AGRITOX | Interreg Atlantic Area European Regional Development Fund | EUROPEAN UNION

Newsletter
Issue 2: May 2020

Prevention and Mitigation of Mycotoxin Contamination of Food and Feed Caused by Climate Change

Introduction

In the 1st e-newsletter, introduction to Argitox project, objectives, detailed description of the six Agritox work packages and the latest progress (project meetings, stakeholder events, workshops, conference presentations and publications by project partners) were described. Agritox is a new collaborative project, funded under Interreg Atlantic Area Priority 3: Strengthening the territory's resilience to risks of natural, climate and human origin. Agritox carries out research on mycotoxins in food and feed. The main goal of Agritox is to advance the development of a mycotoxin warning network that will increase consumer safety, as well as establish some possible indicator of risks related to climate change.

This 2nd e-newsletter focuses on the analytical methods for mycotoxin detection and the capacity of Agritox partners and describes the latest progress achieved by the researchers working on the project.

Detection of mycotoxins and determination of their concentrations are usually performed using several instrumental and non-instrumental methods. However, over the past decades, these methods were mostly focused on routine analysis of a single analyte or groups of structurally related mycotoxins which mainly based on immunochemical techniques such as ELISA or less sensitive analytical instruments such as liquid chromatography coupled with ultraviolet/diode array and/or fluorescence detectors. The recent advances in the analytical instrumentations by coupling liquid chromatography to tandem mass spectrometry (LC-MS/MS) enabled scientists to develop several highly sophisticated LC-MS/MS-based methods for the simultaneous identification and quantification of different classes of mycotoxins in various food and feed matrices at (ultra)trace levels. These methods are preliminary aimed at saving the time and labour and reducing the overall cost of analysis, in addition to gaining more insight into the potential natural co-occurrence of mycotoxins. Also, due to the expected extreme weather conditions as a result of climate change, migration of toxigenic fungi to new geographic areas is expected occur which hypothesizes unexpected findings of mycotoxins i.e. emerging mycotoxins. This will require developing sensitive and reliable analytical methods.

For this purpose, a validated analytical LC-MS/MS method for the quantitative detection of 42 mycotoxins including the regulated mycotoxins (aflatoxins, fumonisins, ochratoxin A, zearalenone, T-2 toxin and deoxynivalenol), the emerging mycotoxins (beauvericin, alternariol, alternariol-methyl-ether and enniatins) and two masked mycotoxin metabolites (deoxynivalenol-3-glucoside and T-2-glucoside) in cereal was developed by Agrtiox partner at Teagasc Institute[1]. Quick, easy, cheap, effective, rugged, and safe (QuEChERS)–based extraction procedure was evaluated and optimised in order to rapidly extract mycotoxin residues from wheat and oat samples. The developed analytical method meets the all required criteria listed under Commission Regulation 1881/2006 and Commission Recommendation 165/2013. Furthermore, Agritox partner at University of Santiago de Compostela has optimized QuEChERS–based extraction procedure with dispersive solid-phase

Continued >

This project has received funding from the Interreg ATLANTIC AREA Subsidy Contract EAPA_998/2018 - Agritox.

Partners

Continued from page 1

extraction (dSPE) clean-up step to extract 24 different mycotoxins including regulated, emerging and masked compounds from beers. Following the extraction and purification of the target analytes, an LC-MS/MS method was applied to detect and quantify the target mycotoxins[2]. This developed analytical method was also validated for the detection of the same mycotoxins in several feedstuffs and other food matrices such as milk, maize, wheat and barley by the same research group. Previously, multi-analyte LC-MS/MS methods which allowed the (semi)quantitative or qualitative analysis of 300 (now reached 500) mycotoxins, other microbial metabolites, or mycotoxin degradation products in a single analytical run were developed[3]. On the other hand, there is a recent trend for using high resolution LC-MS instruments to cover a wider range of mycotoxins and other toxins in different matrices, and to allow the discovery and elucidation of novel mycotoxins or other fungal and microbial metabolites.

Footnotes:

1 De Colli *et al.*, 2020. Determination of 42 mycotoxins in oats using a mechanically assisted QuEChERS sample preparation and UHPLC-MS/MS detection. Journal of Chromatography B. Doi:10.1016/j.jchromb.2020.122187.

2 González-Jartín *et al.*, 2019. A QuEChERS based extraction procedure coupled to UPLC-MS/MS detection for mycotoxins analysis in beer. Food Chemistry, 275, 703-710.

3 Sulyok *et al.*, 2020. Validation of an LC-MS/MS-based dilute-and-shoot approach for the quantification of > 500 mycotoxins and other secondary metabolites in food crops: challenges and solutions Analytical and Bioanalytical Chemistry, 412:2607–2620

Agritox Analytical Capacity

Partner 1: Food Safety Department, Teagasc Food Research Centre (Dublin, Ireland)

The Teagasc residues laboratories at Ashtown, Dublin are an EU National Reference Laboratory that carry out analysis and research on detection of multiple classes of food and feed contaminants such as veterinary drugs, pesticides and mycotoxins. The laboratories also carry out extensive research to enhance the safety and quality of Irish foods including research on disinfectant agents, minerals and vitamins.

To accomplish these tasks, the laboratories have been equipped with a range of state-of-the art instrumentations for organic and inorganic chemical analysis including three Waters UHPLC–MS/MS (QqQ) systems, two Agilent UHPLC-MS/MS (QqQ), two ABSCIEX UHPLC-QTRAP-MS and Agilent 7900 ICP-MS systems. In, 2019, the 5500+ Qtrap system was purchased which has been specifically developed by SCIEX for the analysis of mycotoxins. Teagasc has also purchased the 6500+ Qtrap MS system contains novel Selexion (ion mobility separation module) that may enhance selectivity for some mycotoxins (**Fig 1.1**).

Fig 1.1. 6500+ Qtrap MS system for targeted analysis.

The laboratory is well equipped with equipment for bio-analytical sample preparation including automated solid phase extraction, microwave digestors, centrifuges, shaking/agitation equipment and nitrogen evaporation system (**Fig 1.2**).

Fig 1.2. Microwave digestors and nitrogen evaporation systems.

Recently, a rapid analytical UHPLC-MS/MS method for the quantitative measurement of 40 mycotoxins and other fugal metabolites, including all the regulated mycotoxins (European Commission 1881/2006 & European Recommendation 165/2013), the emerging Enniatins and the masked metabolites D3G and T2G, in cereal samples, was developed and validated. The sample preparation procedure, which is QuEChERS-based, was evaluated and optimised in order to rapidly extract mycotoxin residues from wheat and oat samples. Therefore, up to 55-60 samples a day can be extracted and analysed by a single laboratory analyst. This sensitive analytical method will be used at Teagasc to analyse several cereal samples for mycotoxins contamination in the framework of Agritox project.

Partner 2: Institute for Global Food Security, Queen's University Belfast (Belfast, Northern Ireland)

Recently, the Institute for Global Food Security (IGFS) moved into state-of-the-art laboratories within the new School of Biological Sciences building including a first class analytical laboratory that houses numerous mass spectrometers including; LC-MS and GC-MS systems, LC-HRMS, ICP-MS systems and ambient mass spectrometry that includes a DART-MS and REIMS-QToF MS.

Of the instrumentation at hand, the two triple quadrupole systems (LC-MS/MS), the hybrid quadrupole-time of flight system (LC-QToF) and ambient mass spectrometer (DART-MS) can all be used in the analysis of mycotoxins, for both quantitative and qualitative analyses. The two LC-MS/MS systems (Waters) include a TQ-MS and Xevo TQ-S (**Fig 2.1**) that allows quantification of mycotoxins in animal feed to ascertain their exposure, with our current method validated for the quantification of more than 50 mycotoxins, including the regulated ones.

The enhanced sensitivity of the Xevo TQ-S also facilitates the analysis of mycotoxin metabolites in biological matrices such as plasma, serum, urine and faeces. This allows us to try and understand how the animals metabolise the ingested mycotoxins as they detoxify them for excretion.

Fig 2.1. Waters Xevo TQ-S coupled to an Acquity i-class LC system for targeted analysis.

The LC-HRMS, an Agilent 6545 LC-QToF is another useful tool in the qualitative analysis of mycotoxins, particularly in biomarker studies for probing the metabolism of mycotoxins. Some metabolites are known and can be purchased, therefore allowing their presence to be verified by comparison against a known standard.

However, the advantage of HRMS is that ions can still be confirmed qualitatively by comparing against a database based on the exact mass. Most analyses conducted on a HRMS such as the 6545 LC-QToF (**Fig 2.2**) have minimal sample preparation bar a protein precipitation step and therefore there are minimal losses of analytes, allowing a global picture of the analytes present.

As the analysis is more often than not untargeted, it allows the data to be mined at a later date for any analytes, exogenous or endogenous, that have may have been identified retrospectively.

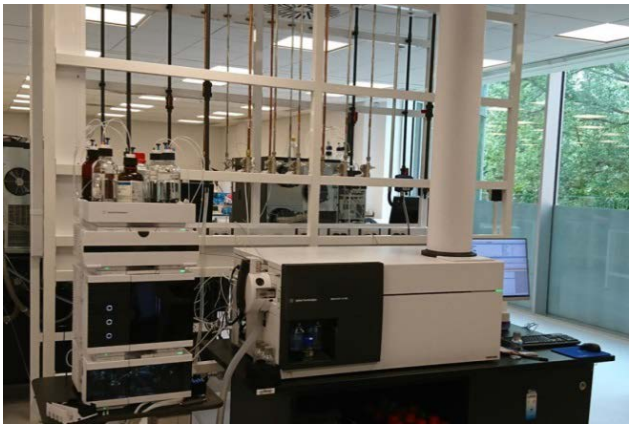

Fig 2.2. Agilent 6545 LC-QTof for untargeted analysis.

Our DART-QDa system (Waters & IonSense) has the potential for screening animal feed for various mycotoxins. After a generic sample extraction procedure on feed, the extract could be screened for the presence of mycotoxins on the DART-QDa (**Fig 2.3**) before deciding whether the sample(s) needs to be analysed further on one of the more expensive LC-MS/MS systems.

DART-QDa system employs a low resolution MS, which gives a lower sensitivity in matrix, the mycotoxin levels permitted in animal feed may allow for their detection using this platform. If successful, any sample that is below the cut-off value would not have to be analysed further on one of the more expensive LC-MS/MS systems. This screening approach can save time and money in not having to analyse samples that were either negative for the mycotoxins of interest, or that are below the permitted level, giving a quicker turnaround time as well as being cost effective for both the lab and industry partner.

Fig 2.3. DART-QDa system for screening of animal feed extracts for mycotoxins.

Partner 3: Faculty of Veterinary, University of Santiago de Compostela, (Lugo Campus, Spain)

The University of Santiago de Compostela (USC) laboratory is equipped with the latest generation of instrumentations for chemical analysis including ultra-performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS) and high-resolution time-of-flight mass spectrometry (UHPLC-HRMS-TOF). At USC, several analytical methods were developed for the simultaneous detection of a wide range of mycotoxins including regulated, emerging, and modified compounds.

All these methods are based on QuEChERS extraction followed by mass spectrometry analysis. In this sense, the UHPLC-MS/MS methods have been successfully validated so far for the analysis of 24 mycotoxins in various matrices such as beer, milk, maize, wheat, barley, alfalfa, and several feedstuffs. These analytical methods have good performance characteristics since are precise, selective, and accurate over a wide range of concentrations. In addition, the UHPLC-HRMS-TOF detection method allows the USC to perform a qualitative detection of more than 500 mycotoxins and other fungal metabolites.

Behind Agritox Project

This 2nd Agritox newsletter was prepared by Dr. Mohamed F. Abdallah (Teagasc Food Research Centre, Ireland), Dr. Brett Greer (Queen's Belfast University, Northern Ireland, UK) and Dr. Jesús M. González-Jartín (University of Santiago de Compostela, Spain).

Mohamed F. Abdallah

Mohamed F. Abdallah has recently joined the Teagasc residues laboratory located in Ashtown, Dublin, which is led by Dr. Martin Danaher, as analytical scientist/technologist after obtaining his PhD from Ghent University, Belgium. During his PhD, he was focusing on management of mycotoxins using UPLC-MS/MS and UPLC-QTOF/MS for targeted and un-targeted analyses of mycotoxins, respectively.

The research results obtained during his post graduate studies have been published in several peer-reviewed international journals. In 2019, he was awarded the Young Scientist Award during the EURACHEM meeting held in Tartu, Estonia.

Brett Greer

Brett Greer is a post-doctoral research fellow in the Institute for Global Food Security, School of Biological Sciences at Queen's University Belfast, Northern Ireland. His main research interests are in the development of detection methods for the analysis of natural toxins such as mycotoxins and cyanotoxins using LC-MS based technologies and various extraction methodologies. His work also includes the development of detoxification and mitigation strategies for mycotoxins in animal feed. He has worked on several natural toxin based projects of late and has published work in several peer-reviewed journals.

Jesús M. González-Jartín

Jesús M. González-Jartín is a research associate in the Department of Pharmacology, Pharmacy and Pharmaceutical Technology at the University of Santiago de Compostela, Spain.

His main research interests are development of detection methods for the analysis of mycotoxins, phycotoxins and cyanotoxins and development of detoxification procedures, using the nanotechnology. Dr. González-Jartín has collaborated on National and EU funded projects and published several papers in peer-reviewed journals.

Project Progress

Presentations

14th of May 2020

Agritox general presentation has been released and published on Agritox website.

Brochures

14th of May 2020

Agritox project brochure has been released and published on Agritox website. The brochures are available in four languages (English, Spanish, Portuguese and French).

Workshops

5th of March 2020

Agritox workshop held Workshop on Mycotoxins in Silages and Oats at the Teagasc Food Research Centre, Ashtown, Dublin 15, Ireland.

Publications by Project Partners

De Colli, L., Elliott, C., Finnan, J., Grant, J., Arendt, E.K., McCormick, S.P., Danaher, M. Determination of 42 mycotoxins in oats using a mechanically assisted QuEChERS sample preparation and UHPLC-MS/MS detection. Journal of Chromatography B 2020, Doi:10.1016/j.jchromb.2020.122187. [Journal Link]

González-Jartín, J.M., de Castro Alves, L., Alfonso, A., Piñeiro, Y., Yáñez Vilar, S., González Gomez, M., Vargas Osorio, Z., Sainz, M.J., Mercedes R. Vieytes, M.R., Rivas, J. Botana, L.M. Detoxification agents based on magnetic nanostructured particles as a novel strategy for mycotoxin mitigation in food. *Food chemistry 2019*, 294, 60-66. [Journal Link]

González-Jartín, J.M., Alfonso, A., Sainz, M.J., Vieytes, M.R., Aguín, O., Ferreiroa, V., Botana, L.M. First report of Fusarium foetens as a mycotoxin producer. *Mycotoxin research 2019*, 35(2), 177-186. [Journal Link]

González-Jartín, J.M.,Alfonso, A., Rodríguez, I., Sainz, M.J., Vieytes, M.R., Botana, L.M. A QuEChERS based extraction procedure coupled to UPLC-MS/MS detection for mycotoxins analysis in beer. *Food Chemistry 2019*, 275, 703-710. [Journal Link]

Oliveira, M., Vasconcelos, V. Occurrence of Mycotoxins in Fish Feed and Its Effects: A Review. *Toxins* 2020, 12, 160. [Full Text]