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Rapid methods for detection of anti-protozoan drugs

Key external stakeholders:

Meat, egg and poultry sectors; feed mills; regulatory agencies, e.g. DAFF, FSAI, IMB

Practical implications for stakeholders:

The objective of this research was to develop and validate a range of rapid methods for detection of three key anti-protozoan drug residues namely, diclazuril, halofuginone and toltrazuril. As the available technologies for residue detection are often highly specialised (and costly) and generally not suitable for application within industry, low-cost, effective means of screening such components is beneficial to food producers. Significantly, a comprehensive liquid chromatography method was developed to detect 21 anti-protozoan and anticoccidial residues in eggs and meat and validated to meet EC 2002/657 Criteria.

Anti-protozoan drugs are used in the treatment of Eimeria and Cryptosporidium parvum infections in poultry, pigs, lambs and calves. Residues of these drugs can occur in food because of feed contamination or failure to observe withdrawal periods following administration. To date, there has been little knowledge on the incidence of antiprotozoan drug residues in food of animal origin due to the lack of suitable analytical methods and the difficulty in analysing these substances. This new development therefore has significant implications for meat, egg and poultry sectors through its application for detection of anti-protozoan drug residues within food at factories, feed mills, or on-line processing monitoring at large-scale food production.

Main results:

- Novel antibodies were developed on the project to halofugionone and diclazuril.
- A range of biosensor assays were developed for these residues including a novel multiplex immunoassay, capable of simultaneous detection of diclazuril, halofuginone and toltrazuril.
- A comprehensive liquid chromatography method was developed and validated to detect 21 anti-protozoan and anticoccidial residues in eggs and meat.

Opportunity:

A new analytical test was developed and validated to detect 21 anti-protozoan and anticoccidial residues in eggs and meat. This comprehensive test is currently the best available for these residues and is now available as a commercial service to the Irish Food Industry to ensure that there are in compliance with HACCP and their produce is safe.

Collaborating Institutions:

DCU.

Contact Dr. Martin Danaher Email: martin.danaher@teagasc.ie

Teagasc project team: Dr. Martin Danaher (Project Leader/PI),
Dr. Mary Moloney
Ms. Lesa Clarke.
External collaborators: Prof. Richard O'Kennedy (DCU)
Ms Jenny Fitzgerald (DCU).

1. Project background:

Diclazuril, halofuginone and toltrazuril are three anti-protozoan drugs widely used in the treatment of Eimeria and Cryptosporidium parvum infections in food producing animals. Few analytical methods are available for detecting anti-protozoan drug residues and the majority are based on liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS).

The objective of this research was to address the gap in anti-protozoan analysis, to improve food safety and assure consumer protection. This was achieved through development of screening and LC-MS/MS based methods. DCU researchers were responsible for the developing a suite of antibodies, leading to more sensitive and selective assays. Researchers at Teagasc were responsible for development and validation of analytical methods.

2. Questions addressed by the project:

- Can immunochemical assays be developed and validated to detect anti-protozoan drug residues in food?
- Is Irish food free of antiprotozoan drug residues?

3. The experimental studies:

Researchers at DCU developed antibodies to diclazuril and halofuginone drug residues. The halofuginone antibody was genetically engineered to produce a single chain variable fragment antibody that was 185-fold more sensitive than the starting antibody. A sensitive biosensor assay was subsequently developed by DCU to detect halofuginone residues in eggs.

Teagasc developed biosensor test methods to detect halofuginone and diclazuril residues in liver tissue. They also developed a biochip array assay capable of simultaneous detection of diclazuril, halofuginone and toltrazuril residues.

A more extensive LC-MS/MS method was developed to detect a wide range of anti-protozoan and anti-coccidial drug residues in eggs and meat. This method was successfully transferred to National Reference Laboratory at Ashtown, where more extensive validation trials were conducted in egg and avian muscle. Standard operating procedures and validation reports have now been completed for this assay. The assay was submitted for ISO17025 accreditation and was inspected in June 2011. It is expect that this will be fully accredited before the autumn of 2011.

4. Main results:

- An LC-MS/MS test was developed and validated to detect 21 anti-protozoan and anticoccidial residues in eggs and muscle tissues.
- This test is now accredited to ISO17025 standard.
- Antibodies were developed on this project to diclazuril and halofuginone molecules. These antibodies have the potential for exploitation in commercial diagnostic kits.
- Biosensor assays were developed to detect diclazuril and halofuginone residues in eggs and meat.
- A novel biochip array assay was developed, capable of the simultaneous detection of three anti-protozoan residues from one sample extract.

5. Opportunity/Benefit:

- The antibodies and kit reagents developed on this project have potential for exploitation in commercial diagnostic kits.
- An analytical method was developed for detecting 21 anti-protozoan and anticoccidial residues in eggs and meat. The method has been validated according to the new Maximum Residue Limits that were set for anticoccidial residues. The resulting test puts Ireland in the lead regarding the testing of these residues.

Contact Dr. Martin Danaher Email: martin.danaher@teagasc.ie

- The Irish Food Industry can avail of this test and be confident that their product is compliant with current EU limits. This will allow Ireland to continue to command premium prices for their produce because of its excellent safety reputation.

6. Dissemination:

Main publications:

1. Darcy, E., Leonard, P., Fitzgerald, J., Danaher, M., O'Kennedy, R. Purification of antibodies using affinity chromatography. Methods in Molecular Biology. 681 (2011) 369-382.
2. Fitzgerald, J., Leonard, P., Darcy, E., Danaher, M., O'Kennedy, R. (2010). Light-chain shuffling from an Antigen-biased Phage Pool Allows 185-fold improvement of an Anti-Halofuginone Single-Chain Variable Fragment. Biochemistry 410 (2010), 23-33.
3. Fodey, T., Leonard, P., O'Kennedy, R., O'Mahony, J., Danaher, M. (2010). Developments in the production of biological and synthetic binders for immunoassay and sensor-based detection of small molecules. TrAC 30 (2010) 254-269.

Conferences:

1. Fitzgerald, J., Leonard, P., Darcy, E., Danaher, M., Crooks, S., Fodey, T., Elliott, C., and O'Kennedy, R. (2010) SPR Biosensor Detection of Halofuginone Residues in Egg. Poster presented at: The Sixth International Symposium on Hormone and Veterinary Drug Residue Analysis, 2010 June 01-04; Ghent – Belgium.
2. Clarke, L., O'Kennedy, R., Danaher, M. (2010). Detection of diclazuril in liver by SPR Biosensor. Advancing Beef Safety and Quality through Research and Innovation. 6 - 7 October 2010. In: An International Conference organised by ProSafeBeef. IBERS, Aberystwyth University, Wales, 06-Oct-2010, 0 23530 B2.
3. Moloney, M., Danaher, M. (2010). Determination of eight anticoccidials by UPLC-MS/MS. Poster presented at: The Sixth International Symposium on Hormone and Veterinary Drug Residue Analysis, 2010 June 01-04; Ghent – Belgium.

7. Compiled by: Dr. Martin Danaher