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RESEARCH PAPER

A Novel Molecularly Imprinted Fluorescence Test Strip for Detection of Cimaterol

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Abstract: A novel test strip for rapid detection of cimaterol was prepared by combining highly specific selectivity of molecularly imprinted technology with convenient rapid response performance of test strip. The cimaterol molecularly imprinted polymers (MIPs) were prepared through chemical polymerization method, and nitrocellulose membrane was immersed into MIPs clouding solution. After that, the template molecules were eluted and then a clipping step was conducted for obtaining the test strip. After re-adsorption of cimaterol, fluorescent dye Eosin Y was added onto the surface reaction zone on the strip. Qualitative and quantitative analysis would be realized via determining fluorescence quenching in its reaction zone. The experimental results showed that the fluorescence quenching had a good relationship with cimaterol concentration ranging from 0.01–100 μg mL^{-1} with the limit of detection of 0.01 μg mL^{-1}. The molecular imprinting test strips provided convenient way in practical application especially for the detection of pork and fodder sample on site.

Key Words: Test strip; Molecularly imprinted; Cimaterol; Fluorescence; Eosin Y

1 Introduction

Cimaterol is one of a class of clenbuterol, which is usually added in fodder and animal drinking water to increase lean percentage. It could also be used as antiasthmatic to treat respiratory diseases in clinical. Several researches showed that a high cimaterol intake could cause heart palpitations, flustered, nausea, vomiting, muscle tremors and other symptoms, even be life-threatening[1]. Cimaterol was included in the disabled list by Ministry of Agriculture of the People's Republic of China, and a ban showed that cimaterol added in animal's production and food animals was prohibited[2]. In order to ensure the food safety and people's health, the monitoring and supervision for clenbuterol term in acquisition of live pigs, processing and marketing process should be strengthened. Reported methods for determination of cimaterol include enzyme-linked immunosorbent assay (ELISA)[3], high performance liquid chromatography (HPLC)[4], gas chromatography-mass spectrometry (GC-MS)[5], liquid chromatography-mass spectrometry (LC-MS)[6], electrochemical analysis method (EC)[7], etc. However, these methods usually suffer from many shortcomings, such as expensive instruments, high cost and time consuming, which can't meet the requirements of rapidity, simplicity and cheapness in market supervision. Thus, developing accurate, rapid and convenient analytical methods with high specificity for cimaterol is of great significance.

Immunochromatographic strip technique, e.g. test strip, has attracted much attention and been widely applied in food safety monitoring, water quality analysis, medicine because of

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its advantages of simple preparation, easy implementation, simple operation and rapid determination, etc[8–10]. Song[11] and Xu[12] used fluorescent silica nanoparticles and doped $Ru(phen)_3^{2+}$ silica nanoparticles instead of colloid gold in preparation of cimaterol immunochromatographic strip respectively. By utilizing competitively combining of cimaterol and secondary antibodies with antibody, the qualitative analysis of cimaterol was achieved by monitoring the change of fluorescence color on detection line. Despite the high sensitivity (0.037 and 0.6 ng mL^{-1}, respectively), these immunochromatographic strips exhibited high cost and poor anti-interference ability. Furthermore, fluorescent change on control line and detection line was often not obvious with high miss rate.

Molecularly imprinted technique (MIT) has attracted much attention and been widely explored in many applications because of its excellent selectivity[13,14]. In this study, a new test for rapid detection of cimaterol was developed by combining high selectivity of MIT and convenient rapid response performance of test strip method. Molecularly imprinted polymers (MIPs) were prepared by chemical polymerization method while cimaterol was chosen as the template. Nitrocellulose membrane was immersed into MIPs clouding solution. Ethanol was used as the eluent to elute template molecules after drying, and then molecular imprinted strip with specific recognition ability for cimaterol was obtained. After re-adsorption of cimaterol in sample, fluorescent dye Eosin Y was added and a quenching reaction occurred with cimaterol. Qualitative and semi-quantitative analysis was realized via determining fluorescence quenching extent in reaction zone. Color intensity of reaction zone was simply defined by color picker of Photoshop software, and quantitative analysis was realized.

2 Experimental

2.1 Instruments and reagents

The following instruments were used in this study: DX86841 micropipettor (20–200 μL, Dragon laboratory instruments limited, China), PL303 precision electronic balance (Mettler Toledo, Shanghai, China), UVA365 Ultraviolet ray lamp (Longpro Co. Ltd., Guangzhou, China), Deli 0111 QD-L (Guangzhou capable group Co. Ltd., China) and Canon 660d digital camera (Canon lnc., Japan).

Cimaterol standard was purchased from Witega laboratories, Garmany. Ethanol and Eosin Y were purchased from sinopharm chemical reagent Co., Ltd. α-Methylacrylic, *N,N*'-methylenebisacrylamide and ammonium persulphate were obtained from Xilong Chemical Co. Ltd, China. Nitrocellulose membrane and plastic substrate were purchased from PALL Co. Ltd, USA. All reagents were of analytical grade. Double distilled water was used throughout the experiment. The pork was bought in local supermarket.

2.2 Preparation of MIPs and non-molecularly imprinted polymers (nMIPs)

Cimaterol stock solution (100 μg mL^{-1}) was prepared by dissolving cimaterol standards in double distilled water, and series of 10.0, 1.0, 0.1 and 0.01 μg mL^{-1} cimaterol solutions were prepared by serial dilution, respectively.

For the synthesis of MIPs, 25 μL of α-methylacrylic was added into 5 mL of 100 μg mL^{-1} cimaterol solution, and was ultrasonically mixed for 10 min. After that, 0.065 mM *N,N'*-methylenebisacrylamide was added and subjected to ultrasonication for 30 min, and then the mixture was allowed to stand overnight. 0.088 mM $(NH_4)_2S_2O_8$ solution was added as an initiator, while MIPs was obtained after ultrasonication for 10 min. The fabrication procedure of nMIPs was the same as MIPs without the addition of cimaterol.

2.3 Preparation of cimaterol molecular imprinted strip

Nitrocellulose membrane was immersed into cimaterol MIPs for 30 min and then placed in oven at 50 °C, 30 min. Plastic substrate of 3.0 cm × 1.0 cm was cut out as the substrate of strip, and double-sided adhesive was pasted on the back. Round hole (area = 38.46 mm^2) was obtained by piercing base and double-sided adhesive. The nitrocellulose membrane covered with cimaterol MIPs was pasted on base, and then a clipping step was conducted to obtain the test strip. 100 μL of ethanol was used as eluent to elute reaction zone (the round hole in strip, Fig.1) for removing the template molecule (20 μL per time). The strip was put in blast drying oven at 50 °C, 1 h to remove the ethanol, and then molecular imprinted test strip was obtained. The production process is shown in Fig.1.

2.4 Qualitative, semi-quantitative and quantitative determination methods

Approximately 20 μL of different concentrations of cimaterol solution was added in reaction zone and allowed to stand for 5 min. After that, 100 μL of double distilled water was used to elute reaction zone (20 μL each time, area was 38.46 mm^2). Then 20 μL of 0.346 g L^{-1} Eosin Y solution was evenly added on reaction zone and allowed to stand for 5 min. The strip was put in drying oven for 8 min at 50 °C, and then

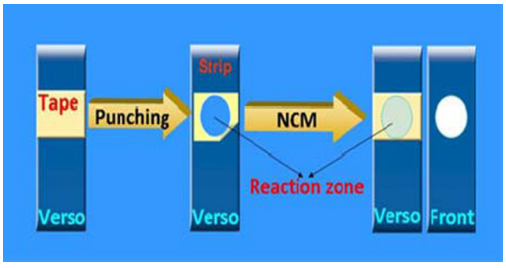

Fig.1 Preparation process of test strip