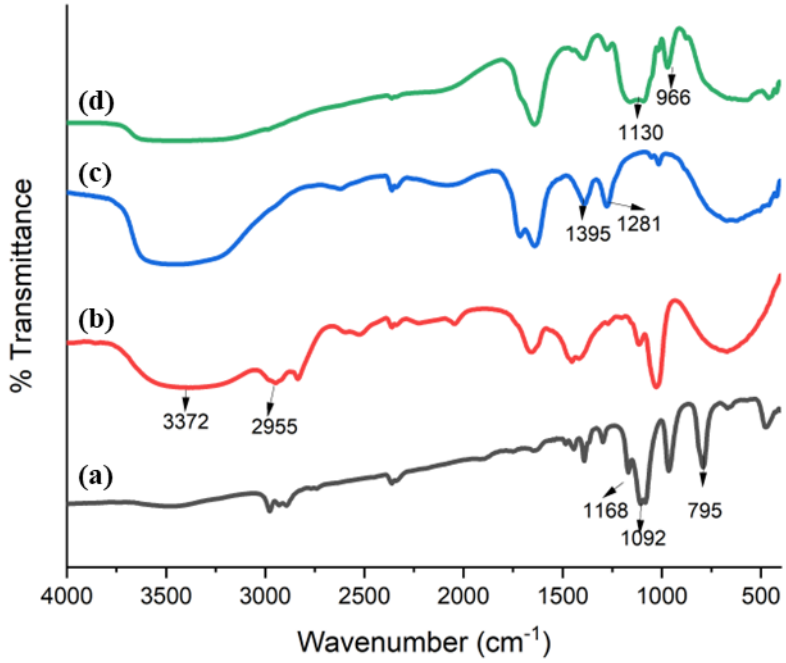

Figure 2. FTIR spectra, (a) TEOS; (b) methanol; (c) Dragendorff's reagent; and (d) wet gel

The sol-gel process can be studied using infrared spectroscopy, as shown in Figure 2. The TEOS spectra showed specific peaks. All the peaks located at 1168 cm^{-1}, 1092 cm^{1} and 798 cm^{-1} correspond to C-H crocking in CH_3 of TEOS, C-O stretching, and SiO_4 asymmetric stretching, respectively (Rubio, 1998). Figure 2b shows the methanol spectra, consisting of O-H stretching at 3372 cm^{-1} and asymmetric C-H stretching at 2955 cm^{-1}. Dragendorff's reagent spectra were observed at 1395 cm^{-1} and 1281 cm^{-1} which refer to the NO_3^- group from bismuth nitrate and Bi-OH, respectively (Astuti *et al.*, 2016).

Different bands of the synthesized wet gel followed the hydrolysis process. The wet gel spectra have some specific absorption peaks at 1130 cm^{-1} indicating the absorption of asymmetric Si-O-Si and 966 cm^{-1} indicating Si-O-Si stretching.

The decrease in the intensity of the TEOS spectra (1168 cm^{-1} and 1092 cm^{-1}) was accompanied by an increase in new bands in the wet gel spectra (Rubio *et al.*, 1998; Mujiyanti *et al.*, 2020).

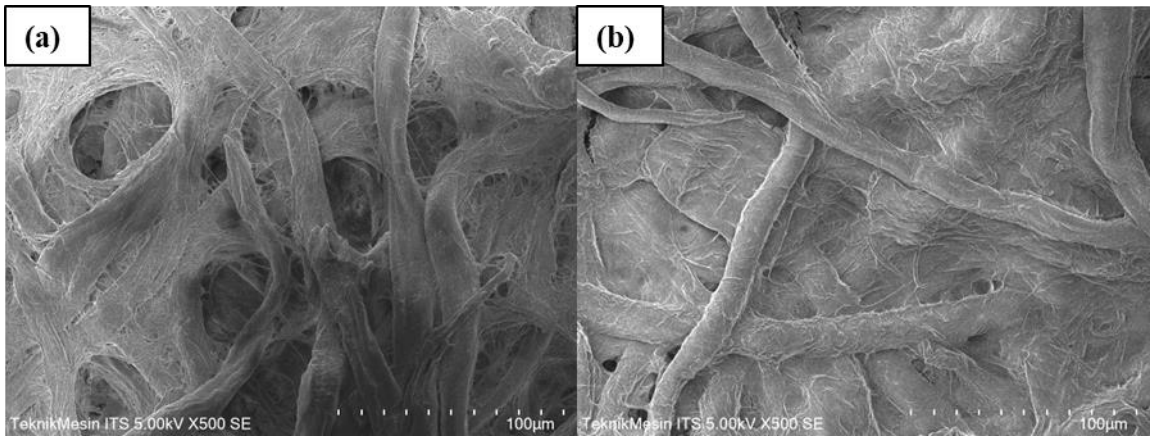

Figure 3. SEM image, (a) bare cellulosic paper; and (b) cellulosic paper after immobilization of reagent by sol-gel method

The characterization of the test strip was further reviewed based on the morphology using SEM analysis. Figure 3 shows the difference in surface between bare cellulosic paper and paper coated with xerogel. The bare cellulosic paper had large cavities. The cavities in the SEM image in Figure. 3b was covered with a xerogel (Thohir *et al.*, 2022). The results confirmed that the test strip was fabricated using the sol-gel method.

Qualitative and quantitative analysis

To sense sibutramine HCl qualitatively, Dragendorff's reagent was used as a chemical recognition agent. Dragendorff's reagent consists of bismuth subnitrate and potassium iodide to form BiI_4^- in a yellow solution (Santos *et al.*, 2017). Excess BiO_4^- react with protonated sibutramine HCl to form a complex compound with an orange-red color, as shown in Figure 4. The chemical reaction is shown in Eq. (4) (Karamahito *et al.*, 2021).

Figure 4. The color formation of sibutramine HCl (0,6 mM) detection

$$C_{17}H_{27}ClN + Bi_5O(OH)_9(NO_3)_4 + 20\ KI + 12\ CH_3COOH \rightarrow C_{17}H_{27}ClNH^+ + 4\ KNO_3 + 4(BiI_4)K + 12\ CH_3COOK + 10\ H_2O \quad (4)$$

The intensity of the color change product can be employed to quantify sibutramine HCl using a scanner and ImageJ software. The test strip was captured using a scanner, and the image was analyzed by converting the color change to an RGB value as Δintensity. The increase in color intensity resulted in an increase in Δintensity, which exhibited a linear trend as the concentration of sibutramine HCl increased.

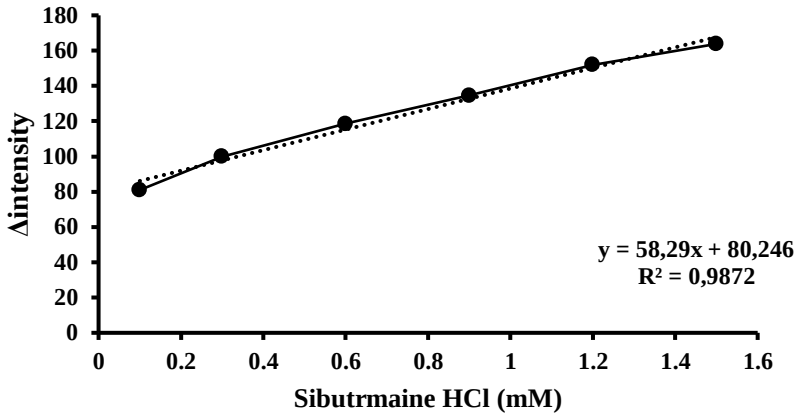

Figure 6. The standard curve of sibutramine HCl

Based on Figure 6, we can determine the relationship between the concentration of the analyte and Δintensity. The obtained standard curve showed good linearity (R^2= 0,9872) in the sibutramine HCl concentration 0,1 mM to 1,5 mM. Other validation parameters were observed, and the data are presented in Tables 1 and 2 for the precision of the analysis.

Table 1. Analysis parameters of test strip with sibutramine HCl

Parameter of Validation	Result
Range concentration/(mM)	0,1–1,5
Coefficient correlation	0,9872
Limit of Detection/(mM)	0,2 mM
Limit of Quantification/(mM)	0,8 mM
Sensitivity/(Δintensity)	80,24

Table 2. The precision of analysis in difference concentration *(intraday)*

Concentration (mM)	%RSD
0,6	1,3
0,9	1,5
1,2	3,5

The test strip was applied to slimming herbal medicines from two commercially available samples, namely samples A and B. Neither sample consisted of sibutramine HCl, as shown on the label of the product. The sample measurement was obtained by the spiked method, and the analysis accuracy was determined by comparing the measured sibutramine HCl concentration with three theoretical concentrations (0,9 mM; 1,2 mM and 1,5 mM).

Table 3. Accuracy of sibutramine HCl from A and B samples, determined by test strip

Sample	%Accuracy		
	0,9 mM	1,2 mM	1,5 mM
A	84	81	87
B	90	98	82

The measurement results for both sample solutions were not obtained with sibutramine HCl. This is appropriate, based on what was stated on the label. Thus, samples of slimming herbal products are safe to consume. Based on Table 3, the resulting % errors are approximately 12%, suggesting that the method has good accuracy.

Conclusions

An optical sensor-based test strip was synthesized using the sol-gel method. Based on the performance of measurement, the test strip has good

linearity (R^2=0,9872) with range concentration 0,1-1,5 mM, sensitivity (80,24 Δintensity), LOD (0,2 mM), LoQ (0,8 mM), precision (RSD < 5%) and 81-98% accuracy. This study describes a new, fast, efficient, economical, and easy technique for detecting sibutramine HCl in slimming herbal products. This test strip can be used for sibutramine HCl screening and is useful for consumers to safely consume herbal products. Future research on this method is needed to improve the performance of this test strip, especially for selectivity with any interference or the use of the type of agent recognition for more sensitive detection.

Acknowledgment

This research was funded by the DIPA of the Directorate General of Higher Education, Education and Culture Ministry, 2022.

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