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Production of polyclonal antibodies and development of fluorescence polarization immunoassay for sulfanilamide

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Abstract

N-sulfanil-4-aminobutyric acid (SAB), which mimics common parts of the sulfonamides' structure, was synthesized and used to produce antibodies to sulfanilamide. Rabbit polyclonal antibodies have been raised using SAB conjugates with ovalbumin (OVA) or soybean trypsin inhibitor (STI). The immunogen based on SAB-STI could yield higher affinity anitbodies against sulfanilamide. The same SAB derivative was used for synthesis of a fluorescein-labeled tracer with fluorescein-thiocarbamyl ethylendiamine. A fluorescence polarization immunoassay (FPIA) for sulfanilamide was developed. The limits of detection sulfanilamide were 0.07, 0.10, and 0.07 μg mL -1 for water, diluted milk, and precipitated milk samples, respectively. The developed FPIA exhibited sensitivities below the respective maximal residue limits (MRLs) for individual sulfonamides (0.1 μg mL -1). The coefficients of variation of results for milk samples were lower than 5%. Total time for simple sample pretreatment and measurement is about 10 min for one sample. High cross-reactivity with sulfaguanidine (96%), sulfamethoxypyridazine (75%), and sulfachloropyridazine (28%), which have planar structures, could be suitable for simultaneous detection of these sulfa drugs in milk and developed fluorescence polarization immunoassay could be classified as a group-selective assay.

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Keywords

Fluorescence polarization immunoassay, Milk quality control, N-sulfanil-4- aminobutyric acid, Selectivity of antibodies, Sulfanilamide