UNIVERSIDADE D
COIMBRA

Ana Rita Lopes Santos

Relatórios de Estágio e Monografia intitulada "State of the art of the evaluation of mycotoxins in rice: analytical methods, occurrence and detoxification strategies" referentes à Unidade Curricular "Estágio", sob a orientação da Dra. Ana Maria Rico, do Dr. Francisco Baptista e da Professora Doutora Ana Teresa Sanches Silva apresentados à Faculdade da Farmácia da Universidade de Coimbra, para apreciação na prestação de provas públicas de Mestrado Integrado em Ciências Farmacêuticas.

Setembro de 2021

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Setembro de 2021

Eu, Ana Rita Lopes Santos, estudante do Mestrado Integrado em Ciências Farmacêuticas, com o n.º 2016225444, declaro assumir toda a responsabilidade pelo conteúdo do Documento Relatórios de Estágio e Monografia intitulada "State of the art of the evaluation of mycotoxins in rice: analytical methods, occurrence and detoxification strategies" apresentados à Faculdade de Farmácia da Universidade de Coimbra, no âmbito da unidade de Estágio Curricular.

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Coimbra, 16 de setembro de 2021.

Ana Rita Lopes Santos

(Ana Rita Lopes Santos)

Agradecimentos

À minha família, em especial aos meus pais e avós por todo o incentivo e apoio incondicional, e pelo esforço para que pudesse concretizar os meus sonhos.

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Obrigada!

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PARTE I

RELATÓRIO DE ESTÁGIO EM FARMÁCIA COMUNITÁRIA

Farmácia Central – Coimbra

Sob orientação da Dra. Ana Maria Rico

Lista de Abreviaturas

FC – Farmácia Comunitária

MICF – Mestrado Integrado em Ciências Farmacêuticas

MNSRM – Medicamentos não Sujeitos a Receita Médica

PIM – Preparação Individualizada da Medicação

SWOT – *Strenghts, Weaknesses, Opportunities, Threats*

1. Introdução

A Farmácia Comunitária (FC) representa muito mais do que um local de dispensa de medicamentos, sendo usualmente considerada o primeiro contacto da população com os cuidados de saúde. Esta relação deve-se à confiança e proximidade estabelecidas entre o farmacêutico e o utente ao longo dos tempos, e à sua constante acessibilidade.[1]

O Mestrado Integrado em Ciências Farmacêuticas (MICF) pretende formar os seus estudantes para que se tornem mestres do medicamento e, embora existam várias áreas pelas quais podem enveredar, a Farmácia Comunitária constitui não só aquela com mais visibilidade, mas também a que concentra um maior número de farmacêuticos.[1] Os 5 anos que compõem o curso culminam com um estágio curricular em que a Farmácia Comunitária constitui uma área obrigatória. Este estágio surge como uma oportunidade para os estudantes aplicarem os conhecimentos adquiridos durante o curso, e representa muitas das vezes o primeiro contacto com a realidade profissional.

Realizei o meu estágio em FC na Farmácia Central, em Coimbra, entre os dias 11 de janeiro e 30 de abril, sob orientação da Dra. Ana Maria Rico. Durante este período tive a oportunidade de aplicar e aprimorar os conhecimentos adquiridos ao longo do curso e de conhecer de perto a realidade do trabalho em FC.

O presente relatório constituiu uma reflexão sobre esta experiência, acompanhada de uma análise SWOT, identificativa dos Pontos Fortes (*Strenghts*), Pontos Fracos (*Weaknesses*), Oportunidades (*Opportunities*) e Ameaças (*Threats*).

2. Farmácia Central

A Farmácia Central representa uma das farmácias mais antigas da cidade de Coimbra, estando em funcionamento desde 1830. O fornecimento de medicamentos é assegurado por três fornecedores diários e, pontualmente, são realizadas encomendas diretamente aos laboratórios.

É composta pelo espaço de atendimento, dois gabinetes de apoio ao utente, uma sala de *back office*, uma casa de banho, e pelo espaço de arrumação dos medicamentos.

Para além da cedência e aconselhamento de medicamentos e outros produtos de saúde e dermocosmética, esta farmácia reúne ainda um conjunto de outros serviços farmacêuticos, tais como a medição de parâmetros bioquímicos e a preparação individualizada da medicação (PIM). Acresce ainda a visita de uma nutricionista semanalmente para a realização de consultas de nutrição.

3. Análise SWOT

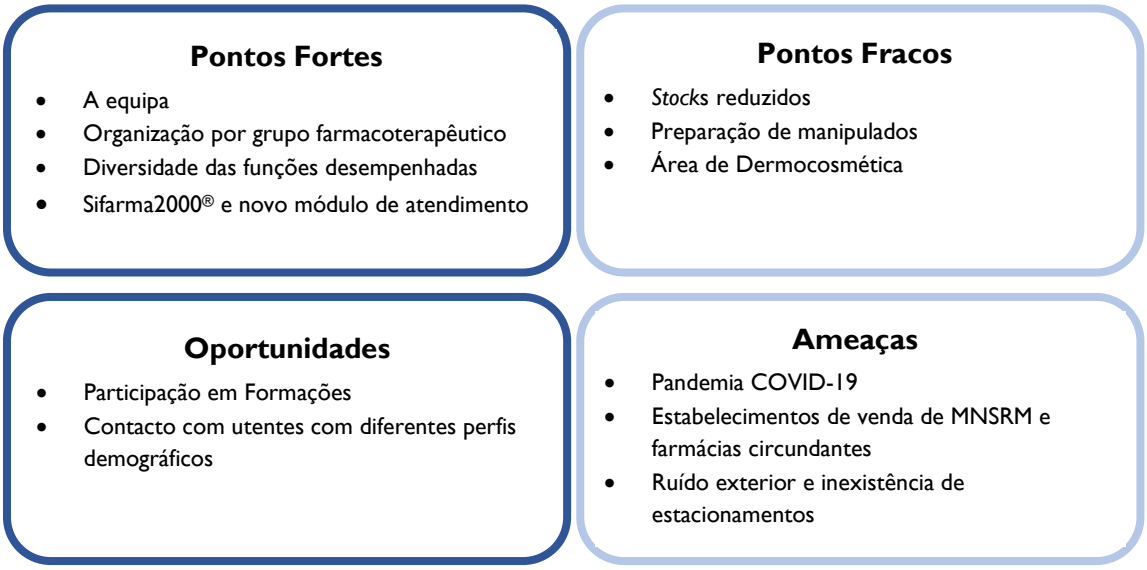

Figura 1 - Análise SWOT do estágio realizado na Farmácia Central.

3.1. Pontos Fortes

3.1.1. A equipa

A equipa da Farmácia Central é composta por 4 farmacêuticos e 1 técnica de Farmácia, que compõem uma equipa dinâmica e onde o bom ambiente é constante. No início do estágio, devido à Pandemia COVID-19, e de modo a não comprometer o funcionamento da farmácia caso ocorresse uma contaminação, a equipa foi dividida em dois turnos.

O bom ambiente vivido na farmácia e a disponibilidade permanente para me auxiliar quando necessário, fez com que depressa me sentisse integrada na equipa, tendo sido sempre incentivada a colocar as minhas questões.

É ainda importante destacar a presença de outros dois estagiários na farmácia, o que a meu ver também foi um ponto forte, dado que existiu uma grande entreajuda entre nós.

Tudo isto foi fundamental para a minha evolução e para o aumento da minha confiança na realização das tarefas ao longo do estágio.

3.1.2. Organização da Farmácia (grupo farmacoterapêutico)

Na Farmácia Central não existe um robot e os medicamentos estão arrumados em gavetas organizadas por grupo farmacoterapêutico (Figura 2), e não por ordem alfabética, como é comum na maioria das farmácias.

Inicialmente, aquando da fase de receção de encomendas e arrumação dos medicamentos, senti alguma dificuldade em adaptar-me a esta organização, uma vez que desconhecia alguns princípios ativos e nomes comerciais. A equipa da Farmácia Central foi fundamental para ultrapassar esta dificuldade, dado que todos se mostraram disponíveis a esclarecer qualquer dúvida que tivesse, e disponibilizaram um Prontuário Terapêutico, para que consultasse quando necessário. O Sifarma 2000® foi também uma grande ajuda, pois em caso de dúvida, ao consultar a ficha do produto ou durante o atendimento, era possível rapidamente consultar a gaveta em que o medicamento estava arrumado.

Assim sendo, ao longo do tempo, este método de organização mostrou-se benéfico, uma vez que me permitiu associar com maior destreza um princípio ativo (PA) ou nome comercial à finalidade terapêutica do medicamento.

Figura 2 - Organização dos medicamentos por grupo farmacoterapêutico.

3.1.3. Diversidade das funções desempenhadas

Ao longo do estágio fui desempenhando uma série de atividades que fazem parte do quotidiano de um farmacêutico comunitário. Estas tarefas foram surgindo por fases, à medida que me ia adaptando ao ritmo e realidade de trabalho da farmácia.

Comecei por rececionar as encomendas e arrumar os produtos. Esta fase é de extrema importância, já que condiciona o funcionamento de toda a farmácia. Aquando da receção é fundamental verificar os prazos de validade e o preço, bem como conferir se a quantidade encomendada corresponde à quantidade fornecida, para evitar a ocorrência de erros de *stock*. Esta fase permite ainda uma familiarização com os medicamentos e com o aspeto da embalagem, auxiliando ainda na associação entre o nome do princípio ativo e o nome comercial do medicamento, o que demonstrou grande utilidade na posterior fase de atendimento. Na fase de arrumação é ainda importante respeitar o princípio *first in first out*.

Após esta primeira fase, passei então para a fase de atendimento ao público. Inicialmente comecei por observar os atendimentos, mas fui depois ganhando autonomia e realizando eu própria os atendimentos. Com isto desenvolvi várias competências, incluindo a simplificação da linguagem científica e a adaptação do discurso ao tipo de doente.

Tive também oportunidade de realizar outras atividades de *back office* fundamentais ao funcionamento da farmácia, incluindo a gestão de *stocks*, o controlo de prazos de validade e a regularização de devoluções.

3.1.4. Sifarma2000® e novo módulo de atendimento

O Sifarma2000® é o *software* existente em grande parte das farmácias portuguesas, constituindo uma ferramenta crucial nos processos de gestão e atendimento do utente. Em 2020 foi lançado um novo módulo de atendimento, que irá substituir a versão anterior.

Ao longo do estágio contactei com ambas as versões deste *software*, o que considerei vantajoso, uma vez que aprendi a dominar as funcionalidades de ambos. O novo módulo acaba por ter algumas vantagens relativamente à versão anterior, como por exemplo o facto de ser mais intuitivo, permitir a aplicação de descontos internos e campanhas promocionais e ainda permitir a adição de novos componentes ao atendimento, mesmo quando já se atingiu a etapa de pagamento.

Uma vez que este sistema ainda se encontra em implementação nas farmácias, considero que ter estagiado numa farmácia que possui este sistema como um ponto forte do meu estágio, e que poderá ter bastante utilidade no futuro numa farmácia.

3.2. Pontos Fracos

3.2.1. *Stocks* reduzidos

Assegurar a existência de um *stock* mínimo e máximo de cada produto é um fator chave para assegurar a satisfação das necessidades do utente numa farmácia. Devido ao reduzido espaço de armazenamento de medicamentos na Farmácia Central, estavam disponíveis poucas embalagens de cada medicamento, o que resultava por vezes em atendimentos não finalizados na totalidade.

Considerei este aspeto um ponto fraco do meu estágio, pois estas situações refletiam-se na insatisfação dos utentes, mesmo existindo a possibilidade de realizar uma encomenda instantânea e muitas vezes garantir a dispensa do medicamento no próprio dia.

3.2.2. Preparação de Manipulados

De acordo com o Decreto-Lei n.º 95/2004, de 22 de abril, um medicamento manipulado é definido como "qualquer fórmula magistral ou preparado oficinal preparado e dispensado sob a responsabilidade de um farmacêutico".[2]

Devido à crescente evolução da indústria farmacêutica, e ao aumento de variedade de formulações no mercado, a prescrição de medicamentos manipulados tem vindo a diminuir, deixando de existir em múltiplas farmácias. Devido ao reduzido número de requisições e à baixa rentabilidade associada à gestão de *stocks* das matérias-primas, na Farmácia Central não se preparam medicamentos manipulados.

Considerei este aspeto como um ponto fraco, pois os medicamentos manipulados constituem um fator preponderante na obtenção de preparações que ainda não existem no mercado, ou na realização de ajustes de dose. Para além disto, não me permitiu colocar em prática os conhecimentos adquiridos em "Farmácia Galénica", o que seria uma atividade desafiante e com provável utilidade num futuro profissional.

3.2.3. Área de Dermocosmética

Nos dias de hoje há uma crescente preocupação das pessoas com uma imagem mais cuidada, sendo a Dermocosmética uma área farmacêutica em constante crescimento. A aposta nesta área na Farmácia Central é muito limitada, dispondo de alguns produtos de diferentes marcas, sendo que apenas a Bioderma® apresenta uma gama completa. Apesar

disto, não se justifica uma maior diversidade, dado que não há uma grande procura destes produtos por parte dos utentes.

Apesar da baixa diversidade, existe o cuidado de que os produtos existentes em *stock* vão de encontro às necessidades e gosto dos utentes.

Embora compreenda o porquê de não haver uma maior aposta por parte desta farmácia na área da Dermocosmética, como estagiária considero ter sido um dos pontos fracos do meu estágio, dado que gostava de ter tido oportunidade de ter uma maior experiência no aconselhamento destes produtos e um maior contacto com diferentes gamas.

3.3. Oportunidades

3.3.1. Participação em Formações

Ao longo do estágio tive oportunidade de participar em diversas formações dirigidas por especialistas, onde se podem destacar palestras acerca de "Nutrição clínica no tratamento oncológico", onde foram dados a conhecer e explicados os produtos da Fresubin®; Frequentei também uma palestra designada "Como ajudar os seus utentes a dormir melhor", uma problemática que está a tornar-se cada vez mais relevante, devido ao aumento de pessoas que passaram a precisar de suplementação ou medicação para dormir, devido à ansiedade e *stress* derivados da pandemia COVID-19. Entre muitas outras, quero ainda destacar uma apresentação a que fui acerca do coronavírus, um dos temas mais preocupantes atualmente, e do papel do farmacêutico na testagem e educação da população. Devido ao contexto pandémico estas formações ocorreram em regime *online*, mas tive também possibilidade de participar em algumas formações presenciais ministradas na farmácia por delegados de informação médica.

Estas formações são fundamentais, não só para consolidar conhecimentos, mas também para conhecer melhor alguns produtos, o que constitui uma mais-valia no momento do aconselhamento farmacêutico.

Sendo que a área farmacêutica é uma área em constante evolução, estas formações surgem também como uma oportunidade para que os profissionais de saúde se mantenham atualizados acerca dos novos produtos existentes, de modo a poder prestar o melhor aconselhamento aos utentes.

3.3.2. Contacto com utentes com diferentes perfis demográficos

A Farmácia Central, devido à sua localização na baixa de Coimbra, é frequentada por uma grande diversidade de utentes. Isto permitiu-me contactar com utentes com necessidades distintas, havendo a necessidade de adaptar a minha linguagem a cada situação. A faixa etária mais comum entre os utentes corresponde aos idosos, os quais necessitam de uma atenção especial, uma vez que muitos vivem e passam grande parte do tempo sozinhos, acabando por procurar um apoio e companhia no farmacêutico. Muitos deles frequentam a farmácia já há muitos anos, e ao longo do período de estágio comecei a tratar alguns deles pelo nome, estabelecendo uma relação mais próxima.

É também comum encontrar utentes mais carenciados e toxicodependentes. São pessoas mais complicadas de lidar, e mostrou-se fundamental aprender a comunicar com eles, mantendo sempre uma postura profissional, de modo a evitar conflitos.

Devido à sua localização na zona central da cidade, esta farmácia acaba também por ser procurada por turistas, o que me deu oportunidade de experienciar atendimentos noutros idiomas, particularmente em inglês.

3.4. Ameaças

3.4.1. Pandemia COVID-19

A pandemia COVID-19 resultou em alterações significativas no quotidiano de toda a população. Em janeiro, quando iniciei o estágio, Portugal entrou numa fase de confinamento. Embora o estágio não tenha sido interrompido, houve uma reorganização da equipa da farmácia, e passamos a trabalhar por turnos. Houve também uma notória diferença no volume de utentes da farmácia, os quais apenas aí se deslocavam com o intuito de adquirir o que fosse estritamente necessário, no menor período de tempo possível, impossibilitando a execução de um aconselhamento mais personalizado. Com o decorrer do estágio, e devido ao avanço da vacinação e ao progressivo desconfinamento, em meados de março o volume de clientes voltou ao normal e terminou o trabalho por turnos. Apesar disso, o uso de máscara obrigatório, o distanciamento dos utentes ao balcão e a existência de acrílicos de proteção são fatores que embora necessário para evitar a propagação do vírus, constituem uma barreira na comunicação entre o utente e o farmacêutico.

3.4.2. Estabelecimentos de Venda de MNSRM e farmácias circundantes

Na Baixa de Coimbra existe um grande número de farmácias com grande proximidade entre si. Esta grande oferta num pequeno raio da Farmácia Central constitui uma ameaça, tornando necessária a criação de estratégias para atrair os utentes. Estas estratégias passam por um atendimento de qualidade, pela criação de campanhas e pela prestação de serviços diferenciadores.

Para além das farmácias circundantes, existem também locais de venda de MNSRM nas imediações da farmácia. Isto constitui uma notória ameaça, uma vez que o grande volume de compras destes locais possibilita a aquisição de produtos a um preço inferior e, consequentemente, a prática de preços mais acessíveis comparativamente às farmácias. Assim, torna-se imperativo uma diferenciação no atendimento prestado na farmácia, através de um aconselhamento mais personalizado que vá de encontro às expectativas e necessidades do utente, destacando o valor do farmacêutico na sociedade.

3.4.3. Ruído exterior e inexistência de estacionamentos

O facto de a Farmácia Central se localizar numa rua muito movimentada e ter sempre duas portas abertas para o exterior faz com que o barulho do tráfego se faça sentir ao longo do dia. O prédio ao lado também se encontra em obras, o que resulta num grande volume de ruído. Isto constitui uma ameaça ao normal funcionamento da farmácia, uma vez que existia uma grande dificuldade em compreender os utentes, havendo por vezes a necessidade de repetirem o que estavam a dizer.

Também o facto de não existir estacionamento na farmácia ou nas imediações constitui uma grande ameaça, pois uma pessoa que se desloque de carro terá de o deixar bastante longe. Isto faz com que os utentes que frequentam a farmácia sejam na sua maioria pessoas que se deslocam a pé ou em transportes públicos, pois as pessoas que se descolam de carro acabam muitas vezes por preferir uma farmácia com melhor acessibilidade.

4. Casos Práticos

Caso 1:

Uma utente grávida dirigiu-se à farmácia, apresentando dor de cabeça e congestão nasal. Referiu sofrer de sinusite e tomar habitualmente o Sinutab® (paracetamol 500mg + pseudoefedrina 30mg), mas que o médico a informou que não poderia tomar este

medicamento durante a gravidez. Assim sendo, e após me assegurar de que a utente não sofria de problemas hepáticos, recomendei como medidas não farmacológicas o repouso e o aumento da ingestão de líquidos. Para aliviar a dor de cabeça, sugeri a toma de paracetamol na posologia de 1 comprimido de 8/8h em SOS. Como descongestionante nasal aconselhei a utilização de uma água do mar hipertónica. Expliquei que deveria realizar 1 a 2 pulverizações em cada narina até 3 vezes por dia, por um período máximo de 4-5 dias, dado que a utilização contínua pode causar secura nasal, e o uso por um período superior a 10 dias resulta num efeito *rebound*. No caso de não se sentir melhor ao fim de 5 dias, referi que deveria dirigir-se ao médico, para averiguar a necessidade de um corticosteroide nasal.

Caso 2:

Um utente do sexo masculino com cerca de 30 anos dirige-se à farmácia e revela sentir dificuldade em adormecer. Anuncia que vai começar um novo emprego, e que ao longo da vida sempre sentiu dificuldade em adormecer associada a alguma ansiedade em situações de mudança. Questionei se apresentava mais algum sintoma associado à ansiedade como enjoos ou mal-estar, ao que respondeu negativamente.

Face a esta situação, aconselhei a toma de Valdispertstress® na posologia de um comprimido três vezes por dia. Trata-se de um medicamento não sujeito a receita médica (MNSRM) indicado em situações de *stress*, como é o caso do utente. É um medicamento à base de plantas, constituído por extrato de raiz de valeriana (associado a uma sensação de calma e relaxamento) e estróbilo de lúpulo (possui uma ação calmante, auxiliando no alívio dos sintomas de stress e ansiedade).

Aconselhei ainda algumas medidas não farmacológicas que poderiam ajudar a reduzir o tempo até adormecer, como evitar a utilização de aparelhos eletrónicos na hora de dormir, dormir num ambiente confortável e evitar praticar exercício físico 2 horas antes de dormir.

Referi também que caso a dificuldade em adormecer não passasse ao fim de duas semanas, deveria consultar o médico.

Caso 3:

Uma jovem com cerca de 25 anos dirige-se à farmácia com uma receita de Fosfomicina®, indicada no tratamento de infeções urinárias. Apresentava-se queixosa, dizendo tratar-se de uma situação recorrente, sendo já a segunda vez no decorrente ano,

que estava a tomar medicação para este problema. Após colocar algumas questões de modo a perceber as causas e sintomas, a utente admitiu que raramente bebe água ao longo do dia e não utiliza um produto específico para a higiene genital. Referiu ainda que não se encontra a tomar mais nenhum medicamento.

Assim sendo, alertei para a importância das medidas não farmacológicas, como a ingestão abundante de água e aconselhei o Lactacyd® para uma adequada higiene genital. Referi também que deveria evitar o uso de pensos diários e a utilização de roupa interior sintética, e aconselhei a que esvaziasse completamente a bexiga quando urina.

Aquando da cedência do medicamento garanti ainda que a utente sabia como deveria tomá-lo: dissolver o conteúdo da saqueta num copo com água e ingeri-lo numa só toma com o estômago vazio.

5. Considerações Finais

Durante os 5 anos do MICF adquirimos conhecimentos teóricos que nos permitem exercer a profissão de farmacêutico, mas a farmácia comunitária surge como muito mais que um espaço onde pomos em prática esses conhecimentos. É também um espaço onde nos relacionamos com os utentes e com toda a equipa de trabalho.

Este estágio constituiu o meu primeiro contacto com a realidade profissional e permitiu-me aplicar os conhecimentos que adquiri ao longo do curso, mas também desenvolver novas competências, que decerto terão grande impacto num futuro profissional.

É um trabalho muito desafiante, mas também muito gratificante, sendo fundamental estarmos seguros dos nossos conhecimentos, para que possamos transmitir essa confiança ao utente.

Resta-me deixar um obrigada a toda a equipa e estagiários da Farmácia Central por toda a disponibilidade e boa disposição, por me terem recebido tão bem e por todos os conhecimentos transmitidos.

6. Bibliografia

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PARTE II

RELATÓRIO DE ESTÁGIO EM INDÚSTRIA FARMACÊUTICA

Laboratórios Basi - Mortágua

Sob orientação do Dr. Francisco Baptista

Lista de Abreviaturas

CTD – *Common Technical Document*

FFUC – Faculdade de Farmácia da Universidade de Coimbra

FI – Folheto Informativo

IF – Indústria Farmacêutica

INFARMED, I.P. – Autoridade Nacional do Medicamento e Produtos de Saúde, I.P.

MICF – Mestrado Integrado em Ciências Farmacêuticas

RCM – Resumo das Características do Medicamento

SWOT – *Strenghts, Weaknesses, Opportunities, Threats*

1. Introdução

O Mestrado Integrado em Ciências Farmacêuticas é constituído por um plano curricular abrangente, que permite aos seus alunos enveredar pelas diversas áreas do medicamento. No segundo semestre do 5º ano é realizado um Estágio Curricular e a FFUC oferece, para além do estágio obrigatório em Farmácia Comunitária, a possibilidade de estagiar noutras áreas através de protocolos estabelecidos com diferentes entidades.

Assim sendo, devido à curiosidade sobre o mundo farmacêutico e ao interesse suscitado pelas Unidades Curriculares de Assuntos Regulamentares do Medicamento e Tecnologia Farmacêutica, optei por realizar um estágio em Indústria Farmacêutica nos Laboratórios Basi, para além do estágio em Farmácia Comunitária. Realizei um estágio na área de Gestão de Produto, mais concretamente no departamento de Alterações e Renovações Regulamentares, o qual decorreu entre os dias 3 de maio e 30 de julho de 2021, sob orientação do Dr. Francisco Baptista.

O presente relatório tem como objetivo apresentar uma análise SWOT, identificativa dos Pontos Fortes (*Strenghts*), Pontos Fracos (*Weaknesses*), Oportunidades (*Opportunities*) e Ameaças (*Threats*) desta experiência.

2. Laboratórios Basi – Indústria Farmacêutica, S.A.

Os Laboratórios Basi, sediados em Mortágua, estão integrados no Grupo FHC | Farmacêutica, o qual é constituído por empresas que estão presentes em todas as etapas do ciclo de vida do medicamento.[1]

Os Laboratórios Basi contam já com mais de 50 anos de história e assentam a sua atividade nos pilares flexibilidade, inovação, competitividade e eficiência. Possuem um portefólio com mais de 240 produtos farmacêuticos, abrangendo 17 áreas terapêuticas. Estes produtos têm um alcance global, estando presentes não só na Europa, como também na África e Médio Oriente, somando um total de 60 países.[2]

As suas instalações destinam-se ao desenvolvimento de formas líquidas e semissólidas e estão divididas em duas unidades: unidade de líquidos e semissólidos (LSM), e unidade de injetáveis. Trata-se de uma indústria certificada em termos de Qualidade pela norma ISO 9001:2015, em termos ambientais pela norma ISO 14001:2015, e em termos de Higiene e Segurança do trabalho pela norma ISO 45001:2018.[3] Esta IF engloba, para além da Produção e Gestão e Controlo de Qualidade, as áreas de Investigação e Desenvolvimento, Assuntos Regulamentares, Logística e Manutenção.

O departamento de Gestão de Produto mais propriamente dito engloba 3 subdepartamentos: *Codification & Artwork Management*, *Launch and Tech Transfer* e *Variations and Renewals*. Neste departamento tive oportunidade de explorar todo o circuito de Gestão de Produto, desde os lançamentos e transferências de fabrico, até às alterações e renovações, passando também pela elaboração de *artworks*.

3. Análise SWOT

Figura 3 - Análise SWOT do estágio realizado nos Laboratórios Basi.

3.1. Pontos Fortes

3.1.1. Formação inicial de integração

No primeiro dia de estágio tive a oportunidade de participar nas formações de integração, de modo a adquirir uma ideia geral sobre o funcionamento da IF, bem como a forma como os seus departamentos estão estruturados física e organizacionalmente. A esta formação seguiu-se uma visita às instalações, inclusive às áreas de produção, o que me permitiu ter uma visão mais global acerca da IF. De seguida participei em algumas formações mais específicas, nomeadamente de modo a perceber o funcionamento de alguns programas e bases de dados, cuja utilização se mostrou ser útil ao longo do estágio. O conhecimento que me foi transmitido nestas formações foi fundamental para o meu desempenho ao longo do estágio e para a realização das tarefas que me foram atribuídas em conformidade com os padrões e exigências de qualidade da empresa.

3.1.2. A equipa de trabalho

Os Laboratórios Basi são compostos por uma equipa jovem e dinâmica, que se encontra em constante colaboração. A proximidade entre as idades dos estagiários e dos colaboradores proporcionou uma rápida integração e facilitou o processo de aprendizagem. Fui recebida de forma calorosa e todos os elementos do departamento mostraram estar disponíveis para me auxiliar nas tarefas sempre que precisasse, e encorajaram-me a expor as minhas dúvidas.

3.1.3. Aplicação de Conhecimentos de MICF nas tarefas realizadas

Tendo sempre presente as bases teóricas que me foram fornecidas durante o MICF, o estágio nos Laboratórios Basi permitiu-me colocar em prática os meus conhecimentos, enquanto experienciava a realidade do ambiente de uma IF.

Um ponto forte que quero salientar do meu percurso em IF foi a preparação de documentação para a submissão de alterações a entidades regulamentares, o que envolveu a elaboração de *cover letters* e o preenchimento de *application forms*, bem como a atualização da parte envolvida do documento técnico comum (CTD).

Apesar de na cadeira de Assuntos Regulamentares do Medicamento nos ser dada uma ideia da estrutura do CTD de um produto, durante o estágio pude contactar com inúmeros CTD, o que me permitiu compreender de forma prática o modo como este documento está organizado e ter uma melhor perceção das secções que é necessário consultar, para encontrar uma dada informação.

Considero também que os conhecimentos que nos são transmitidos durante o MICF acerca da forma de pesquisa no site do INFARMED foram uma mais-valia durante o estágio, dado que consegui utilizar esta plataforma de forma autónoma.

Outra tarefa que realizei durante o estágio foi a elaboração de materiais promocionais. Estes constituem uma forma de apresentar os medicamentos e outros produtos de saúde Basi aos profissionais de saúde e resultam de uma associação entre informações científicas do produto e um *design* apelativo.

3.1.4. Desenvolvimento de Competências

Para além da aplicação dos meus conhecimentos de MICF, este estágio permitiu-me desenvolver outras competências, nomeadamente a nível de pesquisa, linguístico e

informático. Relativamente às competências desenvolvidas a nível de pesquisa, estas prenderam-se com a necessidade de encontrar artigos científicos para a elaboração de materiais promocionais. O uso constante de plataformas como a *pubmed* e ICH levou a um melhoramento da minha capacidade de encontrar informação científica fidedigna e atualizada. O desenvolvimento de competências linguísticas está relacionado com o facto de a grande maioria dos artigos científicos e diretrizes, estarem escritos em inglês. Isto levou a um desenvolvimento da minha aptidão nesta língua, destacando a evolução a nível de vocabulário técnico-científico.

Relativamente ao desenvolvimento informático, este prendeu-se com o contacto constante com o Microsoft Word e Excel. É ainda importante referir a utilização constante da plataforma do INFARMED, IP (Infomed), de modo a conseguir aceder aos RCM e FI dos diferentes produtos.

3.1.5. Contacto com outros Departamentos

Outro ponto forte que acho relevante destacar é o contacto entre departamentos. Os departamentos de Assuntos Regulamentares, Gestão de Produto e Gestão de Qualidade encontram-se todos no mesmo *open space*, o que facilita a comunicação entre os elementos dos diferentes departamentos. Esta comunicação é imprescindível, dado que todos os departamentos se encontram relacionados.

Durante o meu estágio contactei com elementos dos outros subdepartamentos, como por exemplo com a equipa de *Artwork & Management*, na elaboração dos materiais promocionais, e com o departamento de Assuntos Regulamentares, o que me permitiu ter uma visão mais alargada das atividades realizadas nos restantes departamentos.

3.2. Pontos Fracos

3.2.1. Diversidade das tarefas realizadas

Como ponto fraco deste estágio destaco a pouca diversidade de tarefas realizadas. O elevado ritmo de trabalho por parte dos próprios colaboradores limita o tempo de que dispõem para nos atribuir uma maior diversidade de tarefas, pelo que acabei por passar grande parte do tempo a atualizar e inserir informação em bases de dados.

Apesar disso houve um esforço durante todo o estágio para que nos sentíssemos acompanhados e para que as nossas dúvidas fossem esclarecidas.

3.3. Oportunidades

3.3.1. Estágio em Indústria Farmacêutica – um Estágio diferenciador

O estigma de que um estudante de MICF tem como saídas profissionais exclusivamente Farmácia Comunitária e Farmácia Hospitalar está a desvanecer devido ao progressivo crescimento da Indústria Farmacêutica. O crescente interesse por esta área está relacionado com a enorme quantidade de departamentos e áreas que engloba, bem como com a diversidade de funções que é possível ocupar.

A FFUC distingue-se das Faculdades de Farmácia das restantes universidades ao possibilitar a realização de um estágio opcional em Indústria Farmacêutica, permitindo aos estudantes alargar a sua visão e aprofundar conhecimentos nesta área. Este estágio caracteriza-se assim como um ponto de distinção face aos colegas das outras universidades, assumindo-se como uma vantagem num mercado de trabalho cada vez mais competitivo.

3.3.2. Bases de dados e Rede Interna da Empresa

A IF, e mais concretamente o departamento de Assuntos Regulamentares e Gestão de Produto, assenta muito a sua informação em bases de dados, de modo a conseguir consolidar informação e responder eficientemente a pedidos de elementos, não só a nível interno, como a nível externo. Assim, durante o meu estágio auxiliei na criação e organização de bases de dados, com o objetivo de compilar a informação que de outra forma estaria dispersa nas pastas da rede interna.

A procura de informação para integrar a base de dados contemplou a análise de vários CTD, documentação referente a alterações e renovações e a consulta de bases de dados nacionais e internacionais, o que me permitiu expandir os conhecimentos neste ramo, permitindo que esta pesquisa com o passar do tempo se tornasse cada vez mais autónoma.

Neste processo de busca de informação tive contacto com documentos e ofícios de diferentes países, elaborados por diversas autoridades, o que considero ter sido uma ótima oportunidade para a aquisição de valências que não teria sido possível a nível académico.

3.4. Ameaças

3.4.1. Competitividade com profissionais de outras áreas

Durante o meu estágio foi possível contactar com profissionais com diferentes formações académicas nos departamentos de Assuntos Regulamentares e Gestão de Produto, o que poderá estar relacionado com a discrepância entre a evolução do plano curricular de MICF e o progressivo crescimento da Indústria Farmacêutica.

Este fator é uma ameaça, dado que dele resulta uma maior competitividade e dificuldade de acesso a este mercado cada vez mais exigente. Porém, é da nossa responsabilidade transmitir aquilo que nos diferencia dos outros profissionais nesta área, e salvaguardar a nossa posição, apostando em formações contínuas, para garantir a manutenção de informação atualizada.

4. Considerações Finais

É de louvar a oportunidade que tive de estagiar nos Laboratórios Basi, dado que foi o meu primeiro contacto com a IF, permitindo-me entender melhor a sua dinâmica e organização e a posição do farmacêutico nesta área.

Ao longo desta experiência tive oportunidade de consolidar e aprofundar os conhecimentos que adquiri ao longo do curso, mas também de desenvolver *soft skills*, como a gestão de tempo, as metodologias de trabalho e proatividade, que terão com certeza grande utilidade no futuro. Foi uma experiência enriquecedora e desafiante a nível profissional e pessoal, que me permitiu descobrir algo que teria todo o gosto em fazer no futuro.

Durante o estágio fui orientada por profissionais competentes, que demonstraram sempre total disponibilidade e me transmitiram conhecimentos e valores. Não posso então terminar sem deixar um grande obrigada a toda equipa de Gestão de Produto e Assuntos Regulamentares dos Laboratórios Basi.

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PARTE III

MONOGRAFIA

"State of the art of the evaluation of mycotoxins in rice: analytical methods, occurrence and detoxification strategies"

Sob orientação da Professora Doutora Ana Teresa Sanches Silva

List of Abbreviations

AFs – Aflatoxins
AFB1 – Aflatoxin B1
AFB2 – Aflatoxin B2
AFG1 – Aflatoxin G1
AFG2 – Aflatoxin G2
AFM1 – Aflatoxin M1
APCI – Atmospheric Pressure Chemical Ionization
APPI – Atmospheric Pressure Photo-Ionization
ASE – Accelerated solvent extraction
a_w – Water Activity
BEA – Beauvericin
CIT – Citrinin
CL – Chemiluminescence
CO_2 – Carbon dioxide
DAD – Diode array detector
DNA – Deoxyribonucleic acid
DON – Deoxynivalenol
DON-3G – Deoxynivalenol-3-glucoside
EC – European Commission
ECD – Electron capture detector
EFSA – European Food Safety Authority
ELISA – Enzyme-linked immunosorbent assay
ENNs – Enniatins
ESI – Electrospray Ionization
EO – Essential Oils
EU – European Union
FAO – Food and Agriculture Organization
FB1 – Fumonisin B1
FB2 – Fumonisin B2
FB3 – Fumonisin B3
FDA – Food and Drug Administration
FID – Flame ionization detector
FIIA – Flow injection immunoassay
FLD – Fluorescence detector
FUMs – Fumonisins
GC – Gas chromatography
GEMS – Global Environment Monitoring System
HBV – Hepatitis B virus
HCC – Hepatocellular carcinoma

HPLC – High Performance Liquid Chromatography
IAC – Immunoaffinity column
IARC – International Agency for Research on Cancer
INE – National Institute of Statistics (Portuguese)
LC – Liquid Chromatography
LFIA – Lateral Flow Immunoassay
LOD – Limit of Detection
LOQ – Limit of Quantification
MAE – Microwave-assisted extraction
MON – Moniliformin
MS – Mass spectrometry
MT – Million tons
OTA – Ochratoxin A
PFCD – Portuguese Food Composition Database
PGI – Protected Geographic Indication
PSA – Primary/ Secondary amine
QqQ – Triple quadrupole
QuEChERS – Quick, easy, cheap, effective, reliable and safe
RASFF – Rapid Alert System for Food and Feed
RH – Relative Humidity
RNA – Ribonucleic acid
ROS – Reactive Oxygen Species
SFE – Supercritical fluid extraction
SLE – Solid-liquid extraction
SPE – Solid Phase Extraction
SPME – Solid-phase microextraction
STC – Sterigmatocystin
TCs – Trichothecenes
TLC – Thin Layer Chromatography
TDI – Tolerable Daily Intake
TOF – Time-of-flight
UV – Ultraviolet
WHO – World Health Organization
ZEA – Zearalenone
ZEA-14G – Zearalenone-14-glucoside

Resumo

A prevalência de micotoxinas no meio ambiente está associada à potencial contaminação dos alimentos, principalmente no que respeita aos cereais, o que resulta num inevitável aumento da exposição humana. O arroz, sendo o segundo cereal mais consumido no mundo, constitui uma importante fonte de contaminação por micotoxinas, podendo ser contaminado por várias micotoxinas simultaneamente.

Devido à elevada frequência de alertas de contaminação por micotoxinas notificadas pelo portal *Rapid Alert System for Food and Feed* (RASFF) da Comissão Europeia, e à publicação de estudos que reportam níveis de micotoxinas acima dos limites regulamentares estabelecidos, este trabalho pretendeu fazer uma compilação de alguns desses estudos e rever as principais técnicas utilizadas na deteção e quantificação destes compostos em arroz. Para além disso foi realizada uma revisão dos efeitos tóxicos associados à contaminação por micotoxinas, bem como das técnicas utilizadas atualmente na tentativa de mitigar essa contaminação.

Apesar de um grande número de países já ter uma legislação definida com limites máximos definidos, existe ainda um longo caminho a percorrer nesta área, tendo sido este tema considerado uma prioridade pela Autoridade Europeia para a Segurança Alimentar (*European Food Safety Authority*, EFSA), sobretudo devido à crescente preocupação com as alterações climáticas, que favorecem o desenvolvimento de fungos em alimentos e potenciam a contaminação desses mesmos alimentos por micotoxinas. É necessário um maior número de estudos para poder definir a real exposição da população, bem como as consequências e potenciais sinergismos relacionados com a co-ocorrência de micotoxinas.

Palavras-chave: Aflatoxinas, Arroz, Co-ocorrência, Cromatografia líquida de alta resolução, Espetrometria de massas, Micotoxinas, Mitigação, QueChERS.

Abstract

The prevalence of mycotoxins in the environment is associated with potential crops contamination, mostly cereals, which results in an unavoidable increase of human exposure. Rice, being the second most consumed cereal worldwide, constitutes an important source of contamination by mycotoxins, since it is usually contaminated with several mycotoxins simultaneously.

Due to the increasing number of notifications reported by the Rapid Alert System for Food and Feed (RASFF) portal of the European Commission, and the publication of studies that reported the occurrence of mycotoxins in levels above the legislated limits, this work intends to compile some of those studies and review the main methods used on the detection and quantification of these compounds in rice. Moreover, the toxic effects associated with mycotoxins contamination were also reviewed, along with the techniques used in order to attempt to minimize that contamination.

Although a great number of countries already have defined legislation with specified maximum levels, much more needs to be done, since this thematic was considered by the European Food Safety Authority (EFSA) as a priority, mostly due to the growing concern with climatic changes. Climatic changes are proven to be associated with fungal growth in foodstuffs, leading to mycotoxins' contamination. Further investigation is still required to establish the real exposition to these contaminants, as well as the consequences and possible synergistic effects due to the co-occurrence of mycotoxins.

Keywords: Aflatoxins, Co-occurrence, High Performance Liquid Chromatography, Mass spectrometry, Mitigation, Mycotoxins, QuEChERS, Rice.

1. Introduction

Mycotoxins are secondary products resultant from toxigenic fungal metabolism. They consist in low molecular weight metabolites and are mostly produced by the genus *Aspergillus*, *Fusarium* and *Penicillium*.[1] Over 400 types of mycotoxins have been identified, but attention is mainly given to those with greatest public health relevance, such as aflatoxins (AFs), ochratoxin A (OTA), fumonisins (FUMs), trichothecenes (TCs) and zearalenone (ZEA).[1,2] Their structural diversity results in different chemical and physicochemical properties, and they are associated with the development of acute and chronic problems such as carcinogenicity, teratogenicity, mutagenicity and hepatotoxicity.[1,3]

Due to their worldwide prevalence and their association with health disorders, mycotoxins have been recognized as a major health and economic issue.[4] In fact, these toxins are considered by EFSA as a threat and are one of the most reported hazards on RASFF.[5]

The European Commission has established a regulation where the maximum levels allowed for some mycotoxins are established, but many studies have reported cases where those limits are exceeded.[6] Therefore, and due to climate change, a strict control is required, as well as the development and validation of suitable analytical methods.[7]

It is almost impossible to avoid the presence of mycotoxins in the food chain, but their levels can be controlled by the implementation of good agriculture practices and decontamination processes.[1]

This monography consists of a review of the most commonly found mycotoxins in rice and the main methods used for their extraction, detection and quantification, as well as the techniques used in decontamination processes.

1.1. Portugal as rice producer

Around 90% of worldwide rice production takes place in Asia, with China being de greatest rice producer. According to FAO (Food and Agriculture Organization), in 2019, China produced about 210 million tons (MT) of rice, followed by India (177 MT) and Indonesia (54 MT). In the 27 member countries of the European Union (EU), around 472 thousand hectares are occupied with rice cultivation. The average rice production in those countries is around 3.1 MT, and 1.1 MT are imported annually. In Portugal, the area devoted to rice cultivation rounds 30×10^3 ha (hectares), which results in about 180 thousand tons of rice per year, which corresponds to only 6% of total EU production.[8, 9]

Portugal is the fourth major rice producer in Europe, following Greece, Spain and Italy, and its production generates around 60 million euros every year. In Portugal, rice is cultivated under irrigated conditions, which explains their location in lowlands along the Tejo river, and along the Sado and Mondego river valleys.[9]

Rice crop's may be influenced by some factors, such as high temperatures, water availability and sun radiation.[10] Those factors affect rice quality and reputation and allow their classification as "Protected Geographic Indication" (PGI). In Portugal, to date, only two brands were classified as PGI: *Arroz Carolino* from Lezírias Ribatejanas and *Arroz Carolino* from Baixo Mondego.[11] After being collected and packaged, it is of main importance that rice remains with low humidity levels (less than 14%) in order to avoid fungal growth and eventual mycotoxins' contamination.[12]

1.2. Rice intake in Portugal and globally

Rice (*Oryza sativa* L.) plays an important role in economy and society, since it's considered a staple food and is consumed worldwide.[13] According to World Health Organization (WHO), in 2003, the Global Environment Monitoring System/Food Contamination Monitoring and Assessment Programme (GEMS/Food) revealed that rice was the second cereal with bigger consumption/per capita, right after wheat.[14]

Rice ingestion is found to be a great source of micronutrients, and its consumption in Asian countries is around 100-150 Kg per capita/year. In the rest of the world, rice consumption rounds 60 Kg per capita/year.[15] Portugal is considered the greater rice consumer in Europe. According to INE (Portuguese National Institute of Statistics), in 2019 were ingested approximately 14.9 Kg per capita, which is about three times above the EU average.[11,16]

A great amount of rice produced in Portugal resembles to the round grain variety "Carolino" (Japonica), and the characteristically elongated grain "Agulha" (Indica).[15,9]

After being collected, rice can suffer some physical processes, like drying, peeling and bleaching.[11] It can also suffer thermal treatment during industrial processing, and in that case it is called vaporized rice.[15] Rice processing involves milling to multiple degrees of coarseness, in order to obtain different final products. Brown rice is obtained through the removal of the hulls from rough rice. Milling allows the removal of the outer bran from brown rice, which results on white rice.[17] Brown rice was only submitted to drying and

peeling. This kind of rice is a great source of fibre, minerals and vitamins in comparison with other types of rice, since some of these compounds are lost during bleaching.[15]

The presence of gradient levels of temperature and moisture and its mineral composition, make rice a potential target for mycotoxins' contamination. These properties, associated with its great consumption worldwide, constitute an important source of exposure for humans.[17]

1.3. Rice: Nutrients and bioactive compounds

From a nutritional point of view, rice plays an important role in both human and animal nutrition and provides over 20% of the calories consumed worldwide.[4,18] According to the Portuguese Food Composition Database (PFCD), rice is a great source of B and E vitamins, riboflavin, thiamine, niacin and iron and other important minerals. Among cereals, rice is the one with the higher value of carbohydrates, and lower protein, lipid and fibre contents.[10,19] The bioactive compounds of rice include polyphenols, namely anthocyanins and flavonoids.[18] The composition described in PFCD is similar to the one found in the FoodData central, the American Food Composition database (US Department of Agriculture).[20]

The nutritional composition differs depending on the type of rice, which varies in grain length, colour, aroma, and growing conditions. After being harvested and processed, rice can be either categorized as white or brown rice, depending on if it went through a milling process or not, respectively.[18] The milling process is associated with a reduction of mineral, vitamins and fibre levels, which is why brown rice has a better nutritional value in comparison with milled white rice, although the last one is a major source of calories.[10,11] Some countries prepare parboiled rice, which is submitted to an industrial process where is submerged in water, vaporized, and then dried. This type of rice is a great source of fibre and minerals.[18] Therefore, rice seems to play an important role, not only at nutrition, but some studies have also reported that the presence of anthocyanins may have an impact in modulating inflammatory responses.[18]

2. Mycotoxins

Over 400 mycotoxins have been identified to date, but only a few represent a concern to human health, including aflatoxins (AFs), ochratoxin A (OTA), deoxynivalenol (DON), T-2/HT-2 toxins, fumonisins (FUMs) and zearalenone (ZEA).[1]

2.1. Aflatoxins

Aflatoxins are a family of mycotoxins produced by fungus of the genus *Aspergillus* (mainly *A. flavus* and *A. parasiticus*), which can be found in many food matrices, including rice.[2] These fungi are distributed worldwide with optimal growth conditions of 30 °C temperature and 80-85% relative humidity, being most prevalent in tropical and subtropical regions.[2,21]

Among all classes of mycotoxins, aflatoxins are thought to be the most toxic, and the greatest concern, not only at economic level (mainly in the United States and European Union), but also in health terms, contributing to hundreds of hepatocellular carcinoma cases every year in developing countries.[22,23]

The most relevant aflatoxins are aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), aflatoxin G2 (AFG2) and aflatoxin M1 (AFM1), with aflatoxin B1 being the most occurring and toxic one.[24] AFB1 and AFB2 are produced by both *A. flavus* and *A. parasiticus*, while AFG1 and AFG2 are only produced by the last mentioned.[25] Besides being widely distributed, this class of structurally related toxins, is also very stable and resistant when submitted to some processes in the food processing chain, like roasting, extrusion and cooking, which makes them a paramount concern in public health and economic interests.[26]

2.2. Ochratoxins

Ochratoxins are produced by *Aspergillus* or *Penincillum*, mainly *A. ochraceus* and *P. verrucosum,* under variable environmental conditions. Although *P. verrucosum* is the responsible for the growth of ochratoxins in colder areas, *A. ochraceus* is the responsible for their prevalence in tropical regions. Ochratoxin A is known to be the most toxic and prevalent among this class.[24,25] Ochratoxins are found to be stable in acidic conditions and elevated temperatures. This thermal resistance makes them difficult to eliminate under normal cooking conditions.[25] OTA is considered a global contaminant and has been reported in many food commodities, with a considerable prevalence in cereals.[21]

2.3. Fumonisins

Fumonisins are fungal toxins produced by *Fusarium* spp. (mainly *F. verticillioides* and *F. proliferatum*), found most frequently in maize and cereals. This type of mycotoxins is widely distributed, as their producers are able to grow under a wide range of temperatures and

relative humidity.[27] This class of mycotoxins is known to be non-fluorescent and hydrophilic, unlike other classes, that can be completely dissolved in organic solvents.[25]

There are more than over 28 known fumonisins, divided into four main groups: A, B, C and P. The fumonisins B group is the most frequent in nature and comprises fumonisin B1 (FB1), fumonisin B2 (FB2) and fumonisin B3 (FB3), with FB1 being the most toxic and frequent member of the family (70-80% of all fumonisins).[28]

2.4. Trichothecenes

Among all the mycotoxins that have been identified so far, about 200 belong to the trichothecenes family. Trichothecenes (TCs) are a group of mycotoxins mainly produced by fungal species of *Fusarium* spp. This family is organized in four groups: the trichothecenes A, B, C and D, each with structurally related toxins. Types A and B TCs are the most frequent of the group.[29]

Type A TCs are the most toxic and include T-2 and HT-2 toxins. These toxins are mainly produced by *F. lansehtiae*, *F. sporotrichioides*, *F. poae* and *F. acumninatum*, and have been detected in many food matrices including barley, oat, wheat, rice, and maize.[28]

Type B TCs include nivalenol and deoxynivalenol (DON), with the last one being the most frequent, although less toxic, of the group. DON is predominantly produced by *F. culmorum* and *F. graminearum* and can be found in cereal and cereal-based products, widely distributed.[27]

Of all classes of mycotoxins, trichothecenes are the most structurally diversified, and mainly contaminate cereals, like maize, rice, oats, wheat, and barley.[30]

2.5. Zearalenone

Zearalenone (ZEA) is a macrocyclic lactone produced by multiple species of *Fusarium,* mainly *F. graminearum*, *F. sporotrichioides* and *F. semitectum.* It is usually associated with maize crops, but it can also be found in other cereals like wheat, barley, rice, and oat. This toxin tends to appear mostly in temperate and warm countries with high humidity levels. [28] ZEA´s contamination usually occurs concurrently with DON or, less frequently, with aflatoxins. This mycotoxin can be partially eliminated under elevated temperatures but is stable under normal cooking conditions.[31]

2.6. Co-occurrence

Co-occurrence consists in the occurrence of multiple mycotoxins within the same food matrix.[2] This multiple exposure is very frequent, being even more common than the presence of a single mycotoxin.[24] Although there is still a lot to know, the co-occurrence of mycotoxins may result in additive or synergistic effects, increasing the toxicity of the contaminated material.[2]

In rice, the occurrence of different mycotoxins and their metabolites is unavoidable due to the simultaneous infection with multiple fungi, that are toxigenic, i.e. they are able to produce multiple mycotoxins.[17]

AFB1 and AFB2 are the most frequently documented as co-occurring mycotoxins, but it has also been reported the co-occurrence of mycotoxins produced by different fungi species.[17] It has been described in several studies the combined effects of mycotoxins; however it is still unknown the nature of the observed effects, the relative potencies of each mycotoxin and the way those interactions could enhance their respective toxic effects.[24,32]

2.7. Emerging mycotoxins

Emerging mycotoxins can be defined as a group of mycotoxins that has not been routinely determined or legislatively regulated, but the evidence of their incidence has been rapidly increasing in the last few decades.[33]

Enniatins (ENNs) and beauvericin (BEA) are structurally related mycotoxins that belong to this class, produced by many filamentous fungi. ENNs are mainly produced by *Fusarium* spp, *Alternaria* spp, *Halosapheia* spp and *Verticillum* spp, while BEA is mostly produced by *Beauveria* spp., *Paecilomyces* spp., *Polyporus* spp., and *Fusarium* spp..[32,34] These emerging mycotoxins have been reported in several matrices in recent publications, but their toxic effects have not yet been well established. The main source of contamination of these mycotoxins are cereals (including maize, wheat, barley and rice), not only for being ideal matrices for fungal growth, but also because of their great consumption among the population.[32]

To date, 29 enniatin analogues have been reported, with enniatin A (ENN A), A_1 (ENN A_1), B (ENN B), B_1 (ENN B_1) and B_4 (ENN B_4) being the most prevalent, but there have also been found lower amounts of enniatins C, D, E and F. Their structural differences are responsible for the distinct bioactivities of these analogues.[34]

Studies have shown that emerging mycotoxins are prevalent worldwide and are able to co-occur with other classes of mycotoxins. Therefore, they might be an hazard for human and animal health. There has not been found reports on mycotoxicosis caused by BEA and ENNs, although some studies have described possible risks associated with their ingestion due to their ionophoric properties. Further investigation needs to be done in order to evaluate their health risk and eventually come up with regulatory levels.[32,34]

Moniliformin (MON) and sterigmatocystin (STC) are also emerging mycotoxins that have also already been reported in rice. STC has the particularity of being a precursor of AFB1, and so they share a similar mechanism of toxicity, by forming DNA adducts and generating reactive oxygen species (ROS). This can lead to false negatives or underdetermination of AFB1, since STC can be later converted into its successor, considered by many authors as the most toxic and concerning mycotoxin.[33] *Alternaria* toxins, such as alternariol and tenuazonic acid, and citrinin (CIT) are other examples of emerging mycotoxins, mostly detected in fruits and vegetables.[33]

2.8. Masked mycotoxins

Masked mycotoxins are produced by plant enzymes involved in detoxification processes or during food processing through conjugation with polar substances like glucose, sulfate and amino acids. This structure modification leads to a difficulty in their detection by conventional analytical methods.[32,35]

Deoxynivalenol-3-glucoside (DON-3G) and ZEA-14-glucoside (ZEA-14G) are among the most commonly detected conjugates. Those conjugations are an attempt of the plants to make the compounds more soluble in water for faster elimination, and they usually exhibit lower toxicity in comparison with parent forms.[36]

When metabolized, the masked mycotoxins suffer hydrolysation, and release the original mycotoxin. This can also happen during processing and constitutes a concern, because masked mycotoxins are not being accounted by analytical methods and a food commodity that was judged as compliant might become non-compliant at a later stage, because of the release of the mycotoxin.[35]

2.9. Mycotoxins-producing fungi

Mycotoxin-producing fungi mostly belonging to the genera *Aspergillus*, *Fusarium* and *Penicillium* are among the organisms able to contaminate rice.[27]

Fungi growing conditions are dependent of many factors, like temperature, humidity, water activity, pH and nutritional composition of the food product, and so their relevance is different around the world. Even inside the same genera, different species may grow during different stages of production.[27]

Aspergillus grows predominately in tropical countries, with high temperatures paired with high values of relative humidity (RH) and water activity (a_w). For example, rice in tropical Asia is mostly contaminated with *Aspergillus* fungi (like *A. flavus* and *A. ochraceus*) because of the conditions during harvest and postharvest stages.[17] *Fusarium* spp. grows under high temperature and moisture and is the major cause of a decline in rice quality during cultivation due to environmental conditions. [27] *Penicillium* spp. is not found in the field during the growing period, and their contamination is usually associated with rice storage conditions.[27]

It is well known that not all fungi are threatening and not all their secondary metabolites are toxic, since mycotoxins' toxicity depends not only on their producer, but also on their interaction with each other and with other microorganisms, on the edaphoclimatic conditions and on the system of farm management (organic versus conventional) they are submitted to.[17] Moreover, fungal contamination of a certain food matrices, is not a synonym of its contamination with mycotoxins, since fungi only produce these metabolites under specific circumstances as a strategic defensive mechanism. Therefore, the production of mycotoxins might not be associated with the presence of the fungal itself, but with the presence of other fungi or microbes, or even with the fluctuation of the environmental conditions (like water availability and temperature).[37]

2.10. Factors associated with rice contamination by mycotoxins

Food contamination by mycotoxins is dependent on the presence of fungal, the application of unsuitable agricultural practices and the conditions of harvesting and storage. Since most mycotoxins are thermostable and consequently able to persist under food processing and cooking temperatures, the key to their absence must be based on the prevention of their occurrence.[38]

Mycotoxins' contamination may occur in different stages, since pre-harvest to post-harvest steps, during processing, packaging, distribution or even storage. Usually, mycotoxins' contamination is associated with fungal growth due to improper storage conditions.[30]

Rice is one of the food crops that is more susceptible to contamination after harvesting, since it is harvested in subtropical environments (under warm and humid conditions), and then stored for large amounts of time before its consumption. When stored under inappropriate conditions it constitutes a great substrate for fungal growth. According to FAO, every year around 15% of the rice harvest are lost due to fungal growth and mycotoxins' contamination.[39,40]

Rice development is strongly dependent on temperature, since it has a great impact on the plant photosynthesis, which when is submitted to temperature stress suffers a reduction on the physiological activity. Therefore, climate change may have a substantial impact on rice production. Along with temperature increase, projections point to a decrease in precipitation along the Mediterranean basin area, which should have a negative impact on this crop, since it is very dependent on water supply.[9]

Climate changes are also increasing mycotoxins' contamination. Earth temperature is expected to increase 1.5 to 4.5 °C until the end of the 21st century. Global warming boosts water evaporation from the surface, which results in an increasing of moisture in the atmosphere. Consequently, it is expected an increase in fungal population and mycotoxins' occurrence since temperature and humidity are key factors for their growth.[41]

In addition to global warming, the rising levels of carbon dioxide (CO_2) also presents an impact in food safety since it affects the availability and nutritional quality of rice. Specifically, it seems to be associated with a reduction on the levels of B vitamins, protein, zinc and iron.[9]

2.11. Toxicity and mechanisms of action of mycotoxins

Mycotoxins' contamination is associated with multiple risks to human health due to their toxicity, in particularly their carcinogenicity. In order to avoid these risks, taking into account epidemiological, experimental and mechanism studies, the International Agency for Research on Cancer (IARC) has come up with a scale of hazard assessment of mycotoxins in human health.[42]

Mycotoxin ingestion can result in both acute and chronic toxicity. Acute toxicity is associated with a rapid toxic response, while chronic toxicity is a result of a low dose exposure over a long period. While chronic toxicosis has been found to be a global problem, acute toxicosis is more common in developing countries, particularly in Africa.[22,42]

Aflatoxins have carcinogenic, mutagenic, hepatotoxic, teratogenic, and immunosuppressive effects, with liver being the most affected organ. AFB1 is the most toxic of all aflatoxins, with AFB2, AFG1 and AFG2 having, respectively, 50, 20 and 10% of its toxigenic power.[32] Aflatoxins have been classified by IARC as a Group 1 carcinogen, due to the high risk of development of hepatocellular carcinoma (HCC) after a chronic exposure. AFM1 is a result of AFB1's biotransformation and has been classified as a Group 2B (possibly carcinogenic to humans). In humans, acute aflatoxicosis usually results in abdominal pain, vomiting, pulmonary and cerebral edema, coma, convulsions or even death.[42,43]

After being ingested, aflatoxins are biotransformed in the liver by a family of enzymes called CYPP450. These are responsible for turning AFB1 into its carcinogenic form: AFB-8,9-epoxide. This metabolite is able to form adducts with cellular macromolecules, such as DNA, which results in a modification of its structure and biological activity, and therefore in the carcinogenic and mutagenic effects of the toxin. A mutation of gene p53 seems to be the base of the association between aflatoxins and HCC, and this type of cancer is found to be more prevalent in regions with high consumption of aflatoxins.[44]

In countries with high rate of hepatitis B virus (HBV), the exposure to AFB1 may constitute an even bigger issue, since the risk of liver cancer development after exposure to aflatoxins in HBV-positive people is about 30 times superior than in HBV-negative people.[38]

Ochratoxin A is a fat-soluble mycotoxin that has been classified by IARC as Group 2B (possible human carcinogen) and is associated with immunotoxicity, neurotoxicity, genotoxicity and embryotoxicity in both human and animals.[43,45] Its toxicity seems to be related with its structural similarity with phenylalanine, an essential amino acid. OTA inhibits proper protein synthesis in the kidney and liver, by interfering with phenylalanine hydroxylase. It also seems to interfere with DNA and RNA synthesis.[45]

Fumonisins are classified by IARC as belonging to Group 2B (possibly carcinogenic to humans) and seem to be associated with esophageal tumours and liver toxicity.[26,43] FB1 is found to be the most abundant and toxic of the group, followed by FB2 and FB3. Recent studies have been focusing on FUM's mechanism of action, and their similarity to sphinganine and sphingosine has come to attention with their possible role on the inhibition of

sphingolipids biosynthesis. These sphingolipids are allocated on the membrane of eukaryotic cells and are responsible for the formation of secondary messengers, involved in the regulation of several cellular processes such as gene expression and proteins activation/deactivation. By disrupting these mechanisms, this class of mycotoxins might contribute to many effects at a cellular level like apoptosis induction and carcinogenic effects.[46]

Some studies have correlated the levels of FBs in food with the development of esophageal cancer in humans. Moreover, they also seem to be associated with brain and spinal cord neural tube defects, when ingested in high levels during pregnancy.[46]

ZEA is frequently described as an estrogenic mycotoxin due to its structural similarity to estrogens. Because of that, ZEA and its metabolites are able to bind competitively to estrogen receptors, activate the estrogen gene, and induce reproductive disorders. Long-time exposure to ZEA has also shown to be associated with liver lesions and hepatocarcinoma development in worst cases.[47] ZEA is associated with cytotoxic, hematologic, genotoxic, hepatotoxic and immunotoxic effects, and has been classified by IARC as a group 3 carcinogenic (not classified as human carcinogenic), due to reduced evidence in experimental animals and inadequate evidence in humans.[5,43]

Trichothecenes can easily penetrate cell membranes and react with cellular organelles and nucleic acids, which justify their high toxicity. The major mechanism described consists on the inhibition of ribosomal protein synthesis, followed by disruption of DNA and RNA synthesis.[48]

DON has been found to be immunosuppressant and genotoxic, but due to lack of evidence of carcinogenicity, was classified by IARC as group 3 carcinogenic (not classified as human carcinogenic).[5,26,43] Nausea, vomiting, diarrhea, dizziness and fever are some of the reported effects after human exposure to DON-contaminated grains.[49]

T-2 toxins have also been classified as group 3 by IARC, and along with HT-2 toxin have been associated with a reduction of body weight, liver and kidney toxicity, immunotoxicity, neurotoxicity and haematotoxic effects.[5,43]

2.12. Mycotoxins legislation with special focus at EU level

Due to the global toxic effects of mycotoxins, a vast number of governmental authorities (including FDA, WHO, EFSA, FAO) are paying attention and setting maximum levels for mycotoxins in foodstuffs, in order to protect human health.[24] The availability of

toxicological information and dietary exposure, along with the distribution of mycotoxins and the available analytical methods are among the factors that influence the regulated levels.[50]

In Europe, and more specifically in Portugal, the maximum levels of mycotoxins are established for the most known and frequently detected ones in the section 2 of the Commission Regulation (EC) No. 1881/2006 of 19 December 2006 and its amendments that sets maximum levels for certain contaminants in foodstuffs. Those limits were fixed according to mycotoxins' prevalence and toxicity, and are established for several molecules, such as AFs, OTA, DON, ZEA and FMs in many food matrices.[6] This regulation was amended in 2010 by the Commission Regulation (EU) No. 165/2010 of 26 February 2010 which established new AFs maximum levels in foodstuffs. Before the milling process, the levels are expected to be slightly higher, due to the greater fraction of mycotoxins in bran, that are removed during this process, lowering the concentrations to acceptable levels.[17,51] The levels established for cereals by the Commission Regulation (EC) No. 1881/2006 of 19 December, are described in Table 1.

Table 1 - Maximum permitted levels (µg/kg) established for mycotoxins in cereals

	Maize	Other cereals and derivates	
Mycotoxins	**Maximum levels (µg/kg)**	**Matrix**	**Maximum levels (µg/kg)**
AFB1	5	Cereals and derivates	2
Sum of AFB1, B2, G1 and G2	10		4
OTA	3-5	Unprocessed cereals (including raw rice)	5
		Products derived from unprocessed cereals	3
DON	750 - 1750	Unprocessed cereals, not including durum wheat, oats, and maize	1250
ZEA	20 - 200	Unprocessed cereals, except maize and rice	100
T-2 and HT-2 toxin	200 (indicative TDI level)	Unprocessed cereals and cereal products	-
Fumonisins	200 - 2000	Rice	-

Legend: Adapted from Commission Regulation (EC) No. 1881/2006 and its amendments, establishing the maximum permitted levels of mycotoxins in cereals.[6] TDI – Tolerable Daily Intake.

As a result of the low levels of *Fusarium* toxins detected in rice, this matrix does not have the same maximum permitted levels as other cereals. No maximum levels of *Fusarium* toxins (ZEA, FUMs, T-2 and HT-2 toxin) are established for rice or rice products.[6] Due to the harmful effects related to the presence of T-2 and HT-2 toxin in feed and foodstuff, the European Commission came out with a recommendation ("Commission Recommendation of 27 March 2013 on the presence of T-2 and HT-2 toxin in cereals and cereal products"), where are established the tolerable daily intake (TDI) for some food matrices. Rice and rice products are not included in those matrices because these toxins occur in very low levels in this matrix, and so it was excluded from this Recommendation, since it doesn't seem to constitute an health concern.[52]

Outside the European Union, levels of mycotoxins are regulated according to different legally binding documents, or have no limits at all, depending on the type of mycotoxin and foodstuff. All these limits were described in "Worldwide regulations for mycotoxins in food and feed in 2003" by FAO (2004).[53]

China and India, the main rice producers in the world, have established maximum levels, although those are much higher than those of EU. China sets a maximum of 10 μg/Kg to AFB1 and 20 μg/Kg to the sum of AFs. In India, the limits for AFs are set in 30 μg/Kg, which constitutes a matter of concern to the consumers health.[53]

One of the greatest limitations in this regulation is associated with the fact that the maximum limits are set according with the mycotoxins' individual toxicity, not taking in account their co-occurrence and potential synergism.

Maize has regulated levels for more mycotoxins than rice, and those levels are higher for maize as we can conclude by the analysis of Table 1. Since rice is the second most consumed cereal worldwide, after wheat, and a great number of studies have reported its contamination by several unregulated mycotoxins, the establishment of maximum limits for more mycotoxins in rice seems to be required.

3. Analytical methodologies to determine mycotoxins

Since their first discovery, many methods have been developed for the analysis of mycotoxins in food, despite the frequent analytical challenges. These challenges include difficulties associated with low-level contamination, complex matrices where contamination occurs, evolving complex extraction procedures, the structurally diversity of mycotoxins as well as their co-occurrence. In order to face these challenges, many analytical methods have

been developed, although they require continuous improvements in order to support mycotoxin legislation and protect human's health and the food and feed industry.[4]

Mycotoxin determination in food samples is usually associated with common steps, that include sampling, homogenization, sample preparation (extraction generally followed by clean-up) and lastly detection and quantification.[4]

3.1. Sampling

Sampling is considered a key step in mycotoxins analysis, since it is fundamental to ensure the accuracy of the results and to decide if the whole food batch is compliant or not.[4,54]

Mycotoxins are not distributed homogeneously in food; therefore, the implementation of a rigorous sampling protocol is of great importance, to guarantee that the analysed sample is representative of the entire bulk. Considering consumers safety and producers protection, many sampling plans have been established.[54] These plans are instituted by regulatory entities, such as FDA and the European Commission (EC), that came up with the Commission Regulation No. 401/ 2006 where the sampling and analysis methods (such as the number and amount of samples) for the official control of mycotoxins in foodstuffs are described.[8]

Processed products usually require simpler sampling procedures, since mycotoxins are less heterogeneously distributed in these products than in raw agricultural commodities.[27]

3.2. Extraction and clean-up

Extraction is a step required before most quantification analytical methods.[26] This step is of great importance and consists on the separation of the analytes of interest from the food matrix, followed by a clean-up phase to eliminate possible interferences. In the case of solid food samples, like rice, the first step consists on the extraction of compounds of interest into a liquid phase, followed by a clean-up step in order to enhance the specificity and sensitivity of the detection method.[55]

The mycotoxins' chemical properties, the nature of the food matrix and the final method for detection that will be used are three main factors that should be considered in the selection of the methods for extraction and clean-up.[4]

The most frequently used extraction technique consists in the extraction using organic solvents: liquid-liquid extraction (in case of a liquid sample) and solid-liquid extraction (in case of solid samples).[56] Solid-liquid extraction (SLE) is commonly used for mycotoxins extraction from grains and cereals, such as rice. The solvent selection must rely on the polarity of the mycotoxins of interest and on the type of matrix. Mycotoxins are usually soluble in organic solvents (like chloroform, acetone, methanol, and acetonitrile), but barely soluble in water. Fumonisins are an exception and present high-water solubility. A mixture of organic solvents with water or acidic solvent is commonly used since water enhance the penetration of the organic solvents in the food matrix and the acidic solvent has the ability to break the strong bonds between the analyte and protein and sugar present on the food matrix.[27,57] This method is associated with high recoveries; however, the use of large amounts of sample and organic solvents and the necessity to apply tedious purification processes in order to eliminate interferences during determination are important disadvantages.[58]

Recent studies have been using solvent extraction methods, such as supercritical fluid extraction (SFE), microwave-assisted extraction (MAE) and accelerated solvent extraction (ASE). In comparison with SLE, these methods are faster, require smaller volumes of chemical solvents and are associated with better extraction efficiencies, although they might be costly. Before further clean-up steps, sample filtration and centrifugation are required to eliminate possible interfering particles.[57]

The clean-up step plays an important role allowing eliminating the substances that may interfere with the detection of mycotoxins, and consequently improve accuracy and precision. Some clean-up methods have been described, including solid phase extraction (SPE), immunoaffinity columns (IAC), solid-phase microextraction (SPME), matrix solid-phase dispersion and the emergent quick, easy, cheap, effective, reliable, and safe (QuEChERS) method.[56]

SPE consists of extracting mycotoxins dissolved in an extract (mobile phase) passing through a solid support (stationary phase), where the mycotoxins are absorbed. After elution with an organic solvent, the mycotoxins remain retained in the solid support (because of the presence of an adsorbent), and the matrix interferences are eluted. The solid phase selection depends on the polarity of mycotoxins and type of matrix.[57,59] This technique is described as safe, efficient and reproducible, although it has some limitations, such as the fact that the sample has to be in a liquid phase, the low selectivity due to matrix effects and the impossibility of using the same solid support for all mycotoxins.[59]

Immunoaffinity columns are composed by an activated solid phase support, bound to a given antibody. When the sample extract passes through the column, mycotoxins bind selectively to the column antibodies, while interferents and other matrix components are removed by a subsequent washing step. After that, the mycotoxin is eluted with a miscible solvent, like methanol, removing them from the column.[60] This method has great selectivity, although also presents some disadvantages, like the high cost, the column is limited to a single use, and is only able to isolate a given type of mycotoxins, or a group of structurally related mycotoxins. Besides that, there is also the risk of antibody denaturation, while in contact with some organic solvents, or the possibility of cross-reactivity and establishment of non-specific interactions.[32,60] IAC are available for the extraction of the most common mycotoxins, like AFs, ZEA, OTA, FUMs and DON, and some columns allow the simultaneous extraction of different classes of mycotoxins.[60] For more complex samples, sometimes it is required the combination of IAC with other extraction method like SPE.[61]

The emergent sample preparation method QuEChERS has been used for extraction and clean-up of different food matrices prior to the detection of mycotoxins. This technique includes two different phases: an extraction step (solvent extraction) followed by a purification one (dispersive-SPE).[57,62] The first step is based on a solvent extraction, using acetonitrile in the presence of salts like magnesium sulphate ($MgSO_4$) and sodium chloride (NaCl), in order to remove water from the organic phase and reduce the amount of polar interferences, respectively.[57] For the second phase, a primary/secondary amine (PSA) or C18, are frequently used to retain co-extracted compounds like lipids, sugars, organic acids, or even some pigments. As described in the name itself, this is a fast, simple, and inexpensive method, that uses small amounts of solvent comparing with other methods.[57]

A compilation of studies that reported mycotoxin's occurrence around the world is presented in Annex 1, along with the respective extraction and purification methods. The most frequently used methods for the extraction step on the compiled studies were QuEChERS, immunoaffinity columns and SPE, but in the last few years there is a growing preference for QuEChERS method.

OTA contamination was found in levels higher than those permitted in cereals, in multiple studies.[2,63-65] Aflatoxins levels were also found to be above the permitted limits, according to some studies.[2,38.64-70]

By exploring Annex 1 table, we are once again threatened with the prevalence and unavoidability of mycotoxins' contamination, since more than one study reported the contamination with at least one mycotoxin in over 80% of the analysed samples.[64,68]

Moreover, methods that have shown to be efficient in removing fungal from foodstuffs, might not be efficient in removing mycotoxins, since Ruadrew *et al.* found 1/3 of the analysed samples were contaminated with aflatoxins, in the absence of Aspergillus.[66]

The sample with the greatest mycotoxins levels found in this literature review was reported by Suarez-Bonet *et al.* in a sample of rice from Spain.[67] The maximum levels of AFB1 and total aflatoxins were respectively, 91.7 and 138.6 μg/Kg, which far exceed the regulated limits, and the fact that those samples were cultivated in Spain enhances the fact that this is a worldwide problem, with a huge presence even in developed countries.[67] The highest contamination with OTA was reported by Manizan *et al.* in a sample with 15 μg/Kg.[2] Furthermore, Manizan also emphasized the co-occurrence of mycotoxins, by finding 8 different mycotoxins in two rice samples.[2]

3.3. Analytical Methods

3.3.1. Chromatographic Techniques

Chromatographic methods are the most frequently used for mycotoxins analysis in food samples.[54]

Thin layer chromatography (TLC) is commonly used as a rapid screening technique in the analysis of some mycotoxins. Thus, recent investigation has been focusing on the application of methods that allow the detection and quantification of multiple mycotoxins with high selectivity and sensitivity, and the achievement of more accurate results.[59]

In order to accomplish that, many other techniques have been developed like high performance liquid chromatography (HPLC) coupled with mass spectrometry (MS), fluorescence (FLD), diode array (DAD), or ultraviolet (UV) detectors. Moreover, gas chromatography (GC) coupled with MS, flame ionization (FID) or electron capture (ECD) detectors have been applied in identification and quantification of volatile mycotoxins like TC. GC is rarely used in the analysis of mycotoxins with low volatility and high polarity, since it requires a prior derivatization step.[3,71]

Liquid chromatography (LC) is able to separate thermolabile, non-volatile and substances with different polarities Moreover, it can differentiate substances with structural similarities, without the need of derivatization steps, that are required in GC.[72] The solid phases placed inside the analytical column in LC can be classified as normal or reverse phases. LC in normal phase consists in the elution of mycotoxins through a solid phase (composed by free or covalent-bounded particle of phenyl, aluminium or silica), using a low

polarity solvent like acetonitrile. Although it has been used for the analysis of aflatoxins, reverse phase is nowadays the method of choice. Reverse phase consists on hydrocarbonated solid phases (C_8, C_{18} or short chain of phenyl, cyanopropyl and n-alkyl bound to silica surface), through which mycotoxins are eluted using binary mixtures of water and organic solvents.[58]

Regarding the most frequently used detectors in LC, UV detectors have been losing popularity, due to the lack of selectivity and sensitivity, since a lot of interferences absorb in this zone of the spectrum, along with mycotoxins. Diode array detector, although allows a complete spectrum of all wavelengths, is associated with low sensitivity levels. For mycotoxins that present natural fluorescence (some aflatoxins and OTA), or for those that are fluorescent after derivatization, fluorescence detectors are also a good option, since they present high sensitivity and selectivity levels. In spite of those benefits, FLD is being replaced by MS.[56]

HPLC coupled to mass-spectrometry has allowed great advances in mycotoxins' analysis, since it offers higher sensitivity and selectivity in comparison with other methods, as well as structural information of the analysed mycotoxin metabolites or degradation products. That is why an increasing number of researchers have been using this technique, not only for identification and quantification, but also for toxicokinetic and metabolism studies.[73,74] The mass spectrometer ionizes the molecules and identifies them based on their mass-to-charge ratio (m/z). Based on the ionization technique, different interfaces have been applied in the detection of mycotoxins, such as atmospheric pressure chemical ionization (APCI), atmospheric pressure photo-ionization (APPI) and electrospray ionization (ESI).[75] Moreover, there are multiple types of mass analysers, like triple quadrupole (QqQ), time-of-flight (ToF) and ion trap. Each mass spectrometer presents advantages and disadvantages, and their selection is dependent on the purpose of the analysis. QqQ is mainly used in routine analysis, due to its selectivity, robustness, and repeatability, although it is not able to determine unknown compounds. For that purpose, there are other developed instruments such as ToF detectors (which provides exact mass) or ion trap detectors (offers fragmentation schedule, allowing an unambiguous identification of the compound).[32] Triple quadrupole, ESI and ToF are the most commonly used in mycotoxin analysis. ToF and Orbitrap analysers are becoming more popular due to their high resolution and high accuracy.[4]

HPLC coupled to mass-spectrometry was initially applied to the analysis of single mycotoxins, but to date it is possible to simultaneously quantify many mycotoxins belonging

to various chemical families in a single run, which makes it the method of choice for detecting multiple mycotoxins. The simultaneous detection of multiple mycotoxins is particularly desirable because of the co-occurrence of multiple mycotoxins in food. These modern chromatographic methods may also reach sub-ppb levels of limit of detection, when used following suitable preparation and purification steps.[76]

3.3.2. Immunochemical Methods

The immunoassay technology has proven to offer many advantages in mycotoxins determination, through the development of simple, efficient, and sensitive methods, based on antibody-antigen reactions. Among these methods are included enzyme-linked immunosorbent assay (ELISA), flow injection immunoassay (FIIA), lateral flow immunoassay (LFIA), flow immunoassay and chemiluminescence (CL).[77]

CL has already been applied in the determination of mycotoxins in maize samples and consists in the production of fluorescence as a result of a chemical reaction. The most reported advantages are the use of simple instrumentation and the low detection limits obtained.[78,79]

ELISA is probably the most frequently used of all published immunological based methods for mycotoxins determination. ELISA kits are available for detection and quantification of all major mycotoxins and provide rapid screening results, without the need for clean-up and concentration steps, which makes possible its use in field conditions.[60]

This technique is based on the interaction between mycotoxins and antibodies marked with toxin-enzyme conjugate for multiple binding sites. The level of colour developed is dependent on the amount of antibody-bound toxin-enzyme conjugates. There are two types of ELISA tests: direct and indirect. Direct ELISA provides quick results and, because it uses only one antibody, it reduces cross-reactivity reactions. However, the direct method is associated with less sensitivity, due to the difficulty of signal amplification on the primary antibody. Indirect ELISA recurs to labelled secondary antibodies, providing higher sensitivity, due to signal amplification.[71] This method is specific, rapid, and easy to use, although it has some disadvantages, including the possibility of cross-reactivity occurrence and dependence on a specific matrix (since matrix effect or interference may induce under or overestimation of mycotoxins) and contamination level.[27] Moreover, each kit is designed for a single use and detects only one mycotoxin. Also, it can become costly when there is

the need to identify various mycotoxins and perform multiple tests. HPLC analysis are often used as confirmation method after ELISA.[60]

In Annex II, a summary of relevant detection/quantification analytical methods to determine mycotoxins in rice and rice products is presented.

Liquid chromatography coupled with mass spectrometry or fluorescence were the most used techniques. Although HPLC-FLD is preferred for single mycotoxin determination, HPLC-MS/MS is the preferred method for simultaneous determination of multiple mycotoxins, and according to the studies compilation in Annex II, through the years there is a tendency to employ this method.

New technologies are being applied for mycotoxin determination, such as Orbitrap and ToF detectors. These new technologies allow the obtainment of more accurate results, and specifically, quadrupole-Orbitrap has the ability to confirm the presence of a certain compound by its exact mass, and to identify metabolites or compounds that have not yet been monitored.[80] Quadrupole-ToF detectors are also being used in mycotoxin determination, since they provide exact mass information and determine the presence of unknown compounds in real samples.[81]

The used methods seem to be suitable, since both LODs and LOQs are below the maximum limits set by EU. Moreover, we can observe that through the years, LOD and LOQ levels are becoming lower, which is associated with the evolution of the used techniques, which are becoming more sensitive. By the analysis of the table of Annex 11, we can also conclude that the lower LOD and LOQ levels were obtained when using liquid chromatography coupled to triple quadrupole MS, which is the current method of election for mycotoxins' determination in food.

Internal standards are chemical compounds that present a similar behaviour to the target substance, and that are not present in the sample, but intend to minimise process losses (like extraction losses). Internal standards are not frequently used in these studies, and only two of the studies did use these standards in their works.[26,69] The internal standards selected were sulfamethoxazole and labelled stable isotopes, due to their chemical and chromatographic similarity to the target toxins.[26,69]

4. Mycotoxin contamination in rice

In the EU, the RASFF allows a quick and simple share of information, between food safety entities and the European Commission members, about food and feed hazards, like

contamination by mycotoxins, pesticide residues, pathogenic microorganisms, or heavy metals.[4] Every time a contamination by mycotoxins or other food hazard is found, the RASFF member state that discovered it, releases a market notification.[82] RASFF notifications can be provided by different entities, such as non-official market controls, industrial companies controls, border controls, consumers, or they might even be reported by countries outside EU.[83]

RASFF is a valuable tool, not only because it allows the identification of emerging food safety risks, but it is also possible to check the most frequent occurrences in a certain period.[82]

According to RASFF, mycotoxins are the basis of a great number of notifications, being one of the main cited hazards during the last decade. In 2019, 553 notifications were emitted referring to mycotoxins in foodstuffs, and around 84.6% corresponded to AFs contamination.[82,84]

Rice is one of the main cereals contaminated by mycotoxins, and Table 2 summarizes the reported notifications since 2019. According to this table, since 2019 over 40 occurrences classified as a serious risk were reported, which means the samples contamination levels exceed the legislated levels, and so they were removed from market. The highest AFB1 levels reported in this period were found in a batch imported from Pakistan to Netherlands, where 44 μg/kg was reported for AFB1 and 49 μg/kg for total AFs. These values far exceed the levels regulated by the European Commission for these mycotoxins (2 μg/kg for AFB1, and 4 μg/kg for AFs).[6,84]

All these findings emphasize the presence and relevance of mycotoxins in food safety discussion, and the need of a rigorous control. Moreover, looking at the results we can conclude that there is a higher incidence of notifications in basmati and organic rice. This raises a question: are these types of rice more susceptible to mycotoxin contamination? Also, most of the contamination samples were original from countries outside de EU, which emphasizes the need of a stricter control of food products coming from those countries.

Table 2 – RASFF notifications due to mycotoxins contamination from 2019 to 2021.

Date	Country	Origin Country	Product	Mycotoxin	Levels (µg/Kg)
22/02/2019	Italy	Pakistan	Basmati rice	AFB1	4.3
22/02/2019	Belgium	Italy	Organic brown rice	OTA	14.1
01/03/2019	Belgium	Pakistan	Basmati rice	AFB1	6.8
01/03/2019	Italy	Pakistan	Basmati rice	AFB1	19.9
				AFs	21.6
22/03/2019	Austria	Germany	Organic brown rice	AFB1	7.1
22/05/2019	France	Italy	Basmati rice	AFB1	4.49
02/08/2019	Germany	Netherlands	Basmati rice	AFB1	3.60
05/09/2019	Poland	Myanmar	Parboiled brown rice	AFB1	4.09
24/10/2019	Portugal	Myanmar	Rice	AFB1	19
28/11/2019	Switzerland	Sri Lanka	Roasted red rice flour	AFB1	15.6
				AFs	19
18/12/2019	Switzerland	Sri Lanka	Roasted red rice flour	AFB1	6.8
				AFs	8.2
27/02/2020	Switzerland	Sri Lanka	Parboiled rice	AFB1	3.4
15/06/2020	Sweden	Cambodia	Organic brown rice	AFB1	20.6
03/07/2020	Greece	Pakistan	Basmati rice	AFB1	5.6
				AFs	5.6
07/07/2020	Greece	Pakistan	Basmati rice	AFB1	6.3
				AFs	6.3
07/07/2020	Greece	Pakistan	Basmati rice	AFB1	6.0
				AFs	6.0
31/07/2020	Poland	Pakistan	Long grain brown rice	AFB1	6.54
				AFs	6.54
21/08/2020	Greece	Pakistan	Basmati rice	AFB1	4.6
				AFs	4.6
21/08/2020	Switzerland	United Kingdom	Basmati rice	OTA	8.3
01/09/2020	Switzerland	Sri Lanka	Red rice	AFB1	8.9
				AFs	11
				OTA	10.3
15/10/2020	Germany	India	Basmati rice	OTA	6.23
20/10/2020	Germany	Pakistan	Organic brown basmati rice	AFB1	14.3
				AFs	15.4
02/12/2020	Netherlands	India	Brown basmati rice	AFB1	24
				AFs	27
05/01/2021	Spain	Pakistan	White rice	AFB1	2.2 - 3.1
21/01/2021	Spain	Pakistan	White rice	AFB1	3
22/01/2021	Greece	Pakistan	Basmati rice	AFB1	3.1
28/01/2021	Netherlands	Pakistan	Organic brown basmati rice	OTA	11.2
04/03/2021	Netherlands	Pakistan	Organic brown basmati rice	AFB1	9.1
17/03/2021	Germany	Netherlands	Basmati rice	OTA	5.26
27/04/2021	Germany	Netherlands	Rice flour	AFB1	5.7 ± 2.5
27/05/2021	Germany	India	Basmati rice	OTA	4.94 ± 0.41
06/08/2021	Netherlands	Pakistan	Brown rice	AFB1	44
				AFs	49
10/08/2021	Belgium	Pakistan	Broken rice	AFB1	8.6

Legend: Notifications of mycotoxins contamination in rice and rice products from 2019 to 2021; Adapted from RASFF portal

5. Contamination Mitigation

Since mycotoxin-producing fungi may affect rice in multiple stages, many strategies to overcome this problem have been developed, from prevention of their occurrence to decontamination methods.[85]

One of the developed strategies to reduce mycotoxigenic fungi in the field is chemical control. Although chemicals have shown to be successful in crop protection, they are associated with undesirable effects. By acidifying the soil, they may interfere with the plant's growth, as they decrease the occurrence of beneficial organisms. Furthermore, nowadays, there is an increasing pressure to reduce the use of insecticides, fungicides, and herbicides, in order to achieve higher agricultural sustainability levels.[17]

Postharvest strategies are associated with the application of proper storage conditions. Most losses because of mycotoxins' contamination are associated with inadequate storage. Therefore, the application of suitable packaging practices (such as the use of ultra-hermetic airtight containers), temperature and humidity control, and ventilation efficiency are essential to avoid fungal growth and mycotoxins accumulation.[85]

The distribution and concentration of mycotoxins, as well as their physical and chemical properties, suffer modifications during processing, which may lead to a variation of their toxicity levels.[86] Therefore, it is of great importance to understand the impact and phases where those variations occur. Some studies have found higher levels of AFB1 and AFB2 in brown rice and bran, and lower levels in white rice, suggesting the most relevant step to overcome this mycotoxin is bran removal.[87]

Since in some cases mycotoxins' occurrence cannot be avoided, some decontamination methods have been developed. These methods must be safe, environmentally friendly, and effective and have a good cost-benefit relationship. A decontamination strategy, to be considered effective, must be able to inactivate, remove or destroy the mycotoxins, and retain the nutritional properties of the foodstuff. Moreover, it must not alter the product's technological properties, or form other toxic substances or metabolites.[88]

In the case of aflatoxins, several detoxification strategies have been proposed, such as physical methods of separation, thermal inactivation, irradiation, adsorption from solution, irradiation, solvent extraction, microbial inactivation, and fermentation, as well as chemical detoxification methods.[88]

In summary, three types of decontamination methods may be applied: physical, chemical, or biological. Physical methods involve processes of density or mechanical separation, heat inactivation, ionization or UV radiation, or solvent extraction.[89] Other alternative are chemical methods, based on the destruction of the mycotoxins' chemical structures. These methods include the use of chlorination agents, oxidants, or hydrolytic agents, and also the use of plant extracts and essential oils.[90,91]

Although quite a few synthetic preservatives have been identified, their continuous use has been associated with some disadvantages, like health and environmental issues, an increase in fungal resistance and allergic reactions. Therefore, the tendency to use natural compounds, such as essential oils, to preserve foodstuffs has been increasing in the last decades.[91] Essential oils have shown to exhibit biological antifungal, antibacterial and antioxidant properties, and have already been applied in a wide range of industries, including the pharmaceutical, agricultural and food ones.[92] Some studies have been performed in order to establish essential oils (EOs) effects on mycotoxigenic fungi and mycotoxin synthesis, and the results indicated that thyme and oregano EOs have been commonly used against *A. flavus* and *A. parasiticus*.[91,93] Moreover, cinnamon and cinnamaldehyde have revealed to present antifungal activity against *Aspergillu*s and *Fusariu*m genera, and significant antimycotoxigenic activity against DON, AFB1, ZEA and OTA. Great results using oregano extracts have also been reported against OTA.[94,95,96] Regardless all these advantages, essential oils also present some issues, like the occurrence of undesirable organoleptic effects and their low potency. As an attempt to overcome their undesirable organoleptic effects, research studies have developed new approaches such as encapsulation and coating. Their low potency is being overcome through their association with other antimicrobial compounds, to obtain synergistic effects.[91]

Both physical and chemical methods present disadvantages, since the complete decontamination is not achieved, and these methods are associated with high costs and nutritional loss.[90]

Lastly, another strategy developed to reduce mycotoxigenic fungi contamination, comprises the use of microorganisms. This biocontrol method is based on multiple mechanisms, including their ability to compete with pathogens for space and nutrients, to produce antimicrobial compounds, to induce host resistance to the disease, or to directly antagonize the pathogen. Lactic acid bacteria have been used as biocontrol agent, since they seem to have a great potential to control fungal diseases. A couple strains of *Streptomyces*

corchorusii and *Burkholderia gladioli* have also been studied because of their abilities to produce cell wall degrading enzymes and to inhibit *A. flavus* growth, respectively.[17]

Some of these methods have already been applied in rice in order to mitigate mycotoxin contamination, through the application of field and postharvest good practices. Rice processing also constitutes an important step and seems to reduce mycotoxin content, although it can't fully eliminate these contaminants.[17] Essential oils have also already been applied in rice, in order to manage mycotoxin formation and fungal growth, and seem to constitute an effective technique. One of the studies was performed by Wan, in order to evaluate the effects of thyme, lemongrass, cinnamon, peppermint and clove essential oils in the production of DON in contaminated rice. These samples were incubated for 5 days in the presence of the previously referred EOs and, by the end of that period, the results indicated a several reduction on mycotoxin production.[97]

6. Conclusions and Future Perspectives

Mycotoxins and their fungal producers constitute a great public health issue, with AFB1 being in the spotlight of those concerns, since it was considered by IARC as a group 1 carcinogen.

Since mycotoxins contamination is unpredictable, the key to minimize their occurrence must be based on prevention and control. To do so, the implementation of good agricultural and production practices, along with the adoption of proper process, transport, and storage conditions with control analysis of critical points is fundamental.

Although agricultural practices and control methods are in constant evolution, a large number of RASFF notifications are still reported every year due to mycotoxins contamination, with some of the values being far above the legislated levels. Some studies related with this problematic have been developed in order to evaluate the levels of mycotoxins in food samples, and the results are a concern. Suarrez-Bonet found levels of AFB1 up to 91.7µg/Kg and total aflatoxins up to 138.6µg/Kg in rice samples. These values far exceed the 2µg/Kg and 4µg/Kg, respectively legislated for AFB1 and total aflatoxins for rice by the Commission Regulation (EC) No. 1881/2006 of 19 December 2006.

To minimize the exposure to mycotoxins, more sensitive and accurate analytical methods for their determination have been developed. IAC and QuECHERS are the preferred for extraction and purification and HPLC-MS/MS is the preferred for quantification purposes. Considering the continuous methods evolution, it is expected that these

techniques get replaced by high resolution mass spectrometers like Orbitrap and ToF. These detectors are still very expensive, but there is a perspective that in the future they will be less expensive and progressively more frequent in routine laboratories. The development screening methods with greater precision and sensibility able to be employed in the field is also expected.

Further investigation is still required in this field in order to better understand the effects of mycotoxins co-occurrence and their potential synergism. Moreover, climate changes have been found to be problematic in this research area, since higher temperature and humidity levels are favourable conditions for fungal growth and mycotoxin production. Therefore, it would be of great importance to carry out more studies in order to evaluate the impact of climate changes in rice contamination by mycotoxins.

The legislation itself also requires to be updated, since it establishes the maximum levels for mycotoxins in cereals, but for some mycotoxins, rice is excluded. In this line, it would be of great importance to establish maximum mycotoxins levels in rice, for the more common mycotoxins but also for emergent and masked mycotoxins. This is of high relevance, since rice is one of the most consumed cereals worldwide, resulting in a large exposure to these contaminants, and posing a serious risk for human health.

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Annex I – Occurrence of mycotoxins and the main used extraction and clean up methods

Type of sample	Mycotoxins analyzed	Extraction method	Extraction Conditions	Number of samples	Sampling period	Levels of contamination (µg/kg)	Conclusions of the study	Ref.
Organic Rice	OTA	Extraction with MSPD	Sample was blended with the solid phase C8 (2.5g/1.5g) until achieving a homogeneous mixture. The mixture was eluted through a column (100 mm x 9 mm i.d. glass column with a coarse frit) using MeOH: FA (99:1, v/v). The eluate was concentrated using a N_2 steam, filtered and then centrifuged.	9	April 2005 - November 2005	Mean: 2.57 ± 3.43 Range: 2.10–7.60	OTA was present in 4 out of the 9 samples.	63
Rice	AFs	SPE	Solvent: ACN: H2O: acetic acid (79:20:1 v/v/v). The supernatant was centrifuged, and a purification step was conducted, diluting the final extract with ACN:water:acetic acid (20:79:1). After a second purification by filtration, the final sample was injected into the UHPLC-MS/MS.	40	January - March 2010	0.15 - 4.42 (10/40 samples)	80% of the cereal samples were contaminated with at least one mycotoxin; 4% of the samples exceeded the EU regulatory levels for AFs and OTA (4 and 5 ng/g respectively)	64
	OTA					0.2 - 4.34 (6/40 samples)		
	ZEA					1.5 - 51.1 (5/40 samples)		
	DON					6.15 - 34.92 (8/40 samples)		
	FB1					12.59 - 33.25 (3/40 samples)		
	FB2					12.36 - 31.19 (3/40 samples)		
	T2					5.88 - 55.35 (3/40 samples)		
	HT-2					48.18 (1 sample)		
Jasmine Rice	AFs	Immunoaffinity columns	Sample extract: MeOH:H20 (60:40 v/v) and NaCl. The sample was diluted in distilled water and filtered. IAC: The column was buffered with PBS at a flow rate of 5ml/min. The sample was then eluted using MeOH and distilled water, at a flow rate of 2 ml/min, and collected in an amber glass vial.	-	-	Mean: 11.4 of total aflatoxins (in the absence of *Aspergillus*)	1/3 of the analyzed samples exceeded the levels of AFs tolerated in the EU.	66

Annex I – Occurrence of mycotoxins and the main used extraction and clean up methods (Continuation)

Type of sample	Mycotoxins analyzed	Extraction method	Extraction Conditions	Number of samples	Sampling period	Levels of contamination (µg/kg)	Conclusions of the study	Ref.
Rice	AFB1	Immunoaffinity column	Sample extract: MeOH:H20 (80:20 v/v) and NaCl. After filtration, the extract was diluted in phosphate buffered saline (PBS), ad filtered again. IAC: The column was buffered with PBS and then the filtered sample was eluted through the column with ACN at a flow rate of 5 ml/min. The column was washed twice with distilled water and air-dried. After that, the eluate was dried and derivatized, and an aliquot was used for the HPLC analysis.	67	-	<LOD - 91.7	Most of the analyzed samples exceeded the levels of AFB1 and AFs (2 and 4 µg/kg, respectively) tolerated in cereals in the European Community	67
	AFB2					<LOD - 12.1		
	AFG1					<LOD - 78.7		
	AFG2					<LOD - 31.0		
	AFs					<LOD - 138.6		
Rice	Total mycotoxins	QuEChERS	Extraction step: Solvent: ACN:HOAc (99:1 v/v) Salts: mixture of anhydrous $MgSO_4$, NaCl, $(CH_2COONa)_2 \cdot 2H_2O$ and $C_6H_6Na_2O_7 \cdot 1.5H_2O$ (4:1:1:0.5). After being vortexed and centrifuged, the supernatant was collected in a PTFE tube for the purification step, containing anhydrous magnesium sulfate and a C18 sorbent (This process is imperative to reduce the quantity of lipids and eliminate the excess of water, simplifying the evaporation). After centrifugation, the supernatant was evaporated and reconstituted in MeOH:H_2O (70:30 v/v). After filtration, the extract was collected into a LC vial.	24	2013	ND	The target mycotoxins were not detected in any of the samples.	5

Annex I – Occurrence of mycotoxins and the main used extraction and clean up methods (Continuation)

Type of sample	Mycotoxins analyzed	Extraction method	Extraction Conditions	Number of samples	Sampling period	Levels of contamination (µg/kg)	Conclusions of the study	Ref.
Rice	AFB1, AFB2, AFG1, AFG2, OTA, DON, ZEA, FB1, FB2, HT2, T2	d-SPE, QuEChERS	Extraction step: Solvent: water + 10% FA in ACN Salts: mixture of anhydrous $MgSO_4$, NaCl, tri-Na and di-Na Purification step (d-SPE) ACN extract + MgSO4 + C18 + Al-N + PSA. After centrifugation, the extract was evaporated to dryness under a N_2 steam, and reconstituted using mobile phase A:B (1:1 v/v). The samples were then filtered and collected in a vial for injection.	20	-	ZEA was detected in 2 rice samples and AFB1 was detected in 6 rice samples	The contamination levels were below the EU limits for typical foods and feeds.	98
Rice	AFB1	SPE	The samples were extracted with 20mL ACN/water/glacial acetic acid (79:20:1, v/v/v. Aliquots of 500 µL extracts were transferred into glass vials containing an equal volume of ACN/water/acetic acid (20:79:1, v/v/v).	65	April 2010 - April 2011	<LOQ - 30.83	All the samples were contaminated with at least one mycotoxin. 3 rice samples exceeded the limit established in EU and Iran for AFB1 (5µg/Kg); ZEA was detected in 19 out of 65 samples in high levels.	68
	AFB2					0.6 - 1.26		
	FB1					54.48 - 176.58		
	OTA					0.65 - 11.54		
	ZEA					4.95 - 215.46		
Rice	T-2 toxin	SPE using multi - walled carbon nanotubes as sorbents	The samples were macerated using 10 mL of ACN/water (84:16, v/v) and then ultrasonicated. After centrifugation, the supernatant was collected and dried using nitrogen gas. The residues were reconstituted in ACN/water (20:80, v/v) and then diluted with water. This solution	10	-	6.13 (1/10 samples)	EFSA has established a TDI of 100 ng/Kg body weight for the total of T-2 and HT-2 toxins	99

Annex I – Occurrence of mycotoxins and the main used extraction and clean up methods (Continuation)

Type of sample	Mycotoxins analyzed	Extraction method	Extraction Conditions	Number of samples	Sampling period	Levels of contamination (µg/kg)	Conclusions of the study	Ref.
	HT-2 toxin		was passed through the multi-walled carbon nanotubes sorbents. The cartridges were eluted with MeOH containing 1% FA, and the eluate was evaporated using nitrogen gas. The residues were re-dissolved in ACN/water containing ammonium acetate (30:70, v/v), filtered and collected in a vial for injection.			11.81 (1/10 samples)		
White rice	AFB1	SPE, Immunoaffinity columns	AFs: Solvent: ACN:water (90:10 v/v) After filtration, the supernatant was diluted with deionized water. IAC: the dilute filtrate was eluted at a flow rate of 3-4 drops/s using HPLC grade MeOH and washed with water. After evaporation under a nitrogen stream, a mixture of ACN:water (1:9 v/v) was added to the vials. OTA: Solvent: ACN:water (90:10 v/v) After filtration, the sample was mixed in PBS and filtered using a glass microfiber. After filtration, 10 ml of filtrate were mixed with acetic acid and passed through the IAC. IAC: The sample was eluted with MeOH and collected in a vial.	34	August 2012 - March 2013	7.70 ± 0.89	25% of the samples of brown rice were above the maximum permitted level at EU for AFB1, and 32% for total AFs. 19% samples of rice and rice products were found positive and 14% were found above the EU maximum content for OTA (5µg/kg)	65
	AFs					11.9 ± 1.20		
	OTA					8.50 ± 0.60		
Brown rice	AFB1			28		8.91 ± 1.20		
	AFs					12.4 ± 0.98		
	OTA					7.84 ± 0.90		
Rice flour	AFB1			30		3.51 ± 1.20		
	AFs					5.20 ± 0.82		
	OTA					4.91 ± 1.53		
Sweet puffed Rice balls	AFB1			22		2.90 ± 0.85		
	AFs					4.30 ± 1.25		
	OTA					3.87 ± 0.75		
Rice cookies	AFB1			28		3.18 ± 0.40		
	AFT					5.40 ± 0.92		
	OTA					3.18 ± 0.60		
Rice sweets	AFB1			21		4.10 ± 1.30		
	AFT					5.70 ± 0.80		
	OTA					5.10		
Rice noodles	AFB1			20		3.60 ± 0.85		
	AFT					3.60 ± 0.85		
	OTA					ND		
Rice bread	AFB1			25		2.40 ± 0.43		
	AFT					2.40 ± 0.43		
	OTA					ND		

Annex I – Occurrence of mycotoxins and the main used extraction and clean up methods (Continuation)

Type of sample	Mycotoxins analyzed	Extraction method	Extraction Conditions	Number of samples	Sampling period	Levels of contamination (µg/kg)	Conclusions of the study	Ref.
Brown rice	AFT	QuEChERS	Extraction step: Solvent: water and HOAc in ACN (10% v/v) Salts: mixture of anhydrous $MgSO_4$, NaCl, $(CH_2COONa)_2 \cdot 2H_2O$ and $C_6H_6Na_2O_7 \cdot 1.5H_2O$ Centrifugation in order to separate the aqueous phase from the organic phase and then collection of the supernatant for the Purification step: C18 silica sorbent, anhydrous magnesium sulfate, PSA and silica. After centrifugation, the supernatant was collected into a vial. After evaporating the remaining ACN and reconstituting in water with a 1:1 (v/v) ratio of 0.1% (v/v) FA:MeOH, the sample was filtered and collected in the UHPLC-MS/MS vial	14	-	N.D	6 samples were contaminated with one or more mycotoxins. The levels determined were below the maximum limits of EU regulation.	26
	OTA					N.D		
	DON					N.D		
	FB1					2.49- 5.41		
	FB2					4.33		
Infant cereals based on rice	AFB1	SPE	Solvent: ACN:water: FA (80:19.9:0.1 v/v/v) After centrifugation, the supernatant was transferred into an HPLC vial and a [13C] labelled working solution was added.	20	March 2012 - June 2012	1/20 (5.9)	1 sample exceeded the EU limit for AFB1; 2 samples exceeded the EU limits for OTA.	69
	AFB2					4/20 (1.1 - 5.0)		
	AFG1					ND		
	AFG2					ND		
	DON					7/20 (1.4 - 55.0)		
	HT-2 toxin					ND		
	T-2 toxin					3/20 (1.1 - 3.6)		
	FB1					ND		
	FB2					ND		
	OTA					2/20 (1.3 - 1.4)		
	ZEN					1/20 (9.0)		
Rice wine	OTA	VADLLME (Vortex-assisted dispersive liquid-liquid microextraction)	After centrifugation, the sample pH was adjusted to 4.0 – 4.3 using 4M NaOH or HCL solutions. Extraction solvent: dichloromethane Dispersive solvent: ACN The mixture was vortexed. After	8	2016	0.20µg/L (1/8 sample)	The contamination levels did not exceed the maximum residue limit set by Eu (2 µg/L)	100

Annex I – Occurrence of mycotoxins and the main used extraction and clean up methods (Continuation)

Type of sample	Mycotoxins analyzed	Extraction method	Extraction Conditions	Number of samples	Sampling period	Levels of contamination (µg/kg)	Conclusions of the study	Ref.
	AFs		centrifugation, the sediment phase was evaporated to dryness using a nitrogen stream at 50 °C. The residues were reconstituted in a MeOH/water solution (50:50, v/v) and filtrated through a nylon filter membrane.			ND		
Brown rice	AFB1	Immunoaffinity column	Sample extract: MeOH:Water (80:20, v/v) with NaCl. After filtration, the solution was diluted in phosphate buffered saline (PBS). IAC: The solution was applied to the IAC at a flow rate of 2–3 mL/min. The column was washed with distilled water, and the sample was eluted with MeOH and diluted with milli Q water	187	-	<LOD - 0.069	Less than 14% of the rice samples were contaminated with aflatoxins, but two of the market samples were well above the maximum tolerable limit.	70
	AFB2					<LOD		
	AFG1					< LOD		
	AFG2					<LOD		
	AFs					<LOD - 0.069		
Red rice	AFB1					< LOD - 63.32		
	AFB2					<LOD - 8.591		
	AFG1					<LOD		
	AFG2					<LOD		
	AFs					<LOD - 70.91		
Rice	AFs	IAC	Sample extract: Sodium chloride and LC grade MeOH 70%. After filtration, the mixture was diluted in PBS and then filtered again. IAC: elution of the sample with 100% LC grade MeOH and LC grade water	100	2017	4,9 (1 sample)	The level is in accordance with legislated levels.	39
	DON ZEA	Stable isotope dilution assay	Solvent: ACN:water:FA (80:19.9:0.1 v/v/v). After centrifugation, the supernatant was resuspended in a mobile phase composed by 70% of water:MeOH:acetic acid (94:5:1, v/v/v) and 30% of water:MeOH:acetic acid (2:97:1, v/v/v).			ND (0/100 samples) 15/100 samples (90,56 - 126,31)	ZEA levels were higher in 36% of the samples, than the current maximum limit established by Brazilian and European regulation	

Annex I – Occurrence of mycotoxins and the main used extraction and clean up methods (Continuation)

Type of sample	Mycotoxins analyzed	Extraction method	Extraction Conditions	Number of samples	Sampling period	Levels of contamination (µg/kg)	Conclusions of the study	Ref.
Rice	AFB1	QuEChERS	Extraction step: Solvent: ACN Salts: mixture of $MgSO_4$ and NaCl. Centrifugation in order to separate the aqueous phase from the organic phase and then collection of the top organic phase for the Purification step: C18 silica sorbent and magnesium sulfate After centrifugation, the supernatant was collected into a vial. After evaporating the remaining ACN and adding MeOH, the sample was filtered and collected in a new vial.	47	April 2013	Mean: 3.9 (<LOQ - 14)	Most samples were contaminated with more than one mycotoxin (8 different mycotoxins were detected in 2 rice samples). Contamination levels higher than the EU limit for AFB1 were found in 42% of rice samples and for Aft in 32% of the same samples. OTA levels were also higher than the regulated from the EU.	2
	AFG1					Mean: 3.3 (<LOQ - 17)		
	AFs					Mean: 5.8 (<LOQ - 33)		
	OTA					Mean: 6.3 (<LOQ - 15)		
	FB1+FB2					Mean: 6.0 (2.7 - 13)		
	ZEA					Mean: 6.6 (<LOQ-7.5)		
Ready to eat rice	DON	QuEChERS	Extraction step: Solvent: ACN Salts: mixture of $MgSO_4$ and NaCl. Purification step: Anhydrous $MgSO_4$ and a C18 silica sorbent. After centrifugation, the extract was filtered using a syringe nylon filter, into the LC-MS/MS vial; For GS-MS/MS the supernatant was evaporated to dryness using a nitrogen flow.	38	September 2016 - December 2016	0.29	All levels were in accordance with the EU legislation	101
	HT-2 toxin					3.47		
	T-2 toxin					0.52		
	ZEA					0.13		
	AFG2					0.17		
Polished rice	AFB1	QuEChERS	Extraction step: Solvent: ACN aqueous solution (95:5, v:v) Salts: anhydrous magnesium sulfate and sodium chloride. Purification step: After vortex and centrifugation, the supernatant was collected and filtered into the LC-MS/MS vial	78	-	2 samples (0.003-0.14	The levels of AFB1 were lower than the regulation limit in EU (2µg/Kg)	21
Unhusked rice				22		N.D.		

Annex I – Occurrence of mycotoxins and the main used extraction and clean up methods (Continuation)

Type of sample	Mycotoxins analyzed	Extraction method	Extraction Conditions	Number of samples	Sampling period	Levels of contamination (µg/kg)	Conclusions of the study	Ref.
Rice	AFB1	QuEChERS	Extraction step: Solvent: ACN containing 1% acetic acid Salts: mixture of anhydrous magnesium sulfate and sodium chloride. Purification step: Anhydrous magnesium sulfate and a C18 sorbent. After vortex and centrifugation, the supernatant was collected and filtered into the LC-MS/MS vial	144 (bulk sample > 0.5kg)	October 2016 - September 2017	13/144 samples (ND - 93µg/Kg)	The levels of AFB1 were lower than the regulation limit in Vietname (5µg/Kg), but higher than the EU limits (2µg/Kg)	38
	FB1					3/144 samples (ND - 675)		
	OTA					ND		
	ZEA					ND		

Legend: ACN - acetonitrile; AFB1 – Aflatoxin B1; AFB2 – Aflatoxin B2; AFG1 – Aflatoxin G1; AFG2 – Aflatoxin G2; AFs – Total aflatoxins; C8- octysilica; $(CH_2COONa)_2 \cdot 2H_2O$ - sodium citrate tribasic dihydrate; $C_6H_6Na_2O_7 \cdot 1.5H_2O$ - sodium citrate dibasic sesquihydrate; DON – Deoxynivalenol; d-SPE – Dispersive Solid Phase Extraction; EFSA- European Food Safety Authority; EU – European Union; FA – Formic Acid; FB1 – Fumonisin B1; FB2 – Fumonisin B2; GC- Gas Cromatography; HCl – hydrogen chloride; HOAc – Acetic Acid; HPLC – High Performance Liquid Chromatography; IAC – Immunoaffinity Column; LC – Liquid Chromatography; LOD – Limit of Detection; MeOH- methanol; $MgSO_4$ – Magnesium Sulfate; MSPD -matrix solid phase dispersion; NaCl – Sodium Chloride; NaOH – Sodium hydroxide; ND – Not Detected; OTA – Ochratoxin A; PBS- phosphate buffered saline; PSA- Primary/Secondary amine; SPE – Solid Phase Extraction; TDI – Tolerable Daily Intake; UHPLC- MS/MS – Ultra High Performance Liquid Chromatography coupled with tandem mass spectrometry; ZEA – Zearalenone

Annex II – Compilation of techniques used for the determination of mycotoxins in rice and rice products

Mycotoxins analyzed	Analytical technique	Conditions	Analytical Column	LOD and LOQ (µg/Kg)	Ref.
OTA	LC-FD	Mobile phase: - FA 0.1M (70:30 v/v) Flow rate: 0.7 ml/min $\lambda_{Excit\ max}$: 333 nm and $\lambda_{Emis\ max}$: 460 nm	C18 column (150 x 4.6 mm, 5 µm)	LOD: 0.05; LOQ: 0.19	63
AFT (AFB1, AFB2, AFG1 and AFG2)	LC - MS/MS	Mobile phase: A - MeOH; B - water with 0.1% acetic acid; Elution: Gradient; Column temperature: 30 °C; Injection volume: 10 - 30 µL; Flow: 0.25 mL/min; Electrospray ionization (ESI); Capillary potential: 3 kV; Nebulizing, desolvation and cone gas: nitrogen; Desolvation gas temperature: 400 °C; Source temperature: 120 °C;	C18 column (2.1 x 50 mm, 1.9µm)	LOD: 0.01 - 25; LOQ: 0.02 - 40	64
OTA					
ZEA					
DON					
FB1					
FB2					
T2 toxin					
HT-2 toxin					
Aft (AFB1, AFB2, AFG1 and AFG2)	Fluorescence detector	HPLC-FD Mobile phase: MeOH: Water [40:60 v/v] adjusted with 350µl of 4 M nitric acid and 119 mg of potassium bromide per 1L of mobile phase. Column temperature: 40 °C; Injection volume: 100 µL; Flow: 1 mL/min; $\lambda_{Excit\ max}$ =362 nm, and $\lambda_{Emis\ max}$ =426 nm (for AFB1 and AFB2) and $\lambda_{Emis\ max}$ = 256 nm for AFG1 and AFG2)	Inertsil ODS-3V C18 column (4.6 x 150mm, 5µm)		66
Aft (AFB1, AFB2, AFG1 and AFG2)	Fluorescence detector	HPLC - FD: Mobile phase: Water:ACN:MeOH [65:15:20 v/v/v] degassed for 30 min using vacuum filtration Column temperature: 20 °C; Injection volume: 20 µL; Flow: 1.0 mL/min; $\lambda_{Excit\ max}$ = 360 nm, and $\lambda_{Emis\ max}$ =450 nm	Reverse phase C18 column (4.6mm x 250mm, 5µm)	LOD: 0.4 -0.6; LOQ: 1.2 - 1.9	67

Annex II – Compilation of techniques used for the determination of mycotoxins in rice and rice products (Continuation)

Mycotoxins analyzed	Analytical technique	Conditions	Analytical Column	LOD and LOQ (µg/Kg)	Ref.
Total mycotoxins (AF, OTA, T-2 and HT-2 toxins, DON, ZEA, FB1)	LC-ESI-MS/MS	Mobile phase: H2O:MeOH 9:1 with 5 mM ammonium acetate; Elution: Gradient; Column temperature: 30 °C; Injection volume: 20 µL; Flow: 0.3 mL/min; Electrospray ionization (ESI); Ionization mode: Positive; Capillary potential: 2.9 kV; Nebulizing, desolvation and cone gas: nitrogen; Collision gas: argon Cone gas flow: 80 L/h Flow of desolvation gas: 650 L/h; Desolvation gas temperature: 350 °C; Source temperature: 140 °C;	Silica-based reversed-phase C18 Atlantis T3 (150 mm× 2.1mm x 5µm)	LOD: 0.11 - 59.9; LOQ: 0.37 - 199	5
AFB1, AFB2, AFG1, AFG2, OTA, DON, ZEA, FB1, FB2, HT2, T2	UHPLC-MS/MS (micromass quattro premier XE triple-quadrupole mass spectrometer)	Mobile phase: A - 0.5% FA in 5mM aqueous ammonium formate; B – ACN:MeOH (1:1, v/v) Elution: Gradient; Column temperature: 40 °C; Injection volume: 5 µL; Flow: 0.25 mL/min; Electrospray ionization (ESI); Ionization mode: Positive (except for ZEA)	C18 column (1.7µm, 100 x 2.1 mm), with a pre-column (1.7µm, 5 x 2.1 mm)	LOD: 0.5–15; LOQ: 1.7 - 50	98
AFB1	HPLC - ESI - MS/ MS	Column temperature: 25 °C; Nebulizing, desolvation and cone gas: nitrogen; Source temperature: 550 °C	C18 column (5 µm, 30 x 2 mm)	LOD: 0.03 - 2.5; LOQ: 0.3	68
AFB2				LOD: 0.03 - 2.5; LOQ: 0.6	
FB1				LOD: 0.03 - 2.5 LOQ: 7	
OTA				LOD: 0.03 - 2.5 LOQ: 0,6	
ZEN				LOD: 0.03 - 2.5 LOQ: 2	

Annex II – Compilation of techniques used for the determination of mycotoxins in rice and rice products (Continuation)

Mycotoxins analyzed	Analytical technique	Conditions	Analytical Column	LOD and LOQ (µg/Kg)	Ref.
T-2 toxin	UHPLC-MS/MS	Mobile phase: A - Water with 5mmol/L ammonium acetate; B - MeOH Elution: Gradient; Column temperature: 40 °C; Injection volume: 5 µL; Flow: 0.4 mL/min; Electrospray ionization (ESI); Ionization mode: Positive; Flow of desolvation gas: 1000 L/h; Flow of cone gas: 30L/h Desolvation gas temperature: 500 °C; Source temperature: 150 °C;	C18 column (100 x 3.0 mm, 2.7µm)	LOD: 0.01; LOQ: 0.02	99
HT-2 toxin				LOD: 0.03; LOQ: 0.10	
Aflatoxins	HPLC-FD	Mobile phase: ACN:MeOH:water [20:20:60 v/v/v] Flow rate: 1ml/min $\lambda_{Excit\ max}$: 360nm and $\lambda_{Emis\ max}$: 440nm	C18 (4.6 x 250mm, 5µm)	AFB1: LOD 0.04; LOQ 0.20; AFB2: LOD 0.10; LOQ 0.30; AFG1: 0.04; LOQ 0.20 AFG2 LOD 0.10; LOQ 0.30	65
OTA		Mobile phase: ACN:water:acetic acid [47:51:2 v/v/v] Flow rate: 1ml/min $\lambda_{Excit\ max}$ = 333nm and $\lambda_{Emis\ max}$ =460nm		LOD: 0.06; LOQ: 0.18	
Aft (AFB1, AFB2, AFG1, AFG2)	HPLC - ESI - MS/ MS	Mobile phase: A - 0.5% (v/v) FA in water containing 5mM ammonium formate; B - MeOH Elution: Gradient; Column temperature: 40 °C; Injection volume: 10 µL; Flow: 0.3 mL/min; Electrospray ionization (ESI); Ionization mode: Negative and Positive Collision energy: 25eV Cell accelerator voltage: 3V Capillary voltage: 3 kV; Nozzle voltage: 1000V Gas flow: 16L/min; Gas temperature: 150 °C	C18 column (100 x 2.1 mm, 1.8 µm)	LOD: 0.27 - 0.39; LOQ: 0.82 - 1.2	26
OTA				LOD: 0.47; LOQ: 1.5	
DON				LOD: 5.0; LOQ: 15	
FB1, FB2				LOD: 0.48; LOQ: 1.5	

Annex II – Compilation of techniques used for the determination of mycotoxins in rice and rice products (Continuation)

Mycotoxins analyzed	Analytical technique	Conditions	Analytical Column	LOD and LOQ (µg/Kg)	Ref.
AFB1	HPLC - ESI - MS/ MS	Mobile phase: A - 0.1% FA in water; B - 0.1% FA in MeOH, both containing 5mM ammonium formate; Elution: Gradient; Column temperature: 35 °C; Flow: 0.3 mL/min; Electrospray ionization (ESI); Ionization mode: Positive Flow of desolvation gas: 10 L/min; Desolvation gas temperature: 300 °C Nebulizer: 45 psi Sheath gas temperature: 350 °C Flow rate:11L/min Capillary voltage: 3500V; nozzle voltage: 0V	C18 column (100 x 2.1 mm, 1.8 µm)	LOD: 0.1; LOQ: 0.5	69
AFB2				LOD: 0.5 LOQ: 1.0	
AFG1				LOD: 0.1; LOQ: 0.5	
AFG2				LOD: 0.5 LOQ: 1.0	
DON				LOD:10.0; LOQ: 50.0	
HT-2 toxin				LOD: 1.0; LOQ: 5.0	
T-2 toxin				LOD: 0.05; LOQ: 0.1	
FB1				LOD: 5.0; LOQ: 10.0	
FB2				LOD: 1.0; LOQ: 5.0	
OTA				LOD: 0.1; LOQ: 0.5	
ZEA				N.D.	
AFB1	HPLC - MS/MS	Mobile phase: A - MeOH; B - water with 0.1% FA Elution: Gradient; Column temperature: 40 °C; Injection volume: 5 µL; Flow: 0.3 mL/min; Electrospray ionization (ESI); Ionization mode: Positive Capillary potential: 4.0 kV; Vaporizer temperature: 300 °C Capillary temperature: 350 °C	C18 column (100 x 3.0 mm, 2.7µm)	LOD: 0.05; LOQ: 0.1	100
AFB2				LOD: 0.05; LOQ: 0.1	
AFG1				LOD: 0.1; LOQ: 0.2	
AFG2				LOD: 0.05; LOQ: 0.1	
OTA				LOD: 0.05; LOQ: 0.1	

Annex II – Compilation of techniques used for the determination of mycotoxins in rice and rice products (Continuation)

Mycotoxins analyzed	Analytical technique	Conditions	Analytical Column	LOD and LOQ (µg/Kg)	Ref.
AFB1	HPLC-FD	Mobile phase: water:ACN:MeOH (6:2:3, v/v/v), containing KBr and nitric acid Elution: Gradient; Injection volume: 20 µL; Flow: 1 mL/min; $\lambda_{Excit\ max}$ = 362nm and $\lambda_{Emis\ max}$ = 455nm (for AFG1 and AFG2) and 425 (for AFB1 and AFB2)	C18 column (4.6 ×150 mm, 5 µm)	LOD: 0.016; LOQ: 0.054	70
AFB2				LOD: 0.012; LOQ: 0.039	
AFG1				LOD: 0.011; LOQ: 0.038	
AFG2				LOD: 0.004; LOQ: 0.012	
DON	LC-MS/MS	Mobile phase: water:MeOH:ACN (600:200:200, v/v/v) was added to119 mg potassium bromide and 47.6 µL nitric acid Elution: Gradient; Flow: 1 mL/min; Electrospray ionization (ESI); Ionization mode: Positive Capillary temperature: 208 °C; Vaporizer temperature: 338 °C ; Spray voltage: 4500 V; Sheath gas pressure: 60 bar	RP - C18 column (4.6 x 150 mm, 5µm)	LOD: 0.005; LOQ: 0.025	39
ZEA				LOD: 0.01; LOQ: 0.025	
AFB1	UHPLC-MS/MS	Mobile phase: A – 0.1% FA in water; B - MEOH:ACN (1:1 v/v) Elution: Gradient; Column temperature: 40 °C; Injection volume: 1 µL; Flow: 0.4 mL/min; Electrospray ionization (ESI); Ionization mode: Positive and negative Capillary potential: 1.5 kV; Flow of desolvation gas: 1000 L/h; Desolvation gas temperature: 500 °C; Source temperature: 150 °C;	C18 column (1.6µm, 2.1 x 100 mm)	LOD: 0.05; LOQ: 0.25	2
AFG1				LOD: 0.12 LOQ: 0.25	
Aft (AFB1, AFB2, AFG1 and AFG2)				-	
OTA				LOD: 0.25; LOQ: 0.62	
FB1 + FB2				LOD: 0.5; LOQ: 1	
ZEA				LOD: 2.5; LOQ: 5	

Annex II – Compilation of techniques used for the determination of mycotoxins in rice and rice products (Continuation)

Mycotoxins analyzed	Analytical technique	Conditions	Analytical Column	LOD and LOQ (µg/Kg)	Ref.
DON HT-2 toxin T-2 toxin ZEA AFG2	LC-MS/MS	Mobile phase: A - MeOH (5mM ammonium formate and 0.1% FA); B - water (5mM ammonium formate 0.1% FA; Elution: Gradient; Column temperature: 25 °C; Injection volume: 20 µL; Flow: 0.25 mL/min;	Reverse analytical column C18 (3µm, 150 x 2mm ID) and a guard column C18 (4 x 2mm ID, 3µm)	LOD: 0.04 - 1.5; LOQ: 0.13 - 5	101
AFB1	LC- MS/MS	Mobile phase: A - aqueous FA solution with ammonium formate; B - ACN Elution: Gradient; Injection volume: 5 µL; Ionization: electrospray ionization (ESI)	ShimadzuShim-pack XR-ODS III column	LOD: 0.03 LOQ: 0.5	21
AFB1 FB1 OTA ZEA	LC - MS/MS	Mobile phase: A - MeOH; B- ammonium acetate 10mM Elution: Gradient; ESI mode: positive (for AFB1 and FB1) and negative (for OTA and ZEA) Ionization: electrospray ionization (ESI)	C18 column (4.6 x 150 mm, 2.7µm)	LOD: 0.1 LOQ: 0.3	38

Legend: ACN – acetonitrile; AFB1 – Aflatoxin B1; AFB2 – Aflatoxin B2; AFG1 – Aflatoxin G1; AFG2 – Aflatoxin G2; AFs – Total aflatoxins; DON – Deoxynivalenol; ESI – Electrospray Ionization; FA – Formic Acid; FB1 – Fumonisin B1; FB2 – Fumonisin B2; FD – Fluorescent Detector; H_2O – Water; HPLC – High Performance Liquid Chromatography; LC – Liquid Chromatography; LOD – Limit of Detection; LOQ – Limit of Quantification; MeOH – Methanol; MS/MS – Tandem mass spectrometry; OTA – Ochratoxin A; RP – Reverse Phase; UHPLC – Ultra High Performance Liquid Chromatography; ZEA - Zearalenone