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Enzyme-linked immunoassay based on imprinted microspheres for the detection of sulfamethazine residue

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ABSTRACT

This study attempts to develop an enzyme-linked immunoassay (ELISA) using a molecularly imprinted polymer (MIP) as an artificial recognition element. The MIP microspheres were prepared using precipitation polymerization with SM_2 as the template molecule, methacrylic acid as the functional monomer and ethylene glycol dimethacrylate as the cross-linker. After the microspheres were coated in microtiter plate wells, the molecular imprinting ELISA (MI-ELISA) method was established based on the direct competition between free SM_2 and horseradish peroxidase (HRP)-labelled SM_2 in heterogeneous mode. The linear regression analysis data for the calibration curve showed a good linear relationship with a regression coefficient of 0.999 in the concentration range of 100 $\mu g\,L^{-1}$–3200 $\mu g\,L^{-1}$. Furthermore, following the selective solid-phase extraction (SPE) with bulk SM_2 MIPs as the sorbent and MI-ELISA detection, the limits of detection and quantification were 6.8 $\mu g\,kg^{-1}$ and 20.4 $\mu g\,kg^{-1}$, respectively, for SM_2 in swine muscle. For the first time, MI-ELISA combined with molecular imprinting SPE was developed to determine trace SM_2 in real samples, and the results show that it can be a useful analytical tool for quick detection in residue analysis.

1. Introduction

Sulfamethazine (SM_2, Fig. 1) is a chemotherapeutic agent that is widely used in the clinical veterinary field for the treatment of bacterial and protozoan infections [1]. However, several monitoring programmes have shown the presence of SM_2 at trace levels in animal food products, which implies a human health risk [2]. For this reason, the European Union (EU) has adopted a maximum residue level (MRL) of 100 $\mu g\,kg^{-1}$ for SM_2 in foodstuffs with animal origins, including milk [3]. In addition, the Codex Alimentarius Commission (CAC) declared that the residue content of SM_2 in milk should not be more than 25 $\mu g\,kg^{-1}$, and in other edible tissues, the MRL should not exceed 100 $\mu g\,kg^{-1}$ [4].

Several analytical methods have been developed for rapid screening for SM_2 residues, such as microbiological assays and antibody-based immunoassays including the enzyme-linked immunoassay (ELISA), immunochromatographic assay, and colloidal gold immunoassay strips and sensors. Although microbiological determination is cost-effective and has high throughput, it has low selectivity. Among these immunological assays, the ELISA based on polyclonal or monoclonal antibodies is now a well-established technique. The simplicity of detection, high sensitivity, good specificity and short analysis times are some of the characteristics that make the method very attractive. Considering the fact that the other immunoassays are not yet perfect, ELISA is still the most prevalent technique in SM_2 residue screening.

As an excellent screening tool, ELISA is traditionally performed based on the recognition element of polyclonal or monoclonal antibodies, which presents several fundamental limitations due to the nature of the biological antibody involved. Antibodies produced by animals are relatively physically and chemically unstable, which reduces their shelf time and limits their use in extreme environ-

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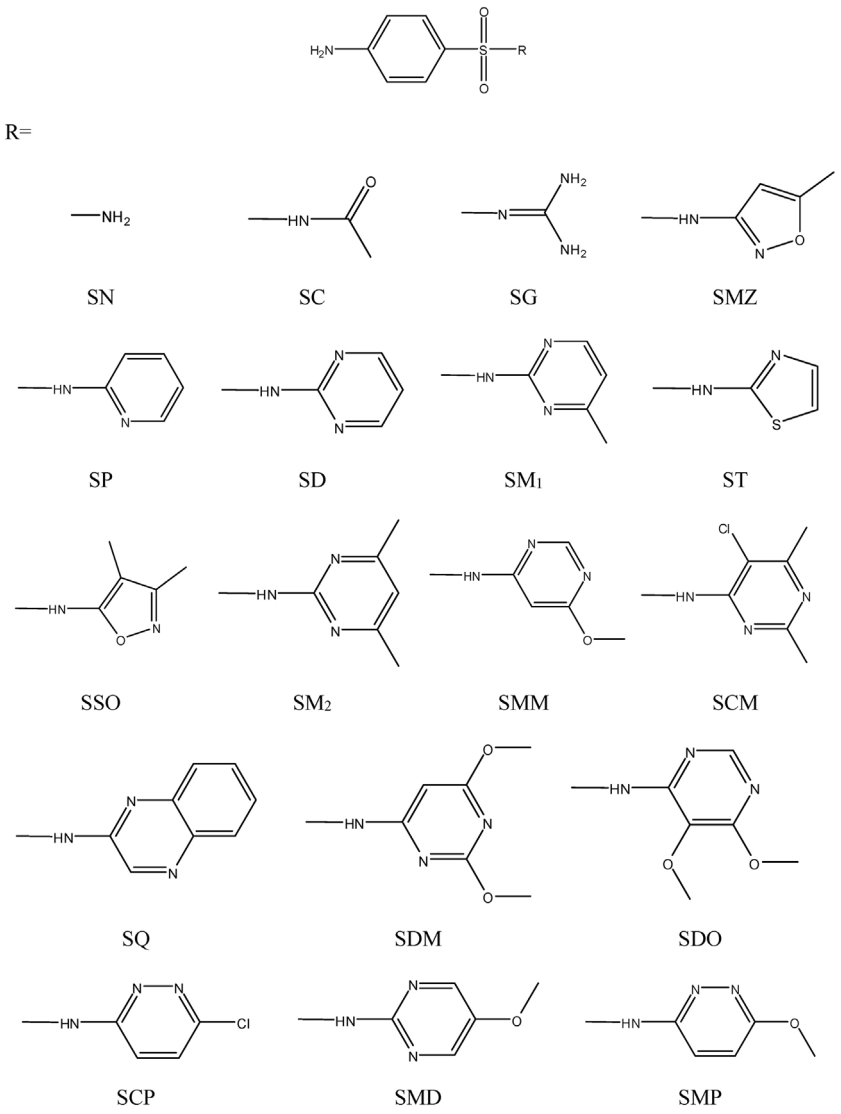

Fig. 1. The structures of the generally used SAs. SN, sulphanilamide; SC, sulfacetamide; SG, sulfanilyl guanidine; SMZ, sulfamethoxazole; SP, sulfapyridine; SD, sulfadiazine; SM_1, sulfamerazine; ST, sulfathiazole; SSO, sulfisoxazole; SM_2, sulfamethazine; SMM, sulfamonomethoxine; SCM, sulfaclomide; SQ, sulfaquinoxaline; SDM, sulfadimethoxine; SDO, sulfadoxine; SCP, sulfachlorpyridazine; SMD, sulfametoxydiazine; SMP, sulfamethoxypyridazine.

mental conditions. Furthermore, in the case of small molecules, it is not possible to prepare immunogenic derivatives of all potential analytes [5]. In addition, changes in the antibody properties and affinities will occur due to the influences of the different immune procedures and antigens. Although researchers make considerable efforts to improve antibody stability and the preparation process, the current techniques may still be tedious, time consuming, laborious and expensive.

Molecular imprinting is known as a template polymerization method, producing "tailor-made", highly selective synthetic receptors for given molecules [6]. In recent studies, owing to their high selectivities, inherent stabilities, low costs, and simple preparation procedures, molecularly imprinted polymers (MIPs) have already been identified as stable receptor or enzyme mimics that are suitable for substitution for the natural antibodies in assays or sensors [7]. The first molecularly imprinted sorbent assay (MIA) was based on a competitive radioligand-binding measurement. Unfortunately, this format involves the utilizing of radioactive material, which is undesirable and therefore slows the progress of assays based on enzyme labels. However, MIPs also face some undeniable challenges, such as a lack of water compatibility and difficult coating procedures for microplates. This is especially true for the immobilized method, which limits the application of MIPs in ELISA where this format is preferable. Here, we fixed the MIP microspheres in microtiter plates (96 wells) using poly(vinyl alcohol) (PVA) as glue. The MI-ELISA method was then developed successfully and has good analytical characteristics for SM_2 standards. However, the RSD of the results was somewhat high in the real sample analysis. Although we have made considerable progress in the development of the MI-ELISA method, it needs further study for optimization. Undoubtedly, the MI-ELISA has great promise in SM_2 residue analysis.

To the best of our knowledge, there has not been an MI-ELISA method reported for SM_2 residue analysis. The purpose of this work was to develop a rapid and selective enzyme-linked assay coupled with MIPs for the simultaneous determination of trace SM_2 in animal tissues. First, the MIP microspheres are coated on microplates. Next, the sample is selectively treated with molecular imprinting solid-phase extraction (MI-SPE) with direct competition between free SM_2 and SM_2-HRP conjugates. Then, the amount of polymer-bound SM_2 was determined using an ELISA reader, and thus, the trace residue of SM_2 can be calculated. In this study, the funda-