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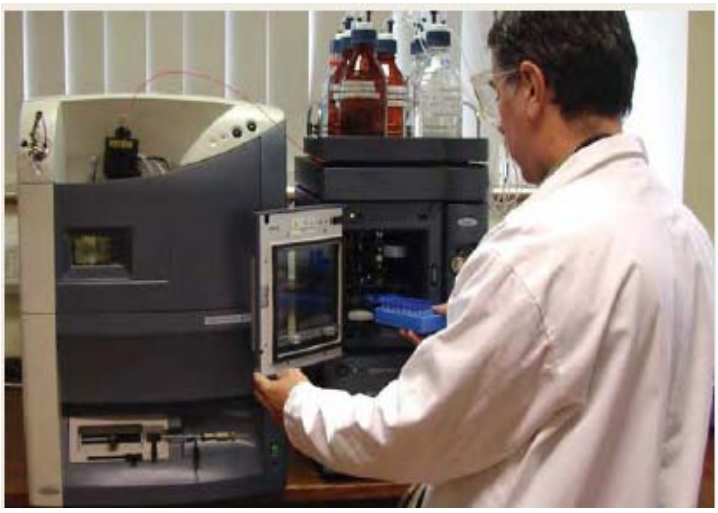
# Detection of flukicide residues in milk and meat



## Key external stakeholders:

Dairy, beef and sheep farmers; meat and milk processors; Irish baby food industry; regulatory agencies e.g., DAFF, FSAI, IMB.

## Practical implications for stakeholders:

- The first analytical test to detect all of the major anti-parasitic drug residues has been developed.
- A new group of residues in milk and meat samples were detected for the first time; nitroxynil, closantel, triclabendazole and rafoxanide were detected in milk at low levels. However, with setting of provisional Maximum Residue Limits (MRLs) for some flukicides in milk, this will become less of a problem from 2011 on.
- The main recommendation for primary processors is that flukicide residues should be monitored in milk, particularly during the spring period post-calving.

The technology developed under this funding has been comprehensively validated according to international guidelines and was accredited to the ISO 17025 standard and the technology has been applied to some 3000 test samples.

## Main results:

- A sensitive test was developed and validated to detect 38 anti-parasitic drug residues in milk and animals tissue.
- Technology was satisfactorily evaluated through application in inter-laboratory studies.
- Technology was accredited to ISO17025 standard in 2009.
- Technology has been applied to approximately 3000 test samples.

## Opportunity / Benefit:

This technology is now available as a tool to monitor the safety of milk and meat products, preventing contaminated product entering the food chain and potential product recalls, with all of the economic fallout this entails.

## Collaborating Institutions:

USDA-EARC

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**Teagasc project team:** Dr. Martin Danaher
**External collaborators:** Dr. Steve Lehotay, USDA-EARC

## 1. Project background:

Flukicide veterinary drugs are widely used by Irish farmers for controlling infections caused by liver and stomach fluke in cattle and sheep. Active ingredients used in these veterinary products, include albendazole, clorsulon, closantel, nitroxynil, oxyclozanide, rafoxanide and triclabendazole. Remaining drugs do not show activity against both immature and mature stages of the parasite. Many of these substances are potent pharmaceutical agents and teratogenic or goitrogenic. Therefore, it is important to monitor these residues in foodstuffs to ensure food safety and support the export of Irish produce into the EU.

Prior to the instigation of research, no multi-residue analytical methods were available to detect this range of flukicide residues in milk or meat. As a result, there was a need to develop a new method for milk, meat and liver. This technology had to be comprehensively evaluated through in validation and inter-laboratory studies. In order to allow the technology to be applied in official food inspection purposes, it would have to be accredited to ISO17025 standard.

## 2. Questions addressed by the project:

- Can methodology be developed to detect flukicide residues in food?
- Is the technology suitable for application in reference laboratories?

## 3. The experimental studies:

Initially the focus of the project was to investigate the following flukicide drug residues only, nitroxynil, closantel, oxyclozanide, triclabendazole (plus its two metabolites) and niclosamide. In the initial months of the project, methodology was extended to a further 32 drug residues at no additional cost.

It was decided to apply a rapid sample preparation procedure, which was developed by the USDA for pesticide residues. A PhD student, Brian Kinsella, was recruited and spent a period of 12 months developing a QuEChERS (quick, easy, cheap, effective, rugged and safe) sample preparation procedure for the extraction of anthelmintic residues from bovine milk and tissues. Liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) was used for detection and quantification of residues, which included the benzimidazoles (BZs), macrocyclic lactones (MLS) and the generally overlooked flukicides.

Initial work focused on the optimisation of a QuEChERS method to effectively extract anthelmintic residues from milk and liver. The method involves the extraction of residues with acetonitrile (MeCN) in the presence of salts (MgSO4 and NaCl) to induce phase separation. After shaking and centrifugation, a portion of supernatant undergoes dispersive solid-phase extraction (d-SPE). The purified extract was analysed by LC-MS/MS using two 24.5 min injection to cover the positively and negatively ionised compounds. The method was successfully validated at the maximum residue level (MRL) according to the 2002/657/EC guidelines and met acceptability criteria in all but a few cases.

The method was subsequently transferred to the Teagasc laboratories at Ashtown, where an improved method was developed for the detection and quantification of anthelmintic residues in milk and tissues. Two different protocols of the same method were used to detect residues at the MRL and non-MRL levels. The improved method involves the addition of a concentration step when analysing in the low $\mu g.kg^{-1}$ (ppb) range. Ultra-high performance liquid chromatography (UHPLC) coupled to MS/MS was used for detection and quantification. All 38 anthelmintic residues could to be detected in <13 min using a single injection and to ≤2 $\mu g.kg^{-1}$. A dual validation approach was proposed to validate the two protocols at MRL and non-MRL levels. The method met acceptability criteria in all but a few cases. The inclusion of 19 internal standards, including 14 isotopically labelled internal standards, improved accuracy, precision, decision limits (CCα) and detection capability (CCβ).

Stability studies on anthelmintic residues were carried out in fortified tissues and sample extracts (MeCN and dimethyl sulphoxide). Samples spiked at two concentrations (2 and 500 µg kg-1) were stored for different periods of time and at different temperatures (+4°C and/or -20°C). The majority of residues were found to be stable in tissue samples and extracts. However, DMSO extracts stored at -20°C were not very stable. The stability of solvent standards stored at +4°C, -20°C and -30°C over 9 months was also investigated. Solvent

standards were found to be most stable when stored in a freezer, although there is no advantage to storing standards at -30°C compared to -20°C. The stability of 20 anthelmintic residues in incurred bovine muscle, kidney and liver tissue was examined during 6 months storage at -20ºC. The majority of residues were found to be stable in all three tissues. No interconversion of the benzimidazoles and their metabolites was observed.

Finally, UHPLC-MS/MS was used for the qualitative and quantitative analysis of the new anthelmintic monepantel and its sulphone metabolite in goat's milk. Sample preparation was carried out using a modified QuEChERS method, which included a concentration step to enable detection of residues to ≤1 µg kg-1. The method was successfully validated according to 2002/657/EC.

## 4. Main results:

- A sensitive test was developed and validated to detect 38 anti-parasitic drug residues in milk and animals tissue.
- Technology was satisfactorily evaluated through application in inter-laboratory studies.
- Technology was accredited to ISO17025 standard in 2009.
- Technology has been applied to approximately 3000 test samples.

## 5. Opportunity/Benefit:

- The nature of the technology developed on this project is such that it can be applied in any laboratory that has equivalent equipment and therefore is not suitable for patenting or licensing.
- The technology that has been developed on this project was transferred to the National Reference Laboratory at Teagasc, where it was validated and accredited according to ISO17025 standard.
- It has since been applied to monitor the safety of Irish produce in order to satisfy Directive 96/23/EC. This is essential for the continued export of Irish food into the EU.
- The technology is also being used to support policy in the area of flukicide residues. Two provisional maximum residue limits have been agreed by the CVMP for closantel and triclabendazole in milk.

## 6. Dissemination:

### Main publications:

Kinsella, B., Lehotay, S.J., Mastovska, K., Lightfield, A.R., Furey, A. and Danaher, M. (2009) New method for the analysis of flukicide and other anthelmintic residues in bovine milk and liver using liquid chromatography-tandem mass spectrometry, *Analytica Chimica Acta* 637: 196-207

Kinsella, B., O'Mahony, J., Malone, E., Moloney, M., Cantwell, H., Furey, A. and Danaher, M. (2009) Current trends in sample preparation for growth promoter and veterinary drug residue analysis, *Journal of Chromatography A* 1216: 7977-8015.

Kinsella, B., Whelan, M., Cantwell, H., McCormack, M., Furey, A., Lehotay, S.J. and Danaher, M. (2010) A dual validation approach to detect anthelmintic residues in bovine liver over an extended concentration range, *Talanta* 83: 14-24.

## 7. Compiled by: Dr. Martin Danaher