CONffIDENCE: Contaminants in food and feed: Inexpensive detection for control of exposure

DEVELOPMENT OF IMMUNOCHEMICAL METHODS (ELISA AND IMMUNOSENSOR) FOR DETERMINATION OF PARAQUAT RESIDUES IN FOOD SAMPLES

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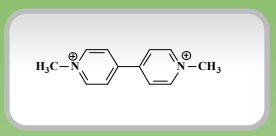
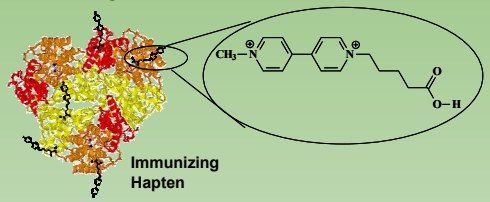
Introduction

Paraquat is a broad-spectrum herbicide used for weed control, as defoliant (cotton, hops) and for destruction of potato haulms. Since 2007 its use has been banned in the EU. Maximum Residue Limit (MRLs) have been set by ECC Council Regulation, No 396/2005), however the compound is not amenable to pesticide multi-residue methods due to the cationic nature. Rapid screening methods are desirable to verify compliance with Good Agricultural Practices and for monitoring purposes. The present communication reports antibody production for paraquat and the development and evaluation of a competitive enzyme-linked immunosorbent assay (ELISA) to determine residues of this pesticide in different matrices.

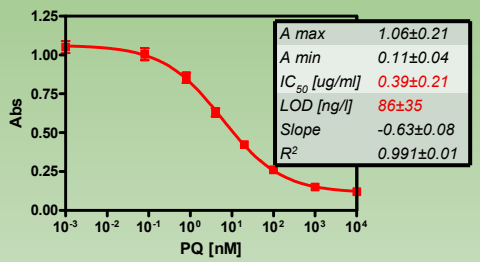


Antibody production



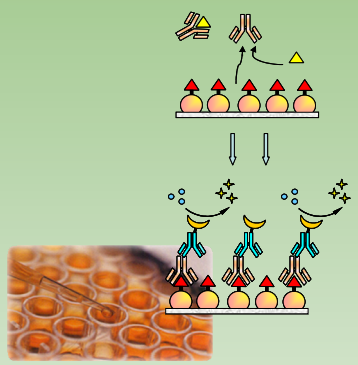
A immunizing hapten (PQ1) was synthesized that preserves all the most important chemical functionalities of paraquat. The hapten was covalently coupled to horseshoe crab hemocyanin (HCH) and the bioconjugate used to produce polyclonal antibodies in white New Zealand rabbits after an appropriate immunizing protocol.

ELISA calibration curve



Obtained in PBS (25%MeOH))
As198 (1/16000) vs. PQ-BSA (0.0625µg/mL)

Indirect ELISA format



Immunochemical Analysis of Wheat, Barley and Potato Extracts

Extraction Method

5g wheat/barley or 10g potato + 10mL water

↓ Mix 2 min, Settle 10 min

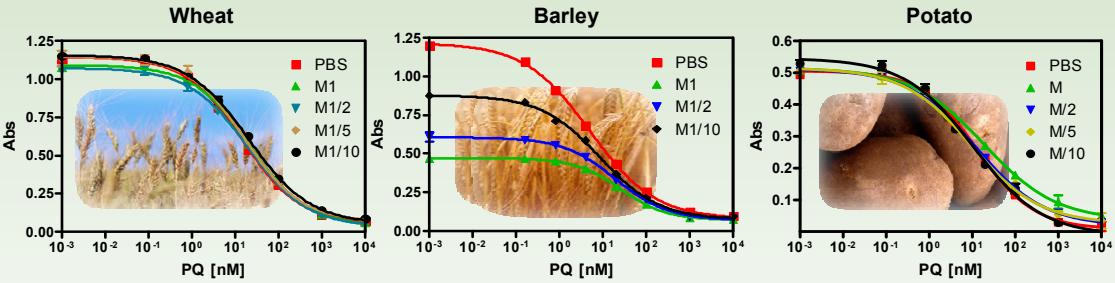
+10mL MeOH:HCl 0.1M (1:1)

↓ Mix 2 min, Centrifuge 5 min

Filter & Buffer (PBS 100mM)

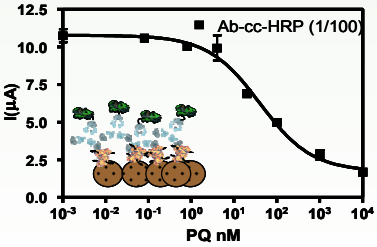
↓

ELISA

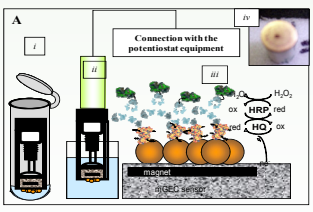


The assays have been made with As198 (1/16000) and PQ-BSA (0.0625 µg/mL).
Only the extracts prepared in barley show a slight matrix effect that can be removed by dilution of the matrix.

Electrochemical Magneto Immunosensor



PQ calibration curve obtained in PBS (25%MeOH). As198-HRP (1/100) vs. MP-PQ-BSA (0.125 mg/mL)



Scheme of the immunosensor

CONCLUSIONS

Antibodies for Paraquat have been produced and used to develop a microplate-based ELISA and a magneto electrochemical Immunosensor.

➢ The ELISA limits of detection are very good, without dilution (wheat: 0.85 mg/Kg, barley: 7.86 mg/Kg, potatoes: 3.85 mg/Kg). The assay works in compliance with the EC regulations regarding the possibility to measure PQ below the maximum residue limits established.

➢ The immunosensor uses antigen derivatized magnetic particles and HRP (horseradish peroxidase) labelled PQ specific antibodies, that catalyzed the oxidation of the substrate producing an amperometric current. The immunosensor was able to reach a limit of detection of about 0.5 ug L-1.

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