Pesticides in Food – Immunochromatographic Detection of Thiabendazole and Methiocarb

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Abstract: In this work preliminary studies on application of immunochromatographic method to detection of pesticides in food samples, in particular fruit juices, are presented. The aim was to develop immunochromatographic technique on a porous membrane for rapid detection of the pesticides thiabendazole and methiocarb in fruit juices. For detection colloidal carbon conjugated with secondary antibody was used. The tests were evaluated visually. Our results show that in the fruit juices samples examined the detection limit for thiabendazole covered the MRL (maximum residue limit) for food of plant origin established by the legislative and for methiocarb the method has to be further optimised in order to decrease the detection limit below the MRL.

Keywords: immunochromatography; pesticide; thiabendazole; methiocarb; antibody

INTRODUCTION

Pesticides are substances used to get rid of various pests, which can be of microbiological, plant or animal origin. They are aimed at protection of plants, store supply, technical products, our homes as well as animals and humans. Of the largest importance, however, is their implication in agriculture. Since in many cases they can be toxic to non-target organisms as well as to humans and can be transferred into regions distant from the site of application the efforts to introduce control measurements arise. For pesticide detection the chromatographic methods are generally used, such as High Performance Liquid Chromatography and Gas Chromatography, which, however, require purification step(s) prior to analysis. Recently, immunochemical methods for pesticide detection developed, which represent a very suitable alternative to the classic chromatography. The immunochemical methods are quick and specific and can be used for rapid screening of pesticide presence in samples (ABAD *et al*. 1999, 2001). In this study we present an immunochromatographic technique which enables to test pesticides on their presence in food, in particular fruit juices, due to their maximum residue limit (MRL) given by the legislative. The examined pesticides were thiabendazole, a benzimidazole fungicide, which can be found mainly in apples, pear, bananas and citrus fruits and methiocarb, an N-methylcarbamate pesticide, which can be found in strawberries and vegetable. The MRL for food of plant origin established by the legislative is 0.05–15 mg/kg for thiabendazole and 0.05–2 mg/kg for methiocarb (ANONYMOUS 2006).

MATERIAL AND METHODS

For pesticides detection the immunochromatographic technique on a porous membrane based on the principles of paper immunochromatography was used (WANG *et al*. 2005). In this work it was modified to the indirect competitive format. On the membrane two lines are applied: control line (C-line) with immobilised rabbit anti-swine antibody (RASw) and the test line (T-line) with the conjugate ovalbumine-thiabendazole (OVA-TBZ) or ovalbumine-methiocarb (OVA-MET), respectively. Instead of colloidal gold detection colloidal carbon was used (Van Amerongen *et al.* 1993), which was conjugated with the swine anti-mouse antibody (SwAM; antibody against specific anti-pesticide monoclonal antibody). The pesticide from the sample competed with the immobilised pesticide for the limited number of binding sites on the antibody molecule, which was in this work the specific monoclonal antibody against the given pesticide. The intensity of the test line indirectly correlated with the amount of pesticide in the sample. In case of negative control, no pesticide was used. The fruit samples for thiabendazole were apple, pear and orange juices and for methiocarb strawberry and red wine juices. Prior to assays, all the juices were centrifuged 10 min at 16 000 rpm and their supernatants were applied on the membrane. The reaction mixture applied on the membrane contained the optimally diluted fruit juice sample 2-times more diluted. The immunochromatografic tests were evaluated visually.

RESULTS AND DISCUSSION

The developed immunochromatographic technique for pesticide detection in fruit juice samples is specific, sensitive and rapid. It enables to gain results within several minutes. For detection the colloidal carbon was used, because the differences between the lines were better distinguishable.

In the first step the negative effect of sample matrix on the immunoassay was reduced. The negative effect of the matrix may result in lines that are not sharp or in change in membrane appearance. Various sample dilution were prepared: 2%, 5%, 10%, 20% and 50% fruit juice (Figure 1). Due to the visual evaluation of the lines and membrane quality 5% fruit juice was chosen as the most suitable dilution for strawberry and red wine juices and 20% fruit juice for apple, pear and orange juices.

Afterwards, the immunochromatographic test was optimised for detection of pesticides thiabendazole and methiocarb in the optimally diluted fruit juices. The most optimal immunoreagents concentrations were: for thiabendazole OVA-TBZ 100 μg/ml, monoclonal antibody against thiabendazole 50 μg/ml and RASw 100 μg/ml and for methiocarb OVA-MET 125 μg/ml, monoclonal antibody against methiocarb 6.25 μg/ml and RASw 100 μg/ml. Under these conditions artificially contaminated model samples with the examined pesticides in the concentration range 0 ng/ml – 1 mg/ml were prepared and their detection limit was set (Figure 2).

As the detection limit the concentration of pesticide on the first strip is considered where the test line fainting is well recognisable. This concentration was then calculated on pesticide concentration in original sample expressed in mg/kg. For thiabendazole the detection limit in all examined juice samples was below the MRL given by the legislative, in particular 0.005 mg/kg for apple juice, 0.5 mg/kg for pear and orange juices in comparison with the MRL for the given crop 5 mg/kg. Therefore this method could be very suitable for food monitoring and control regarding this pesticide but further studies on signal refining are needed. As for methiocarb, the work is still in progress in order to decrease the detection limit below the MRL value.

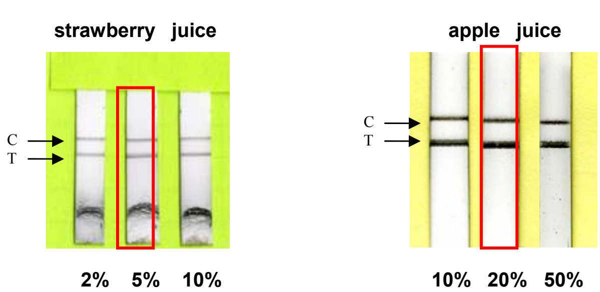

Figure 1. Example of optimal fruit juice dilution choice for strawberry (methiocarb) and apple juices (thiabendazole). The optimal juice dilution is highlighted with the rectangular. C = control line, T = test line

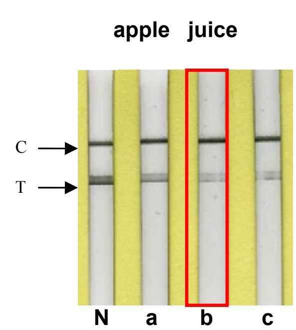

Figure 2. Artificially contaminated apple juice sample with the following concentrations of thiabendazole: N) 0 ng/ml, a) 0.1 ng/ml, b) 1 ng/ml, c) 10 ng/ml. The detection limit concentration is highlighted with the rectangular. C = control line, T = test line

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References

Abad A., Manclús J.J., Moreno M.J., Montoya A. (2001): Determination of thiabendazole in fruit juices by a new monoclonal enzyme immunoassay. Journal of AOAC International, **84**: 156–161.

Abad A., Moreno M.J., Pelegrí R., Martínez M.I., Sáez A., Gamón M., Montoya A. (1999): Determination of carbaryl, carbofuran and methiocarb in cucumbers and strawberries by monoclonal enzyme immunoassays and high-performance liquid chromatography with fluorescence detection. An analytical comparison. Journal of Chromatography A, **833**: 3–12.

Anonymous 2006. Commission Directive 2006/60/CE. In: Official Journal of the European Union, 27. 7. 2006, L206/1-L206/11.

Van Amerongen A., Wichers J.H., Berendsen L.B.J.M., Timmermans A.J.M. *et al*. (1993): Colloidal carbon particles as a new label for rapid immunochemical test methods: Quantitative computer image analysis of results. Journal of Biotechnology, **30**: 185–195.

Wang S., Zhang C., Wang J., Zhang Y. (2005): Development of colloidal gold-based flow-through and lateral-flow immunoassays for the rapid detection of the insecticide carbaryl. Analytica Chimica Acta, **546**: 161–166.