

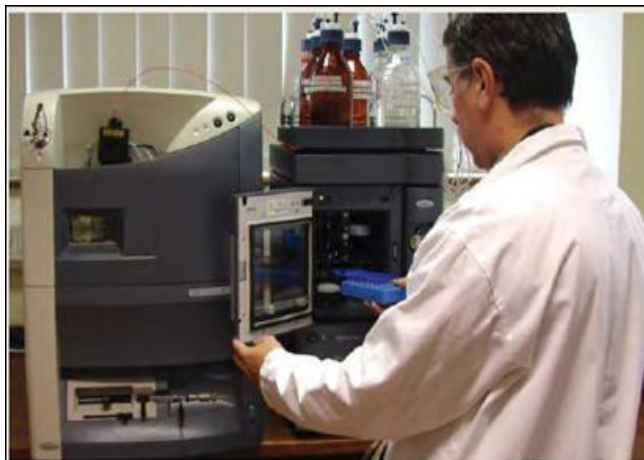
Project number: 6591

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Food and The Marine

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Detection of β -lactam antibiotics and quaternary ammonium compounds in food



Key external stakeholders:

- Regulatory agencies: DAFF, FSAI and The HPRA.
- Primary meat and milk processors.
- Irish baby food industry.
- Dairy, beef and sheep farmers.

Practical implications for stakeholders:

The project produced the most comprehensive method that has been developed for the analysis of beta-lactam antibiotic residues in milk and meat and should have widespread use in testing laboratories around the world. The application of this methodology demonstrated that the cephalosporin drug cefquinome did not degrade significantly during dairy products manufacture. Therefore, it is important to monitor antibiotic residues at farm and factory level to prevent food contamination. A sensitive method was developed to analyse QAC residues in milk using a novel chromatographic separation process. This method allows sensitive analysis of two different QAC residues in milk in the low part per billion range.

Main points

- Chemical residues can contaminate food at farm or processing level, and can remain in the product until consumed.
- Therefore, continued surveillance of antibiotic and QAC residues by industry and regulatory laboratories is critical to prevent product contamination.
- The application of the more comprehensive tests that were developed on this project for beta-lactam antibiotics and QAC residues will protect the pure image of Irish meat and dairy products.

Main results:

Analytical methods were developed to measure ≥ 30 beta-lactam antibiotic residues in meat and milk. The application of the methodology to the analysis of cefquinome residues in different dairy products showed the importance of monitoring antibiotic residues in food, as they can remain in the final product. A method was also established for the determination of 15 QACs in milk samples. Its demonstrated that the incidence of QAC residues in milk is low. However, further research is required for high fat dairy products.

Opportunity / Benefit:

The methods developed for beta-lactam residues in meat and milk are very important for monitoring the compliance of Irish and imported food with EU regulatory limits. The methodology for QAC residues can help to identify the potential source and the occurrence of these residues in Irish farms.

Collaborating Institutions:

CIT

Teagasc project team: Martin Danaher (PI)
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External collaborators: Ambrose Furey, Cork Institute of Technology

1. Project background:

Preliminary research carried out by Teagasc, identified the presence of quaternary ammonium compound (QAC) residues in milk and dairy products. QACs are used in many detergent and disinfectant products to improve the antimicrobial effectiveness of the cleaning agent. Some products may be used inadvertently at farm or processing level to maintain a high standard of plant hygiene. This project was undertaken to assess the frequency of occurrence and the potential source of QACs in dairy products. The project also carried out research on cephalosporin residues in meat, milk and dairy products. Cephalosporins belong to the beta-lactam group and are important antibiotics used in human and veterinary medicine. The European Medicinal Agency (EMA) advise that 3rd and 4th generation cephalosporins should only be administered systemically to animals in the event that clinical conditions respond poorly to narrow spectrum antibiotics. This is because resistant bacteria might transfer resistance genes to human pathogens. At the start of the project, no chemical methods were available nationally to measure the presence of cephalosporin residues in food. Rapid low-cost inhibition tests are used to screen food samples for antibiotics but these tests cannot tell what drug is present or quantity it. In the event that a positive sample is found, residues should be confirmed by LC-MS/MS (liquid chromatography coupled to tandem mass spectrometry). This project aimed to address this deficiency by establishing LC-MS/MS tests for cephalosporin residues in meat and milk.

2. Questions addressed by the project:

- Qs1: Can improved analytical methods be developed to detect beta-lactam antibiotics and QAC residues in food?
- Qs2: Can beta-lactam residues impact on dairy starter cultures and even transfer as residues into products?
- Qs3: Can QAC residues be detected in Irish milk?

3. The experimental studies:

The project was coordinated by the Teagasc Food Research Centre (TFRCA), Ashtown, Dublin. TFRCA were responsible for the development and validation of analytical methods for the measurement of beta-lactam antibiotics and QAC residues using LC-MS/MS in collaboration with the VPHRL (Veterinary and Public Health Laboratory, Baskweston, Celbridge, Co. Kildare) (Tasks 1 to 3). Researchers in the Teagasc Animal & Grassland Research and Innovation Centre (AGRIC), Moorepark, Co Cork were responsible for investigation of QAC contamination on Irish farms through sampling of bulk milk tanks (Task 4). The milk samples collected for QAC analysis were tested using the method developed at TFRCA. The new analytical methods for the determination of beta-lactam antibiotics were assessed through inter-laboratory studies by exchanging samples between VPHRL and TFRCA (Task 5). In addition, external proficiency test samples were provided by RIKILT based in the Netherlands. The final standards operating procedures (SOPs) for meat and milk methods were transferred to the VPHRL by Teagasc. In addition, one month was spent by Teagasc staff at Backweston to transfer the methodology. Researchers based in the Teagasc Food Research Centre (TFRCA), Moorepark were responsible for assessing the impact of the nine cephalosporin antibiotic residues on 7 *L. lactis* starter cultures and 8 *S. thermophilus* strains (Task 6). As part of this task 6, the stability of a cephalosporin, namely cefquinome, was assessed in the manufacturing of different dairy products (milk, 4 / 12 skim milk, cream, cheese, curd, whey, buttermilk and butter). TFRCA developed and validated a bespoke test to test residues in the samples collected on Task 6. The research activities on the project was disseminated on Task 7 through e-Newsletters, workshops, seminars, national conferences, international conferences and peer reviewed publications.

4. Main results:

Tests were developed and validated to measure ≥ 30 beta-lactam residues in meat and milk. The developed methods were fully validated according to EU Commission Decision 2002/657/EC. The methods are far superior to existing published methods because of the range of analytes covered and the simplicity of the sample preparation procedures used. Methodology was evaluated through inter-laboratory studies and SOPs were transferred to the VPHRL, who were trained on the methods. An UPLC-MS/MS method was established for the simultaneous determination of 15 QACs in milk samples based on a method developed

by the EURL. The method was applied to analyse bulk milk tank samples collected from Irish farms showing that only 8 out of 316 samples contained measurable QAC residues. The concentration of QACs in milk samples were ≤ 0.012 mg/kg. The impact of nine cephalosporin antibiotics on 7 *L. lactis* starter cultures and 8 *S. thermophilus* strains was studied. The results showed that the minimum inhibitory concentration (MIC) varied between the antibiotics tested, did not vary much between the *L. lactis* starter cultures tested, but did vary between the *S. thermophilus* strains tested. For cefadroxil and cephalexin the MIC was 50-100 mg/L for *L. lactis* starters, 20 mg/L for cefoperazone and 1.5-5 mg/L for the remaining six antibiotics. For the *S. thermophilus* cultures the MIC was generally about 5 mg/L, Apart from one strain that had an MIC of 8 mg/L, the others were < 1 mg/l. The fate of the cephalosporin, cefquinome, was investigated in milk samples showing that cefquinome residues were stable and could be detected in all of the dairy products analysed following the production of dairy products from contaminated milk. Cefquinome residues were found to mainly concentrate in the polar dairy fractions including skim milk, whey and buttermilk.

5. Opportunity/Benefit:

Two new sensitive and accurate tests were developed, which allow the combined analysis of 30 or more cephalosporins, penicillins and carbapenem residues in meat and milk. The research carried out during the development of this method has produced many key findings that improve the analysis of these residues including stability improvements, sample preparation and improved chromatographic separation. This method is very important for monitoring compliance with EU regulatory limits and providing new knowledge on the occurrence of these residues in food. The methodology can also be potentially applied to monitor residues in the environment to combat antimicrobial resistance. The residues of beta-lactams are unlikely to impact on starter cultures used in dairy manufacturing processes but residues have been shown to survive processing and can transfer into products. Thus the application of the newly developed tests is important to ensure milk is free of residues to prevent product contamination and protect the reputation of Irish dairy products.

A sensitive method was developed to analyse two different groups of QAC residues in milk. QACs are an emerging group of residues and research has shown that the frequency of occurrence in Irish farm milk is low. However, further research is required to investigate the fate of their occurrence in different processed dairy products.

6. Dissemination:

Di Rocco, M., Moloney, M., O'Beirne, T., Earley, S., Berendsen, B., Furey, A., Danaher, M. (2016) Development of a multiresidue method for β -lactam antibiotics in bovine muscle using UHPLC-MS/MS. In the proceedings of EuroResidue VII conference. Egmond aan Zee, The Netherlands. 23 – 25th May, 2016.

Ramkumar, Abilasha & Danaher, Martin. (2015). Evaluation of various QuEChERS sample preparation methods for the determination of Quaternary ammonium compounds in milk (Poster). In the proceedings of HPLC 2015: 42nd International Symposium on High Performance Liquid Phase Separations and Related Techniques. Geneva, Switzerland, June 21–25th 2015. Di Rocco, M., Furey, A., Danaher, M. Development of a multiresidue method for β -lactam antibiotics in food of animal origin by UHPLC-MS/MS. 43rd Annual Food Research Conference. University College Dublin, 10-11th December 2014.

Danaher, M. Recent developments in the analysis of residues in milk and dairy products. Teagasc Gateways – Advanced Analytical Methodologies for the Food Industry. Teagasc Moorepark, 16th November 2017.

Danaher, M. Development and application of modern methods for the detection and quantification of residues and contaminants in milk. IDF Scientific Symposium, Dublin, 31st March 2018.

Di Rocco, M., Moloney, M., O'Beirne, T., Earley, S., Berendsen, B., Furey, A., Danaher, M. Detection of β -lactam compounds in meat and milk by UHPLC-MS/MS. DAFM Workshop. Backweston, 31st March 2018.

Main publications:

Di Rocco, M., Moloney, M., O'Beirne, T., Earley, S., Berendsen, B., Furey, A., Danaher, M. (2017) Development and validation of a quantitative confirmatory method for 30 β -lactam antibiotics in bovine muscle using liquid chromatography coupled to tandem mass spectrometry. Journal of Chromatography A. 1500: 121 – 135.

Rocco, M., Moloney, M., Haren, D., Gutierrez, M., S. Earley, S., Berendsen, B., Furey, A., Danaher, M. (2020) Improving the chromatographic selectivity of β -lactam residue analysis in milk using phenyl-column chemistry prior to detection by tandem mass spectrometry. Analytical and Bioanalytical Chemistry. <https://doi.org/10.1007/s00216-020-02688-4>

7. Compiled by: Martin Danaher