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Simultaneous detection of sulfamethazine, streptomycin, and tylosin in milk by microplate-array based SMM–FIA

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Abstract

This paper presents an approach to simultaneously detect sulfamethazine, streptomycin, and tylosin in milk by indirect competitive multianalyte Fluorescence immunoassay (FIA). Microscope glass slides modified with agarose were used for the preparation of small molecule microarrays (SMMs). Bovine serum albumin (BSA) conjugates of the haptens were immobilized on glass slides. The system consists of four glass slides containing 96 wells formed by an enclosing hydrophobic mask, which precisely matches a standard microplate. All liquid handling and sample processing were fully automated as 96-wells ELISA format. Monoclonal antibodies against sulfamethazine, streptomycin, and tylosin allowed the simultaneous detection of the respective analytes. Antibody binding was detected by a second antibody labeled with Cy5 generating fluorescence, which was scanned with chip scanner. The detection limits for three analytes were 3.26 ng/ml (sulfamethazine), 2.01 ng/ml (streptomycin), and 6.37 ng/ml (tylosin), being far below the respective MRLs. The system proved to be the first SMM–FIA platform having the potential to test for numerous antibiotics in parallel, such being of considerable interest for the control of safety in the food industry.
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Keywords: FIA; Microarray; Drug residue; Sulfamethazine; Streptomycin; Tylosin

1. Introduction

Veterinary drug are widely used in domestic animals for the prevention and treatment of infectious diseases and for growth promotion. The improper application can lead to the contamination of foodstuffs at the farm level. The veterinary drug substances in food result in the risk of undesirable health effects for the consumer. Therefore the regulatory authorities worldwide have enacted maximum residue limits (MRLs) for a number of veterinary drugs in food (Commission Regulation (EC), 1990; Food & Drug Administration, 2004). These reasons make it important to effectively control and detect veterinary drug residues in animal food. Various methods for the detection of antibiotic residues have been established, such as microbiological (Nouws et al., 1999), chromatographic (Schenck & Callery, 1998), and immunoassay methods (Strasser, Dietrich, Usleber, & Märtlbauer, 2003). However, microbiological tests are time consuming, lack sensitivity for diverse groups of antibiotics, and do not allow substance identification. Chromatographic methods are expensive and thus restricted to confirmatory purposes. Enzyme linked immunosorbent assay (ELISA) and fluorescence immunoassays (FIA) are excellent survey tools because of their high-throughput, user friendliness, and field portability. These important characteristics make immunoassays attractive tools for food testing by regulatory agencies to ensure food safety. Immunoassay is traditionally performed as individual test, however in many cases it is necessary to perform a panel of tests on each sample (detection of drug residues). To address this requirement, microarray-based immunoassay technologies have been developing utilizing microarray platform (multianalyte analysis) and classic immunoassay

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(multi-samples analysis). A numbers of research groups have developed immunoassays potentially useful for antibiotics multiplexed analysis. Michael et al. (Bertram et al., 2004) developed a parallel affinity sensor array (PASA)-based ELISA for the rapid automated analysis of 10 antibiotics in milk. Claus et al. (Erik, Martin, Jens, Leif, & Claus, 2003) investigated factors that influence the sensitivity of the immunomicroarray for pesticide analysis. Randox system Evidence (Randox Laboratories Ltd., Crumlin, UK) developed a biochip technology for multi-analyte detection. Zuo and Ye (2006) described the small molecule microarrays (SMMs) as analytical tools for drug residue detection. The SMMs are the array-based detection systems that use small molecules as probes immobilized on a variety of surfaces (Falsey, Li, & Lam, 2000; MacBeath, Koehler, & Schreiber, 1999; Reddy & Kodadek, 2005; Ye, Anthony, & Joydeep, 2003). It was effectively employed to study the interaction between small molecules and proteins.

In this paper, we report a microplate-array-based SMM–FIA system to simultaneously detect sulfamethazine, streptomycin, and tylosin in milk. In this system, we constructed SMM by printing these drug small molecules which had conjugated with carrier protein onto four agarose film-coated modified glass slides according to 96-well plate, so these drug small molecules covalently bound to glass slides by carrier protein and retain their ability to interact specifically with corresponding antibodies in solution. The indirect competitive immunoassay format was employed to quantitatively detect drug residues. The optimization and performance of this system are discussed.

2. Experimental

2.1. Microplate-array based SMM–FIA assay setup

A scheme of the microplate-array-based SMM–FIA system is depicted in Fig. 1. The slide frame with sealing mat provides 24 separate locations for individual microarrays on a single glass in the standard 8×12 configuration at 9 mm centers (Fig. 1a). Four glass slides each having 24 wells are assembled to a standard microplate containing 96 wells formed by an enclosing hydrophobic frame (Fig. 1b). The 96-well microarray format is compatible with automated operation systems of the standard ELISA. After reaction and washing, the slides are disassembled out, and scanned with a ScanArray Lite laser confocal scanner (PerkinElmer Life Science, Boston, MA, USA).

2.2. Reagents and chemicals

Sulfamethazine monoclonal antibody (mAb) CH2027, streptomycin mAb CH 2025, and tylosin mAb CH 2023 were purchased from Biodesign (Monrovia, ME). The hapten BSA conjugates for sulfamethazine, streptomycin, and tylosin were delivered by Biodesign together with the antibodies. Sulfamethazine, streptomycin sulfate, tylosin hemitartrate dehydrate, $NaIO_4$, and agarose were from Sigma (St. Louis, MO, USA). Cy5-labeled goat anti-mouse antibody (PI-2000; lot N0904) was purchased from Rockland (Burlingame, PA). All chemicals and solvents were purchased from Sigma and Gibco-BRL (Gaithersburg, Maryland), unless stated, otherwise, and used without additional purification. All solutions were prepared in deionized and sterilized water.

All monoclonal antibody and Cy5-labeled antibody solutions were prepared with phosphate-buffered saline (PBS; 80 mM potassium phosphate buffer with 145 mM NaCl, pH 7.4) containing 0.5% BSA. The washing buffer was a PBST buffer (PBS buffer containing 0.05% Tween 20). Each antibiotics stock (1 mg/mL in PBS) was 10-fold serially diluted (100, 10, 1.0 and 0.1 ng/mL) into PBS buffer.

2.3. Preparing activated agarose film-coated glass slides

The glass slides were cleaned ultrasonically in succession with a 1:10 dilution of detergent in warm water for 5 min, repeatedly rinsed in distilled water and 100% methanol followed by drying in oven at 60 °C. A 1% agarose solution was prepared by adding 100 mg agarose to 10 ml deionized distilled water, mixing and boiling for 5 min. Then 2 ml of the agarose solution was poured over each of the silanized glass slides (Dako, Glostrup, Denmark) which were preheated at 60 °C. After gelation of the agarose, the slides were dried at 37 °C in a dryer overnight. The dried slides can be stored at 4 °C for future use. Before immobilization of the hapten BSA conjugates, the agarose films were activated by immersion in 20 mM $NaIO_4$ in 0.1 M phosphate-buffered saline (PBS), pH 7.4, for 30 min at room temperature, then thoroughly rinsed with deionized distilled water and dried.

2.4. Chip production and microarraying

The veterinary drug small molecules were first conjugated them with carrier protein such as BSA. The BSA conjugates and BSA (as negative control) were suspended in PBS buffer containing 20% glycerol and printed on activated agarose film-coated glass slides by ProSys 5510 spotting workstation (Cartisian Technologies, Ann Arbor, MI) with 150 μm diameter and 250 μm spacing. The robot-mounted print head consists of four CMP3 Chipmaker pin (TeleChem International, Sunnyvale, CA), precisely arranged as a line with spacing of 9 mm. This print head can simultaneously and precisely print same solutions to four designated well locations of slide. Twenty-four subarrays in a 7×4 pattern of three different kinds of conjugates and negative control (BSA) were printed on each slide in three columns. Each conjugate was printed with seven spot replicates. Printing was performed in a cabinet at 25 °C and 60% humidity. Covalent hapten conjugate surface immobilization was established through a Schiff's base reaction at 37 °C overnight. Following immobilization, the microarray substrate surface chemistry was blocked by incubating the slides for 60 min in PBS supplemented with 1% (w/v) BSA

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