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Juliana Scariot Munaretto

DETERMINAÇÃO DE CONTAMINANTES ORGÂNICOS EM MATRIZES COMPLEXAS UTILIZANDO MÉTODO QuEChERS E CROMATOGRAFIA LÍQUIDA ACOPLADA À ESPECTROMETRIA DE MASSAS DE ALTA RESOLUÇÃO

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Orientador: Prof. Dr. Renato Zanella

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Santa Maria, RS
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(Arthur Schopenhauer)

RESUMO

DETERMINAÇÃO DE CONTAMINANTES ORGÂNICOS EM MATRIZES COMPLEXAS UTILIZANDO MÉTODO QuEChERS E CROMATOGRAFIA LÍQUIDA ACOPLADA À ESPECTROMETRIA DE MASSAS DE ALTA RESOLUÇÃO

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A presença de contaminantes orgânicos no meio ambiente e em alimentos têm se tornado uma grande preocupação devido aos efeitos nocivos aos seres vivos. Tais contaminantes podem ser desde agrotóxicos empregados na agricultura para o controle de pragas, produtos de transformação formados no meio ambiente ou através de processos químicos, como o tratamento de água, e mais recentemente fármacos e produtos de cuidado pessoal. Com isso, tem-se a necessidade de empregar procedimentos de preparo de amostra e sistemas de detecção capazes de detectar uma variedade de contaminantes orgânicos em nível de traços presentes em matrizes complexas como alimentos e amostras ambientais. Portanto, o presente trabalho tem como objetivos: (1) abordar o emprego da espectrometria de massas de alta resolução (HRMS) para a identificação e quantificação de contaminantes orgânicos em filé de peixe utilizando dois modos de aquisição de dados (*full scan* e *all ions* MS/MS) e otimizar o método QuEChERS para o preparo da amostra (ARTIGO 1); (2) apresentar uma segunda aplicação quantitativa do uso de QuEChERS e HRMS para amostras de frutas (maçã, pera e uva) no modo *full scan*, bem como aplicar o método proposto em amostras reais de frutas (ARTIGO 2) e (3) otimizar e empregar o método QuEChERS para a extração de antimicrobianos ionóforos em cama de frango, seguido de quantificação por LC-MS/MS e identificação de produtos de transformação por LC-QToF/MS após três diferentes processos de compostagem (ARTIGO 3). Os três artigos apresentados abordam a importância da otimização do procedimento de preparo de amostra, QuEChERS, a fim de obter recuperação adequada de contaminantes orgânicos, além de minimizar os efeitos de matriz causados pelos interferentes presentes no extrato. Assim como o uso de HRMS, a qual demonstrou ser uma ferramenta analítica bastante eficiente, por combinar alta resolução e exatidão de massa, para identificação e quantificação de contaminantes orgânicos alvo, produtos de transformação e metabólitos em matrizes complexas utilizando uma análise cromatográfica rápida.

Palavras-chave: Contaminantes Orgânicos. Matrizes Complexas. QuEChERS. HRMS.

ABSTRACT

DETERMINATION OF ORGANIC CONTAMINANTS IN COMPLEX MATRICES USING QuEChERS METHOD AND LIQUID CHROMATOGRAPHY COUPLED TO HIGH RESOLUTION MASS SPECTROMETRY

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The presence of organic contaminants in the environment and in food have become a major concern due to the harmful effects to living organisms. Such contaminants may be pesticides used in agriculture for the control of pests, transformation products formed in the environment or through chemical processes, such as water treatment, and more recently pharmaceuticals and personal care products. Therefore, it is necessary to use sample preparation procedures and detection systems able to detect a variety of organic contaminants in trace level present in complex matrices, such as food and environmental samples. Thus, this study aims to: (1) address the use of high resolution mass spectrometry (HRMS) for the identification and quantification of organic contaminants in fish fillet using two acquisition data modes (full scan and all ions MS/MS) and to optimize QuEChERS method as sample preparation (PAPER 1); (2) present a second quantitative application of the use of QuEChERS method and HRMS for fruit samples (apple, pear and grape) in full scan mode, as well as to apply the proposed method for commercial fruit samples (PAPER 2) and (3) optimize and use QuEChERS method for extracting ionophore antimicrobials in poultry litter, followed by its quantification by LC-MS/MS and identification of transformation products by LC-QToF/MS after three different composting processes (PAPER 3). The three papers presented the importance of sample preparation optimization, QuEChERS, in order to obtain proper recovery of organic contaminants, besides to minimize the matrix effects caused by interferences in the extract. As the use of HRMS, which proved to be a very effective analytical tool for combining high resolution and mass accuracy, for identification and quantification of target organic contaminants, transformation products and metabolites in complex matrices using a rapid chromatographic run.

Keywords: Organic Contaminants. Complex Matrices. QuEChERS. HRMS.

LISTA DE ILUSTRAÇÕES

LISTA DE TABELAS

LISTA DE ABREVIATURAS E SIGLAS

4MBC	4-metil-benzilidina-camfor
AHTN	Toxalida
ANVISA	Agência Nacional de Vigilância Sanitária
CB	Carbamatos
CE	Energia de colisão, do inglês *colision energy*
CID	Dissociação induzida por colisão, do inglês *collision-induced dissociation*
csv	Separados por vírgula, do inglês *comma separated values*
DC	Corrente direta, do inglês *direct current*
DDT	Diclorodifeniltricloroetano
DEET	N, N-dietil-m-toluamida
DL_{50}	Dose letal mediana
d-SPE	Extração em fase sólida dispersiva, do inglês *dispersive solid phase extraction*
ECs	Contaminantes emergentes, do inglês *emerging contaminants*
EDCs	Disruptores endócrinos, do inglês *endocrine disrupting compounds*
EFSA	Autoridade Européia para a Segurança dos Alimentos, do inglês *European Food Safety Authority*
EHMC	2-etil-hexil-4-trimetoxicinamato
EIC	Cromatograma do íon extraído, do inglês *extracted ion chromatogram*
ESI	Ionização por eletronebulização, do inglês *electrospray ionization*
eV	Elétrons volt
EUA	Estados Unidos da América
GC-ECD	Cromatografia gasosa com detecção por captura de elétrons, do inglês *gas* chromatography with *electron capture detection*
GC-MS	Cromatografia gasosa acoplada à espectrometria de massas, do inglês *gas chromatography coupled to mass spectrometry*
GC-MS/MS	Cromatografia gasosa acoplada à espectrometria de massas em série, do inglês *gas chromatography coupled to tandem mass spectrometry*
GPC	Cromatografia com permeação em gel, do inglês *gel permeation chromatography*
HCH	Hexaclorociclohexano
HE	Alta energia, do inglês *high energy*
HHCB	Galaxolida
HLLE	Extração líquido-líquido homogênea
HPAs	Hidrocarbonetos poliaromáticos
HPLC	Cromatografia líquida de alta eficiência, do inglês *high performance liquid chromatography*
IBGE	Instituto Brasileiro de Geografia e Estatística
IDA	Ingestão diária aceitável
INMETRO	Instituto Nacional de Metrologia, Normalização e Qualidade

	Industrial
IPAs	Antimicrobianos ionóforos, do inglês *ionophore antimicrobials*
IS	Padrão interno, do inglês *internal standard*
IUPAC	União Internacional de Química Pura e Aplicada, do inglês *International Union of Pure and Applied Chemistry*
K_{ow}	Coeficiente de partição octanol/água
LAS	Lasalocida
LC/MS	Cromatografia líquida acoplada à espectrometria de massas, do inglês *liquid chromatography coupled to mass spectrometry*
LC-MS/MS	Cromatografia líquida acoplada à espectrometria de massas em série, do inglês *liquid chromatography coupled to tandem mass spectrometry*
LC-ToF/MS	Cromatografia líquida acoplada à espectrometria de massas por tempo de voo, do inglês *liquid chromatography coupled to time of flight mass spectrometry*
LC-QToF/MS	Cromatografia líquida acoplada à espectrometria de massas quadrupolo-tempo de voo, do inglês *liquid chromatography coupled to quadrupole-time of flight mass spectrometry*
LE	Baixa energia, do inglês *low energy*
LOD	Limite de detecção, do inglês *limit of detection*
LOQ	Limite de quantificação, do inglês *limit of quantification*
LMR	Limite máximo de resíduo
m/z	Razão massa/carga
mDa	MilliDalton
MAD	Maduramicina
MAPA	Ministério da Agricultura, Pecuária e Abastecimento
ME	Efeito matriz, do inglês *matrix effect*
MeCN	Acetonitrila
MON	Monensina
MS	Espectrometria de massas, do inglês *mass spectrometry*
MS/MS	Espectrometria de massas em série, do inglês *mass spectrometry in tandem*
NaCl	Cloreto de sódio, do inglês *sodium chloride*
NAR	Narasina
NH_3	Amônia
OCs	Organoclorados
OFs	Organosfosforados
OMS	Organização Mundial da Saúde
PARA	Programa de Análise de Resíduos de Agrotóxicos em Alimentos
PBDE	Éter difenil polibromado, do inglês *polybrominated diphenyl ethers*
PCBs	Bifenilas policloradas, do inglês p*olychlorinated biphenyls*
PFCs	Compostos perfluorados, do inglês *perfluorinated compounds*
PLE	Extração por líquido pressurizado, do inglês *pressurized liquid extraction*
PPCPs	Fármacos e produtos de cuidado pessoal, do inglês *pharmaceuticals and personal care products*

PNCRC	Plano Nacional de Controle de Resíduos e Contaminantes
PR	Piretróides
PSA	Amina primária secundária, do inglês *primary secondary amine*
PTFE	Politetrafluoretileno
q2	Segundo quadrupolo ou cela de colisão
Q1	Primeiro quadrupolo
Q3	Terceiro quadrupolo
QToF	Quadrupolo-tempo de voo, do inglês *quadrupole-time of flight*
QuEChERS	Rápido, fácil, econômico, robusto e seguro, do inglês *quick, easy, cheap, rugged and safe*
r^2	Coeficiente de determinação
RF	Radiofrequência
RSD	Desvio padrão relativo, do inglês *relative standard deviation*
RSD_{pi}	Desvio padrão relativo para precisão intermediária
RSD_r	Desvio padrão relativo para repetitividade
SAL	Salinomicina
SANCO	Saúde e Proteção do Consumidor, do francês *Santé et Protection des Consommateurs*
SDL	Limite de detecção de varredura, do inglês *screening detection limit*
SRM	Monitoramento de reação selecionada, do inglês *selected reaction monitoring*
TIC	Cromatograma total de íons, do inglês *total ion chromatogram*
ToF	Tempo de voo, do inglês *time of flight*
TP	Produtos de transformação, do inglês *transformation products*
TPP	Trifenilfosfato, do inglês *triphenyl phosphate*
TR	Triazinas
t_R	Tempo de retenção
TQ	Triplo quadrupolo
SS	Padrão de substituição, do inglês *surrogate standard*
SPE	Extração em fase sólida, do inglês *solid phase extraction*
UHPLC-MS/MS	Cromatografia líquida de ultra-alta eficiência acoplada à espectrometria de massas em série, do inglês *ultra high performance liquid chromatography coupled to tandem mass spectrometry*
UHPLC-QToF/MS	Cromatografia líquida de ultra-alta eficiência acoplada à espectrometria de massas quadrupolo-tempo de voo, do inglês *ultra high performance liquid chromatography coupled to quadrupole-time of flight mass spectrometry*
U.S.	Estados Unidos, do inglês *United States*
USDA	Departamento de Agricultura dos Estados Unidos, do inglês *United States Department of Agriculture*
US-MMSPD	Dispersão da Matriz em Fase Sólida Minituarizada Assistida por Ultrassom, do inglês *Ultrasonic Assisted Miniaturized Matrix Solid-Phase Dispersion*

SUMÁRIO

1 INTRODUÇÃO

Nos últimos anos, intensificou-se a investigação de contaminantes orgânicos nas mais diversas matrizes, sejam elas alimentos ou ambientais. Isso é decorrente da constante preocupação devido aos potenciais riscos causados aos seres vivos e ao meio ambiente. O termo “contaminante orgânico” inclui desde compostos sintéticos e/ou naturais, assim como seus produtos de transformação, os quais podem ser formados no meio ambiente ou através de processos químicos, como o tratamento de água (BRACK et al., 2016). Destes, destacam-se os agrotóxicos, devido ao seu amplo uso na agricultura para o controle de pragas, cuja finalidade seja alterar a composição da flora ou da fauna, a fim de preservá-las da ação danosa de seres vivos considerados nocivos (BRASIL, 2002; LeDOUX, 2011; FAO, 2013a). Vale ainda salientar a presença de fármacos e produtos de cuidado pessoal (PPCPs, do inglês *pharmaceuticals and personal care products*). Sendo estes, um grupo de compostos amplamente diversificados utilizados na medicina veterinária, na agricultura, na saúde humana e no cuidado pessoal (fragrâncias, loções, protetores solares, dentre outros) (DEBLONDEA et al., 2011; GUTIÉRREZ et al., 2016). Os efeitos negativos dos PPCPs são devido a sua emissão contínua no meio ambiente, não havendo a necessidade de serem compostos de característica persistente (GRACIA-LOR et al., 2012; BU et al., 2013).

Devido a sua complexidade e a fim de tornar viável a identificação e quantificação em nível de traços desses contaminantes em matrizes complexas como alimentos e amostras ambientais, é necessário uma etapa de preparo da amostra (RIDGWAY; LALLJIE; SMITH, 2007; PRESTES et al., 2013) seguido da análise empregando cromatografia líquida acoplada à espectrometria de massas (LC-MS, do inglês *liquid chromatography coupled to mass spectrometry*). O preparo de amostra é de suma importância, principalmente, devido ao efeito matriz, o qual pode causar aumento e/ou supressão do sinal dos analitos devido a presença de interferentes no extrato da matriz, e consequentemente afetar a capacidade de detecção, a seletividade, repetibilidade, exatidão, linearidade de resposta e o limite de quantificação do método (MARÍN et al., 2009; GOSETTI et al., 2010; FERRER et al., 2011). Após o preparo de amostra, a análise empregando LC-MS no modo de monitoramento de reação selecionada (SRM, do inglês *selected reaction monitoring*) é uma técnica fundamental para a análise multirresíduo em matrizes complexas

utilizando espectrometria de massas de baixa resolução, entretanto o emprego de métodos de varredura por espectrometria de massas de alta resolução tem ganhado espaço na identificação tanto de compostos alvo quanto de não-alvo, como na identificação de possíveis produtos de transformação (GARCÍA-REYES et al., 2007; GÓMEZ et al., 2010; MALATO et al., 2011).

Sendo assim, este trabalho aborda o emprego do método QuEChERS para o preparo de amostra de matrizes complexas e o uso da cromatografia líquida acoplada à espectrometria de massas de baixa e alta resolução para a determinação de contaminantes orgânicos em filé de peixe, frutas (maçã, pera e uva) e em cama de frango. Três artigos são apresentados na sequência, sendo o objetivo do "ARTIGO 1": (1) otimizar o preparo de amostra para a determinação de contaminantes orgânicos em filé de peixe empregando o método QuEChERS; (2) realizar a validação qualitativa e quantitativa de contaminantes orgânicos empregando dois modos de aquisição de dados (*full scan* e *All Ions* MS/MS) para a determinação de contaminantes orgânicos empregando cromatografia líquida acoplada à espectrometria de massas de alta resolução do tipo quadrupolo-tempo de voo (LC-QToF/MS, do inglês *liquid chromatography coupled to quadrupole-time of flight mass spectrometry*) e (3) comparar os resultados obtidos por cada um dos modos de aquisição de dados utilizados empregando o procedimento QuEChERS de preparo de amostra otimizado.

Visando uma segunda aplicação quantitativa da espectrometria de massas de alta resolução e QuEChERS, o "ARTIGO 2" teve como objetivo: (1) empregar o procedimento de preparo de amostra QuEChERS acetato nas matrizes de pera, uva e maçã para a determinação de agrotóxicos utilizando LC-QToF/MS no modo *full scan* e (2) aplicar o método proposto em amostras reais das frutas estudadas.

O "ARTIGO 3" teve como objetivo: (1) desenvolver um procedimento de preparo de amostra robusto empregando o método QuEChERS para a extração de antimicrobianos ionóforos em cama de frango, seguido da quantificação por cromatografia líquida acoplada a espectrometria de massas em série (LC-MS/MS, do inglês *liquid chromatography coupled to tandem mass spectrometry*); (2) quantificar os antimicrobianos ionóforos presentes em cama de frango antes e após três diferentes procedimentos de compostagem em escala piloto (aeração, viragem e combinando aeração e viragem) e (3) identificar produtos de transformação estáveis formados durante a compostagem da cama de frango empregando LC-QToF/MS.

2 REFERENCIAL TEÓRICO

2.1 PRODUÇÃO E IMPORTÂNCIA DO PESCADO NA ALIMENTAÇÃO

O Brasil é banhado por uma costa marítima de 8,5 mil quilômetros e possui 12% de toda a água doce do planeta. Possui 8,2 bilhões de metros cúbicos de água distribuídos em rios, lagos, açudes e represas; e devido as condições ambientais e climáticas favoráveis, tem potencial para se tornar um dos maiores produtores de pescado no mundo (MPA, 2010).

A pesca industrial no Brasil é composta por cerca de 1.600 embarcações e envolve cerca de nove mil trabalhadores dentro destas embarcações. Os principais produtos capturados são o camarão rosa, a piramutaba, o pargo e as pescadas na região Norte; os atuns no Nordeste; a sardinha, a corvina, a tainha e o bonito listrado (matéria prima da indústria do atum enlatado) nas regiões Sudeste e Sul (MPA, 2010).

A atividade de pesca industrial costeira no Brasil tem apresentado dificuldades devido a defasagem tecnológica associada às diversas etapas da cadeia produtiva, seja pelo excesso de esforço na pesca ou pela baixa qualidade dos produtos pesqueiros. Por outro lado, a pesca industrial oceânica, aquela voltada para a captura de grandes peixes pelágicos, ainda constitui uma fronteira de desenvolvimento à pesca no país (MPA, 2010).

A aquicultura (definida como o cultivo de organismos aquáticos para o consumo) teve um papel de destaque no crescimento da produção de pescado no país. No ano de 2011 a criação de pescado atingiu 628,7 mil toneladas, o que representa um crescimento de 31,1% em relação ao ano anterior. No continente as espécies de peixe mais criadas foram a tilápia e o tambaqui (67% do total). O crescimento da aquicultura foi bastante forte em todas as regiões do país, mas em especial na região Norte, onde houve um crescimento em média de 126% em relação ao ano anterior, passando de 41.839 para 94.718 toneladas. Nas regiões Nordeste e Sudeste o crescimento foi de 35 e 21%, respectivamente, Minas Gerais apresentou um aumento de produção de 123%. As regiões Sul e Centro-Oeste apresentaram menores aumentos na produção (15 e 8%, respectivamente) (MPA, 2011).

Para algumas espécies a produção nacional de 2011, marítima e no continente, foi de: atum (1.718 toneladas), carpa (450,9 toneladas), jundiá (354,7 toneladas) e tilápia (9.681,6 toneladas). Já o salmão foi o terceiro produto mais importado, sendo o Chile o maior fornecedor (em torno de 33 mil toneladas). A produção na aquicultura nacional de carpa, jundiá e tilápia foi, respectivamente, 38.079,1; 1.747,3 e 253.824,1 toneladas (MPA, 2011).

A balança comercial brasileira de pescado no ano de 2011 apresentou exportações de US$ 271.193.147 e importações de US$ 1.262.888.212, ou seja, um déficit de aproximadamente US$ 991 milhões, representando uma elevação de 32,5% em relação a 2010 (MPA, 2011).

A carne de peixe caracteriza-se como alimento funcional, ou nutracêutico, pois além de nutrientes básicos, possui propriedades de prevenção ou diminuição dos sintomas de determinadas doenças devido a presença de ácidos graxos poliinsaturados chamados de ômega-3. Os principais encontrados no peixe são o ácido eicosapentaenóico e o ácido docosahexaenóico, sendo estes os responsáveis pelos efeitos de proteção à saúde humana (SUÁREZ-MAHECHA et al., 2002; KRIS-ETHERTON; HARRIS; APPEL, 2003). A Organização Mundial da Saúde (OMS) recomenda o consumo per capita de 12 kg de peixe por ano por habitante, sendo que a média global é de 18 kg/ano, porém na América Latina e Caribe esse número é menor, 9 kg/ano (FAO, 2013b).

2.2 PRODUÇÃO DE FRANGO E O USO DE CAMA DE FRANGO COMO FERTILIZANTE

No Brasil, a produção de carne de frango chegou a 13,058 milhões de toneladas em 2011, um crescimento de 6,8% em relação a 2010. Sendo também o principal país exportador de frango. Com este desempenho o país se aproxima da China, hoje o segundo maior produtor mundial, cuja produção de 2011 teria somado 13,2 milhões de toneladas, abaixo apenas dos Estados Unidos, com 16,757 milhões de toneladas (BRAZILIAN CHICKEN, 2012; USDA, 2012a).

Ao lado do crescimento dos índices produtivos, cresce também a preocupação com os efeitos das criações intensivas de aves sobre o meio ambiente, principalmente no que diz respeito à geração e disposição dos resíduos produzidos (ORRICO JÚNIOR; ORRICO; LUCAS JÚNIOR, 2010). O resultado, em regiões de

cultivo intensivo de aves, é a aplicação de estrume em excesso, além do que as culturas necessitam, enriquecimento dos ecossistemas devido a presença diversos nutrientes e eutrofização (YONKOS et al., 2010).

Enquanto, esterco de frango refere-se somente aos excrementos do animal, a cama de frango contém uma mistura de dejetos, material do leito da cama (serragem, apara de madeira, etc.), penas, descamações da pele das aves e restos de alimento que caem dos comedouros (YONKOS et al., 2010; VIRTUOSO et al., 2015). A cama de frango é indispensável para proteger os animais das intempéries climáticas e do atrito mecânico com o piso, sendo considerada todo material distribuído sobre o piso de galpões para servir de leito às aves (VIRTUOSO et al., 2015).

O uso de cama de frango como fertilizante vem sendo a melhor solução para manejar as grandes quantidades de resíduos gerados. Além disso, a quantidade de nutrientes presentes na cama de frango, como nitrogênio, fósforo e potássio, são bem mais elevadas do que em outros resíduos animais, sendo bastante vantajoso para o uso na agricultura (ABRAHAM; KEPFORD, 2000; BISWAS; MCGRATH; SAPKOTA, 2012; CRIPPEN et al., 2016).

A compostagem é o processo que transforma o resíduo orgânico em um produto estável, possível de ser aplicado no solo, de menor densidade e volume, além de apresentar redução de odor e toxinas. A aplicação no solo do produto de cama de frango após compostagem apresenta menor risco de contaminação quando comparado com o uso sem realização desse procedimento (ABRAHAM; KEPFORD, 2000).

2.3 PRODUÇÃO DE MAÇÃ, PERA E UVA

A produção mundial de maçãs, em 2012, segundo dados do *United States Department of Agriculture* (USDA), foi de 67,8 milhões de toneladas, onde a produção chinesa correspondeu a 56%. Nas últimas 6 safras, a produção aumentou 53% na China e 10% na União Europeia. Em termos mundiais, a produção de maçãs é crescente (28% em 6 anos) (USDA, 2012b; MAPA, 2013).

O Brasil produziu, em 2011, 1,3 milhão de toneladas de maçãs, o que o classifica como 9º maior produtor mundial. O valor da produção de maçãs foi calculado pelo Instituto Brasileiro de Geografia e Estatística (IBGE) em R$ 851,7

milhões. A produção brasileira ocupa 38 mil hectares, 96% desses pomares estão em Santa Catarina (18 mil hectares) e Rio Grande do Sul (17 mil hectares). No estado do Paraná, a pomicultura ocupa uma área de 1.800 hectares. São Paulo, Minas Gerais e Bahia figuram, com 105, 157 e 60 hectares de pomares de macieiras, respectivamente. A produção dos três estados, juntos, não chega a 0,5% do total (MAPA, 2013).

A balança comercial brasileira de maçãs frescas oscila entre positiva e negativa, dependendo do ano. Em 2011, a balança apresentou o pior resultado desde o ano 2000, com um saldo negativo de US$ 48,4 milhões. Ao contrário, o saldo da balança comercial dos sucos de maçã é sempre positivo e crescente, tendo atingido seu melhor resultado em 2012, com US$ 51,6 milhões (MAPA, 2013).

A pereira é considerada, há muito tempo, uma alternativa importante para diversificação da produção de frutas de clima temperado na região Sul do Brasil. Baseado em dados coletados entre 2001-2012, a área colhida de pera no Brasil passou de 1.952 hectares para 1.668 hectares, evidenciando uma redução média anual de 1,32%. O Estado do Rio Grande do Sul figura como o maior produtor do país, apresentando 56,30% da área plantada e 50,12% da produção nacional (MELLO, 2013a; FIORAVANÇO & OLIVEIRA, 2014).

A China é o maior produtor mundial, sendo responsável por 67,26% da produção global. O segundo maior produtor mundial, a Itália, foi responsável por apenas 3,25% da produção mundial, em 2010, e apresentou redução de 23,51% da sua produção em relação ao ano de 2001. Em relação a quantidade de pera exportada, a Argentina liderou esse segmento até 2008. A partir de 2009, a China passou a liderar as exportações de pera, com 437.929 toneladas, em 2010 (MELLO, 2013a).

A viticultura brasileira ocupa, atualmente, uma área de 81 mil hectares, com vinhedos desde o extremo Sul até regiões próximas à linha do Equador. Duas regiões se destacam: o Rio Grande do Sul por contribuir, em média, com 777 milhões de quilos de uva por ano, e os polos de frutas de Petrolina/PE e de Juazeiro/BA, no Submédio do Vale do São Francisco, responsável por 95% das exportações nacionais de uvas finas de mesa (MAPA, 2016). De acordo com os dados estatísticos disponíveis no portal do IBGE, em 2012, houve uma redução de 0,52% na produção de uvas no Brasil em relação ao ano de 2011 (MELLO, 2013b). Em 2012, a produção de uvas destinadas ao processamento (vinho, suco e

derivados) foi de 830,92 milhões de quilos, o que representa 57,07% da produção nacional. O restante da produção (42,93%) foi destinado ao consumo *in natura* (MELLO, 2013b).

Os principais destinos da uva brasileira são a União Europeia (75%) e os Estados Unidos (12%). Já o Brasil importa grande quantidade de vinhos finos, originários de uvas européias, do Chile, Argentina e Itália. Também são importados espumantes da Itália, Argentina e França, além de uvas secas e frescas, principalmente da Argentina e do Chile (MELLO, 2013b).

2.4 CONTAMINANTES ORGÂNICOS

Devido ao surgimento de equipamentos analíticos cada vez mais sensíveis, os quais possibilitam a detecção em nível de traços de substâncias em matrizes complexas, juntamente com o avanço do conhecimento sobre seus efeitos ecotoxicológicos, tem-se intensificado o monitoramento de resíduos de contaminantes orgânicos (WILLE et al., 2012). Dentre estes contaminantes, dois subgrupos serão abordados nos itens subsequentes, os agrotóxicos e os fármacos e produtos de cuidado pessoal (PPCPs, do inglês *pharmaceuticals and personal care products*).

2.4.1 Agrotóxicos

Segundo o *Codex Alimentarius*, agrotóxico refere-se a qualquer substância ou mistura que tenha como objetivo prevenir, destruir ou controlar qualquer tipo de praga incluindo espécies de plantas ou animais que devam estar presentes durante a produção, estocagem, transporte, distribuição ou processamento de alimentos e rações animais para o controle de ectoparasitas. O termo inclui substâncias utilizadas como reguladores de crescimento para plantas, desfolhantes, dessecantes, agentes promotores de amadurecimento de frutos, inibidores de germinação e substâncias que são aplicadas aos grãos antes e depois da colheita para evitar a deterioração do alimento durante a estocagem e transporte. Sendo excluídos desse conceito os fertilizantes, nutrientes animais e/ou vegetais, aditivos alimentares e medicamentos de uso veterinário (FAO, 2013a).

A legislação brasileira conceitua agrotóxicos como sendo produtos e agentes de processos físicos, químicos ou biológicos, destinados ao uso nos setores de produção, no armazenamento e beneficiamento de produtos agrícolas, nas pastagens, na proteção de florestas, nativas ou plantadas, e de outros ecossistemas e de ambientes urbanos, hídricos e industriais, cuja finalidade seja alterar a composição da flora ou da fauna, a fim de preservá-las da ação danosa de seres vivos considerados nocivos, bem como as substâncias e produtos empregados como desfolhantes, dessecantes, estimuladores e inibidores de crescimento (BRASIL, 2002).

Os agrotóxicos podem ser classificados de diferentes maneiras de acordo com sua aplicação: inseticidas (combatem insetos), herbicidas (combatem plantas daninhas), fungicidas (combatem fungos), acaricidas (combatem ácaros), moluscocidas (combatem moluscos), rodenticidas (combatem bactérias), entre outros (BAIRD, 2002).

A composição química dos agrotóxicos é bastante diversa, porém muitos deles apresentam características em comum, sendo classificados dentro de um mesmo grupo. Os principais grupos são os organofosforados, organoclorados, carbamatos, piretróides e triazinas (BARBOSA, 2004).

Organoclorados (OCs): foram amplamente utilizados, desde os anos 40 devido ao forte efeito no controle de pragas e doenças. OCs foram a primeira classe de agrotóxicos orgânicos sintetizados testados na agricultura, e o clorodifeniltricloroetano (DDT) e hexaclorociclohexano (HCH) foram, provavelmente, os mais conhecidos. A produção e o uso intenso na agricultura desses compostos resultaram em uma ampla contaminação do meio ambiente (KALYONCU; AGCA; AKTUMSEK, 2009). Apresentam alta estabilidade, baixa volatilidade, são apolares, lipofílicos, e, por consequência, são consideravelmente persistentes no meio ambiente com tendência a bioacumulação levando a contaminação de alimentos, em especial aqueles com alto teor de gordura (LeDOUX, 2011).

Organofosforados (OFs): foram os primeiros a substituírem os organoclorados e são derivados dos ácidos: fosfórico, fosfônico e fosfínico. Suas propriedades físico-químicas e solubilidade em água variam amplamente (LeDOUX, 2011; TERRY Jr., 2012). Desde o início muitos compostos têm sido sintetizados para sua utilização como inseticidas (malationa, diazinona, etc.), antihelmínticos (triclorfom), herbicidas

(tribufós), dentre outras aplicações. São compostos biodegradáveis e, portanto, apresentam curta persistência no solo (JOKANOVIC, 2001; TERRY Jr., 2012).

Carbamatos (CB): são agrotóxicos orgânicos derivados do ácido carbâmico e podem ser inseticidas, herbicidas ou fungicidas. Geralmente são compostos instáveis e vários fatores influenciam sua degradação, como umidade, temperatura, luminosidade e volatilidade (BARBOSA, 2004).

Piretróides (PR): são derivados sintéticos das piretrinas, as quais são inseticidas naturais produzidas por algumas espécies de crisântemos. Os piretróides agem como neurotoxinas e atuam no sistema nervoso dos insetos alvo (WOUDNEHA; OROS, 2006). Como principais características, são lipofílicos desde apolares até de pouca polaridade.

Triazinas (TR): são os herbicidas mais utilizados na agricultura, sendo a maioria derivados da s-triazina (1,3,5-triazina) e alguns da 1,2,4-triazina. Triazinas são degradadas em hidroxitriazinas e são levemente básicas, apresentam pouca solubilidade em água, são estáveis no meio ambiente e, portanto, persistentes (LeDOUX, 2011).

A avaliação e a classificação do potencial de periculosidade ambiental de um agrotóxico são baseadas em estudos físico-químicos, toxicológicos e ecotoxicológicos. Assim, a Tabela 1 abaixo demonstra o sistema de classificação quanto à toxicidade dos agrotóxicos com valores de DL_{50} em rato via oral e dérmica.

Tabela 1 - Classificação dos agrotóxicos de acordo com os efeitos à saúde humana.

Classificação	Toxicidade	DL_{50} via oral (mg kg^{-1} peso corporal)	DL_{50} via dérmica (mg kg^{-1} peso corporal)
Ia	Extremamente tóxico	< 5	< 50
Ib	Altamente tóxico	5-50	50-200
II	Moderadamente tóxico	50-2000	200-2000
III	Levemente tóxico	> 2000	> 2000
U	Pouco tóxico	> 5000	

DL_{50}: Dose letal para matar 50% de indivíduos de uma população em teste.
FONTE: WHO, 2009.

Os agrotóxicos podem ser absorvidos pelas culturas, diretamente através de suas folhas, ou indiretamente pelo solo, além da possibilidade de serem

transportados pela chuva e pelo vento de seus pontos de aplicações para culturas vizinhas (BIRKETT; LESTER, 2003; HILDEBRANDT; LACORTE; BARCELÓ, 2007; FENIK; TANKIEWICZ; BIZIUK, 2011). O uso amplamente difundido de agrotóxicos proporciona, além de seu acumulo em diferentes culturas vegetais e em solos, na contaminação de águas e de toda a cadeia alimentar aquática (BIRKETT; LESTER, 2003; FENIK; TANKIEWICZ; BIZIUK, 2011), bem como a bioacumulação de agrotóxicos persistentes em produtos alimentares de origem animal, tais como: carne, peixe, gordura, ovos e leite (LeDOUX, 2011). Esses processos são altamente dependentes do tipo de agrotóxico, do solo, da cultura, das condições climáticas, dos procedimentos de aplicação, e assim, o destino dos agrotóxicos é muito variável (HILDEBRANDT; LACORTE; BARCELÓ, 2007).

Desde 2002, o mercado mundial de agrotóxicos cresceu 93% enquanto o mercado brasileiro cresceu 190%. O Brasil assumiu o posto de maior mercado mundial de agrotóxicos em 2008, passando os Estados Unidos. No ano de 2010, o mercado nacional movimentou cerca de US$ 7,3 bilhões e representou 19% do mercado global (SEMINÁRIO MERCADO DE AGROTÓXICOS E REGULAÇÃO, 2012).

Esse consumo desenfreado implica em diversas consequências, tanto no âmbito da saúde dos trabalhadores devido à exposição ocupacional, como na contaminação alimentar através da ingestão de resíduos de agrotóxicos, o que de fato representa um problema de saúde pública (GARIBOTT, 2012).

2.4.2 Fármacos e Produtos de Cuidado Pessoal

Aproximadamente, há uns 15 anos, um grande número de trabalhos científicos referentes à presença de fármacos no meio ambiente têm sido publicados, principalmente devido ao potencial efeito nocivo dessas substâncias no meio ambiente e a saúde humana (GRACIA-LOR et al., 2012; BU et al., 2013; GARCÍA et al., 2013; LIU; WONG, 2013; GUTIÉRREZ et al., 2016). Os PPCPs são um grupo de compostos amplamente diversificados utilizados na medicina veterinária, na agricultura, na saúde humana e no cuidado pessoal (fragrâncias, sabonetes, loções, cremes dentais, protetores solares, dentre outros). Grandes quantidades de fármacos, de diversas classes são consumidos em todo o mundo para o tratamento ou diagnóstico de doenças. Fármacos, como ibuprofeno e outros

são consumidos em uma faixa de centenas de toneladas por ano em países como a Inglaterra, Alemanha e Austrália (SCHNELL et al., 2009; BU et al., 2013; GARCÍA et al., 2013; GUTIÉRREZ et al., 2016).

PPCPs contém diversos subgrupos de compostos orgânicos, tais como fármacos: antibióticos, hormônios, antiinflamatórios, antipiléticos, β-bloqueadores, citostáticos, etc; e, produtos de cuidado pessoal como almíscares sintéticos, repelentes, conservantes, filtros solares. A Tabela 2 apresenta esses grupos de fármacos e produtos de cuidado pessoal com alguns exemplos de princípios-ativos (LIU; WONG, 2013).

PPCPs são suspeitos de causar elevadas taxas de câncer, deficiência reprodutiva em seres humanos e animais, bem como desenvolvimento e disseminação de resistência antimicrobiana (GRACIA-LOR et al., 2012). Esse grupo de contaminantes não precisa ser persistente no ambiente para causar efeitos negativos devido a sua emissão contínua, e ainda têm potencial de bioacumulação em diferentes níveis tróficos (GRACIA-LOR et al., 2012; BU et al., 2013).

Algumas fontes de contaminação por PPCPs são efluentes domésticos, industriais, hospitalares, chegando às estações de tratamento de esgoto com sua estrutura levemente alterada ou mesmo sem alterações, e em seguida entrando no ambiente aquático. A contaminação ambiental ainda pode ser atribuída à entrada direta devido ao uso em instalações de aquicultura ou indireta através da manipulação durante atividades agrícolas (BU et al., 2013).

Existe uma preocupação constante em relação aos resíduos destes produtos em todo o mundo, principalmente pelo fato de serem produzidos em grande escala. Vários trabalhos relatam que os mesmos não podem ser degradados durante os tratamentos convencionais de água, e como consequência, uma enorme variedade de resíduos de produtos farmacêuticos tem sido detectada em águas residuais e em águas de superfície na faixa de ng L^{-1} a µg L^{-1} (SCHNELL et al., 2009; BU et al., 2013; GARCÍA et al., 2013; LIU; WONG, 2013; FAIRBAIRN et al., 2015). Os PPCPs podem ainda ser encontrados em solo através da irrigação utilizando água de reuso, ou pelo uso de fertilizantes, como cama de frango (CHEN et al., 2013; LI et al., 2015).

Tabela 2 - Classificação dos PPCPs e alguns compostos representativos de cada grupo.

	Subgrupos	Compostos Representativos
Fármacos	Antibióticos	Claritromicina
		Eritromicina
		Sulfametoxazol
		Sulfadimetoxina
		Ciprofloxacino
		Norfloxacino
		Cloranfenicol
	Hormônios	Estrona
		Estradiol
		Etinilestradiol
	Analgésicos e antiinflamatórios	Diclofenaco
		Ibuprofeno
		Paracetamol
	Antiepiléticos	Carbamazepina
		Primidona
	Dislipidêmicos	Clofibrato
		Genfibrozila
	β-bloqueadores	Metoprolol
		Propranolol
	Meios de contraste	Diatrizoato
		Iopromida
	Citostáticos	Ifosfamida
		Ciclofosfamida
Produtos de Cuidado Pessoal	Agentes antimicrobianos	Triclosan
		Triclocarban
	Almiscar sintética/fragrâncias	Galaxolida (HHCB)
		Toxalida (AHTN)
	Repelentes	N,N-dietil-m-toluamida (DEET)
	Conservantes	Parabenos
	Filtros solares	2-etil-hexil-4-trimetoxicinamato (EHMC)
		4-metil-benzilidina-camfor (4MBC)

FONTE: LIU; WONG, 2013

2.5 LEGISLAÇÃO

Limites máximos de resíduos (LMR) são estabelecidos para alguns agrotóxicos e medicamentos de uso veterinário em peixe, porém não há legislação específica para resíduos sólidos, como a cama de frango. LMR também são estabelecidos para amostras de frutas, em legislação nacional e internacional. A

União Européia define que os LMRs devem ser estabelecidos em conformidade com os princípios geralmente reconhecidos de avaliação da segurança do alimento. No estabelecimento do LMR são observados os riscos toxicológicos, a contaminação ambiental e os efeitos microbiológicos e farmacológicos dos resíduos. Além disso, outras avaliações científicas da segurança das substâncias são efetuadas por organizações internacionais (EUROPEAN UNION, 2009a).

Neste sentido, a diferença entre resíduo e contaminante são conceitos que devem ser bem compreendidos. O primeiro, refere-se à uma ou mais substâncias presentes no interior ou à superfície dos vegetais, dos produtos vegetais ou dos produtos comestíveis de origem animal, na água potável ou no ambiente, e resultantes da utilização de um produto fitofarmacêutico, incluindo os respectivos metabólitos e produtos resultantes da sua degradação ou reação (EUROPEAN UNION, 2009b). Já o termo contaminante é definido como qualquer substância que não seja intencionalmente adicionada aos alimentos. Os contaminantes podem estar presentes nos alimentos como resultado das etapas de produção, transformação, acondicionamento, embalagem, transporte e armazenagem do alimento (EUROPEAN UNION, 2010).

O monitoramento de resíduos é avaliado em relação ao LMR, que determina a quantidade legalmente permitida, ou reconhecida, como aceitável e é estabelecido para cada composto aprovado para uso em um determinado alimento. O LMR sempre está correlacionado à Ingestão Diária Aceitável (IDA), que por sua vez é obtida a partir de ensaios de experimentação, avaliando-se a toxicidade, a teratogenicidade e a carcinogenicidade destes compostos não intencionais (DENOBILE; NASCIMENTO, 2004).

No Brasil, a competência para estabelecer LMRs em alimentos para agrotóxicos é do Ministério da Saúde através da ANVISA (2010). Por outro lado, o Brasil não estabelece LMR para medicamentos veterinários, adotando aqueles recomendados pelo Mercosul, *Codex Alimentarius*, União Européia ou Estados Unidos (PACHECO-SILVA; SOUZA & CALDAS, 2014). Além disso, o Ministério da Agricultura, Pecuária e Abastecimento (MAPA) é responsável por monitorar a presença de resíduos de produtos de uso veterinário e contaminantes ambientais em produtos de origem animal por meio do Plano Nacional de Controle de Resíduos e Contaminantes (PNCRC), o qual é responsável por fiscalizar a ocorrência de violações de limites máximos de resíduos ou teores máximos de contaminantes, e

detectar a utilização de produtos de uso veterinário de utilização proibida, para os quais não há limite de tolerância (MAPA, 2010).

2.6 CONTAMINANTES ORGÂNICOS NO MEIO AMBIENTE

Nos últimos anos a preocupação com os contaminantes orgânicos tem sido tanta que diversos trabalhos estão sendo desenvolvidos focando na identificação e quantificação dessas substâncias em solo, sedimento, água e estações de tratamento de esgoto. Além disso, muitos autores estão propondo alternativas a fim de removê-los do meio ambiente, minimizando os efeitos por eles causados. A Figura 1 resume os possíveis caminhos dos contaminantes orgânicos no meio ambiente.

Herrero-Hernández et al. (2013) avaliaram a presença de 58 agrotóxicos, incluindo herbicibas, fungicidas, inseticidas e alguns produtos de degradação em 92 pontos de amostragem de águas naturais da região de La Rioja, Espanha. As águas subterrâneas e superficiais podem estar afetadas devido às atividades agrícolas referente à viticultura. Pode-se detectar 40 agrotóxicos em uma ou mais amostras, sendo os compostos mais encontrados a terbutilazina e seu metabólito (desetil terbutilazina), fluometuron, etofumesato, pirimetanil e tebuconazol. Concentrações acima de 0,1 μg L^{-1} foram detectadas para 37 compostos e em vários casos valores em torno de 18 μg L^{-1}. Os autores observaram a presença de agrotóxicos em 64 e 62% das amostras de águas subterrâneas e superficiais, respectivamente. Sendo assim, a soma de todos os agrotóxicos presentes foi maior de 0,5 μg L^{-1}, o qual é o limite estabelecido pela União Européia para a soma de todos os agrotóxicos detectados em água para consumo humano.

Uma pesquisa conduzida pelo Ministério do Meio Ambiente do Canadá no ano de 2006 teve como objetivo investigar a presença de produtos farmacêuticos, hormônios e bisfenol A em 257 amostras de água potável por 16 meses. Foram analisadas 48 substâncias sendo que 27 foram encontradas nas amostras. A maioria das substâncias encontradas foram antibióticos e bisfenol A, sendo que a presença de hormônios não foi detectada. Os autores relataram que as concentrações encontradas nas águas de Ontário são menores que níveis encontrados anteriormente e relatados em outros trabalhos (KLEYWEGT et al., 2011).

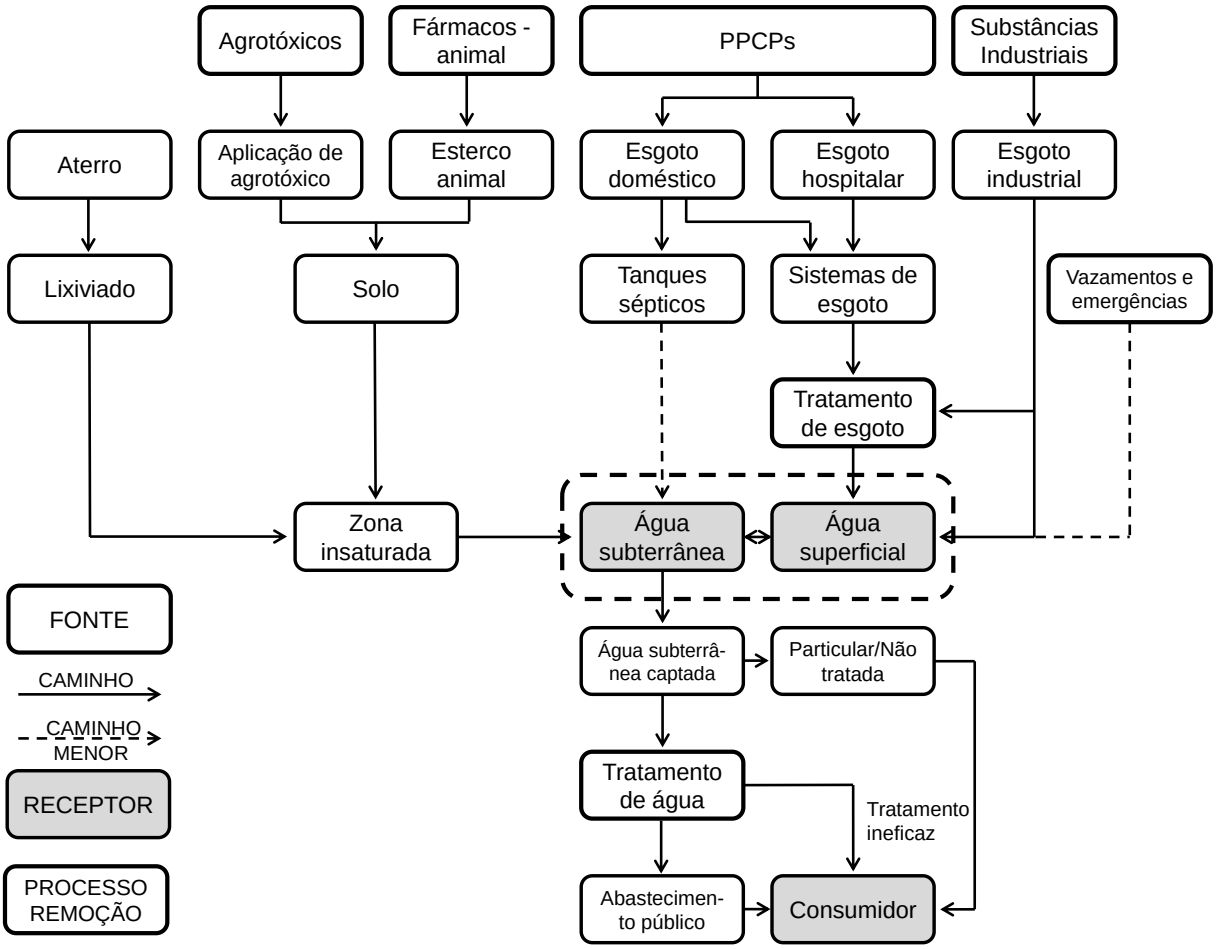

Figura 1 - Esquema dos caminhos possíveis de contaminação do meio ambiente por contaminantes orgânicos, adaptado de STUART et al. (2012).

Combinando diferentes equipamentos, Masia et al. (2013) analisaram 63 amostras de água de superfície e residual de rios da Espanha. As amostras foram analisadas inicialmente para a determinação de 43 agrotóxicos ou produtos de degradação, e desses, 33 foram encontrados nas amostras de água. Após, os autores reanalisaram essas amostras e, com auxílio de uma biblioteca criada no laboratório contendo mais de 1100 poluentes orgânicos, a qual foi utilizada para a identificação dos compostos, sendo que desses cerca de 250 compostos estavam disponíveis como padrões de referência. Após essa análise foram encontrados 5 agrotóxicos e 3 produtos de degradação que não estavam no método empregado anteriormente, além de 13 fármacos e 2 drogas de abuso.

LI et al. (2015) compararam a ocorrência de 15 antibióticos em amostras de solo de 11 produções em estufa na China com o solo de campo aberto, e observaram que as amostras de solo de estufas estavam contaminadas com concentrações maiores que amostras produzidas em campo aberto. As

concentrações, por classes, foram de tetraciclinas (102 μg kg^{-1}), quinolonas (86 μg kg^{-1}), sulfonamidas (1,1 μg kg^{-1}) e macrolídeos (0,62 μg kg^{-1}). Os autores concluíram que o uso de esterco é a principal fonte de contaminação por antibióticos. Outro programa de monitoramento de solo realizado na Húngria coletou 24 amostras no período no inverno e constatou a presença de atrazina em duas amostras nas concentrações de 0,07 e 0,11 μg g^{-1} (OLDAL et al., 2006).

2.6.1 Contaminantes Orgânicos em Peixe

A presença de contaminantes orgânicos no meio aquático acarreta na contaminação dos organismos vivos que nele habitam, estando assim, os peixes expostos a essa contaminação. Diversos trabalhos relacionados a determinação de compostos orgânicos em peixe estão voltados a presença de substâncias persistentes, como agrotóxicos organoclorados, bifenilas policloradas, entre outras. Poucos trabalhos estão disponíveis na literatura com o objetivo de monitoramento, quantificação e/ou varredura de outras classes de contaminantes orgânicos, especialmente PPCPs, nesse tipo de amostra em especial trabalhos envolvendo espectrometria de massas de alta resolução. Por outro lado, trabalhos que envolvam avaliação ecotoxicológica dessas substâncias são bastante encontrados, e é evidente a influência que essas substâncias exercem sobre os organismos aquáticos. Para exemplificar, Nassef et al. (2010) avaliaram a toxicidade de três PPCPs (carbamazepina, diclofenaco e triclosan) através da medida dos efeitos no comportamento de alimentação e velocidade de nado da espécie de peixe japonês medaka (*Oryzias latipes*). Essa espécie foi submetida a doses controladas desses compostos por 9 dias e os comportamentos monitorados durante os dias 5-9. Observou-se que essa exposição afetou o comportamento dos peixes, indicando que os PPCPs apresentam papel de toxicidade em organismos aquáticos.

Amostras de filés de peixes de um córrego dominado por efluente, no Texas (EUA) foram analisadas para 23 fármacos e 2 metabólitos e foram encontrados resíduos de difenidramina, diltiazem, carbamazepina e norfluoxetina em todas as 11 amostras em uma faixa de 0,11 a 5,14 ng g^{-1} (RAMIREZ et al., 2007). Mottaleb et al. (2009) analisaram 10 produtos de cuidado pessoal e 2 alquilfenóis em 11 amostras peixes da mesma localização descrita anteriormente, e os compostos benzofenona, galaxolida, tonalida e triclosan foram detectados em todas as amostras em

concentrações variando de 37 a 90, 234 a 970, 26 a 97 e 17 a 31 ng g^{-1}, respectivamente.

Lazartigues et al. (2011a) desenvolveram um método para a determinação de 13 agrotóxicos em filé de peixe. O método foi aplicado em 50 amostras de filé de carpa e 43 amostras de filé de perca (*Perca fluviatilis*) nas quais foram encontrados resíduos de isoproturon com concentração máxima de 0,50 e 0,85 ng g^{-1}, respectivamente, bem como, resíduos de carbendazim com concentrações máximas de 0,34 ng g^{-1} em carpa e 0,21 ng g^{-1} em perca.

2.6.2 Contaminantes Orgânicos em Cama de Frango

A presença de contaminantes orgânicos em cama de frango é um ponto bastante crítico em termos de contaminação ambiental, uma vez que esse tipo de resíduo é utilizado como fertilizante em solo para a produção de diversas culturas. Poucos trabalhos abordam a presença de resíduos, em especial, fármacos em cama de frango, mas o principal foco são os antimicrobianos ionóforos. Esse grupo de antimicrobianos é amplamente utilizado como aditivo na ração dos animais a fim de controlar a coccidiose, uma doença comum que afeta principalmente aves jovens. Além disso, mais de 80% desses compostos não são absorvidos, sendo excretados intactos através da urina ou fezes dos animais (SUN et al., 2014a). Recentemente, alguns trabalhos vêm focando na busca por produtos de transformação de ionóforos em cama de frango após procedimentos de compostagem, fotodegradação, dentre outros (SUN et al., 2014a; SUN et al., 2014b).

Um método para a determinação de monensina, lasalocida, salinomicina e narasina por LC-MS/MS em cama de frango foi desenvolvido por BISWAS; MCGRATH; SAPKOTA (2012). Os resultados mostraram a presença de monensina (97,8 µg kg^{-1}), lasalocida (19,2 µg kg^{-1}), salinomicina (70,0 µg kg^{-1}) e narasina (57,3 µg kg^{-1}) em cama de frango armazenada por mais de três anos à <5 °C. FURTULA; HUANG; CHAMBERS (2009) também avaliaram a presença de fármacos em cama de frango, e foram encontradas concentrações nas amostras na faixa de 10 a 11.000 µg kg^{-1} dos compostos monensina, salinomicina, narasina e nicarbazina. Amostras de cama de frango de diferentes fazendas do estado da Geórgia (Estados Unidos) foram analisadas para a presença de antimicrobianos ionóforos e, como resultado,

salinomicina foi encontrada em concentrações de 4 até 22 mg kg^{-1}, e monensina e narasina de 0,23 até 4 mg kg^{-1} (SUN et al., 2013).

2.6.3 Contaminantes Orgânicos em Frutas

Sabe-se que agrotóxicos são substâncias usadas na agricultura para proteger as culturas contra insetos, fungos, ervas daninha, bem como outras pestes (WHO, 2015) e, por isso, resíduos de agrotóxicos em alimentos tem de cumprir as normas mais rigorosas para a segurança alimentar (HANOT; GOSCINNY & DERIDDER, 2015). Para garantir que o alimento no mercado seja seguro para consumo, programas oficiais de monitorização são estabelecidos.

Em 2013, 80.967 amostras de alimentos foram analisadas em um monitoramento realizado pelos países da União Europeia, Noruega e Islândia. Em média, as amostras foram analisadas para 200 agrotóxicos, e a maioria delas (55.253 amostras, 68,2%) originárias de países da União Europeia, 22.400 amostras (27,7%) de produtos importados de países de terceiro mundo e 3.314 amostras (4,1%) sem informação de origem. De modo geral, o monitoramento realizado pela *European Food Safety Authority* (EFSA) em 54,6% das amostras não foram detectados resíduos de agrotóxicos, enquanto em 42,8% das amostras analisadas não excederam os valores de LMR permitidos. Um número de 2,6% (2116 amostras) apresentaram resíduos superiores aos LMRs estabelecidos. Mais especificamente, 1610 amostras de maçã foram analisadas, e em 533 (33%) não foram encontrados resíduos de agrotóxicos. Enquanto, em 1077 amostras foram detectados pelo menos um agrotóxico. Até 17 diferentes agrotóxicos foram detectados em uma única amostra de maçã (EFSA, 2015).

No ano de 2015, no Reino Unido, 59 amostras de frutas e vegetais foram analisadas pelo *Department of Health's School Fruit and Vegetable Scheme*. Foram analisadas amostras de maçã (12), banana (9), cenoura (12), pera (10), passas de uva (6) e tangerina (10). Dessas, 4 amostras não apresentaram resíduos de agrotóxicos, 54 amostras continham resíduos abaixo do LMR estabelecido, e somente 1 amostra com concentração maior do que permitido. Para maçã, especificamente, todas as amostras (3 do Reino Unido e 9 da União Européia) continham resíduos abaixo dos valores de LMR estabelecidos e 10 amostras tinham

mais de um agrotóxico presente. Todas as amostras de pera provenientes da União Europeia continham mais de um agrotóxico, porém, abaixo do LMR (PRiF, 2015).

Jardim & Caldas (2012) apresentaram os resultados dos monitoramentos realizados pelo Programa de Análise de Resíduos de Agrotóxicos em Alimentos (PARA) coordenado pela ANVISA e pelo Plano Nacional de Controle de Resíduos e Contaminantes (PNCRC) coordenado pelo MAPA para a presença de resíduos de agrotóxicos no Brasil, entre os anos de 2001 e 2010, nos quais um total de 13556 amostras de 22 frutas e vegetais foram analisadas. Foram encontrados resíduos de agrotóxicos em 48,3% das amostras, desses 13,2% apresentavam alguma irregularidade (uso de substância não autorizada), e menos de 3% das amostras continham resíduos acima do valor de LMR permitido (JARDIM & CALDAS, 2012).

Em geral, o cenário de resíduos de agrotóxicos em alimentos investigados nos programas de monitoramento realizados no Brasil é similar com o que é encontrado em outros países.

2.7 TÉCNICAS DE EXTRAÇÃO DE CONTAMINANTES ORGÂNICOS EM MATRIZES COMPLEXAS

A determinação de contaminantes em nível de traços em matrizes complexas, como alimentos e amostras ambientais, frequentemente, requer uma etapa de preparo da amostra anterior a análise no instrumento. O número de etapas e tempo necessário para o preparo da amostra depende do tipo da amostra e suas propriedades, bem como o analito a ser determinado. As etapas mais comumente empregadas são: amostragem/homogeneização, extração, limpeza e concentração. Muitas vezes a etapa de limpeza é essencial, a fim de isolar o analito de interesse e eliminar possíveis interferentes. A concentração da amostra, por muitas vezes também se faz necessária, com o objetivo de melhorar os limites de quantificação do método utilizado. É importante que o extrato final, obtido após todas as etapas envolvidas de preparo da amostra, seja compatível com a técnica de análise a ser utilizada na determinação do(s) analito(s) de interesse (RIDGWAY; LALLJIE; SMITH, 2007; PRESTES et al., 2013).

O preparo de amostra ideal poderia ser aquele que atendesse todos os itens aqui listados (WILKOWSKA; BIZIUK, 2011):

- Assegurar rapidez nas análises, com um intervalo mínimo entre coleta e a determinação dos analitos na amostra;

- Ser de fácil realização;

- Usar equipamentos e reagentes de baixo custo;

- Permitir a determinação seletiva de analitos;

- Garantir alto nível de automação, minimizando o efeito da influência do analista (erros, etc.) e;

- Empregar pequenas quantidades de solventes e reagentes, limitando a quantidade de resíduos gerados.

Sendo assim, destaca-se o método QuEChERS (do inglês *quick, easy, cheap, effective, rugged* e *safe*), o qual é caracterizado pelo uso de um solvente polar, acetonitrila, para extração e sais para partição (ANASTASSIADES et al., 2003). Essa adição de sais permite que compostos de diferentes polaridades possam ser extraídos. A adição de sulfato de magnésio anidro ($MgSO_4$), promove a separação entre a água e a fase orgânica. Pode-se adicionar cloreto de sódio (NaCl) para auxiliar no efeito *salting out* e acetato de sódio (NaAc) para que haja a formação de tampão acetato quando adiciona-se ácido acético à acetonitrila. Juntamente com o método QuEChERS foi proposto um novo procedimento de limpeza denominado extração em fase sólida dispersiva (d-SPE, do inglês *dispersive solid phase extraction*), onde uma quantia de extrato é colocada em contato com uma mistura de sorventes e/ou sais (ANASTASSIADES et al., 2003;). Esses sorventes podem ser uma amina primária-secundária (PSA), a qual tem elevado efeito quelante devido a presença dos grupamentos amino primário e secundário, acarretando em remoção de ácido orgânicos polares, pigmentos polares, alguns açucares e ácidos graxos (PRESTES et al., 2009; WILKOWSKA; BIZIUK, 2011). Pode-se ainda utilizar C_{18} para remover substâncias interferentes apolares, como lipídeos, e ainda carbono grafitizado para remover esteróis e pigmentos como clorofila (WILKOWSKA; BIZIUK, 2011). O menor tempo de preparo de amostra, a eliminação de etapas de evaporação e a troca do uso de cartuchos tradicionais de SPE por d-SPE são algumas das vantagens oferecidas com o método QuEChERS.

Observando a importância do preparo de amostras na determinação de compostos em concentrações em nível de traços em matrizes complexas, a Tabela 3 apresenta algumas aplicações que abordaram a presença de contaminantes em filé de peixe, cama de frango e frutas.

Tabela 3 - Métodos para a determinação de contaminantes em filé de peixe, cama de frango e em frutas.

Matriz	Analitos	Procedimento de extração	Procedimento de limpeza	Técnica de análise	LOQ (ng g^{-1})	Rec. (%)	Referência
filé de peixe	23 fármacos e 2 metabólitos	extração com solvente	-	LC-MS/MS	0,03 - 10,4	70 - 120	Ramirez et al., 2007
filé de peixe	10 produtos de cuidado pessoal e 2 alquilfenóis	extração com solvente	GPC	GC-SIM-MS GC-MS/MS	2,4 - 397,0	87 - 114	Mottaleb et al., 2009
filé de peixe	13 agrotóxicos	extração com solvente	-	LC-MS/MS	1,0 - 49,0	36 - 115	Lazartigues et al., 2011a
filé de peixe	40 disruptores endócrinos	QuEChERS	d-SPE	GC-MS/MS	1,0 - 25,0	70 - 120	Munaretto et al., 2013
filé de peixe	22 agrotóxicos organoclorados e 7 PCBs	QuEChERS	Criogênia e d-SPE	GC-MS	1,0 - 10,0	70 - 115	Norli; Christiansen; Deribe, 2011
filé de peixe	13 retardantes de chama, 18 agrotóxicos, 14 PCBs, 16 HPAs, 7 PBDE	QuEChERS	d-SPE	GC-MS/MS	0,1 - 10,0	70 - 120	Sapozhnikova; Lehotay, 2013
filé de peixe	18 PCBs, 16 agrotóxicos, 14 PBDE e 25 HPAs	QuEChERS	minicoluna de sílica	GC-MS/MS	0,05 - 10,0	70 - 120	Kalachova et al., 2013
filé de peixe	13 agrotóxicos	QuEChERS	hexano	LC-MS/MS	0,004 - 7,4	33 - 80	Lazartigues et al., 2011b
filé de peixe	6 agrotóxicos	US-MMSPD	HLLE	GC-ECD	1,3 - 4,0	39 - 81	Rezaei; Hosseini, 2011
filé de peixe	107 fármacos	extração com solvente	SPE	LC-ToF/MS	2,6 - 266,7[1]	50 - 118	Peters et al., 2009
filé de peixe	19 disruptores endócrinos	QuEChERS	d-SPE	UHPLC-MS/MS	0,005 - 9,26	40 - 103	Jakimska et al., 2013
filé de peixe	35 antibióticos, 36 agrotóxicos e 11 micotoxinas	ultrassom	-	UHPLC-QToF/MS LC-MS/MS	20 - 100[2]	-	Nácher-mestre et al., 2013
filé de	11 fármacos	PLE	SPE	LC-MS/MS	0,10 - 1,19	19 - 85	Wang; Gardinali,

peixe							2011
cama de frango	monensina, salinomicina e narasina	extração com solvente	-	LC-MS/MS	7,6-12,5[3]	79-83	Sun et al., 2013
cama de frango	monensina, lasalocida, salinomicina e narasina	extração com solvente	SPE	LC-MS/MS	0,67-2,02[3]	92-104	Biswas; McGrath; Sapkota, 2012
frutas	11 agrotóxicos	extração com solvente	-	UHPLC-MS/MS UHPLC-(Q)ToF	0,01[4]	70-110	Grimalt et al., 2010
frutas	15 agrotóxicos	extração com solvente	-	LC-ToF/MS	0,8-50[5]	-	Ferrer et al., 2005
frutas	100 agrotóxicos	QuEChERS	d-SPE	LC-MS LC-MS/MS	0,01-20[5]	-	Núñez et al., 2012
suco de frutas	41 agrotóxicos	d-SPE	-	UHPLC (orbitrap)	0,1-0,5[6]	62-110	Deme; Upadhyayula, 2015

GPC: cromatografia de permeação em gel; HLLE: extração líquido-líquido homogênea; HPA: hidrocarbonetos aromáticos policíclicos; PBDE: éter difenil polibromado; PCB: bifenilas policloradas; PLE: extração por líquido pressurizado; SPE: extração em fase sólida; US-MMSPD: ultrassom assistido com dispersão da matriz em fase sólida miniaturizada; [1]os autores definiram os limites de quantificação considerando os valores de LMR para cada composto, variando de 0,5 a 1,5 o LMR; [2]faixa de valores para varredura; [3]valores de LOQ em µg kg^{-1}; [4]valores de LOQ em mg kg^{-1}; [5]valores de LOD em µg kg^{-1}; [6]valores de LOQ em ng mL^{-1}.

2.8 CROMATOGRAFIA LÍQUIDA ACOPLADA A ESPECTROMETRIA DE MASSAS PARA A DETERMINAÇÃO DE CONTAMINANTES ORGÂNICOS

A cromatografia líquida de alta eficiência (HPLC, do inglês *high performance liquid chromatography*) é uma técnica importante na determinação de compostos orgânicos, sendo o requisito principal para a análise em HPLC a solubilidade dos compostos na fase móvel (HARRIS, 2008). O acoplamento da cromatografia líquida com a espectrometria de massas combina as vantagens da cromatografia (alta seletividade e eficiência de separação) com as vantagens da espectrometria de massas (obtenção de informação estrutural, massa molar e aumento adicional da seletividade) (CHIARADIA; COLLINS; JARDIM, 2008).

O princípio básico da espectrometria de massas (MS, do inglês *mass spectrometry*) é a geração de íons de compostos orgânicos ou inorgânicos e separação através da sua razão entre massa e carga (*m/z*), seguida da detecção qualitativa e quantitativa da respectiva *m/z* e abundância. Essa ionização pode ser realizada termicamente, por campo elétrico, por impacto energético de elétrons, íons ou fótons, dentre outros. De um modo geral, o espectrômetro de massas consiste em uma fonte de íons, um analisador de massas e um detector, sendo que os dois últimos são operados sob condições de alto vácuo. A fonte de íons não necessariamente estará sob vácuo, podendo ficar em pressão atmosférica (GROSS, 2004).

2.8.1 Fontes de Ionização

Tratando-se de fontes de ionização em MS muitas podem ser as opções a serem utilizadas, sendo necessário avaliar as características dos analitos de interesse e desse modo escolher a fonte de ionização mais adequada para a análise. O emprego de cromatografia líquida acoplada à espectrometria de massas (LC/MS, do inglês *liquid chromatography coupled to mass spectrometry*) tem como principal fonte de ionização a eletronebulização (ESI, do inglês *electrospray ionization*) (LANÇAS, 2009).

Na ionização por eletronebulização o líquido no qual o analito de interesse se encontra dissolvido passa através de um capilar, à pressão atmosférica, mantido sob alta tensão. Na saída do capilar são formadas pequenas gotas altamente

carregadas, o *spray*, que são dessolvatadas ao se deslocarem em sentido contrário ao posicionamento de um eletrodo em uma região de pressão atmosférica. A dessolvatação é assistida por um fluxo contínuo de gás seco (geralmente N_2). À medida que ocorre a dessolvatação, o tamanho das gotas é reduzido até o ponto em que a força de repulsão entre as cargas similares fica maior que as forças de coesão da fase líquida (tensão superficial). Neste momento ocorre a chamada “explosão coulômbica”, que gera gotas com tamanhos equivalentes a 10% do tamanho das gotas a partir das quais se originaram. Uma série de explosões passa então a ocorrer até que são produzidos íons do analito a partir destas gotas, os quais são transferidos para o interior do espectrômetro de massas por uma série de dispositivos de focalização (CHIARADIA; COLLINS; JARDIM, 2008), como apresentado na Figura 2.

A ESI possibilita a ionização de compostos sensíveis à temperatura sem que estes sofram degradação, uma vez que, a ionização ocorre diretamente em solução (CHIARADIA; COLLINS; JARDIM, 2008). O emprego de LC/MS utilizando ESI como fonte de ionização é ideal para análise de moléculas mais polares, de maior massa molecular. Ainda, LC-ESI-MS é muito aplicada para determinações de proteínas, aminoácidos, e várias substâncias de interesse na área da bioanalítica, alimentícia e farmacêutica (LANÇAS, 2009).

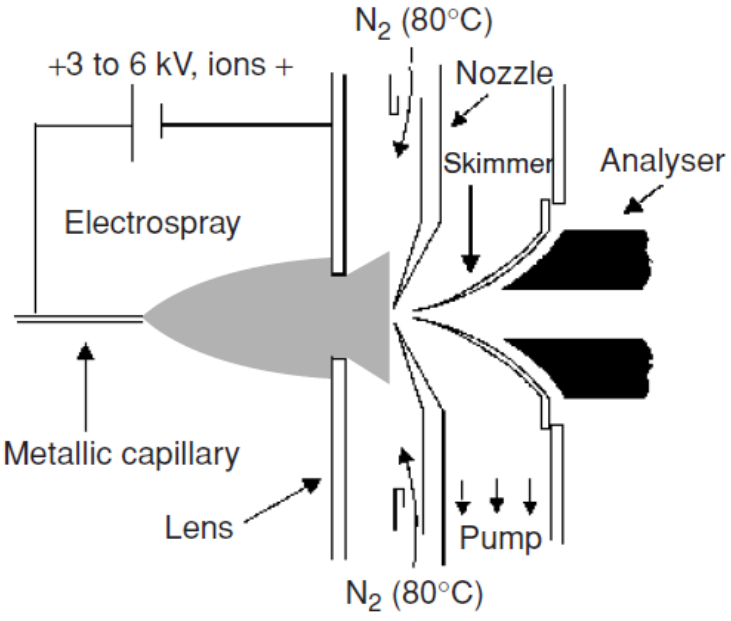

Figura 2 - Esquema prático da fonte de ionização ESI (HOFFMANN; STROOBANT, 2007).

2.8.2 Analisadores de Massas

A separação dos íons nos analisadores de massas ocorre de acordo com a relação existente entre suas massas e cargas, ou seja, a razão *m/z*. Atualmente, existe uma grande variedade de analisadores de massas, e a escolha do mais apropriado depende da aplicação. Para tanto, cinco características medem o desempenho de um espectrômetro de massas, são elas: limite da faixa de massas, velocidade de análise, velocidade de transmissão, exatidão e resolução de massas (HOFFMANN; STROOBANT, 2007).

A faixa de massas determina o limite de *m/z* no qual o analisador de massas pode medir os íons. A velocidade de análise, do inglês *scan speed*, é a razão em que o espectrômetro de massas mede uma faixa de massas determinada, sendo medido em unidade de massa por segundo (u s^{-1}), ou unidade de massas por milissegundo (u ms^{-1}). A transmissão é a razão do número de íons que alcançam o detector e o número de íons que entram no espectrômetro de massas, e geralmente inclui perdas de íons ao longo do percurso do analisador. Deve-se observar para não haver confusão com o termo de ciclo de trabalho, do inglês *duty cycle*. Esse último refere-se a proporção de tempo enquanto um dispositivo ou sistema é utilizado completamente, ou seja, a porção de íons de certa *m/z* produzidos na fonte que são efetivamente analisados. Os ciclos de trabalho se diferem de acordo com o instrumento, mas também analisadores iguais podem ter ciclos diferentes, pois são dependentes do modo de operação. Esses parâmetros estão associados com a sensibilidade do analisador de massas, mas a mesma é melhor descrita pelo termo de eficiência de espectrômetros de massas o que leva em consideração o ciclo de trabalho, a velocidade de transmissão e a eficiência do detector (HOFFMANN; STROOBANT, 2007).

Alta resolução em espectrometria de massas é necessária para separar uma massa de outras e assegura que íons de apenas um tipo contribuam para uma determinada medida, ou seja, o espectrômetro de massas é capaz de distinguir dois picos de *m/z* bastante próximos. Sendo assim, alta resolução é de suma importância para todos os experimentos que envolvam matrizes complexas, as quais contém um significativo número de íons de *background*. Em casos como esse, pode-se diferenciar a presença de analitos em baixa concentração ou não detectá-los devido o efeito de mascaramento por interferentes isobáricos da matriz.

Dois termos bastante importantes e que são facilmente confundidos, devem ser compreendidos, sendo esses: poder de resolução e resolução. O poder de resolução é definido como $M/\Delta M$ na largura total do pico e a meia altura no máximo (FWHM, onde FW: *full width* e HM: *half maximum*). O termo M é a massa medida e ΔM é a largura do pico em unidades de massa em uma determinada altura do pico (meia altura), usado em espectrômetros de massas do tipo quadrupolo e tempo de voo. Uma segunda maneira de calcular é considerando ΔM como a diferença medida em unidades de massa de dois picos próximos expressos com um vale de 10%, esse modo é utilizado no instrumento, setor magnético. A diferença entre esses dois modos de cálculo é um fator de 2, sendo assim quando apresentado um poder de resolução de 10000 empregando o método do vale 10% é igual ao poder de resolução de 20000 por FWHM. Espectrômetros de massas com poder de resolução a partir de 10000 são instrumentos de alta resolução. Portanto, resolução é definida como o inverso do poder de resolução $\Delta M/M$, ou seja, é um número pequeno o qual define a capacidade de resolver dois picos de massas quase iguais. Por exemplo, um instrumento que apresenta um poder de resolução de 10000 (FWHM) para a massa de *m/z* 300 poderia separar massas que diferem em 0,03 unidades de massa (HOFFMANN; STROOBANT, 2007; MARSHALL; HENDRICKSON, 2008; FERRER; THURMAN; ZWEIGENBAUM, 2011).

A quinta característica de suma importância em espectrometria de massas (de alta resolução) é a exatidão de massas, a qual indica a exatidão da *m/z* proveniente do analisador de massas. Ou seja, exatidão de massas é a diferença observada entre a *m/z* teórica a *m/z* medida, que é expressa em partes por milhão (ppm) ou em miliDalton (1 mDa = 0,001 unidades de massa). Esse item está intimamente ligado a estabilidade e a resolução do analisador, instrumentos de baixa resolução não fornecem alta exatidão (HOFFMANN; STROOBANT, 2007; FERRER; THURMAN; ZWEIGENBAUM, 2011). A correção de massas é realizada a partir da introdução contínua na fonte de ionização de uma solução de referência, e esta característica é combinada com o *software* do instrumento, o qual autocalibra constantemente e registra os resultados das massas de referência ao longo da análise (FERRER; GARCÍA-REYES; FERNANDEZ-ALBA, 2005).

A seguir estão descritos dois tipos de espectrômetros de massas de baixa (quadrupolo/triploquadrupolo) e alta resolução (tempo de voo e o espectrômetro híbrido quadrupolo-tempo de voo).

Quadrupolo (qMS): É o analisador de massas mais popular, principalmente, pela sua simplicidade, preço relativamente baixo, boa linearidade, facilidade de ser entendido e operado. O quadrupolo é composto de quatro barras usualmente de metal, disposta em pares nos quais se aplicam uma corrente contínua do tipo DC (corrente direta) e um potencial RF (radiofrequência) alternante. Os íons produzidos na fonte de ionização são focalizados ao centro da região entre os quatro cilindros e atravessam o quadrupolo axialmente. Suas trajetórias são dependentes do campo elétrico produzido onde apenas íons de determinada *m/z* terão essa trajetória estável e chegarão ao detector. A RF é variada para que os íons de diferentes *m/z* obtenham uma trajetória estável ao longo do quadrupolo chegando ao detector, gerando assim o espectro de massas. A trajetória dos íons é um pouco complexa, mas simplificadamente seguem uma trajetória helicoidal (CHIARADIA; COLLINS; JARDIM, 2008; LANÇAS, 2009; STASHENKO; MARTÍNEZ, 2010).

Triplo quadrupolo (TQ): Este instrumento é constituído por três quadrupolos em série, sendo que o segundo quadrupolo (q2 ou cela de colisão) não é utilizado para separar íons de mesma razão *m/z*, mas sim como cela de colisão, na qual ocorre a fragmentação dos íons selecionados no primeiro quadrupolo (Q1) geralmente por dissociação induzida por colisão com um gás inerte (CID, do inglês *collision-induced dissociation*), e também é empregado como direcionador dos íons produzidos ao terceiro quadrupolo (Q3) (CHIARADIA; COLLINS; JARDIM, 2008). A principal vantagem de um analisador do tipo triplo quadrupolo, em relação a um quadrupolo simples é o aumento da relação sinal/ruído, visto que no primeiro selecionam-se somente os íons característicos do analito de interesse. Ou seja, por ser mais seletivo, se torna mais sensível, podendo desse modo obter melhores limites de quantificação para a substância de interesse (STASHENKO; MARTÍNEZ, 2010).

Tempo de voo (ToF): O conceito de analisador de massas por tempo de voo foi desenvolvido por Stephens em 1946 e os primeiros espectros publicados por Cameron e Eggers em 1948. Somente em 1955, Wiley e McLaren publicaram o desenho do que seria o primeiro espectrômetro de massas comercial, mas somente nos anos 80 renovou-se o interesse para esse tipo de analisador (HOFFMANN; STROOBANT, 2007; FERRER; THURMAN, 2009).

O funcionamento do espectrômetro de massas do tipo tempo de voo baseia-se no seguinte princípio, depois de formados os íons na fonte de ionização, esses

recebem um pulso de energia igual (10 kV), mas são acelerados de diferentes maneiras, de acordo com sua *m/z*, chegando ao detector em tempos diferentes. Sendo assim, íons de menor *m/z* terão maior velocidade e chegarão primeiro ao detector, já para *m/z* maiores, o inverso ocorre. Esse tipo de analisador tem alta taxa de transmissão de íons, fazendo com que tenham alta sensibilidade. Espectrômetros de massas lineares têm sua resolução bastante afetada devido à distribuição espacial e a variação da energia cinética dos íons quando recebem o impulso. Devido a isso, opta-se pelo uso de espectrômetros ortogonais, que após seu desenvolvimento, teve seu uso difundido nas mais diversas áreas de aplicação (FERRER; THURMAN, 2009).

Quadrupolo-Tempo de voo (QToF): No final da década de 70 teve início o desenvolvimento de equipamentos híbridos visando combinar características de diferentes analisadores em um único instrumento, a fim de melhorar o seu desempenho. Assim, uniram-se as características de velocidade e sensibilidade do ToF com a alta eficiência do quadrupolo em análises MS/MS, resultando no primeiro instrumento híbrido, quadrupolo-tempo de voo (QToF) (GLISHA; BURINSKY, 2008).

A Figura 3 representa o tipo mais comum de espectrômetro de massas híbrido, o qual inclui um quadrupolo (Q1) e uma cela de colisão quadrupolar (q2) seguidos de um analisador ortogonal por tempo de voo (oa-ToF). Em alguns instrumentos a cela de colisão não precisa ser necessariamente um quadrupolo, podendo ser substituída por um hexapolo, mas que tem a mesma função e operação pelo mesmo princípio (HOFFMANN; STROOBANT, 2007).

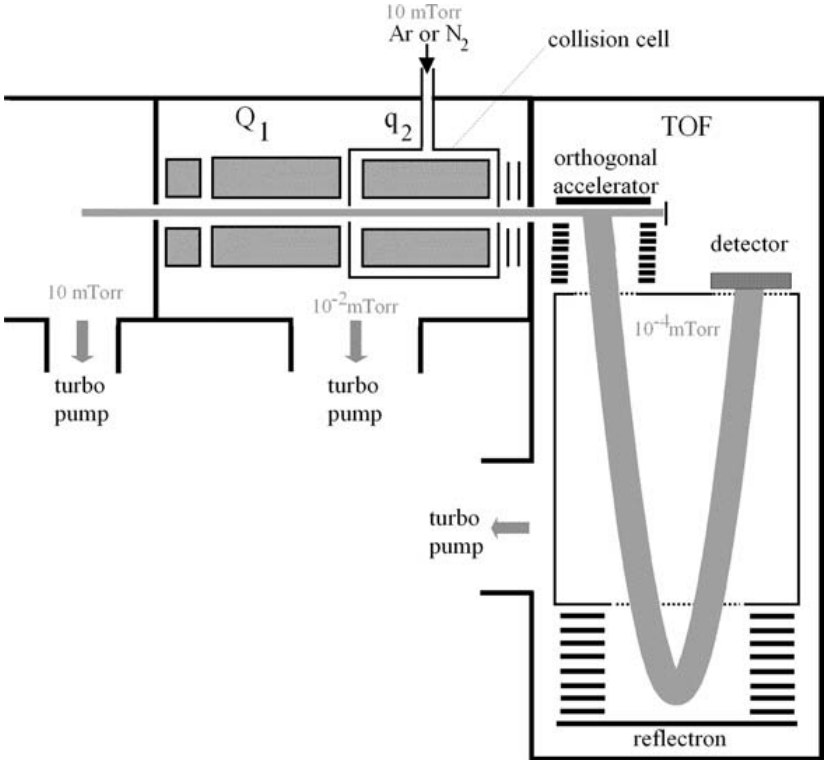

Figura 3 - Representação do sistema híbrido quadrupolo-tempo de voo, empregando colisão quadrupolar e aceleração ortogonal (HOFFMANN; STROOBANT, 2007).

Esse sistema quando operado no modo MS tem Q1 e q2 funcionando somente como guias de íons, funcionando no modo de RF somente. Enquanto isso, o ToF analisa todos os íons que foram acelerados ortogonalmente e é o único analisador de massas operante. No modo MS/MS, Q1 é utilizado para transmitir e selecionar somente os íons precursores de interesse. Na cela de colisão (q2) a fragmentação é induzida pela presença de moléculas neutras de gás (normalmente Ar ou N_2) e os íons produtos gerados são direcionados ao ToF (HOFFMANN; STROOBANT, 2007).

O modo de operação chamado *All Ions* MS/MS para a varredura e identificação de compostos em uma única análise tem como princípio a criação de um método de fácil aquisição, verificação dos resultados através de uma biblioteca de espectros adquiridos no modo MS/MS e quantificação incluindo íons produtos qualificadores. Esse modo de operação necessita que o método contenha uma etapa na qual todos os íons são analisados sem nenhuma energia de colisão e outras duas etapas com valores de energia de colisão, uma mais baixa e outra mais alta. Assim, torna-se possível a realização da varredura e quantificação da amostra em uma única corrida (WÜST; GLAUNER, 2013).

2.8.3 Importância do Acoplamento da Cromatografia com a Espectrometria de Massas de Alta Resolução para a Análise de Contaminantes Orgânicos e seus Produtos de Transformação

Recentemente, o emprego da cromatografia líquida com a espectrometria de massas em série no modo de monitoramento de reação selecionada (SRM) se tornou uma técnica fundamental para a análise multirresíduo em matrizes complexas. Embora desempenhe um excelente papel, trabalhando com esse modo de operação o sistema fica “cego” a outros compostos que possam estar presentes na amostra analisada, bem como pode ficar restrito o número máximo de compostos a serem analisados (GARCÍA-REYES et al., 2007; KAUFMANN et al., 2011). Com isso, o emprego da espectrometria de alta resolução, como por exemplo, por tempo de voo, se tornou uma ferramenta de grande interesse, tanto no ponto de vista da análise no modo de varredura, a fim de identificar compostos desconhecidos ou não-alvo, bem como identificar possíveis produtos de transformação (GARCÍA-REYES et al., 2007; GÓMEZ et al., 2010; MALATO et al., 2011).

O ToF tem como vantagem a capacidade de determinar, teoricamente, um número ilimitado de compostos com alta sensibilidade em uma única corrida. De um modo geral, o empregado do modo de varredura é rápido e fácil, porém os bancos de dados utilizados sejam eles *softwares* comerciais ou bibliotecas criadas no laboratório podem apresentar algumas incertezas nas análises de rotina, e precisam ser superadas. Ou seja, a escolha dos parâmetros de busca e análise devem ser cuidadosamente otimizados (MALATO et al., 2011). A combinação entre alta resolução e exatidão de massas são as grandes vantagens das análises realizadas por espectrometria de massas de alta resolução na determinação de compostos orgânicos em matrizes complexas, bem como na busca por produtos de transformação.

2.8.4 Efeito Matriz

O efeito matriz (ME, do inglês *matrix effect*) é observado como um aumento ou supressão na resposta do detector na presença no analito no extrato da matriz comparado com o mesmo analito presente em solvente orgânico. Quando o resultado for acima de ±20% considera-se que o efeito matriz começa a exercer

influência nas análises qualitativas e quantitativas (PIZZUTTI et al., 2007; PINHO et al., 2009; MARÍN et al., 2009; GOSETTI et al., 2010; FERRER et al., 2011; KWONA; LEHOTAY; GEIS-ASTEGGIANTE, 2012; FACCO et al., 2015; PARRILLA VÁZQUEZ et al., 2015). O efeito matriz exerce um papel bastante importante e por isso deve sempre ser avaliado durante o processo de validação de novos métodos (GOSETTI et al., 2010).

2.8.4.1 Efeito Matriz em Cromatografia Líquida Acoplada à Espectrometria de Massas

Empregando a cromatografia líquida acoplada a espectrometria de massas os fenômenos de aumento ou supressão do sinal decorrente da presença de compostos voláteis na matriz podem ocorrer, sendo a supressão sinal um fator importante ao trabalhar-se com resíduos de contaminantes. No caso de supressão de sinal, tais compostos são capazes de alterar a eficiência de formação de gotas ou evaporação do analito, assim como a quantidade de íons do analito formados na fase gasosa que alcançarão o detector. Este fenômeno afeta consequentemente a capacidade de detecção, a seletividade, repetibilidade, exatidão, linearidade de resposta e o limite de quantificação (MARÍN et al., 2009; GOSETTI et al., 2010; FERRER et al., 2011).

Diferentes espécies químicas podem ser responsáveis pela supressão de sinal como substâncias endógenas da matriz analisada que remanescem mesmo após o procedimento de preparo de amostra. Potenciais íos supressores são: espécies iônicas, substâncias polares e moléculas orgânicas, principamente àquelas com estrutura química semelhante ao analito alvo. Esses coextrativos causam problemas relevantes especialmente quando presentes em altas concentrações e quando coeluem com o analito de interesse (GOSETTI et al., 2010).

Outros fatores que também resultam em supressão do sinal estão relacionados com a concentração do analito, por exemplo, quando o analito está em uma concentração maior a supressão é menor. Por outro lado, quando o analito está em uma concentração menor, a supressão será maior, sendo resultante da maior quantidade de matriz presente. Além disso, a massa e a carga do analito também afetam o processo de ionização e supressão iônica. De um modo geral, moléculas

com massas mais elevadas tendem a suprimir o sinal de moléculas de massas menores (GOSETTI et al., 2010).

O efeito matriz exerce um papel muito importante na espectrometria de massas de alta resolução podendo interferir na efetividade de métodos de varredura porque além de causar a supressão de sinal, pode ainda acarretar em desvios nas medidas de exatidão de massas, bem como interferências por coeluição de interferentes isobáricos. A exatidão de massas, ainda pode ser afetada quando um interferente causa a supressão da(s) massa(s) da solução de referência, a qual é utilizada para corrigir desvios no instrumento através da contínua calibração do eixo de massas. O terceiro efeito associado a presença de interferentes da matriz está relacionado com a resolução de massas associada ao instrumento, e é definido como a habilidade do instrumento em distinguir dois íons de *m/z* similar. Por apresentarem grande número de interferentes, as matrizes complexas têm maior possibilidade de apresentar a coeluição de massas isobáricas, e estas são responsáveis por resultados falso positivos ou falso negativos (MALATO et al., 2011).

Como descrito anteriormente, o efeito matriz em LC-MS é um evento bastante relevante e deve ser devidamente estudado a fim de assegurar confiabilidade aos resultados obtidos. Sendo assim, algumas alternativas podem ser adotadas a fim de minimizar esse problema. A otimização das condições cromatográficas pode ser alterada, melhorando a eficiência de separação, ou ainda com a escolha da estratégia de calibração mais adequada, seja pela adição de padrão interno, calibração externa preparada no extrato "branco" da matriz (mais utilizada) ou ainda calibração interna (MARÍN et al., 2009; GOSETTI et al., 2010; BOTITSI et al., 2011).

2.9 VALIDAÇÃO

Faz-se necessário a validação de métodos analíticos a fim de mostrar a qualidade nas medições químicas, através de sua comparabilidade, rastreabilidade e confiabilidade. A validação de um método é um processo contínuo que começa no planejamento da estratégia analítica e continua ao longo de todo o seu desenvolvimento e transferência (RIBANI et al., 2004).

De acordo com o Instituto Nacional de Metrologia, Normalização e Qualidade Industrial (INMETRO) é sugerido que seja realizado um planejamento da validação

levando em consideração os seguintes itens: definição da aplicação, objetivo e escopo do método; definição dos parâmetros de validação e critérios de aceitação; verificação das características de desempenho do equipamento; qualificação dos materiais (por exemplo, reagentes); planejamento dos experimentos de validação, incluindo o tratamento estatístico, e execução dos experimentos de validação (INMETRO, 2011).

Existem vários guias de validação disponíveis, tanto em nível nacional quanto internacional. No Brasil podem ser adotados guias disponibilizados pela Agência Nacional de Vigilância Sanitária (ANVISA, 2003), através da Resolução - RE nº 899, de 29 de maio de 2003, bem como do INMETRO com o DOQ-CGCRE-008 (revisão 04) de 2011 (INMETRO, 2011). Órgãos internacionais que dispõem de guias são a Conferência Internacional em Harmonização (ICH, 2005), União Internacional de Química Pura e Aplicada - IUPAC (THOMPSON; ELLISON; WOOD, 2002) e SANCO (2013).

A validação de um método é uma exigência na prática das análises químicas, e tem como objetivo demonstrar que o método é apropriado para a finalidade pretendida, ou seja, garantir, através de estudos experimentais, que o método atenda às exigências das aplicações analíticas, assegurando a confiabilidade dos resultados (ANVISA, 2003).

Alguns dos parâmetros envolvidos no processo de validação de métodos analíticos são: curva analítica e linearidade, seletividade, exatidão, precisão (repetitividade), precisão intermediária, limite de detecção e de quantificação. Além desses, é importante determinar a exatidão de massas quando utiliza-se a espectrometria de massas de alta resolução (SANCO, 2013).

3 MATERIAIS E MÉTODOS

Este trabalho está dividido em três artigos, os quais foram desenvolvidos no Laboratório de Análise de Resíduos de Pesticidas (LARP) no Departamento de Química da Universidade Federal de Santa Maria (ARTIGOS 1 e 2) e no *Aga Lab* no Departamento de Química da *The State University of New York at Buffalo* nos Estados Unidos da América (ARTIGO 3). Os artigos desenvolvidos foram submetidos para publicação em revistas internacionais sendo o ARTIGO 1 submetido para a revista *Journal of Chromatography A*, o ARTIGO 2 já foi aceito para publicação na revista *Journal of AOAC International* e o ARTIGO 3 está publicado na revista *Environmental Pollution*, 212 (2016) 392-400.

Nesse item estão descritos procedimentos gerais realizados para ambos os trabalhos desenvolvidos e informações mais específicas referentes a cada trabalho estão detalhadas em seus respectivos artigos.

3.1 INSTRUMENTAÇÃO

A instrumentação utilizada para o desenvolvimento dos trabalhos segue descrita abaixo:

- ✓ Sistema de purificação de água Milli-Q Direct UV3® (Millipore e Barnstead, EUA);
- ✓ Sistema LC-QToF/MS (Agilent Technologies, Santa Clara, CA, EUA) equipado com:
  - Cromatógrafo líquido de alta performance-HPLC 1260;
  - Espectrômetro de massas de alta resolução, do tipo quadrupolo-tempo de voo-6530 com fonte de ionização por eletronebulização;
  - Sistema de geração de nitrogênio NM32LA (Peak Scientific);
  - Processamento de dados qualitativos e quantitativos através dos softwares Qualitative e Quantitative MassHunter B.06.00.
- ✓ Sistema LC-MS/MS (Thermo Scientific, Watham, MA, EUA) equipado com:
  - Cromatógrafo líquido de alta performance-Surveyor;
  - Espectrômetro de massas de tipo triploquadrupolo-TSQ Quantum Ultra™, com fonte de ionização por eletronebulização;
  - Processamento de dados através do software Xcalibur 2.2.

3.2 MATERIAIS UTILIZADOS

- ✓ Água ultrapura, purificada em sistema Milli-Q Direct UV3®, resistividade de 18,2 MΩ cm (Millipore, França);
- ✓ Acetato de sódio anidro (Mallinckrodt, México);
- ✓ Acetona grau HPLC (Mallinckrodt, EUA);
- ✓ Acetonitrila grau HPLC e LC-MS (Mallinckrodt e EMD Millipore, EUA);
- ✓ Ácido acético glacial 100% (J. T. Baker, EUA);
- ✓ Ácido fórmico ≥98,0% (Sigma Aldrich, Alemanha);
- ✓ Cloreto de sódio (Sigma-Aldrich, Alemanha e VWR International, EUA);
- ✓ Extran® neutro (Merck, Brasil);
- ✓ Filtros de nylon de 13 mm e porosidade de 0,2 µm (Vertical Chromatography, Tailândia e VWR International, EUA);
- ✓ Florisil (Restek, EUA);
- ✓ Formiato de amônio ≥99,0% (Fluka Analytical, EUA);
- ✓ Frascos de vidro (*vial*), capacidade de 2 mL;
- ✓ Hexano (95% n-hexano) (J.T. Baker, EUA);
- ✓ Isopropanol grau HPLC (Tedia, EUA);
- ✓ Metanol grau LC-MS (EMD Millipore, EUA);
- ✓ Soluções de referência e calibração para LC-QToF/MS (Agilent, EUA);
- ✓ Sorvente Bondesil C_{18} com tamanho de partícula de 40 µm (Varian, EUA);
- ✓ Sorvente Bondesil PSA com tamanho de partícula de 40 µm (Varian, EUA);
- ✓ Sulfato de magnésio anidro (J.T. Baker, Japão);
- ✓ Tubos de polipropileno, com tampas de rosca com capacidade de 50 e 15 mL (Sarstedt, Alemanha);
- ✓ Padrões de referência dos compostos em estudo;
- ✓ Vidraria comum de laboratório.

3.3 PREPARO DE SOLUÇÕES ANALÍTICAS

Preparou-se individualmente cada padrão de referência, levando-se em consideração a pureza (60,0 a 99,7%) para a pesagem da quantidade necessária para o preparo de uma solução na concentração de 1000 mg L^{-1} em acetonitrila ou metanol grau HPLC. A partir destas soluções estoque preparou-se uma mistura

contendo todos os compostos em uma concentração de 10 mg L^{-1}. Uma solução de todos os analitos na concentração de 1,0 mg L^{-1}, foi utilizada para o preparo das soluções analíticas de trabalho.

3.4 PARÂMETROS AVALIADOS PARA A VALIDAÇÃO DE MÉTODO QuEChERS

Em todos os artigos desenvolvidos foi realizada a validação do procedimento de preparo de amostra empregando o método QuEChERS adaptado para cada matriz, e foram avaliadas as figuras de mérito: seletividade, linearidade da curva analítica, limites de detecção e quantificação, exatidão através do ensaio de recuperação, precisão (repetitividade) e precisão intermediária, bem como avaliação do efeito matriz. Além disso, devido ao uso de espectrometria de massas de alta resolução fez-se a avaliação do erro da exatidão de massa.

3.4.1 Seletividade

A seletividade de um método é a capacidade que o mesmo possui de medir exatamente um composto em presença de outros componentes tais como impurezas, produtos de degradação e componentes da matriz (THOMPSON; ELLISON; WOOD, 2002; ANVISA 2003). Pode-se também dizer que é a habilidade dos métodos de extração, de purificação, do sistema de separação e detecção, em realizar a discriminação entre o analito de interesse e outros compostos (SANCO, 2013).

A seletividade do método foi avaliada fazendo injeções no sistema cromatográfico com a matriz “branco”, ou seja, ausente dos contaminantes em estudo, e da amostra fortificada com os mesmos. A comparação dos cromatogramas obtidos foi realizada a fim de avaliar a ocorrência de coeluição entre as substâncias de interesse e os interferentes da matriz.

3.4.2 Linearidade

A linearidade da curva analítica é a capacidade de um método analítico demonstrar que os resultados obtidos são diretamente proporcionais à concentração do analito na amostra, dentro de um intervalo especificado (ANVISA, 2003; RIBANI

et al., 2004). A quantificação requer que se conheça a dependência entre a resposta medida e a concentração do analito (INMETRO, 2011).

A linearidade das curvas analíticas foi realizada através da injeção das soluções analíticas preparadas de acordo com os relatos de cada artigo. As curvas analíticas foram preparadas em solvente orgânico e no extrato da matriz. Após obteve-se a equação da reta e o coeficiente de determinação (r^2).

3.4.3 Ensaios de Recuperação para Avaliação da Exatidão

A exatidão representa o grau de concordância entre os resultados individuais encontrados em um determinado ensaio e um valor de referência aceito como verdadeiro (RIBANI et al., 2004; SANCO, 2013). Valores aceitos de recuperação para a análise de substâncias em nível de traços é entre a faixa de 70 a 120%. Porém, uma faixa entre 60 e 140% pode ser utilizada em análises multirresíduo de rotina (SANCO, 2013).

A exatidão do método foi avaliada através dos ensaios de recuperação, fortificando as amostras “branco” em diferentes níveis de concentração. Foram realizadas réplicas de extração para cada nível e a recuperação foi calculada de acordo com a equação 1, seguindo o guia de validação do INMETRO (2011).

$$recuperação\,(\%) = \left(\frac{C_1 - C_2}{C_3} \right) \times 100 \qquad (1)$$

Onde:

C_1 = Concentração determinada na amostra fortificada;

C_2 = Concentração determinada na amostra não fortificada;

C_3 = Concentração usada para fortificação.

3.4.4 Precisão (repetitividade) e Precisão Intermediária

A precisão representa a dispersão de resultados entre ensaios independentes, repetidos de uma mesma amostra, amostras semelhantes ou padrões, sob condições definidas, sendo usualmente expressas pelo desvio padrão e pelo desvio padrão relativo (RSD, do inglês *relative standard deviation*), cujos

valores até 20%, são aceitos (THOMPSON; ELLISON; WOOD, 2002; RIBANI et al., 2004; INMETRO, 2011; SANCO, 2013).

A precisão pode ser calculada através da fórmula do desvio padrão relativo (RSD), como mostra a equação 2.

$$RSD\ (\%) = \frac{s}{\overline{X}} \times 100 \qquad (2)$$

Onde:

s = estimativa de desvio padrão absoluto;

$s = \{\sum(x_i - x_m)^2/n\text{-}1\}^{1/2}$;

x_i = valores individuais;

$\overline{X}$ = média das medidas em replicatas;

n = número de medidas.

A precisão, em termos de repetitividade (RSD_r) foi avaliada realizando a extração e análise de réplicas de cada nível de concentração, sendo que cada réplica foi injetada uma única vez. A precisão intermediária (RSD_{pi}) foi avaliada fazendo a injeção da curva analítica dos analitos e das amostras “branco” fortificadas no nível intermediário, sendo estas preparadas em dia diferente da avaliação da repetitividade, ou seja, realizando o procedimento analítico completo em dias diferentes.

3.4.5 Limite de Detecção e Quantificação

O limite de detecção (LOD, do inglês *limit of detection*) representa a menor concentração da substância em análise que pode ser detectada, mas não necessariamente quantificada, utilizando um determinado procedimento experimental. Já o limite de quantificação (LOQ, do inglês *limit of quantification*) representa a menor concentração da substância em análise que pode ser medida, com um grau aceitável de confiança, utilizando um determinado procedimento experimental (RIBANI et al., 2004).

A determinação do limite de quantificação (LOQ) foi realizada adotando a concentração de cada analito que obteve recuperação entre 70 e 120% no ensaio de recuperação (SANCO, 2013). Desse modo, o limite de detecção (LOD) foi determinado como sendo a concentração 3,33 vezes menor que o LOQ.

3.4.6 Avaliação do Efeito Matriz

Para a avaliação do efeito matriz, realizou-se a comparação entre as inclinações das curvas analíticas obtidas, preparadas em solvente orgânico e aquelas preparadas no extrato “branco” da matriz. O cálculo foi efetuado através da equação 3 (FERRER et al., 2011; SOUSA et al., 2013; PARRILLA VÁZQUEZ et al. 2015).

$$Efeito\ matriz = \left(\frac{a_E}{a_S} - 1 \right) \times 100 \qquad (3)$$

Onde:

a_E = inclinação da curva obtida pela injeção das soluções analíticas de cada composto orgânico, preparadas na matriz;

a_S = inclinação da curva obtida pela injeção das soluções analíticas de cada composto orgânico, preparadas em solvente.

3.5 ERRO DE EXATIDÃO DE MASSAS

A determinação do erro de exatidão de massas é importante quando trabalha-se com espectrometria de massas de alta resolução. A medida pode ser determinada de acordo com a equação 4. Para o presente trabalho foram utilizadas as massas *m/z* 121.0509 ($C_5H_5N_4$) e 922.0098 ($C_{18}H_{19}O_6N_3P_3F_{24}$) como referência para a calibração constante do LC-QToF/MS (MALATO et al., 2011).

$$\text{Erro de exatidão de massas (ppm)} = \frac{massa\ medida - massa\ calculada}{massa\ calculada} \times 10^6 \qquad (4)$$

4 ARTIGO 1 – LIQUID CHROMATOGRAPHY WITH HIGH RESOLUTION MASS SPECTROMETRY FOR IDENTIFICATION OF ORGANIC CONTAMINANTS IN FISH FILLET: SCREENING AND QUANTIFICATION ASSESSMENT USING TWO SCAN MODES FOR DATA ACQUISITION

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Abstract

Aquatic environments are often contaminated with complex mixtures of compounds that may pose a risk to ecosystems and human health. As the aquatic environment is usually the last receptacle for these contaminants, it is generally recognized that hazardous chemicals can be potentially transferred into fish fillet. However, among the large number of contaminants, just a few of them have been investigated extensively. This study proposed a strategy to identify and quantify 182 organic contaminants from different chemical classes in fish fillet using liquid chromatography coupled to quadrupole time-of-flight mass spectrometry (LC-QToF/MS). For this purpose, two different scan methods (full scan and all ions MS/MS) were evaluated to assess the best option for screening analysis in spiked fish fillet samples. In general, full scan acquisition was found to be more reliable (84%) in the automatic identification and quantification when compared to all ions MS/MS with 72% of the compounds detected. Additionally, a qualitative automatic search showed a mass accuracy error below 5 ppm for 77% of the compounds in full scan mode compared to only 52% in all ions MS/MS scan. However, all ions MS/MS provides fragmentation information of the target compounds. Undoubtedly, structural information of a wide number of compounds can be obtained using HRMS, but it is necessary thoroughly assess it, in order to choose the best scan mode.

Keywords: Screening detection limit, emerging contaminants, pesticides, fish, HRMS

1. Introduction

Environmental contaminants pose a significant risk to aquatic ecosystems and human health. These contaminants include synthetic and natural compounds as well as their transformation products that are formed in the environment or during technical treatment (e.g. water treatment) [1]. Pesticides are a group of compounds well known as a potential chemical class found in the environment, due to their use as pest control in agriculture to treat crops pre- and post-harvests [2]. Besides this, many works have reported the presence of new compounds in aquatic environments, called emerging contaminants, ECs [3]. ‘Emerging contaminants’ is a denomination for a group of compounds that are not necessarily new, but that were not considered a risk for the environment or human health in the past and only now are their presence and significance being elucidated [4]. Also, these ECs do not present regulatory status and whose effects on environment and human health are unknown [3]. ECs encompass a diverse group of compounds, including brominated and organophosphate flame retardants, plasticizers, endocrine disrupting compounds (EDCs), pharmaceuticals and personal-care products (PPCPs), drugs of abuse, food additives, disinfection by-products, nanomaterials, pesticides and their degradation/transformation products and perfluorinated compounds (PFCs), among others [3,5,6]. Both, the extensive environmental distribution of ECs and their potential ecotoxicological effects at very low concentrations have attracted increasing interest among researchers, regulatory authorities and the public [4].

As the aquatic environment is usually the last receptacle for organic contaminants, many compounds are abundantly present in surface waters and sediments. In aquatic biota, toxic effects, multi-contaminations and bioaccumulation in muscular tissues are regularly observed throughout the food chain, raising many questions about health hazards for fish flesh consumers [7]. It is generally recognized that hazardous chemicals could be potentially transferred to fish fillet through direct uptake of compounds present in the water and fish diet and these contaminants may bioaccumulate [8]. However, among the large number of organic contaminants, just a few of them have been investigated. In light of these concerns, evaluation of levels of these contaminants in fish muscle is an important objective for environmental and health sciences [9].

Due to the large amount of compounds that can be found in the aquatic environment, the number of analytes being monitored for environmental and food safety purposes has steadily increased. Analytical methods using low resolution mass spectrometry, applying selected reaction monitoring (SRM) in MS/MS mode are well-known, but only target compounds can be analyzed, while other possible contaminants present in a sample cannot be detected. In order to overcome the lack of sensitivity and accuracy when seeking untargeted compounds using full scan mode with low resolution mass spectrometers, the use of high resolution mass spectrometry (HRMS) has been the best choice [10]. HRMS instruments as well as quadrupole time-of-flight mass spectrometers (QToF/MS) have gained wide acceptance in the field of environmental and food safety analysis for the rapid screening of organic compounds (target and non-target) due to their high sensitivity in full scan acquisition mode, their ability to provide accurate mass measurements and the high resolution capable of distinguishing signals of two ions with a small *m/z* difference [10-14]. QToF/MS systems can also be used under all ions MS/MS mode, that is, simultaneous acquisition at low (LE) and high collision energy (HE), which provides useful information on the (de)protonated molecule (commonly at LE) and on the main fragment ions (commonly at HE). Based on this information and the isotopic distribution observed in the spectra, a reliable identification of the compounds detected in the samples is feasible [14].

The idea behind screening methods is that data evaluation can be done in an automated, fast and simple way. User-made accurate-mass databases can be built and associated to commercial software, which extracts all the potential compounds of interest from the LC-QToF/MS raw data, and then they can be matched to search targeted compounds in each sample. However, some difficulties have to be overcome for routine application and a study of the benefits and limitations has to be performed, to avoid false negative and/or false positive outcomes. Thus, a careful optimization of the search parameters is required, since they play a determinant role in the selectivity, accuracy and throughput of the whole procedure. In addition, other aspects, such as sensitivity, the matrix effect in the accurate mass measurement, use of isotopic patterns in the compound identification, efficiency of the algorithm search, confirmation criteria, etc., must be evaluated to determine to what extent they affect automatic identification [15].

The complexity of certain matrices can cause problems with ionization efficiency and the detection systems of the analytical instruments and the number and distribution of interfering matrix components varies greatly depending on the particular food or environmental matrix [13,16]. The presence of matrix compounds with similar masses to target analytes could be a major hindrance for unequivocal identification and could thus lead to false positives; isobaric compounds can also cause false negatives. Often matrix components, with similar masses, can co-eluate with analytes, and the instrument might not be capable of fully resolving these two slightly different masses [13]. Even when using the highest resolving power, isobaric interferences can occur, so it is important to process the acquired data to minimize the effect of possible interferences [17].

Considering all the concerns mentioned previously, sample preparation is also a critical step for screening methods because procedures must be as generic as possible in order to widen the scope of the method and to include as many analytes as possible [13,18]. Nácher-Mestre et al. [14] described the development, validation and application of a screening method for the detection and identification of undesirable organic compounds (70 representative compounds, including antibiotics, pesticides, and mycotoxins) in aquaculture products using a generic sample treatment without any purification or pre-concentration step using ultra-high performance liquid chromatography (UHPLC) with QToF/MS. A more elaborate procedure was performed by Peters et al. [19] for the extraction of 100 veterinary drugs for screening identification. Two grams of fish sample were extracted with an acetonitrile/ultrapure water mixture and, after an intensive shaking period and centrifugation the supernatant was collected and diluted. The diluted sample extract was cleaned-up using a solid phase extraction (SPE) column, and elution was achieved with a methanol/acetonitrile mixture. The eluate was evaporated under a stream of nitrogen and re-dissolved in 25 µL of acetonitrile. Formic acid (0.1%) in ultrapure water was added and the extract was analyzed by LC-ToF/MS in full scan mode. Concerning it, the QuEChERS (acronym of quick, easy, cheap, effective, rugged and safe) procedure is the most used methodology for the analysis of a broad group of analytes with a wide spectrum of physicochemical properties using an additional clean-up step based on dispersive solid phase extraction (d-SPE) [20,21].

The lack of information regarding the evaluation of contaminants in fish fillet is a concern nowadays, since most of the literature found focuses on the presence of

persistent compounds in aquatic life. For this reason the main propose of this study is to assess an analytical strategy for target screening determination of organic contaminants in fish fillet, using QuEChERS method for sample preparation and LC-QToF/MS using full scan and all ions MS/MS mode. A custom-made library was optimized for automatic qualitative search comparing the two acquisition modes. Quantification of organic contaminants in fish fillet was also carried out.

2. Experimental

2.1. Chemicals

The analytical standards, of high purity listed in Table 1 were purchased from Dr. Ehrenstorfer (Augsburg, Germany) and Sigma Aldrich (St. Louis, MO, USA). Ultra-pure water 18.2 MΩ was obtained using a Milli-Q Direct UV3® system EMD Millipore (Bedford, MA, USA), acetonitrile HPLC-grade and methanol LC-MS grade were purchased from Mallinckrodt (New Jersey, NJ, USA), isopropanol HPLC-grade from Tedia (Fairfield, OH, USA), formic acid ≥98.0% and ammonium formate ≥99.0% were obtained from Sigma Aldrich. QuEChERS pre-weighed salt kit SampliQ® (veterinary drugs: 4 g of sodium sulfate, Na_2SO_4 + 1 g of sodium chloride, NaCl) was from Agilent Technologies (Santa Clara, CA, USA), and Na_2SO_4 was purchased from J.T. Baker (Center Valley, PA, USA). Nylon syringe filters of 13 mm and 0.2 µm of porosity were obtained from Vertical Chromatography (Bangkok, Thailand), and sorbents C_{18}, and primary secondary amine (PSA) with particle size of 40 µm were purchased from Agilent Technologies.

2.2. Sample preparation

Sample preparation was based on original QuEChERS method using the pre-weighed kit of salts SampliQ®, and capped polypropylene (PP) extraction tubes of 50 mL. Ten grams of sample were used for this procedure followed by addition of 10 mL of acetonitrile with 1 min of shaking. Sample partition was carried out by adding 4 g of Na_2SO_4 and 1 g of NaCl. Manual shaking and centrifugation were performed prior to sample clean-up using dispersive solid phase extraction (d-SPE), which was performed by adding 150 mg of Na_2SO_4 and the sorbents PSA (25 mg) and C_{18} (125 mg) to 1 mL of extract in a 15 mL tube. The final extract was filtered, 10 µL of the internal standard (IS), triphenyl phosphate (1.0 mg L^{-1}) were added and diluted 5

times with ultrapure water. All samples were spiked with 50 µL of the surrogate standard atrazine-d5 (ethyl-d5) to obtain a final concentration in the instrument of 10 µg L^{-1}. In addition, the sample preparation procedure described by Munaretto et al. [22], based on acetate QuEChERS method, for EDCs extraction in fish fillet was also tested in this study to evaluate the accuracy of the studied compounds through recovery tests.

2.3. Instrument conditions

This study was performed using an Agilent (Santa Clara, CA, USA) 1260 LC system coupled to an Agilent 6530 Accurate Mass QToF/MS with jet stream electrospray ionization source operated in positive mode (ESI+). The MS conditions were as follow: capillary voltage 3500 V, drying gas (nitrogen) 10 L min^{-1} (300 ºC), sheath gas flow 10 L min^{-1} (350 ºC), nozzle voltage 1000 V, fragmentor 175 V and skimmer 65 V. Reference masses were *m/z* 121.0509 (purine, $C_5H_5N_4$) and 922.0098 (hexakis (1H, 1H, 3H-tetrafluoropropoxy) phosphazine, $C_{18}H_{19}O_6N_3P_3F_{24}$), and this solution was used in constant flow rate during the run. QToF/MS was operated in 2 GHz mode, extended dynamic range, low mass range over *m/z* 100-1000 and acquisition rate of 1 (full scan) and 3 (all ions MS/MS) spectra/s. Collision energies (CEs) of 0, 20 and 40 eV were used for all ions MS/MS acquisition.

Chromatographic conditions used were: column Zorbax Eclipse Plus C_{18} (50 x 2.1 mm; 1.8 µm particle size) maintained at 35 ºC, with injection volume of 5 µL. Mobile phase used was (A) water:methanol 98:2 (v/v) and (B) methanol, both containing 0.1% (v/v) of formic acid and 5 mmoL L^{-1} of ammonium formate. The gradient program started at 20% B followed by increasing B to 50% in 1 min, and to 98% in 3 min (held for 5.8 min) decreasing to initial condition in 9 min (holding 6 min). The flow rate was 300 µL min^{-1} and the total run time 15 min.

2.4. Automatic search

The target-screening method proposed in this study was started by creating a csv (comma separated values) file with the theoretical monoisotopic exact mass of all 182 compounds to be evaluated in this work, and the retention time (t_R) information to improve automatic search. Retention time information was obtained by injecting a mixer with all the compounds in acetonitrile and solutions with individual compounds were used for those whose identification was not reliable. This database was used

for searching and identifying the target compounds in fish fillet. Automatic detection was performed based on Malato et al. [15] study, retention time window of ±0.4 min, and mass tolerance of 20 ppm were chosen in this study. The Qualitative MassHunter B.06.00 software also includes score values in the search, which are calculated taking into account the accurate masses and the isotopic distribution, where higher values mean a more plausible elemental composition. Score values lower than 70% were not considered as positive findings. Searching of the contaminants was carried out through the formation of protonated ions $[M+H]^+$, adducts of ammonium $[M+NH_4]^+$ and/or sodium $[M+Na]^+$. Confirmation of the compounds was performed with fragments formed for each compound using all ions MS/MS mode.

2.5. Method validation and identification parameters

Quantitative and qualitative validation was performed based on SANCO [16]. Blank samples of catfish (*Rhamdia quelen*) were spiked in four different concentration levels (5, 10, 25 and 50 µg kg^{-1}) with 6 replicates of each level, to evaluate accuracy (recovery tests) and precision (repeatability and intermediate precision (IP)) through relative standard deviation, RSD. Linearity (through the coefficient of determination, r^2) was evaluated by preparing matrix matched analytical curves at 0.5, 1.0, 2.0, 5.0, 10.0, and 20.0 µg L^{-1}. Limit of quantification (LOQ) of each compound was determined as the lowest spiked level that presented adequate recovery (70-120%) and RSD (≤20%) results; even for those compounds with recovery below 70% or above 120% this criteria was applied, since the molecular ion can be identified unequivocally. Limit of detection (LOD) was calculated as being the LOQ divided by 3.33. Due to the complexity of the matrix in relation to the solvent signal of each compound, the matrix effect (ME) was evaluated for all compounds. ME was obtained by comparing the slope of the curves prepared in organic solvent and in matrix, and calculated as follows: ME%=[(slope of matrix-matched curve/slope of analyte in solvent curve)-1]x100. When the change in the chromatographic response is above 20%, the matrix effect is considered to have influence on the analysis [23,24]. Quantification of the compounds was performed using the $[M+H]^+$, $[M+NH_4]^+$ or $[M+Na]^+$ of each compound, using the Quantitative MassHunter B.06.00 software. The mass accuracy was calculated by the software comparing the

theoretical mass and the experimental mass obtained, and the result is given in parts per million (ppm).

For screening methods the confidence of detection of each analyte at a certain concentration level should be established, and this can be achieved using screening methods based on the screening detection limit (SDL) from the validation of a qualitative method. In addition, there are no requirements with regard to recovery of the analytes in the screening method, if it is only intended to be used as a qualitative method. The validation of the screening method in fish fillet was based on analysis of 20 samples spiked at three different concentration levels (10, 25 and 50 μg kg^{-1}) to estimate the SDL of each compound. The SDL of the qualitative screening method is the lowest level at which an analyte has been detected (not necessarily meeting the MS-identification criteria) in at least 95% of the samples (i.e. an acceptable false-negative rate of 5%). The MS-identification criteria established for HRMS operating in the full scan mode is: ≥2 diagnostic ions, preferably including the molecular ion; mass accuracy <5 ppm, and at least one fragment ion [16]. Qualitative MassHunter B.06.00 software was used to process the qualitative data using a custom-made database with all the compounds in study.

Table 1. General information about the compounds studied.

Compounds	CAS-number	Molecular formula	Monoiso-topic mass	$[M+H]^+$	$[M+NH_4]^+$	$[M+Na]^+$	Classifi-cation
Abamectin	71751-41-2	$C_{48}H_{72}O_{14}$	872.4922			895.4814	In/An
Acetamiprid	135410-20-7	$C_{10}H_{11}N_4Cl$	222.0672	223.0745			In
Acrinathrin	103833-18-7	$C_{26}H_{21}NO_5F_6$	541.1324		559.1662		Ac/In
Albendazole	54965-21-8	$C_{12}H_{15}N_3O_2S$	265.0885	266.0958			Fn
Ametryn	834-12-8	$C_9H_{17}N_5S$	227.1205	228.1278			Hb
Aramite	140-57-8	$C_{15}H_{23}O_4SCl$	334.1006		352.1344		Ac
Atenolol	29122-68-7	$C_{14}H_{22}N_2O_3$	266.1630	267.1703			β-blocker
Atrazine	1912-24-9	$C_8H_{14}N_5Cl$	215.0938	216.1011			Hb
Atrazine-d5	163165-75-1	$C_8H_9D_5N_5Cl$	220.1252	221.1324			SS
Avobenzone	70356-09-1	$C_{20}H_{22}O_3$	310.1569	311.1642			SF UVA
Azaconazole	60207-31-0	$C_{12}H_{11}N_3O_2Cl_2$	299.0228	300.0301			Fn
Azamethiphos	35575-96-3	$C_9H_{10}N_2O_5PSCl$	323.9737	324.9809			In
Azinphos-methyl	86-50-0	$C_{10}H_{12}N_3O_3PS_2$	317.0058			339.9950	Ac/In
Azoxystrobin	131860-33-8	$C_{22}H_{17}N_3O_5$	403.1168	404.1250			Fn
Benzophenone 3	131-57-7	$C_{14}H_{12}O_3$	228.0786	229.0859			SF UV
Bitertanol	55179-31-2	$C_{20}H_{23}N_3O_2$	337.1790	338.1863			Fn
Boscalid	188425-85-6	$C_{18}H_{12}N_2OCl_2$	342.0327	343.0399			Fn
Bupirimate	41483-43-6	$C_{13}H_{24}N_4O_3S$	316.1569	317.1647			Fn
Buprofezin	69327-76-0	$C_{16}H_{23}N_3OS$	305.1562	306.1638			In
Caffeine	58-08-2	$C_8H_{10}N_4O_2$	194.0804	195.0877			SCNS
Carbaryl	63-25-2	$C_{12}H_{11}NO_2$	201.0790			224.0682	Ac/In
Carbendazim	10605-21-7	$C_9H_9N_3O_2$	191.0695	192.0766			Fn
Carbofuran	1563-66-2	$C_{12}H_{15}NO_3$	221.1052	222.1127			Ac/In/Ne
Carbofuran-3-hydroxy	16655-82-6	$C_{12}H_{15}NO_4$	237.1001	238.1073			Mt
Carboxin	5234-68-4	$C_{12}H_{13}NO_2S$	235.0667	236.0742			Fn
Carpropamid	104030-54-8	$C_{15}H_{18}NOCl_3$	333.0454	334.0526			Fn

Compounds	CAS-number	Molecular formula	Monoiso-topic mass	$[M+H]^+$	$[M+NH_4]^+$	$[M+Na]^+$	Classifi-cation
Chlorantraniliprole	500008-45-7	$C_{18}H_{14}N_5O_2Cl_2Br$	480.9708	481.9781			In
Chlorbromuron	13360-45-7	$C_9H_{10}N_2O_2ClBr$	291.9614	292.9687			Hb
Chlorfenvinphos	470-90-6	$C_{12}H_{14}O_4PCl_3$	357.9695	358.9768			Ac/In
Chlorpyrifos-oxon	2921-88-2	$C_9H_{11}NO_4PCl_3$	332.9491	333.9564			In
Clarithromycin	81103-11-9	$C_{38}H_{69}NO_{13}$	747.4769	748.4842			Ab
Clenbuterol	37148-27-9	$C_{12}H_{18}N_2OCl_2$	276.0796	277.0869			β2-AA
Clofentezine	74115-24-5	$C_{14}H_8N_4Cl_2$	302.0126	303.0199			Ac
Clomazone	81777-89-1	$C_{12}H_{14}NO_2Cl$	239.0713	240.0785			Hb
Clothianidin	210880-92-5	$C_6H_8N_5O_2SCl$	249.0087	250.0159			In
Cyanazine	21725-46-2	$C_9H_{13}N_6Cl$	240.0890	241.0962			Hb
Cyazofamid	120116-88-3	$C_{13}H_{13}N_4O_2SCl$	324.0448	325.0521			Fn
Cyproconazole	94361-06-5	$C_{15}H_{18}N_3OCl$	291.1138	292.1211			Fn
Cyprodinil	121552-61-2	$C_{14}H_{15}N_3$	225.1266	226.1339			Fn
Danofloxacin	112398-08-0	$C_{19}H_{20}N_3O_3F$	357.1489	358.1561			Ab
Desmedipham	13684-56-5	$C_{16}H_{16}N_2O_4$	300.1110		318.1448		Hb
Diazinon	333-41-5	$C_{12}H_{21}N_2O_3PS$	304.1010	305.1105			Ac/In
Dichlorvos	62-73-7	$C_4H_7O_4PCl_2$	219.9459	220.9532			Ac/In
Dicrotophos	141-66-2	$C_8H_{16}NO_5P$	237.0766	238.0840			In
Dienestrol (diacetate)	84-17-3	$C_{22}H_{22}O_4$	350.1518		368.1856		Hor
Difenoconazole	119446-68-3	$C_{19}H_{17}N_3O_3Cl_2$	405.0647	406.0719			Fn
Difloxacin	91296-86-5	$C_{21}H_{19}N_3O_3F_2$	399.1394	400.1467			Ab
Dimethomorph	110488-70-5	$C_{21}H_{22}NO_4Cl$	387.1237	388.1310			Fn
Dimoxystrobin	149961-52-4	$C_{19}H_{22}N_2O_3$	326.1630	327.1703			Fn
Diniconazole	83657-24-3	$C_{15}H_{17}N_3OCl_2$	325.0749	326.0822			Fn
Diuron	330-54-1	$C_9H_{10}N_2OCl_2$	232.0170	233.0247			Hb/Al
Dodemorph	1593-77-7	$C_{18}H_{35}NO$	281.2719	282.2794			Fn
Emamectin	155569-91-8	$C_{49}H_{75}NO_{13}$	885.5238	886.5311			In

Compounds	CAS-number	Molecular formula	Monoiso-topic mass	$[M+H]^+$	$[M+NH_4]^+$	$[M+Na]^+$	Classifi-cation
Enrofloxacin	93106-60-6	$C_{19}H_{22}N_3O_3F$	359.1645	360.1718			Ab
Epoxiconazole	106325-08-0	$C_{17}H_{13}N_3OClF$	329.0731	330.0803			Fn
Eprinomectin	123997-26-2	$C_{50}H_{75}NO_{14}$	913.5188			936.5080	Ac/In
Erythromycin	114-07-8	$C_{37}H_{67}NO_{13}$	733.4612	734.4685			Ab
Ethion	563-12-2	$C_9H_{22}O_4P2S_4$	383.9876	384.9948			Ac/In
Ethirimol	23947-60-6	$C_{11}H_{19}N_3O$	209.1528	210.1601			Fn
Ethopabate	59-06-3	$C_{12}H_{15}NO_4$	237.1001	238.1074			AnC
Ethoprophos	13194-48-4	$C_8H_{19}O_2PS_2$	242.0564	243.0643			In/Ne
Ethoxysulfuron	126801-58-9	$C_{15}H_{18}N_4O_7S$	398.0896	399.0968			Hb
Etiofencarb sulfoxide	29973-13-5	$C_{11}H_{15}NO_3S$	241.0773	242.0845			In
Etofenprox	80844-07-1	$C_{25}H_{28}O_3$	376.2038		394.2376		In
Etrimphos	38260-54-7	$C_{10}H_{17}N_2O_4PS$	292.0647	293.0727			In
Fenbuconazole	114369-43-6	$C_{19}H_{17}N_4Cl$	336.1142	337.1214			Fn
Fenpropimorph	67564-91-4	$C_{20}H_{33}NO$	303.2562	304.2637			Fn
Fenpyroximate	134098-61-6	$C_{24}H_{27}N_3O_4$	421.2002	422.2074			Ac
Fenthion	55-38-9	$C_{10}H_{15}O_3PS_2$	278.0200	279.0272			In
Fenthion sulfoxide	3761-42-0	$C_{10}H_{15}O_4PS_2$	294.0149	295.0228			In
Flufenacet	142459-58-3	$C_{14}H_{13}N_3O_2SF_4$	363.0665	364.0737			Hb
Fluroxypyr	69377-81-7	$C_7H_5N_2O_3Cl_2F$	253.9661	254.9734			Hb
Flusilazole	85509-19-9	$C_{16}H_{15}N_3F_2Si$	315.1003	316.1076			Fn
Flutolanil	66332-96-5	$C_{17}H_{16}NO_2F_3$	323.1133	324.1214			Fn
Fosthiazate	98886-44-3	$C_9H_{18}NO_3PS_2$	283.0466	284.0538			Ne
Furaltadone	139-91-3	$C_{13}H_{16}N_4O_6$	324.1070	325.1143			Ab
Furathiocarb	65907-30-4	$C_{18}H_{26}N_2O_5S$	382.1562	383.1641			In
Furazolidone	67-45-8	$C_8H_7N_3O_5$	225.0386	226.0458			AnP
Hexythiazox	78587-05-0	$C_{17}H_{21}N_2O_2SCl$	352.1012	353.1085			Ac
Imazalil	35554-44-0	$C_{14}H_{14}N_2OCl_2$	296.0483	297.0562			Fn

Compounds	CAS-number	Molecular formula	Monoiso-topic mass	$[M+H]^+$	$[M+NH_4]^+$	$[M+Na]^+$	Classifi-cation
Imazaquin	81335-37-7	$C_{17}H_{17}N_3O_3$	311.1270	312.1351			Hb
Imazethapyr	81335-77-5	$C_{15}H_{19}N_3O_3$	289.1426	290.1507			Hb
Imidacloprid	138261-41-3	$C_9H_{10}N_5O_2Cl$	255.0523	256.0595			In
Indoxacarb	173584-44-6	$C_{22}H_{17}N_3O_7ClF_3$	527.0707	528.0779			In
Iprovalicarb	140923-17-7	$C_{18}H_{28}N_2O_3$	320.2100	321.2180			Fn
Isoprothiolane	50512-35-1	$C_{12}H_{18}O_4S_2$	290.0647	291.0719			Fn/In
Isoxaflutole	141112-29-0	$C_{15}H_{12}NO_4SF_3$	359.0439	360.0511			Hb
Isoxathion	18854-01-8	$C_{13}H_{16}NO_4PS$	313.0538	314.0610			In
Ivermectin	70288-86-7	$C_{48}H_{74}O_{14}$	874.5079			897.4971	Ac/In
Ketoconazole	65277-42-1	$C_{26}H_{28}N_4O_4Cl_2$	530.1488	531.1560			Af
Lidocaine	137-58-6	$C_{14}H_{22}N_2O$	234.1732	235.1810			LA
Lincomycin	859-18-7	$C_{18}H_{34}N_2O_6S$	406.2138	407.2210			Ab
Mecarbam	2595-54-2	$C_{10}H_{20}NO_5PS_2$	329.0521			352.0413	Ac/In
Mepanipyrim	110235-47-7	$C_{14}H_{13}N_3$	223.1109	224.1182			Fn
Mephospholan	950-10-7	$C_8H_{16}NO_3PS_2$	269.0309	270.0386			In
Mepronil	55814-41-0	$C_{17}H_{19}NO_2$	269.1416	270.1488			Fn
Metacriphos	62610-77-9	$C_7H_{13}O_5PS$	240.0221		258.056		Ac/In
Metalaxyl	57837-19-1	$C_{15}H_{21}NO_4$	279.1471	280.1548			Fn
Metconazole	125116-23-6	$C_{17}H_{22}N_3OCl$	319.1451	320.1536			Fn
Methidathion	950-37-8	$C_6H_{11}N_2O_4PS_3$	301.9619	302.9691			In
Methiocarb sulfoxide	2635-10-1	$C_{11}H_{15}NO_3S$	241.0773	242.0846			In
Methomyl	16752-77-5	$C_5H_{10}N_2O_2S$	162.0463			185.0355	In
Metobromuron	3060-89-7	$C_9H_{11}N_2O_2Br$	258.0004	259.0076			Hb
Metolachlor	51218-45-2	$C_{15}H_{22}NO_2Cl$	283.1339	284.1412			Hb
Metoxuron	19937-59-8	$C_{10}H_{13}N_2O_2Cl$	228.0666	229.0740			Hb
Metsulfuron-methyl	74223-64-6	$C_{14}H_{15}N_5O_6S$	381.0743	382.0816			Hb
Mevinphos	7786-34-7	$C_7H_{13}O_6P$	224.0450	225.0522			Ac/In

Compounds	CAS-number	Molecular formula	Monoiso-topic mass	$[M+H]^+$	$[M+NH_4]^+$	$[M+Na]^+$	Classifi-cation
Miconazole	22916-47-8	$C_{18}H_{14}N_2OCl_4$	413.9860	414.9933			Af
Monensin	22373-78-0	$C_{36}H_{62}O_{11}$	670.4292			693.4184	IPA
Monocrotophos	6923-22-4	$C_7H_{14}NO_5P$	223.0610	224.0679			In/Ac
Monolinuron	1746-81-2	$C_9H_{11}N_2O_2Cl$	214.0509	215.0581			Hb
Myclobutanil	88671-89-0	$C_{15}H_{17}N_4Cl$	288.1142	289.1215			Fn
Nicotine	54-11-5	$C_{10}H_{14}N_2$	162.1157	163.1230			In
Nitenpyram	150824-47-8	$C_{11}H_{15}N_4O_2Cl$	270.0884	271.0956			In
Nuarimol	63284-71-9	$C_{17}H_{12}N_2OClF$	314.0622	315.0695			Fn
Omethoate	1113-02-6	$C_5H_{12}NO_4PS$	213.0225	214.0297			Ac/In
Oxadiazon	19666-30-9	$C_{15}H_{18}N_2O_3Cl_2$	344.0694	345.0767			Hb
Oxadixyl	77732-09-3	$C_{14}H_{18}N_2O_4$	278.1267	279.1339			Fn
Oxamyl	23135-22-0	$C_7H_{13}N_3O_3S$	219.0678			242.0569	Ac/In/Ne
Oxycarboxin	5259-88-1	$C_{12}H_{13}NO_4S$	267.0565	268.0638			Fn
Paraoxon-ethyl	56-38-2	$C_{10}H_{14}NO_6P$	275.0559	276.0631			In/Ac
Penconazole	66246-88-6	$C_{13}H_{15}N_3Cl_2$	283.0643	284.0716			Fn
Pencycuron	66063-05-6	$C_{19}H_{21}N_2OCl$	328.1342	329.1415			Fn
Phenmedipham	13684-63-4	$C_{16}H_{16}N_2O_4$	300.1110	301.1183			Hb
Phenthoate	2597-03-7	$C_{12}H_{17}O_4PS_2$	320.0306	321.0379			In
Phosalone	2310-17-0	$C_{12}H_{15}NO_4PS_2Cl$	366.9869	367.9941			Ac/In
Phosmet	732-11-6	$C_{11}H_{12}NO_4PS_2$	316.9945	318.0018			Ac/In
Picoxystrobin	117428-22-5	$C_{18}H_{16}NO_4F_3$	367.1031	368.1104			Fn
Pirimicarb	23103-98-2	$C_{11}H_{18}N_4O_2$	238.1430	239.1502			In
Pirimiphos-methyl	29232-93-7	$C_{11}H_{20}N_3O_3PS$	305.0963	306.1035			Ac/In
Prochloraz	67747-09-5	$C_{15}H_{16}N_3O_2Cl_3$	375.0308	376.0380			Fn
Procymidone	32809-16-8	$C_{13}H_{11}NO_2Cl_2$	283.0167	284.0239			Fn
Profenophos	41198-08-7	$C_{11}H_{15}O_3PSClBr$	371.9351	372.9424			In
Profoxydim	139001-49-3	$C_{24}H_{32}NO_4SCl$	465.1741	466.1824			Hb

Compounds	CAS-number	Molecular formula	Monoiso-topic mass	$[M+H]^+$	$[M+NH_4]^+$	$[M+Na]^+$	Classifi-cation
Propargite	2312-35-8	$C_{19}H_{26}O_4S$	350.1552		368.1890		Ac
Propiconazole	60207-90-1	$C_{15}H_{17}N_3O_2Cl_2$	341.0698	342.0770			Fn
Propoxur	114-26-1	$C_{11}H_{15}NO_3$	209.1052			232.0944	Ac/In
Propranolol	525-66-6	$C_{16}H_{21}NO_2$	259.1572	260.1645			β-AB
Propyzamide	23950-58-5	$C_{12}H_{11}NOCl_2$	255.0218	256.0290			Hb
Prothiofos	34643-46-4	$C_{11}H_{15}O_2PS_2Cl_2$	343.9628	344.9700			In
Pymetrozine	123312-89-0	$C_{10}H_{11}N_5O$	217.0964	218.1007			AnF
Pyraclostrobin	175013-18-0	$C_{19}H_{18}N_3O_4Cl$	387.0986	388.1067			Fn
Pyrazophos	13457-18-6	$C_{14}H_{20}N_3O_5PS$	373.0861	374.0949			Fn/In
Pyrazosulfuron	98389-04-9	$C_{14}H_{18}N_6O_7S$	414.0958	415.1041			Hb
Pyridaben	96489-71-3	$C_{19}H_{25}N_2OSCl$	364.1376	365.1451			Ac/In
Pyridafenthion	119-12-0	$C_{14}H_{17}N_2O_4PS$	340.0647	341.0729			In
Pyridate	55512-33-9	$C_{19}H_{23}N_2O_2SCl$	378.1169	379.1253			Hb
Pyrifenox	88283-41-4	$C_{14}H_{12}N_2OCl_2$	294.0327	295.0399			Fn
Pyrimethanil	53112-28-0	$C_{12}H_{13}N_3$	199.1109	200.1188			Fn
Quinalphos	13593-03-8	$C_{12}H_{15}N_2O_3PS$	298.0541	299.0613			In/Ac
Quinoxyfen	124495-18-7	$C_{15}H_8NOCl_2F$	306.9967	308.0036			Fn
Rotenone	83-79-4	$C_{23}H_{22}O_6$	394.1416	395.1489			In
Sarafloxacin	91296-87-6	$C_{20}H_{17}N_3O_3F$	385.1238	386.1238			Ab
Simazine	122-34-9	$C_7H_{12}N_5Cl$	201.0781	202.0853			Hb
Spinosad	168316-95-8	$C_{41}H_{65}NO_{10}$	731.4608	732.4702			In
Sulfachloropyridazine	80-32-0	$C_{10}H_9N_4O_2SCl$	284.0135	285.0208			Ab
Sulfadimethoxine	122-11-2	$C_{12}H_{14}N_4O_4S$	310.0736	311.0809			Ab
Sulfamethanize	57-68-1	$C_{12}H_{14}N_4O_2S$	278.0837	279.0910			Ab
Sulfamethoxazole	723-46-6	$C_{10}H_{11}N_3O_3S$	253.0521	254.0594			Ab
Sulfathiazole	72-14-0	$C_9H_9N_3O_2S_2$	255.0136	256.0209			Ab
Tebuconazole	107534-96-3	$C_{16}H_{22}N_3OCl$	307.1451	308.1524			Fn

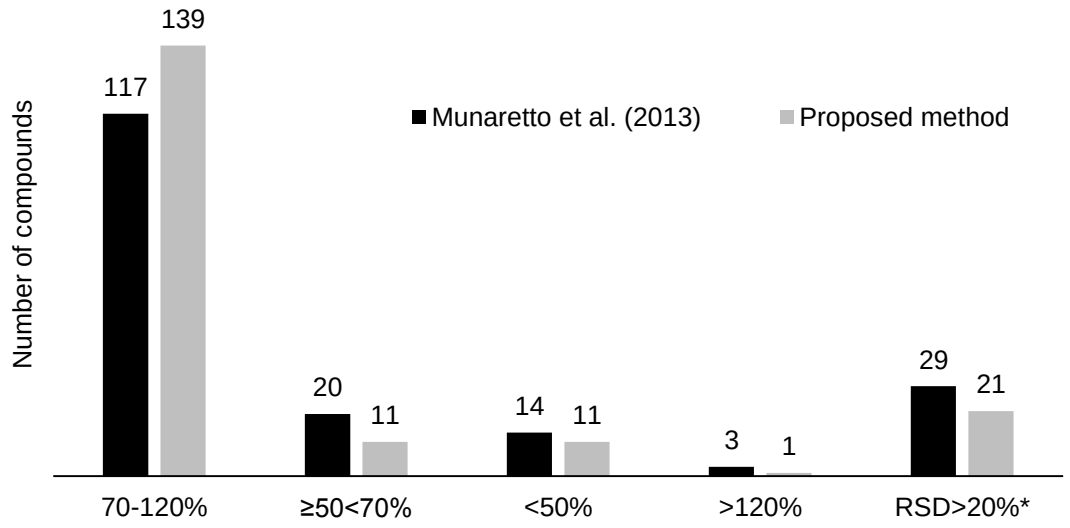
Compounds	CAS-number	Molecular formula	Monoiso-topic mass	$[M+H]^+$	$[M+NH_4]^+$	$[M+Na]^+$	Classifi-cation
Tebufenpyrad	119168-77-3	$C_{18}H_{24}N_3OCl$	333.1608	334.1680			Ac/In
Tetraconazole	112281-77-3	$C_{13}H_{11}N_3OCl_2F_4$	371.0215	372.0288			Fn
Thiabendazole	148-79-8	$C_{10}H_7N_3S$	201.0361	202.0435			Fn
Thiacloprid	111988-49-9	$C_{10}H_9N_4SCl$	252.0236	253.0312			In
Thiamethoxam	153719-23-4	$C_8H_{10}N_5O_3SCl$	291.0193	292.0265			In
Thiobencarb	28249-77-6	$C_{12}H_{16}NOSCl$	257.0641	258.0714			Hb
Thiram	137-26-8	$C_6H_{12}N_2S_4$	239.9883		258.0222		Fn
Tilmicosin	108050-54-0	$C_{46}H_{80}N_2O_{13}$	868.5660	869.5733			Ab
Tolclofos-methyl	57018-04-9	$C_9H_{11}O_3PSCl_2$	299.9544	300.9616			Fn
Trenbolone (acetate)	10161-33-8	$C_{20}H_{24}O_3$	312.1725	313.1798			AS
Triadimefon	43121-43-3	$C_{14}H_{16}N_3O_2Cl$	293.0931	294.1003			Fn
Triasulfuron	82097-50-5	$C_{14}H_{16}N_5O_5SCl$	401.0561	402.0633			Hb
Triazophos	24017-47-8	$C_{12}H_{16}N_3O_3PS$	313.0650	314.0722			Ac/In/Ne
Trichlorfon	52-68-6	$C_4H_8O_4PCl_3$	255.9226	256.9298			In
Tricyclazole	41814-78-2	$C_9H_7N_3S$	189.0361	190.0433			Fn
Tridemorph	24602-86-6	$C_{19}H_{39}NO$	297.3032	298.3113			Fn
Trifloxystrobin	141517-21-7	$C_{20}H_{19}N_2O_4F_3$	408.1297	409.1377			Fn
Triforine	26644-46-2	$C_{10}H_{14}N_4O_2Cl_6$	431.9248			454.9140	Fn
Trimethoprim	738-70-5	$C_{14}H_{18}N_4O_3$	290.1379	291.1452			Ab
Triphenyl phosphate	115-86-6	$C_{18}H_{15}O_4P$	326.0708	327.0781			IS
Tylosin	1401-69-0	$C_{46}H_{77}NO_{17}$	915.5192	916.5264			Ab
Vamidothion	2275-23-2	$C_8H_{18}NO_4PS_2$	287.0415	288.0490			Ac/In
Vinclozolin	50471-44-8	$C_{12}H_9NO_3Cl_2$	284.9959	286.0032			Fn

β2-AA: β2-adrenergic blockers; Ab: Antibiotic; Ac: Acaricide; Af: Antifungal; An: Anthemintic; AnC: Anticoccidial; AnF: Antifeedant; AnP: Antiprotozoal; AS: Anabolic steroid; Fn: Fungicide; Hb: Herbicide; Hor: Hormone; In: Insecticide; IPA: Ionophore Antimicrobial; IS: Internal standard; LA: Local anesthetic; Mt: Metabolite; Ne: Nematicide; SCNS: Stimulant Central Nervous System; SF: Solar Filter; SS: Surrogate standard.

3. Results and discussion

3.1. Sample preparation optimization

Screening methods for qualitative purposes are not required to obtain proper recovery of the analytes. However, sample preparation was evaluated due to the possibility of matrix interferences influence on the identification of compounds, especially because of isobaric substances, which are the most challenging cases in high-throughput screening and also to meet validation parameters for quantification determination [13,16,18]. For this reason two different procedures based on QuEChERS method were evaluated, and the comparison of the obtained results is shown in Figure 1.



Note: atrazine-d5 (SS) results are included; *or not recovered

Figure 1. Comparison of sample preparation methods considering recovery and RSD results.

Initially, the method proposed by Munaretto et al. [22] was tested, in which 117 compounds presented recoveries between 70-120%, 34 compounds showed recovery values lower than 70%, 3 compounds were above 120%, and 29 compounds did not present acceptable values for precision or were not recovered using this extraction procedure. A second method was evaluated (described in Experimental section), and a greater number of compounds were recovered in the

range from 70 to 120%; thus, this sample preparation procedure was chosen for method validation. The main difference reported is the use of Na_2SO_4 instead of $MgSO_4$, which improves recovery percentage of polar compounds. This happens because Na_2SO_4 is a weak drying agent when compared to $MgSO_4$, and when added to the extraction organic solvent (in this case acetonitrile), the amount of water in acetonitrile is higher than when $MgSO_4$ is used, favoring polar compounds extraction [25,26]. The recovery improvement for the polar compounds studied, such as pharmaceuticals, was notable.

3.2. Qualitative validation

Regarding the validation of the qualitative screening method, automatic and manual searches were performed (Table 2) using a custom-made library. All the automatic results were confirmed manually to make sure the automatic detection screening was performed properly. Automatic search was able to detect 84 and 72% of the total number of compounds, while the remaining 16% and 28% were detected manually (including SS and IS) using full scan and all ions MS/MS, respectively. The lack of efficiency for 100% of the compounds in automatic search is most likely due to the complexity of the matrix, caused by the presence of co-extractives that affect proper search [15]. The difference between the automatic search when comparing the two scan modes is due to the scan point collected in each method; while full scan does not provide fragmentation data that is provided using all ions MS/MS mode, this scan mode collects 1 spectra/s, increasing the data points during peak elution. Obviously, the number of points per peak strongly depends on the number of co-eluting target analytes [20]. Therefore, co-eluting matrix interferences, especially those isobaric compounds can reduce the effectiveness of the screening method [15], Figure 2 shows the total ion chromatogram (TIC) of a 10 µg L^{-1} standard solution in acetonitrile, and another one prepared in the blank extract of fish fillet, in full scan and all ions MS/MS scan modes. The presence of several interferences in the obtained extract is notable; fish is known as a complex matrix that contain a large number of matrix components such as lipids and proteins, even after sample clean-up [14].

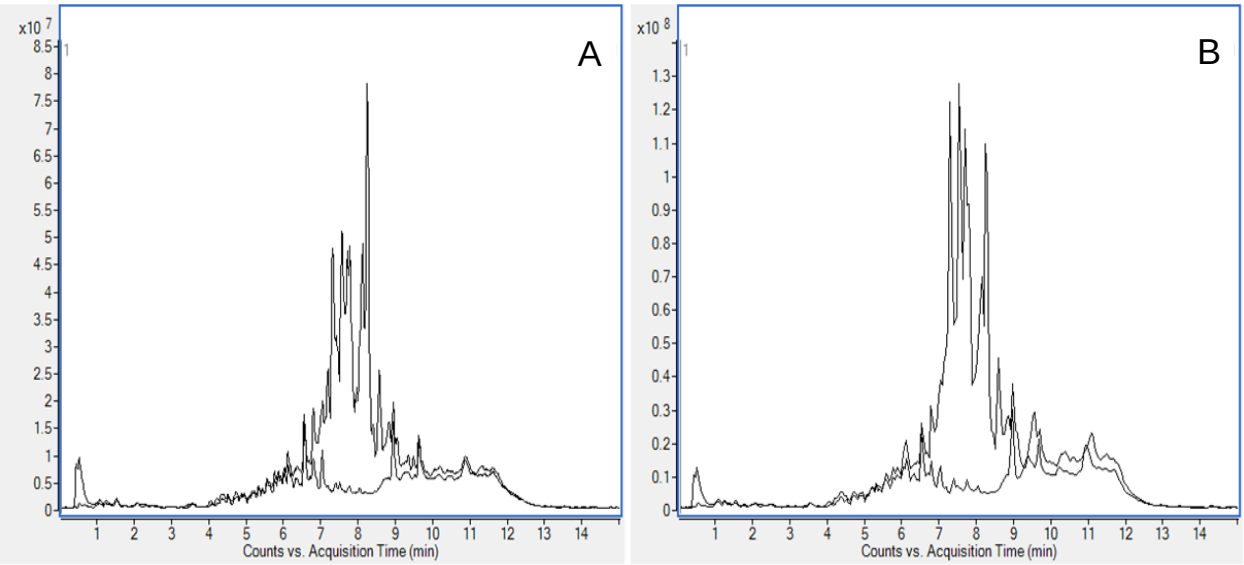


Figure 2. Total ion chromatogram of a 10 µg L^{-1} standard solution prepared in solvent (lower) and in the blank extract of fish fillet (higher) using full scan (A) and all ions MS/MS (B) scan modes.

Screening detection limit (SDL), evaluated for all the contaminants searched in the three spiked concentration levels, through automatic and manual detection, are shown in Table 2. Considering full scan and in all ions MS/MS, respectively, 63.2 and 54.4% of the compounds have a SDL value of 10 µg kg^{-1} in 100% of the spiked samples analyzed, and 4.4 and 6.7% of the compounds in this same SDL showed 5% of false negatives, which is acceptable according to SANCO [16]. SDL values for 17.0 and 15.4% of the compounds were 25 and 50 µg kg^{-1} in full scan mode, respectively. While all ions MS/MS mode showed 20.6 and 3.3% (with 5% of false negative) of compounds with SDL of 25 µg kg^{-1}, and SDL of 50 µg kg^{-1} for 15.0% of the compounds. Automatic search scores were higher than 90% for 76% (full scan) and 32% (all ions MS/MS) of the compounds, including SS and IS. Neither in full scan nor all ions MS/MS modes were score values lower than 70% accepted. The herbicide fluroxypyr and the insecticide prothiofos were not validated using this method because they were not recovered in any of the spiked levels (discussed in the Quantitative validation section).

Matrix interference components with similar masses to target analytes could be a major drawback for unequivocal identification, which could lead to detection of false positives. Most isobaric interferences can be resolved by identifying retention times, which is essential to minimize false positives. In addition, false negatives can occur

and, depending on the resolving power, the instrument might not be capable of fully resolving slightly different masses of co-eluted compounds [13]. These interferences lead to an ion suppression phenomenon of the analytes during ionization in mass spectrometry, reducing their response and thus their detectability [15,27]. For example, the compound pyridate was found manually in the matrix using full scan mode, however, matrix interferences in its retention time were responsible for ion suppression during all ions MS/MS acquisition. It is notable that the matrix plays an important role during screening methods, but in this case the reduced number of data points acquired was very critical because fewer spectra for each compound were obtained. Figure 3 shows the interferences when selected the *m/z* related to pyridate in the blank of the matrix analyzed in all ions MS/MS mode.

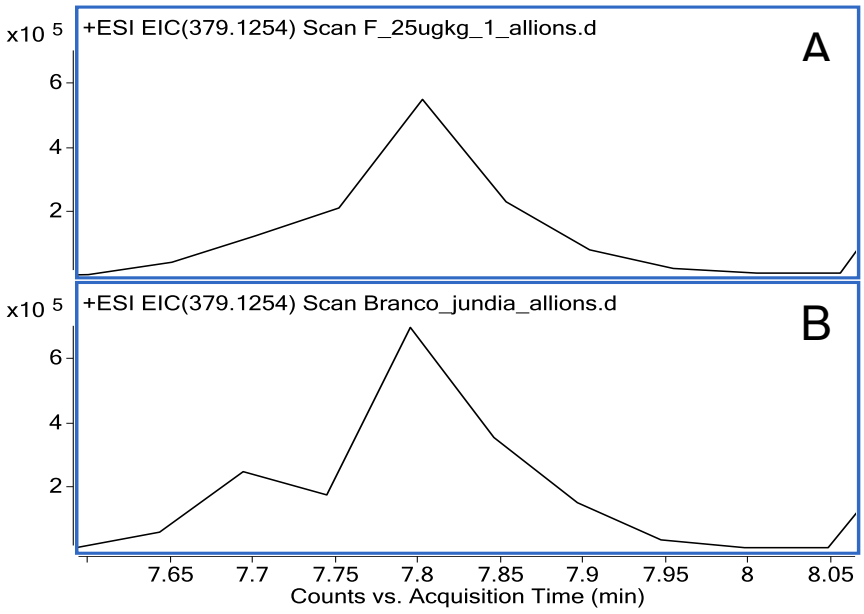


Figure 3. Extracted ion chromatogram (EIC) of the *m/z* 379.1254 (referred to the compound piridate) in the spiked (25 µg kg^{-1}) sample (A) and in the blank extract of fish fillet (B) analyzed by LC-QToF/MS in all ion MS/MS mode.

Table 2. Screening results in full scan and in all ions MS/MS scan modes.

Compounds	t_R (min)	Full Scan Results					All Ions MS/MS Results				
		SDL (µg kg^{-1})			Mass Error (ppm)	Score (%)	SDL (µg kg^{-1})			Mass Error (ppm)	Score (%)
		10	25	50			10	25	50		
Abamectin	7.6			A	-4.0	74.8		M		1.89	-
Acetamiprid	3.6	A			-0.9	92.8	A			-10.2	85.0
Acrinathrin	7.4		A		-2.9	86.4		A		-3.3	81.6
Albendazole	5.6	A			-0.3	91.7		A		12.5	78.6
Ametryn	5.5	A			1.7	95.8	A			7.3	87.5
Aramite	6.8	A*			-6.4	82.9			A	-0.9	91.0
Atenolol	0.6			A	5.0	86.8			M	-10.2	-
Atrazine	5.4	A			0.7	87.2	A			11.7	76.7
Atrazine-d5	5.4	A			2.6	92.4	A			13.3	77.6
Avobenzone	7.8	A			-2.1	90.7		M		-1.3	-
Azaconazole	5.5	A			0.4	95.8	A			5.2	89.9
Azamethiphos	4.9	A			1.0	96.7	A			-5.6	91.4
Azinphos-methyl	5.6	A			11.9	81.2	M			-16.6	-
Azoxystrobin	5.7	A			0.8	97.4	A			6.2	90.1
Benzophenone 3	6.2	A			-0.4	94.0		M		-14.2	-
Bitertanol	6.4			A	-3.5	80.8			M	4.1	-
Boscalid	5.8	A			1.5	95.5	A			4.5	90.2
Bupirimate	6.0			A	-0.1	92.3	M			11.3	-
Buprofezin	6.8			A	-3.9	87.8			A	-3.2	88.1
Caffeine	1.4		A		-3.1	96.7		A		-4.3	85.6
Carbaryl	5.1			M	0.3	-			M	-12.9	-
Carbendazim	1.3	A			1.6	95.0	A			-6.4	90.7
Carbofuran	5.0	M			12.4	-	M			2.6	-
Carbofuran-3-hydroxy	3.6		A		0.4	98.3		A		-3.3	88.3
Carboxin	5.1	A			0.5	96.3	A			-0.5	98.2
Carpropamid	6.3			A	-2.4	86.5		A*		0.6	84.5
Chlorantraniliprole	5.6	A			1.1	97.9	A			6.6	87.4

Compounds	t_R (min)	Full Scan Results					All Ions MS/MS Results				
		SDL (µg kg^{-1})			Mass Error (ppm)	Score (%)	SDL (µg kg^{-1})			Mass Error (ppm)	Score (%)
		10	25	50			10	25	50		
Chlorbromuron	5.8	A			1.9	94.7	A			3.8	90.0
Chlorfenvinphos	6.3	A			1.7	95.3	A			-2.4	93.0
Chlorpyrifos-oxon	6.1	A			1.8	95.4		A*		10.0	82.3
Clarithromycin	5.6	A			0.2	97.8	A			3.1	93.8
Clenbuterol	2.5	A			0.0	93.7	A			-10.0	82.6
Clofentezine	6.5			A	-5.4	90.1	A*			-2.0	89.6
Clomazone	5.6	A			-0.5	95.9		A		12.3	82.0
Clothianidin	2.4	A			1.1	91.1	A*			-9.7	81.2
Cyanazine	4.8	A			-2.9	91.8	A			-8.6	83.4
Cyazofamid	6.1	A			-1.0	94.6	M			4.1	-
Cyproconazole	6.0	A*			5.5	75.6	M			3.2	-
Cyprodinil	6.2		A		1.9	94.3	> 5% false negative			-	-
Danofloxacin	2.0		M		9.2	-		M		6.3	-
Desmedipham	5.6	A			-6.2	90.7	A			2.2	87.4
Diazinon	6.3	M			4.0	-	M			-6.5	-
Dichlorvos	4.9			A	1.6	98.4			A	5.9	91.8
Dicrotophos	2.0	A			-0.4	91.2	A			-10.2	84.7
Dienestrol (diacetate)	6.6	A			-1.8	96.4	A			-2.8	93.5
Difenoconazole	6.5	A			-2.0	95.2	A			-0.7	93.0
Difloxacin	2.5	M			7.2	-			A	0.9	90.0
Dimethomorph	5.8	A			-0.2	94.9	A			1.6	93.8
Dimoxystrobin	6.2		M		12.3	-	M			-1.6	-
Diniconazole	6.5	M			9.7	-	M			0.7	-
Diuron	5.5	A			1.5	96.9	A			6.7	91.6
Dodemorph	5.4	A			3.2	93.9	M			-8.2	-
Emamectin	6.7	M			-0.7	-	M			-0.6	-
Enrofloxacin	2.0	A			1.3	88.9		A		-1.4	92.3
Epoxiconazole	6.1	A*			15.6	79.2		A		8.4	83.4

Compounds	t_R (min)	Full Scan Results					All Ions MS/MS Results				
		SDL (µg kg^{-1})			Mass Error (ppm)	Score (%)	SDL (µg kg^{-1})			Mass Error (ppm)	Score (%)
		10	25	50			10	25	50		
Eprinomectin	7.4			A	1.7	95.8		A		1.4	91.8
Erythromycin	5.2	A			0.8	99.4	A			1.0	98.5
Ethion	6.9			A	-10.3	78.9	M			-10.9	-
Ethirimol	4.4		A		3.6	99.5	A			-6.3	84.7
Ethopabate	4.8			A	0.9	73.2		M		19.2	-
Ethoprophos	6.1	A			-0.2	95.9		A		-1.2	89.2
Ethoxysulfuron	5.9	A			0.7	96.3		A*		2.1	95.6
Etiofencarb sulfoxide	2.3			A	-1.2	94.0		A		-5.5	81.9
Etofenprox	8.1		M		15.9	-		M		-11.9	-
Etrimphos	6.3	A			-0.3	96.0		A		-3.2	93.8
Fenbuconazole	6.1	A			2.4	86.3		A		2.0	87.6
Fenpropimorph	5.6			A	0.6	99.6	M			-12.8	-
Fenpyroximate	7.2		A		-8.7	81.0		M		-15.5	-
Fenthion	6.3		M		12.2	-			M	0.0	-
Fenthion sulfoxide	5.1	A			1.2	96.5	A			-2.3	96.9
Flufenacet	6.0	A			0.1	96.2	A			-7.6	87.1
Fluroxypyr	4.7	-	-	-	-	-	-	-	-	-	-
Flusilazole	6.1	A			-0.4	91.9	M			-11.2	-
Flutolanil	5.8	A			0.3	96.4	A			3.4	93.0
Fosthiazate	5.3	A			0.6	94.5	A			2.1	95.2
Furaltadone	0.7	A			0.9	92.8	A			-4.4	90.7
Furathiocarb	6.7	M			18.1	-			A	4.2	82.3
Furazolidone	1.2	A			-0.1	90.0	A			-9.5	84.5
Hexythiazox	7.0		A		-6.6	82.2			M	-0.6	-
Imazalil	5.2	A			1.7	91.1	A			0.1	98.2
Imazaquin	5.0			A	-0.8	94.7		A		-0.7	80.5
Imazethapyr	4.7		A		1.6	97.8	M			3.2	-
Imidacloprid	2.3		A		-0.9	96.9	A			-11.6	79.3

Compounds	t_R (min)	Full Scan Results					All Ions MS/MS Results				
		SDL (µg kg^{-1})			Mass Error (ppm)	Score (%)	SDL (µg kg^{-1})			Mass Error (ppm)	Score (%)
		10	25	50			10	25	50		
Indoxacarb	6.5	A			-1.1	98.4	A			0.1	97.7
Iprovalicarb	6.0		A		-2.8	92.4			A	-8.6	87.3
Isoprothiolane	5.9	A			-0.6	94.4	A			2.9	91.1
Isoxaflutole	5.4	A			-0.2	93.0	A			1.6	84.3
Isoxathion	6.4			A	-11.1	72.6	M			6.0	-
Ivermectin	8.4	M			2.0	-		M		1.7	-
Ketoconazole	5.3	A			0.7	97.8	A			2.2	95.8
Lidocaine	1.5	M			10.4	-	M			6.2	-
Lincomycin	1.1		A		2.5	95.5		A*		-0.1	89.2
Mecarbam	6.0	M			12.2	-			M	16.3	-
Mepanipyrim	6.0	A			-2.7	85.5	M			10.1	-
Mephospholan	4.9	A			0.8	96.6	A			-6.4	92.4
Mepronil	5.9	M			11.3	-		A*		3.7	93.1
Metacriphos	2.3	M			16.7	-	M			14.7	-
Metalaxyl	5.4	A			1.7	94.6	A			3.1	94.1
Metconazole	6.4		A		0.4	94.0	A			-0.4	87.0
Methidathion	5.6	A			-0.3	93.5		M		-9.4	-
Methiocarb sulfoxide	3.1		A		-0.4	94.1		A		-4.2	84.6
Methomyl	1.2			A	-0.2	95.1		M		8.2	-
Metobromuron	5.3	A			1.6	94.9	A*			7.8	88.3
Metolachlor	6.1	A			0.6	95.8			A	5.2	94.6
Metoxuron	4.5	A			0.8	96.7	A			-8.1	90.6
Metsulfuron-methyl	5.0	A			1.6	96.0	A			-3.0	90.7
Mevinphos	4.3			A	0.1	95.5			A	4.9	85.3
Miconazole	6.2	A			1.8	95.8	A*			7.5	82.8
Monensin	7.8	M			3.0	-	M			-3.2	-
Monocrotophos	1.6		A		1.6	90.6	A*			-9.9	83.3
Monolinuron	5.2	A			1.5	89.6	A			2.6	92.2

Compounds	t_R (min)	Full Scan Results					All Ions MS/MS Results				
		SDL (µg kg^{-1})			Mass Error (ppm)	Score (%)	SDL (µg kg^{-1})			Mass Error (ppm)	Score (%)
		10	25	50			10	25	50		
Myclobutanil	5.9	M			12.4	-		A*		1.5	87.6
Nicotine	1.4	A*			4.7	81.3	A			-0.2	91.9
Nitenpyram	1.0	A			-1.2	95.6	A			-9.9	83.9
Nuarimol	5.7	A			-6.3	94.6			A	0.5	91.9
Omethoate	0.7	A			2.2	89.4	A*			-2.2	87.4
Oxadiazon	6.8			A	-0.1	98.8	M			-16.4	-
Oxadixyl	4.7		A		-0.9	95.1		A		-1.7	90.1
Oxamyl	1.0		M		14.9	-		M		9.1	-
Oxycarboxin	4.1		A		0.4	99.2	A			-12.0	78.5
Paraoxon-ethyl	5.3			A	0.5	97.5		A		7.4	82.4
Penconazole	6.3	A*			1.6	94.9	A			3.3	87.7
Pencycuron	6.5			A	-4.3	89.8	A*			-2.3	90.7
Phenmedipham	4.7		M		3.5	-	M			6.4	-
Phenthoate	6.2	A			4.9	92.6		M		-12.5	-
Phosalone	6.4	A			-0.7	96.5	A			-0.9	96.9
Phosmet	5.6	M			12.0	-	A			-7.0	89.5
Picoxystrobin	6.2		A		-0.4	95.6			A	9.3	81.3
Pirimicarb	4.5	A			4.4	86.1	A			-6.4	86.1
Pirimiphos-methyl	6.4	A			0.0	94.1		A		2.4	87.7
Prochloraz	6.4	A			-0.1	95.1	A			-3.3	90.0
Procymidone	6.1	M			-7.9	-			M	-4.4	-
Profenophos	6.7	M			20.4	-	A			0.2	95.4
Profoxydim	6.6	A			-0.9	92.2	A*			-1.2	91.4
Propargite	6.6	A			7.2	79.1	A			6.1	79.0
Propiconazole	6.3	A			-6.8	94.1		A		-3.8	94.0
Propoxur	4.9	M			17.2	-			A	2.8	78.7
Propranolol	4.7	A			1.6	90.5	A			-5.2	91.1
Propyzamide	5.9	M			11.5	-			M	-0.8	-

Compounds	t_R (min)	Full Scan Results					All Ions MS/MS Results				
		SDL (µg kg^{-1})			Mass Error (ppm)	Score (%)	SDL (µg kg^{-1})			Mass Error (ppm)	Score (%)
		10	25	50			10	25	50		
Prothiofos	7.6	-	-	-	-	-	-	-	-	-	-
Pymetrozine	0.7			M	13.2	-		M		5.7	-
Pyraclostrobin	6.4	A			0.7	96.9	A*			-0.9	96.1
Pyrazophos	6.4	A			0.4	96.4	A			-0.1	97.4
Pyrazosulfuron	6.0	A			1.0	95.6	A			-3.9	91.5
Pyridaben	7.4	A*			-10.0	79.9		A		-5.0	91.1
Pyridafenthion	5.9	A			1.7	96.3	A			6.1	89.1
Pyridate	7.7	M			5.4	-	-	-	-	-	-
Pyrifenox	6.0	A			1.4	96.1	M			-1.4	-
Pyrimethanil	5.6		A		0.5	92.9			M	-18.8	
Quinalphos	6.2	A			3.9	96.2		A		7.8	85.3
Quinoxyfen	7.0	A*			-9.2	80.2			A	0.5	97.0
Rotenone	6.1	A			-0.4	92.4		A		8.9	83.3
Sarafloxacin	2.9			M	18.0	-			M	15.0	-
Simazine	4.9		A		2.5	94.6		A		2.6	86.1
Spinosad	6.2	A			1.9	95.9	A			3.6	90.1
Sulfachloropyridazine	1.9		A		0.0	95.9		A		-2.7	86.2
Sulfadimethoxine	4.3	A			-0.6	94.0	A			-12.2	76.9
Sulfamethanize	1.4	A			-0.3	93.5	A			-9.7	84.0
Sulfamethoxazole	2.1	A			0.1	92.6	A			-9.7	83.5
Sulfathiazole	0.8	A			1.5	93.2	A*			-7.2	82.9
Tebuconazole	6.3	M			11.3	-	M			-4.6	-
Tebufenpyrad	6.8			A	-4.0	80.8	M			-5.2	-
Tetraconazole	6.0			A	5.4	73.1	M			12.1	-
Thiabendazole	1.8	A			0.1	92.8	A			-8.2	87.6
Thiacloprid	4.3	A			0.9	96.5	A			-11.6	82.3
Thiamethoxam	1.4	A			1.0	95.0	A			-8.5	83.9
Thiobencarb	6.5		A		-6.0	87.6	A			-1.4	86.1

Compounds	t_R (min)	Full Scan Results					All Ions MS/MS Results				
		SDL (µg kg^{-1})			Mass Error (ppm)	Score (%)	SDL (µg kg^{-1})			Mass Error (ppm)	Score (%)
		10	25	50			10	25	50		
Thiram	5.9			A	-10.4	74.8			M	5.8	-
Tilmicosin	4.7	A			1.9	94.6	A			1.0	90.5
Tolclofos-methyl	6.5			A	-3.0	93.1			A	-0.6	90.9
Trenbolone (acetate)	6.3		A		4.0	89.2	M			-11.1	-
Triadimefon	5.9		A		3.2	96.2			A	3.9	91.5
Triasulfuron	4.9		A		-0.1	91.9	A*			-5.0	78.9
Triazophos	6.0	A			1.5	95.4	A			2.1	93.2
Trichlorfon	2.9			A	1.0	97.5		A		-6.7	88.2
Tricyclazole	4.4	A			2.2	96.3	A			-8.7	90.5
Tridemorph	6.1	A*			4.6	83.6			A	-2.5	94.8
Trifloxystrobin	6.5	A			-1.4	98.4	A			-0.6	98.8
Triforine	5.6	A			-1.4	89.0	A			6.8	85.1
Trimethoprim	1.1	A			0.7	94.9	A			-8.3	85.5
Triphenyl phosphate	6.3	A			0.8	96.5	A			-3.2	92.3
Tylosin	5.2		A		-0.4	84.4	A*			-0.3	81.4
Vamidothion	3.6	A			0.3	93.4	A			-8.6	85.5
Vinclozolin	5.9	M			14.5	-			M	-1.8	-

t_R: retention time; A: Automatic search (no false negatives); A*: Automatic search (5% false negatives); M: Manual search.

Mass accuracy is a consequence of a very stable accurate mass calibration and the use of dual-sprayer ESI sources for analyte and reference solutions allows for correcting instrument drift by continuous calibration of the mass axis [15,28]. However, reference masses can be also affected by matrix interference. Comparing both scan modes, there was a notably lower mass accuracy error during full scan mode when qualitative automatic search was performed. In this case, 77% of the compounds found automatically showed mass accuracy lower than 5 ppm, while during automatic search using all ions MS/MS mode only 52% presented this error (≤5 ppm). Errors between 10 and 20 ppm were found for 14 and 17% of the compounds in full scan and in all ions MS/MS mode, respectively. During MS/MS acquisition mode the accuracy of (fragment) ions might be lower than accuracies obtained in MS mode, mainly due to energy differences between ions coming from the collision energy cell. It is difficult to focus the kinetic energy of all ions before each pulse, and there are no calibration ions for continuous, on-line, accurate mass-measurement corrections [13].

As reported by Malato [15] problems observed in the automatic search of the parent compounds greatly affect the fragments, which in most of the cases present lower intensity and accurate mass than the protonated molecule. For this reason, an automatic search of the fragment ions was not conducted, however, all the compounds were confirmed by presence of one fragment ion, when using all ions MS/MS. Even when not using the confirmation ions, the proposed validated method showed good reliability in the screening analysis in fish fillet using full scan mode. Combining retention time and structural information, as well isotopic pattern when available, it is possible to identify the presence of organic contaminants in complex matrices, such as fish fillet.

3.3. Quantitative validation

Quantitative analysis was not performed with data obtained in all ion MS/MS scan mode due to the decrease in the data point rate, which negatively affected the chromatographic peak shape for most of the compounds, however, fragmentation information was used to confirm full scan results.

Quantitative validation in the full scan mode was performed, and all results of the parameters evaluated are shown in Table 3. The QToF instrument was operated for the entire analysis in 2 GHz mode-extended dynamic range to make possible the

QToF application for quantification of organic contaminants in trace levels. Linearity was determined by the coefficient of determination (r^2), and values higher than 0.99 were obtained for almost all the compounds, except for a few with unacceptable RSD/recovery, and linear range was obtained for all the compounds from 0.5 to 20 µg L^{-1}. Method limits of quantification (LOQ) and detection (LOD) were from 5 to 25 µg kg^{-1} and from 1.5 to 7.5 µg kg^{-1}, respectively.

Accuracy and precision of the total of 182 compounds (not considering SS and IS) were determined in a repeatability assay (n=6) using blank samples spiked at 5, 10, 25 and 50 µg kg^{-1}, and 76% showed recovery values between 70 and 120% with RSD lower than 20%; 13% of the compounds obtained recoveries lower than 70% or higher than 120% with acceptable RSD; 11% of the compounds did not present good RSD or were not recovered at all. The intermediate precision was evaluated at 25 µg kg^{-1}, and similar results were obtained for the average of the results during validation procedure. The SS, atrazine-d5, showed good recovery (94.0 to 110.2%) and RSD (2.4 to 6.5%) values for all the spiked samples.

The proposed method was not efficient for the extraction of fluroxypyr and prothiofos in the spiked concentration levels in fish fillet. Prothiofos has a high log K_{ow} value of 5.67, which can explain the lack of recovery due to its accumulation in the adipose tissue and low water solubility [29]. The percentage of fat in fish can be highly variable, mainly depending on the presence of fatty acids in the diet and in catfishes this quantity can vary from 2.5 to 5.7% [22,30]. However, fluroxypyr has a dissociation constant (pKa) value of 2.94 [29], suggesting that the extraction method applied is not proper for extraction of acid compounds, being necessary the use of lower pH during sample preparation [31]. The following compounds presented RSD values higher than 20% and were not considered for quantification: abamectin, acrinathrin, atenolol, azinphos-methyl, danofloxacin, desmedipham, enrofloxacin, furathiocarb, hexythiazox, lincomycin, methiocarb sulfoxide, methomyl, paraoxon-ethyl, pymetrozine, pyridate, procymidone, profenophos, quinoxyfen and sarafloxacin. Twenty-three other compounds showed recoveries lower than 70% or higher than 120%, however, RSD values were ≤20%, and for this reason they were considered for quantification and the results are presented in Table 3. In general, recovery values outside the range of 70-120% with RSD values lower than 20% can be accepted when working with a wide number of compounds from different chemical classes and a complex matrix [16]. Due to the main goal of qualitative analysis of this

study, it is possible to affirm that the proposed method was very satisfactory for quantification, meeting all the validation parameters for 139 compounds (including the SS).

The assessment of the matrix effect showed that 19.1% of the compounds have their signal affected by the matrix, considering ME higher than 20% [32]. This shows the importance of using matrix-matched calibration curves, as adopted in this work, to avoid inappropriate quantification caused by matrix interferences. Performing extract dilution is another possible solution to improve this drawback in HRMS, since the matrix effect occurs because of the interaction of compounds co-eluting with the analytes in the ionization process. Ferrer et al. [32] concluded that a dilution factor of 15 would be enough to solve most of the problems related to the presence of matrix components in the extracts, particularly problems of ionization efficiency and saturation of the detection system. The main inconvenience of performing dilutions is in achieving sufficient sensitivity, due to dilution of extracts that implies in a reduction of the analyte amount [13,32]; for this reason the proposed study used a dilution factor of 5 to balance matrix effect and sensitivity of the analytical system.

Table 3. Quantitative results obtained in full scan mode.

Compounds	Spiked level (µg kg^{-1})				25 µg kg^{-1}	Linear range (µg L^{-1})	r^2	LOQ (µg kg^{-1})	LOD (µg kg^{-1})	Mass error (ppm)	ME (%)
	5	10	25	50	%Rec (RSD$_{ip}$)						
	Rec (RSDr), %										
Abamectin	-	-	-	-	-	0.5-20	0.998	-	-	7.8	-61.7
Acetamiprid	95 (6)	100 (7)	100 (2)	98 (3)	105 (6)	0.5-20	0.998	5.0	1.5	2.5	1.5
Acrinathrin	-	-	-	-	-	2.0-20	0.919	-	-	4.3	-20.6
Albendazole	85 (3)	89 (6)	87 (4)	86 (3)	88 (6)	0.5-20	0.999	5.0	1.5	2.0	-6.3
Ametryn	96 (14)	92 (5)	82 (3)	79 (3)	86 (6)	0.5-20	0.999	5.0	1.5	0.7	-2.6
Aramite	91 (18)	93 (14)	81 (12)	81 (12)	83 (19)	0.5-20	0.993	5.0	1.5	5.8	3.3
Atenolol	-	-	-	-	-	0.5-5	0.928	-	-	14.2	-90.9
Atrazine	80 (6)	91 (8)	92 (4)	91 (4)	95 (7)	0.5-20	0.998	5.0	1.5	2.8	-5.2
Atrazine-d5	106 (4)	105 (2)	98 (3)	94 (4)	110 (6)	0.5-20	0.998	5.0	1.5	2.2	-5.8
Avobenzone	-	57 (11)	43 (16)	40 (21)	57 (11)	0.5-20	0.991	10.0	3.0	4.2	-27.6
Azaconazole	92 (6)	97 (8)	96 (2)	94 (3)	105 (5)	0.5-20	0.999	5.0	1.5	2.4	-2.0
Azamethiphos	74 (18)	79 (5)	86 (5)	75 (11)	77 (10)	0.5-20	0.999	5.0	1.5	1.9	-6.0
Azinphos-methyl	-	-	-	-	-	0.5-10	0.839	-	-	14.1	-13.9
Azoxystrobin	110 (13)	107 (6)	98 (7)	98 (3)	105 (5)	0.5-20	0.999	5.0	1.5	-0.9	-3.6
Benzophenone 3	-	75 (10)	81 (5)	82 (5)	85 (13)	0.5-20	0.998	10.0	3.0	-2.7	-3.7
Bitertanol	-	81 (22)	84 (5)	91 (4)	72 (18)	2.0-10	0.994	10.0	3.0	4.8	20.1
Boscalid	-	120 (3)	120 (4)	113 (4)	115 (7)	0.5-20	0.991	10.0	3.0	7.9	-12.0
Bupirimate	87 (17)	93 (8)	89 (4)	88 (4)	86 (8)	0.5-20	0.999	5.0	1.5	3.5	-8.1
Buprofezin	-	67 (20)	55 (18)	70 (17)	67 (20)	0.5-20	0.990	10.0	3.0	10.2	-17.6
Caffeine	82 (14)	82 (6)	81 (2)	76 (2)	80 (16)	0.5-20	0.999	5.0	1.5	7.0	-3.6
Carbaryl	100 (4)	106 (10)	96 (7)	90 (5)	100 (16)	0.5-20	0.998	5.0	1.5	3.8	-1.1
Carbendazim	92 (15)	92 (6)	85 (2)	84 (2)	84 (5)	0.5-20	0.998	5.0	1.5	1.3	-0.3
Carbofuran	112 (18)	114 (9)	117 (3)	112 (6)	117 (7)	0.5-20	0.999	5.0	1.5	2.4	-7.1
Carbofuran-3-hydroxy	100 (8)	100 (12)	96 (5)	94 (8)	103 (5)	0.5-20	0.999	5.0	1.5	3.6	-5.1
Carboxin	93 (15)	98 (8)	92 (6)	92 (5)	95 (7)	0.5-20	1.000	5.0	1.5	0.9	-3.6
Carpropamid	83 (6)	93 (5)	91 (4)	91 (4)	94 (7)	0.5-20	0.998	5.0	1.5	1.3	-9.1
Chlorantraniliprole	94 (7)	103 (6)	104 (3)	99 (2)	109 (5)	0.5-10	0.999	5.0	1.5	-0.2	-0.9
Chlorbromuron	115 (7)	109 (6)	101 (5)	96 (6)	97 (7)	0.5-10	0.995	5.0	1.5	0.8	-13.1
Chlorfenvinphos	92 (6)	96 (7)	93 (5)	92 (4)	98 (7)	0.5-10	0.999	5.0	1.5	1.7	-7.9
Chlorpyrifos-oxon	-	-	78 (6)	70 (14)	72 (14)	0.5-20	0.998	25.0	7.5	1.3	-16.6
Clarithromycin	76 (3)	58 (4)	45 (16)	47 (4)	46 (7)	0.5-20	0.997	5.0	1.5	0.7	-0.2
Clenbuterol	71 (4)	64 (4)	64 (5)	64 (6)	60 (7)	0.5-20	0.998	5.0	1.5	2.0	-4.7
Clofentezine	73(14)	86 (5)	76 (10)	78 (7)	79 (11)	0.5-10	0.995	5.0	1.5	4.3	-11.4
Clomazone	97 (10)	104 (6)	96 (8)	95 (3)	101 (6)	0.5-10	0.999	5.0	1.5	1.9	-4.9
Clothianidin	92 (7)	96 (11)	95 (3)	93 (3)	100 (5)	0.5-20	0.998	5.0	1.5	2.4	-3.0
Cyanazine	94 (18)	104 (8)	101 (3)	98 (4)	83 (11)	0.5-20	0.999	5.0	1.5	3.2	-1.2
Cyazofamid	-	100 (12)	104 (5)	102 (4)	100 (6)	0.5-20	0.992	10.0	3.0	3.7	-20.9
Cyproconazole	86 (18)	84 (14)	86 (8)	88 (9)	95 (11)	0.5-20	0.992	5.0	1.5	10.3	-3.0
Cyprodinil	78 (9)	72 (4)	62 (6)	60 (4)	56 (12)	0.5-20	0.998	5.0	1.5	-0.2	-10.1
Danofloxacin	-	-	-	-	-	0.5-10	0.995	-	-	2.5	479.9
Desmedipham	-	-	-	-	-	0.5-10	0.968	-	-	2.4	-53.1
Diazinon	90 (15)	89 (6)	81 (5)	81 (6)	83 (8)	0.5-20	0.999	5.0	1.5	-5.3	-8.7

Compounds	Spiked level (µg kg^{-1})				25 µg kg^{-1}	Linear range (µg L^{-1})	r^2	LOQ (µg kg^{-1})	LOD (µg kg^{-1})	Mass error (ppm)	ME (%)
	5	10	25	50	%Rec (RSD_{ip})						
	Rec (RSDr), %										
Dichlorvos	98 (17)	102 (20)	107 (6)	101 (15)	88 (13)	0.5-10	0.992	5.0	1.5	-5.1	12.3
Dicrotophos	86 (18)	91 (9)	87 (4)	87 (4)	89 (6)	0.5-20	0.999	5.0	1.5	1.4	-3.5
Dienestrol (diacetate)	104 (7)	87 (7)	79 (7)	81 (8)	82 (10)	0.5-20	0.998	5.0	1.5	1.3	-13.7
Difenoconazole	95 (13)	96 (5)	87 (7)	82 (8)	87 (14)	0.5-10	0.996	5.0	1.5	3.7	-2.3
Difloxacin	67 (12)	52 (20)	52 (16)	50 (19)	53 (16)	0.5-10	0.994	5.0	1.5	0.7	25.0
Dimethomorph	-	120 (4)	113 (4)	105 (2)	101 (5)	0.5-20	0.991	10.0	3.0	0.9	1.2
Dimoxystrobin	-	99 (12)	98 (5)	98 (4)	98 (11)	1.0-20	0.998	10.0	3.0	11.0	-5.5
Diniconazole	83 (15)	91 (7)	83 (9)	81 (6)	86 (9)	0.5-20	0.996	5.0	1.5	8.0	-57.8
Diuron	85 (20)	97 (6)	97 (2)	94 (3)	106 (5)	0.5-20	0.999	5.0	1.5	-0.4	-3.4
Dodemorph	48 (12)	35 (8)	22 (20)	20 (13)	20 (19)	0.5-20	0.996	5.0	1.5	-0.2	-3.8
Emamectin	76 (11)	58 (6)	45 (3)	44 (5)	43 (7)	0.5-20	0.996	5.0	1.5	0.2	4.8
Enrofloxacin	-	-	-	-	-	0.5-10	0.883	-	-	1.0	72.8
Epoxiconazole	92 (11)	102 (9)	99 (6)	99 (5)	98 (8)	0.5-20	0.995	5.0	1.5	-8.0	-5.8
Eprinomectin	108 (15)	91 (18)	75 (10)	74 (16)	78 (8)	0.5-20	0.998	5.0	1.5	7.9	-31.7
Erythromycin	109 (7)	81 (8)	71 (6)	71 (13)	72 (6)	0.5-20	0.993	5.0	1.5	0.0	-10.8
Ethion	84 (18)	74 (20)	99 (19)	97 (18)	74 (20)	0.5-20	0.991	5.0	1.5	13.9	-35.7
Ethirimol	91 (4)	78 (3)	72 (2)	70 (2)	72 (6)	0.5-20	0.999	5.0	1.5	1.4	-1.8
Ethopabate	108 (11)	105 (5)	102 (5)	99 (4)	102 (4)	0.5-20	0.999	5.0	1.5	5.3	-2.3
Ethoprophos	83 (6)	91 (3)	91 (4)	91 (5)	94 (4)	0.5-20	0.999	5.0	1.5	2.0	-6.2
Ethoxysulfuron	96 (6)	95 (9)	93 (3)	93 (2)	96 (12)	0.5-10	0.999	5.0	1.5	0.9	3.2
Etiofencarb sulfoxide	87 (13)	87 (17)	84 (6)	79 (15)	88 (9)	0.5-10	0.998	5.0	1.5	4.9	-10.2
Etofenprox	-	57 (7)	41 (16)	39 (18)	49 (8)	0.5-10	0.997	10.0	3.0	5.2	-56.3
Etrimphos	98 (7)	89 (4)	82 (5)	82 (6)	85 (4)	0.5-20	0.999	5.0	1.5	-0.9	-8.3
Fenbuconazole	86 (14)	101 (11)	102 (4)	99 (2)	104 (4)	0.5-20	0.997	5.0	1.5	-6.4	-9.8
Fenpropimorph	66 (15)	54 (6)	37 (20)	39 (12)	37 (15)	0.5-20	0.996	5.0	1.5	0.2	-5.2
Fenpyroximate	93 (9)	77 (5)	82 (10)	72 (11)	80 (9)	0.5-20	0.998	5.0	1.5	6.8	-17.4
Fenthion	91 (12)	94 (7)	93 (5)	92 (5)	91 (9)	0.5-10	0.994	5.0	1.5	2.5	-11.4
Fenthion sulfoxide	109 (5)	104 (3)	100 (4)	99 (1)	103 (4)	0.5-20	0.999	5.0	1.5	-0.5	-0.4
Flufenacet	100 (8)	104 (2)	99 (4)	94 (4)	101 (3)	0.5-10	0.998	5.0	1.5	2.0	-6.4
Fluroxypyr	-	-	-	-	-	0.5-20	0.988	-	-	-	-2.9
Flusilazole	86 (7)	100 (5)	101 (4)	100 (3)	100 (3)	0.5-20	0.997	5.0	1.5	1.1	-18.6
Flutolanil	94 (6)	101 (2)	104 (4)	103 (4)	104 (3)	0.5-20	0.998	5.0	1.5	-0.9	-6.1
Fosthiazate	99 (17)	101 (3)	92 (16)	96 (4)	101 (4)	0.5-20	0.999	5.0	1.5	1.9	-3.4
Furaltadone	92 (4)	90 (10)	93 (7)	83 (11)	98 (5)	0.5-10	0.997	5.0	1.5	-1.1	13.0
Furathiocarb	-	-	-	-	-	0.5-10	0.866	-	-	17.0	-42.2
Furazolidone	95 (4)	96 (6)	96 (3)	89 (5)	107 (4)	0.5-20	0.999	5.0	1.5	1.3	-1.6
Hexythiazox	-	-	-	-	-	0.5-10	0.981	-	-	13.7	-31.4
Imazalil	93 (7)	87 (4)	78 (7)	77 (8)	77 (7)	0.5-20	0.994	5.0	1.5	-0.8	-16.0
Imazaquin	34 (21)	31 (20)	33 (15)	33 (21)	31 (13)	0.5-20	0.999	5.0	1.5	0.6	-2.2
Imazethapyr	30 (18)	28 (14)	29 (12)	29 (17)	22 (14)	0.5-20	0.999	5.0	1.5	-1.4	-3.9
Imidacloprid	99 (5)	96 (4)	96 (2)	94 (2)	101 (8)	0.5-20	0.999	5.0	1.5	2.1	-0.9
Indoxacarb	105 (5)	99 (7)	96 (5)	95 (3)	101 (8)	0.5-20	0.999	5.0	1.5	2.8	6.4
Iprovalicarb	102 (7)	101 (4)	98 (3)	99 (4)	101 (4)	0.5-20	0.999	5.0	1.5	4.9	-3.9

Compounds	Spiked level (µg kg^{-1})				25 µg kg^{-1}	Linear range	r^2	LOQ	LOD	Mass error	ME
	5	10	25	50	%Rec (RSD_{ip})	(µg L^{-1})		(µg kg^{-1})	(µg kg^{-1})	(ppm)	(%)
	Rec (RSDr), %										
Isoprothiolane	85 (8)	94 (5)	93 (5)	94 (5)	99 (3)	0.5-20	0.998	5.0	1.5	2.1	-6.2
Isoxaflutole	93 (10)	106 (13)	97 (10)	94 (11)	118 (10)	0.5-20	0.994	5.0	1.5	1.7	-8.9
Isoxathion	95 (9)	91 (7)	85 (6)	86 (7)	96 (6)	0.5-20	0.997	5.0	1.5	8.3	-11.1
Ivermectin	71 (15)	61 (10)	53 (8)	54 (10)	56 (11)	0.5-20	0.999	5.0	1.5	5.8	177.7
Ketoconazole	104 (20)	95 (9)	87 (6)	82 (11)	82 (7)	0.5-20	0.992	5.0	1.5	0.5	-19.0
Lidocaine	97 (3)	84 (3)	81 (3)	80 (3)	98 (5)	0.5-20	0.990	5.0	1.5	-0.9	2.3
Lincomycin	-	-	-	-	-	0.5-20	0.997	-	-	0.3	-8.5
Mecarbam	102 (8)	94 (12)	89 (8)	89 (17)	90 (20)	0.5-20	0.998	5.0	1.5	4.0	-27.9
Mepanipyrim	84 (12)	89 (4)	82 (4)	82 (5)	95 (11)	0.5-10	0.999	5.0	1.5	8.7	-9.4
Mephospholan	112 (3)	101 (3)	95 (3)	94 (3)	96 (5)	0.5-20	0.999	5.0	1.5	0.7	-1.3
Mepronil	82 (6)	96 (4)	99 (4)	98 (4)	99 (4)	0.5-10	0.997	5.0	1.5	0.9	-11.8
Metacriphos	98 (9)	98 (5)	97 (4)	97 (5)	92 (4)	0.5-10	0.998	5.0	1.5	11.6	-0.9
Metalaxyl	102 (4)	99 (3)	96 (3)	96 (4)	111 (3)	0.5-20	0.999	5.0	1.5	0.3	-2.5
Metconazole	88 (6)	99 (6)	90 (3)	87 (1)	91 (7)	0.5-20	0.999	5.0	1.5	-1.8	-1.6
Methidathion	105 (18)	101 (13)	104 (6)	96 (6)	96 (8)	0.5-20	0.996	5.0	1.5	2.0	-14.2
Methiocarb sulfoxide	-	-	-	-	-	0.5-20	0.980	5.0	1.5	2.0	-63.7
Methomyl	-	-	-	-	-	2.0-20	0.996	-	-	8.4	14.4
Metobromuron	94 (7)	97 (4)	98 (3)	97 (4)	104 (5)	0.5-20	0.998	5.0	1.5	1.6	-4.0
Metolachlor	89 (4)	89 (11)	88 (4)	88 (5)	91 (4)	0.5-20	0.999	5.0	1.5	2.3	-9.6
Metoxuron	102 (3)	99 (2)	98 (3)	97 (3)	90 (3)	0.5-20	1.000	5.0	1.5	-0.1	-1.7
Metsulfuron-methyl	79 (5)	72 (4)	69 (2)	67 (4)	60 (4)	0.5-20	0.999	5.0	1.5	1.8	-1.8
Mevinphos	99 (8)	107 (8)	103 (3)	99 (4)	103 (15)	0.5-20	0.998	5.0	1.5	5.8	-5.0
Miconazole	67 (10)	61 (13)	51 (9)	51 (13)	42 (19)	0.5-20	0.995	5.0	1.5	2.1	-24.6
Monensin	73 (6)	61 (9)	51 (6)	50 (11)	49 (8)	0.5-20	0.998	5.0	1.5	-0.3	-10.6
Monocrotophos	91 (8)	92 (8)	87 (3)	86 (4)	86 (12)	0.5-20	1.000	5.0	1.5	3.9	-3.8
Monolinuron	95 (7)	98 (7)	96 (7)	97 (4)	104 (6)	0.5-20	0.999	5.0	1.5	2.5	-2.0
Myclobutanil	89 (10)	99 (4)	100 (2)	99 (3)	107 (7)	0.5-10	0.998	5.0	1.5	1.1	-1.8
Nicotine	50 (19)	48 (14)	43 (7)	42 (12)	41 (12)	0.5-20	0.998	5.0	1.5	-0.7	31.5
Nitenpyram	71 (9)	66 (17)	66 (2)	65 (1)	68 (5)	0.5-20	0.999	5.0	1.5	3.5	-5.2
Nuarimol	142 (12)	145 (18)	151 (6)	152 (2)	113 (7)	1.0-10	0.990	5.0	1.5	0.5	-41.8
Omethoate	78 (10)	71 (20)	70 (2)	68 (5)	71 (6)	0.5-20	0.999	5.0	1.5	-0.5	-10.9
Oxadiazon	108 (17)	101 (10)	84 (19)	71 (16)	98 (20)	0.5-20	0.986	5.0	1.5	7.2	23.5
Oxadixyl	101 (8)	100 (18)	101 (2)	99 (3)	100 (4)	0.5-20	0.999	5.0	1.5	4.4	-4.5
Oxamyl	101 (20)	87 (17)	91 (10)	85 (5)	99 (12)	0.5-20	0.997	5.0	1.5	4.4	23.5
Oxycarboxin	109 (11)	103 (11)	97 (5)	93 (12)	104 (6)	0.5-5	0.998	5.0	1.5	1.9	-7.2
Paraoxon-ethyl	-	-	-	-	-	0.5-10	0.996	-	-	1.8	-5.9
Penconazole	89 (10)	85 (17)	85 (2)	83 (3)	88 (7)	0.5-20	0.999	5.0	1.5	-0.1	-9.1
Pencycuron	108 (8)	89 (12)	83 (3)	828 (4)	84 (13)	0.5-20	0.999	5.0	1.5	10.5	-6.7
Phenmedipham	92 (14)	97 (12)	102 (4)	101 (4)	104 (5)	0.5-10	0.998	5.0	1.5	5.2	-0.4
Phenthoate	85 (9)	91 (7)	93 (4)	96 (9)	104 (12)	0.5-10	0.995	5.0	1.5	6.0	-18.1
Phosalone	85 (10)	91 (5)	90 (4)	88 (5)	94 (5)	0.5-10	0.998	5.0	1.5	2.9	-3.1
Phosmet	-	110 (16)	88 (20)	105 (19)	98 (15)	0.5-10	0.962	10.0	3.0	6.4	-34.6

Compounds	Spiked level (µg kg^{-1})				25 µg kg^{-1}	Linear range (µg L^{-1})	r^2	LOQ (µg kg^{-1})	LOD (µg kg^{-1})	Mass error (ppm)	ME (%)
	5	10	25	50	%Rec (RSD_{ip})						
	Rec (RSDr), %										
Picoxystrobin	98 (13)	98 (19)	98 (3)	98 (6)	100 (6)	0.5-20	0.998	5.0	1.5	1.4	-9.9
Pirimicarb	113 (8)	96 (14)	91 (3)	89 (3)	88 (4)	0.5-20	0.998	5.0	1.5	0.0	-1.0
Pirimiphos-methyl	83 (16)	89 (17)	78 (5)	76 (8)	79 (13)	0.5-20	0.998	5.0	1.5	5.9	-10.4
Prochloraz	87 (9)	86 (17)	88 (5)	84 (3)	88 (5)	0.5-20	0.997	5.0	1.5	0.8	-13.2
Procymidone	-	-	-	-	-	0.5-20	0.953	5.0	1.5	-14.4	-14.2
Profenophos	-	-	-	-	-	0.5-10	0.922	-	-	16.8	-43.0
Profoxydim	119 (11)	103 (19)	110 (5)	112 (11)	99 (6)	0.5-20	0.996	5.0	1.5	-1.4	9.8
Propargite	106 (7)	87 (7)	79 (7)	80 (8)	82 (11)	0.5-20	0.998	5.0	1.5	-7.9	-12.7
Propiconazole	88 (9)	90 (17)	94 (2)	91 (3)	97 (5)	0.5-20	0.997	5.0	1.5	0.3	-5.0
Propoxur	103 (14)	105 (7)	98 (4)	96 (6)	90 (15)	0.5-20	0.998	5.0	1.5	4.8	-5.1
Propranolol	50 (4)	44 (4)	45 (6)	46 (6)	39 (12)	0.5-20	0.999	5.0	1.5	-0.5	-5.8
Propyzamide	79 (18)	99 (7)	102 (4)	97 (4)	97 (6)	0.5-10	0.996	5.0	1.5	0.8	-6.7
Prothiofos	-	-	-	-	-	5.0-20	0.992	-	-	5.5	-32.5
Pymetrozine	-	-	-	-	-	0.5-10	0.994	-	-	10.5	-28.4
Pyraclostrobin	105 (7)	95 (20)	88 (3)	88 (5)	93 (6)	0.5-20	0.999	5.0	1.5	-1.6	-10.6
Pyrazophos	96 (9)	88 (15)	92 (5)	87 (3)	94 (10)	0.5-20	0.999	5.0	1.5	-1.0	25.2
Pyrazosulfuron	99 (7)	95 (4)	90 (5)	89 (2)	91 (5)	0.5-20	0.999	5.0	1.5	-2.2	1.5
Pyridaben	88 (17)	73 (10)	71 (14)	70 (17)	72 (12)	0.5-20	0.997	5.0	1.5	10.0	-37.7
Pyridafenthion	98 (10)	98 (18)	104 (2)	100 (3)	109 (5)	0.5-20	0.998	5.0	1.5	-2.4	-1.5
Pyridate	-	-	-	-	-	0.5-20	0.990	-	-	-3.3	-65.4
Pyrifenox	80 (10)	81 (20)	83 (3)	81 (4)	82 (7)	0.5-20	0.998	5.0	1.5	0.5	-10.3
Pyrimethanil	92 (10)	82 (14)	72 (12)	71 (3)	71 (6)	0.5-20	0.999	5.0	1.5	-1.6	-4.4
Quinalphos	90 (11)	91 (3)	86 (4)	87 (5)	88 (8)	0.5-20	0.999	5.0	1.5	3.7	-2.5
Quinoxyfen	-	-	-	-	-	0.5-10	0.971	-	-	14.9	-50.2
Rotenone	88 (8)	101 (2)	97 (5)	93 (7)	95 (5)	0.5-20	0.997	5.0	1.5	0.1	-5.8
Sarafloxacin	-	-	-	-	-	0.5-10	0.989	-	-	18.3	66.5
Simazine	85 (11)	96 (4)	92 (4)	95 (3)	74 (15)	0.5-20	0.996	5.0	1.5	2.0	-9.5
Spinosad	62 (13)	47 (13)	36 (7)	34 (12)	34 (18)	0.5-20	0.996	5.0	1.5	-3.1	-4.0
Sulfachloropyridazine	100 (9)	85 (6)	84 (6)	81 (5)	85 (10)	0.5-20	1.000	5.0	1.5	1.7	-2.5
Sulfadimethoxine	111 (4)	99 (5)	97 (3)	95 (2)	98 (4)	0.5-20	0.999	5.0	1.5	2.7	-1.0
Sulfamethanize	106 (1)	97 (2)	93 (3)	93 (1)	93 (5)	0.5-20	0.999	5.0	1.5	2.3	0.0
Sulfamethoxazole	99 (7)	93 (5)	98 (4)	95 (4)	96 (5)	0.5-20	0.999	5.0	1.5	1.6	-3.4
Sulfathiazole	107 (5)	92 (8)	88 (4)	86 (8)	96 (10)	0.5-20	0.999	5.0	1.5	1.0	-4.3
Tebuconazole	97 (6)	94 (14)	94 (2)	90 (2)	96 (9)	0.5-20	0.998	5.0	1.5	4.2	-1.9
Tebufenpyrad	70 (18)	78 (14)	70 (18)	76 (18)	84 (9)	0.5-10	0.988	5.0	1.5	6.3	-2.5
Tetraconazole	99 (7)	108 (2)	108 (2)	104 (2)	106 (5)	0.5-20	0.998	5.0	1.5	1.8	-7.2
Thiabendazole	106 (6)	95 (1)	87 (2)	84 (1)	89 (3)	0.5-20	0.999	5.0	1.5	0.9	-1.1
Thiacloprid	113 (7)	106 (2)	99 (2)	96 (3)	104 (3)	0.5-20	0.999	5.0	1.5	0.8	-3.4
Thiamethoxam	98 (11)	100 (6)	97 (1)	94 (3)	98 (6)	0.5-20	0.998	5.0	1.5	2.6	-4.7
Thiobencarb	94 (14)	90 (5)	75 (5)	73 (7)	76 (14)	0.5-10	0.997	5.0	1.5	8.0	-5.9
Thiram	-	99 (6)	100 (3)	99 (4)	110 (5)	0.5-10	0.995	10.0	3.0	10.8	-7.9
Tilmicosin	83 (3)	62 (3)	53 (3)	53 (8)	52 (8)	0.5-20	0.997	5.0	1.5	-1.3	189.0
Tolclofos-methyl	89 (16)	87 (16)	77 (7)	76 (13)	71 (15)	0.5-10	0.995	5.0	1.5	5.5	-1.6
Trenbolone	97(19)	95 (6)	87 (5)	82 (3)	86 (9)	0.5-20	0.999	5.0	1.5	1.6	7.1

Compounds	Spiked level (µg kg^{-1})				25 µg kg^{-1} %Rec (RSD$_{ip}$)	Linear range (µg L^{-1})	r^2	LOQ (µg kg^{-1})	LOD (µg kg^{-1})	Mass error (ppm)	ME (%)
	5	10	25	50							
	Rec (RSDr), %										
(acetate)											
Triadimefon	94 (12)	103 (4)	100 (2)	98 (3)	93 (20)	0.5-10	0.998	5.0	1.5	10.7	0.1
Triasulfuron	103 (7)	101 (2)	98 (6)	93 (4)	83 (3)	0.5-20	0.999	5.0	1.5	1.2	-1.4
Triazophos	95 (9)	104 (4)	101 (3)	96 (4)	102 (8)	0.5-10	0.998	5.0	1.5	0.4	-8.9
Trichlorfon	79 (5)	77 (6)	73 (5)	77 (6)	93 (14)	0.5-20	0.958	5.0	1.5	2.0	-45.0
Tricyclazole	92 (8)	92 (2)	87 (2)	86 (2)	89 (7)	0.5-20	0.999	5.0	1.5	1.2	-3.1
Tridemorph	33 (8)	25 (19)	17 (9)	15 (14)	19 (8)	0.5-20	0.997	5.0	1.5	-4.7	-5.7
Trifloxystrobin	119 (7)	105 (4)	87 (10)	85 (13)	91 (11)	0.5-20	0.994	5.0	1.5	1.7	-13.4
Triforine	112 (12)	118 (11)	112 (3)	101 (5)	116 (17)	0.5-5	0.991	5.0	1.5	7.4	-19.5
Trimethoprim	75 (3)	67 (3)	66 (3)	66 (2)	64 (5)	0.5-20	0.999	5.0	1.5	1.0	-2.9
Triphenyl phosphate		-	-	-	-	-	-	-	-	2.0	-
Tylosin	117 (17)	93 (20)	93 (15)	97 (16)	118 (7)	0.5-20	0.994	5.0	1.5	-0.3	10.6
Vamidothion	101 (7)	100 (5)	92 (1)	91 (3)	94 (4)	0.5-20	0.999	5.0	1.5	1.1	-3.0
Vinclozolin	86 (17)	98 (13)	103 (8)	101 (6)	100 (5)	0.5-20	0.996	5.0	1.5	6.2	-0.8

4. Conclusion

The advantages of using liquid chromatography coupled to high resolution mass spectrometry are well-known, especially the focus on screening analysis in complex matrices. Therefore, the proposed working flow for the two scan modes for data acquisition by LC-QToF/MS presents pros and cons, while all ions MS/MS provides more structural information of the compounds due to the fragmentation ions obtained using different collision energies; full scan collects more information data points (1 spectra/s), improving peak shape and SDL limits, presenting lower values of mass accuracy error. However, in terms of working with a complex matrix, such as fish fillet, the automatic search was found to be very efficient for a great number of compounds, being able to detect 84% and 72% of the selected compounds using full scan and all ions MS/MS, respectively. Furthermore, most of the compounds presented a SDL value of 10 µg kg^{-1} in both scan acquisition modes, which is very important as these compounds usually are found in very low concentrations in the aquatic environment as well as, in fish fillet.

In spite of the fact that recovery and validation parameters are not necessary for screening analysis, sample preparation was evaluated to minimize the matrix effect especially caused by isobaric compounds that can interfere in the automatic search, and for quantification purposes. In order to assure a reliable determination of trace levels of organic contaminants, the validation parameters evaluated in fish fillet showed good results for a wide scope. Seventy six percent of the compounds showed recovery values between 70 and 120% with RSD≤20%, which are in accordance with international guides, such as SANCO (2013); and the method was found to be very sensitive with LOQ and LOD values from 5 to 25 µg kg^{-1} and from 1.5 to 7.5 µg kg^{-1}, respectively.

Considering the complexity of the studied matrix and the power of the LC-QToF/MS instrumentation used in this study, it is possible to affirm that screening analyses are critical, even when working with target compounds. Full scan mode was more reliable in target screening as a better peak shape and scan information are provided resulting in automatic identification with low values of mass accuracy errors and high scores. Moreover, quantification can be performed avoiding equivocal errors caused by a low data point rate. However, fragmentation information of the compounds was possible only when using the all ions MS/MS scan mode.

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References

[1] W. Brack, S. Ait-Aissa, R.M. Burgess, W. Busch, N. Creusot, C. Di Paolo, B.I. Escher, L. Mark Hewitt, K. Hilscherova, J. Hollender, H. Hollert, W. Jonker, J. Kool, M. Lamoree, M. Muschket, S. Neumann, P. Rostkowski, C. Ruttkies, J. Schollee, E.L. Schymanski, T. Schulze, T.B. Seiler, A.J. Tindall, G. De Aragão Umbuzeiro, B. Vrana, M. Krauss, Effect-directed analysis supporting monitoring of aquatic environments-An in-depth overview, Sci. Total Environ. 544 (2016) 1073-1118.

[2] M. LeDoux, Analytical methods applied to the determination of pesticide residues in foods of animal origin. A review of the past two decades, J. Chromatogr. A 1218 (2011) 1021-1036.

[3] T. Deblondea, C. Cossu-Leguilleb, P. Hartemann, Emerging pollutants in wastewater: A review of the literature, Int. J. Hyg. Environ. Health. 214 (2011) 442-448.

[4] K. Wille, H.F. De Brabander, L. Vanhaecke, Coupled chromatographic and mass-spectrometric techniques for the analysis of emerging pollutants in the aquatic environment, TrAC-Trends Anal. Chem. 35 (2012) 87-108.

[5] I. Ferrer, M.E. Thurman, Liquid chromatography/time-of-flight/mass spectrometry (LC/ToF/MS) for the analysis of emerging contaminants, TrAC-Trends Anal. Chem. 22 (2003) 750-756.

[6] M. Stuart, D. Lapworth, E. Crane, A. Hart, Review of risk from potential emerging contaminants in UK groundwater, Sci. Total Environ. 416 (2012) 1-21.

[7] A. Lazartigues, C. Fratta, R. Baudot, L. Wiest, C. Feidt, M. Thomas, C. Cren-Olivé, Multiresidue method for the determination of 13 pesticides in three environmental matrices: water, sediments and fish muscle, Talanta 85 (2011a) 1500-1507.

[8] D. Botaro, J.P. Torres, O. Malm, M. F. Rebelo, B. Henkelmann, K.W. Schramm, Organochlorine pesticides residues in feed and muscle of farmed Nile tilapia from Brazilian fish farms, Food Chem. Toxicol. 49 (2011) 2125-2130.

[9] A. Lazartigues, L. Wiest, R. Baudot, M. Thomas, C. Feidt, C. Cren-Olivé, Multiresidue method to quantify pesticides in fish muscle by QuEChERS-based extraction and LC-MS/MS, Anal. Bioanal. Chem. 400 (2011b) 2185-2193.

[10] A. Kaufmann, P. Butcher, K. Maden, S. Walker, M. Widmer, Development of an improved high resolution mass spectrometry based multi-residue method for veterinary drugs in various food matrices, Anal. Chim. Acta 700 (2011) 86-94.

[11] E. Hoffmann de, V. Stroobant, Mass Spectrometry. Principles and Applications, third ed., Wiley. England, 2007.

[12] R. Díaz, M. Ibáñez, J.V. Sancho, F. Hernández, Building an empirical mass spectra library for screening of organic pollutants by ultra-high-pressure liquid chromatography/hybrid quadrupole time-of-flight mass spectrometry, Rapid Commun. Mass Spectrom. 25 (2011) 355-369.

[13] M.M. Gómez-Ramos, C. Ferrer, O. Malato, A. Agüera, A.R. Fernández-Alba, Liquid chromatography-high-resolution mass spectrometry for pesticide residue analysis in fruit and vegetables: Screening and quantitative studies, J. Chromatogr. A 1287 (2013) 24-37.

[14] J. Nácher-Mestre, M. Ibáñez, R. Serrano, J. Pérez-Sánchez, F. Hernández, Qualitative screening of undesirable compounds from feeds to fish by liquid chromatography coupled to mass spectrometry, J. Agric. Food Chem. 61 (2013) 2077-2087.

[15] O. Malato, A. Lozano, M. Mezcua, A. Agüera, A.R. Fernandez-Alba, Benefits and pitfalls of the application of screening methods for the analysis of pesticide residues in fruits and vegetables, J. Chromatogr. A 1218 (2011) 7615-7626.

[16] SANCO, Commission of the European Communities (2013). **Document nº SANCO/12571/2013.** *Method validation and quality control procedures for pesticide residues analysis in food and feed.*

[17] V. Leendert, H.V. Langenhove, K. Demeestere, Trends in liquid chromatography coupled to high-resolution mass spectrometry for multi-residue analysis of organic micropollutants in aquatic environments, TrAC-Trends Anal. Chem. 67 (2015) 192-208.

[18] C. Boix, M. Ibáñez, J. V. Sancho, N. León, V. Yusá, F. Hernández, Qualitative screening of 116 veterinary drugs in feed by liquid chromatography-high resolution mass spectrometry: Potential application to quantitative analysis, Food Chem. 160 (2014) 313-320.

[19] R.J.B. Peters, Y.J. Bolck, P. Rutgers, A.A. Stolker, M.W. Nielen, Multi-residue screening of veterinary drugs in egg, fish and meat using high-resolution liquid chromatography accurate mass time-of-flight mass spectrometry, J. Chromatogr. A 1216 (2009) 8206-8216.

[20] Z. Dzuman, M. Zachariasova, Z. Veprikova, M. Godula, J. Hajslova, Multi-analyte high performance liquid chromatography coupled to high resolution tandem mass spectrometry method for control of pesticide residues, mycotoxins, and pyrrolizidine alkaloids, Anal. Chim. Acta 863 (2015) 29-40.

[21] G. Martínez-Domínguez, R. Romero-González, A. G. Frenich, Multi-class methodology to determine pesticides and mycotoxins in green tea and royal jelly supplements by liquid chromatography coupled to Orbitrap high resolution mass spectrometry, Food Chem. 197 (2016) 907-915.

[22] J.S. Munaretto, G. Ferronato, L.C. Ribeiro, M.L. Martins, M.B. Adaime, R. Zanella, Development of a multiresidue method for the determination of endocrine disrupters in fish fillet using gas chromatography-triple quadrupole tandem mass spectrometry, Talanta 116 (2013) 827-834.

[23] J.F. Facco, M.L. Martins, G. Bernardi, O.D. Prestes, M.B. Adaime, R.Zanella, Optimization and validation of a multiresidue method for pesticide determination in maize using gas chromatography coupled to tandem mass spectrometry, Anal. Methods 7 (2015) 359-365.

[24] P. Parrilla Vázquez, A. Lozano, S. Uclés, M.M. Gómez Ramos, A.R. Fernández-Alba, A sensitive and efficient method for routine pesticide multiresidue analysis in bee pollen samples using gas and liquid chromatography coupled to tandem mass spectrometry, J. Chromatogr. A 1426 (2015) 161-173.

[25] F.J. Schenck, P. Callery, P.M. Gannett, J.R. Daft, S.J. Lehotay, Comparison of magnesium sulfate and sodium sulfate for removal of water from pesticides extracts of foods, J. AOAC Int. 85 (2002) 1177-1180.

[26] A. Kaufmann, P. Butcher, K. Maden, S. Walker, M. Widmer, Multi-residue quantification of veterinary drugs in milk with a novel extraction and cleanup

technique: Salting out supported liquid extraction (SOSLE), Anal. Chim. Acta 820 (2014) 56-68.

[27] F. Gosetti, E. Mazzucco, D. Zampieri, M.C. Gennaro, Signal suppression/ enhancement in high-performance liquid chromatography tandem mass spectrometry, J. Chromatogr. A 1217 (2010) 3929-3937.

[28] A.R. Fernández-Alba, J.F. García-Reyes, Large-scale multi-residue methods for pesticides and their degradation products in food by advanced LC-MS, TrAC-Trends Anal. Chem. 27 (2008) 973-990.

[29] IUPAC, International Union of Pure and Applied Chemistry. http://sitem.herts.ac.uk/aeru/iupac/i ndex.htm, 2015 (accessed 01/14/16).

[30] H.R. Norli, A. Christiansen, E. Deribe, Application of QuEChERS method for extraction of selected persistent organic pollutants in fish tissue and analysis by gas chromatography mass spectrometry, J. Chromatogr. A 1218 (2011) 7234-7241.

[31] M. Kemmerich, G. Bernardi, M.B. Adaime, R. Zanella, O.D. Prestes, A simple and efficient method for imidazolinone herbicides determination in soil by ultra-high performance liquid chromatography-tandem mass spectrometry, J. Chromatogr. A 1412 (2015) 82-89.

[32] C. Ferrer, A. Lozano, A.A. Agüera, J. Girón, A.R. Fernández-Alba, Overcoming matrix effects using the dilution approach in multiresidue methods for fruits and vegetables, J. Chromatogr. A 1218 (2011) 7634-7639.

5 ARTIGO 2 – QUANTITATIVE MULTICLASS PESTICIDE RESIDUES ANALYSIS IN APPLE, PEAR AND GRAPE BY MODIFIED QUECHERS AND LIQUID CHROMATOGRAPHY COUPLED TO HIGH RESOLUTION MASS SPECTROMETRY

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Abstract

Most of the analytical methods currently applied in food control laboratories are focused on the determination of target compounds using liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS), which is an effective technique but low-resolution mass spectrometry is limited. Thus, a method for determination of pesticide multiresidue in fruits (pear, apple, and grape) using modified QuEChERS method and liquid chromatography coupled to quadrupole-time of flight mass spectrometry (LC-QToF/MS) was developed and validated. The proposed method showed good linearity (r^2 >0.99) from 1 to 100 µg L^{-1}. Recovery for blank samples spiked at 0.01, 0.04 and 0.100 mg kg^{-1} were between 66 and 122% with relative standard deviation <28%. Limits of quantification for apple, pear and grape matrices were 0.01 mg kg^{-1} for 112, 120 and 118 compounds and 0.04 mg kg^{-1} for 22, 12 and 17 compounds, respectively, and average mass accuracy error 3.2 ppm. LC-QToF/MS detection using protonated molecular ion and/or adducts, and mass accuracy provided reliability for the method. The proposed method is effective for pesticide residues determination in apple, pear and grape, proving that high resolution mass spectrometry using full scan mode can be a powerful and reliable technique for quantification purposes, being able to attend maximum residue limits values set by different legislations.

Keywords: Pesticide residues, fruits, QuEChERS, LC-QToF/MS, HRMS

Introduction

Analysis of pesticide residues is of paramount importance for protection of human health as for trading and official control purposes. A great number of substances have been used in agriculture and regulatory guidelines set maximum residue limits (MRLs) in food to avoid contamination, and negative health effects [1,2]. In Brazil, the monitoring of pesticide residues in food is regulated by the Ministry of Agriculture, Livestock and Food Supply (MAPA) and by *Codex Alimentarius* in a worldwide view, in both cases minimum MRLs values of 0.01 mg kg^{-1} are established [3,4]. These low MRL values have promoted the development of more powerful and sensitive analytical methods to attend requirements in complex samples, such as food [5]. Thus, most of the analytical methods currently applied in food control laboratories are focus in identification of target compounds [6-10]. Jardim and Caldas [11] presented two Brazilian pesticide residue monitoring programs between 2001 and 2010, which a total of 13556 samples of 22 fruits and vegetables crops were analyzed. Pesticide residues were found in 48.3% of the samples and 13.2% presented some irregularity, mostly non-authorized active ingredient use and less than 3% of the samples showed residue levels above the MRL. In general, the scenario of pesticide residues in foods investigated within the Brazilian governmental monitoring programs is similar to what has been found in other countries [8,11].

Currently, liquid chromatography coupled to mass spectrometry represents the most flexible and effective (i.e. high sensitivity and selectivity) technique employed to determine chemical contaminants in many different food matrices [12-15]. These multiresidue methods are typically carried out using mass spectrometry with triple quadrupole (QqQ) in selected reaction monitoring (SRM) mode [15-19]. Although, LC-MS/MS provides adequate quantification performance and high efficiency for multiresidue analyses, low-resolution sequential mass spectrometry is sometimes limited for trace analysis in complex matrices due to the presence of several interferences, and false negatives can also occur due insufficient selectivity [15]. High resolution mass spectrometry (HRMS) instruments working in full scan mode show high specificity due to high mass accuracy and high mass resolution (resolving power), and they are able to provide greater reduction in chemical noise, thereby

enhancing the selectivity. However, HRMS instruments such as time-of-flight (ToF) or orbitrap have been applied mainly for structure elucidation or confirmation purposes [10,20]. Modern quadrupole time-of-flight (QToF) instruments performing MS/MS analysis generate spectra with better qualitative information, providing enough sensitivity to quantify target contaminants at concentrations below their maximum residue levels [1,21].

Modern residue monitoring programs are based in multiclass/multiresidue pesticide determination and due to it the introduction of new, faster and effective analytical approached are essential for laboratories to improve analytical quality and laboratory efficiency. QuEChERS that stands for quick, easy, cheap, effective, rugged, and safe is a sample preparation method proposed by Anastassiades et al. [22] and it is widely used for determination of pesticide residues in food samples. The modified QuEChERS method described by Lehotay et al. [23] involves an extraction step with acetonitrile containing 1% (v/v) acetic acid and a liquid-liquid partitioning with anhydrous magnesium sulfate ($MgSO_4$) and sodium acetate (NaAc). Combining multiresidue sample preparation method, like QuEChERS, and chromatographic analysis coupled to high resolution mass spectrometry operating in full scan mode gives a less specific procedure in order to comprise a great number of compounds from different chemical classes [13,24].

Ferrer et al. [25] developed a multiresidue method employing LC-ToF/MS for quantitative determination of 15 pesticide residues in different fruit (orange, lemon, apple, and melon) and vegetables (pepper, broccoli, and tomato). Samples were extracted with ethyl acetate and sodium hydroxide, and filtrated in layer of anhydrous sodium sulfate, extracts were evaporated and redissolved in methanol. Authors obtained mass accuracy errors lower than 2 ppm at different concentration levels from 0.01 to 0.5 mg kg^{-1}. According to the authors, this study is a valuable indicator of the potential of LC-ToF/MS for quantitative multiresidue analysis of pesticides in vegetables and fruits. In 2010, Grimalt et al. [26] developed a method for the determination of 11 pesticides in 7 different matrices of fruits and vegetables using UHPLC with QqQ, ToF and QToF. ToF analyzer proved to be an attractive analytical tool for rapid detection and reliable identification of a large number of pesticides thanks to the full spectrum acquisition at accurate mass with satisfactory sensitivity. The last generation of ToF and QToF analyzers present recent developments that improves their capabilities. For example, faster acquisition speed allows lower scan

times without affect the sensitivity. The use of reference sprayers, such as lock-spray, improves robustness in mass accuracy measurement along the time. One of the most interesting advances has been extending the dynamic linear range in ToF analyzers that use time-to-digital converter (TDC) detectors thanks to the so-called dynamic range enhancement (DRE) [26].

Currently more than 900 pesticides are used worldwide, both legally and illegally. Most of these pesticides have MRL values for each crop to protect consumers. Pesticide residues need to be monitored as part of the quality control of food, especially fruits and vegetables; thus, large-scale multiresidue methods covering hundreds of pesticides are needed for quality control. However, the ability to analyze a large number of pesticides in a single analysis is a challenging problem for chromatography and mass spectrometry [13,16]. Thereby, the aim of this work was to optimize and to validate a procedure for quantitative determination of 152 pesticide residues in apple, pear and grape samples using modified QuEChERS acetate method for extraction and analysis by liquid chromatography coupled to quadrupole-time of flight mass spectrometry (LC-QToF/MS) operating in full scan mode. These matrices of fruits were chosen due to their high economical and nutritional importance, their lower complexity when compared to other fresh food (e.g. with high fat content) [27] and, besides this, because processed products of these fruits can present pesticide residues, as it can happen with grapes, grape juice, jam and wine [28,29].

Experimental

Chemicals and apparatus

Analytical standards used in this study are listed in Table 1, and they were acquired from Dr. Ehrenstorfer (Augsburg, Germany), within high purity (94-99.5%). Acetonitrile LC-MS grade and NaAc were purchased from Mallinckrodt (St. Louis, MO, USA), methanol LC-MS grade, glacial acetic acid 100% and anhydrous $MgSO_4$ were obtained from J.T. Baker (Center Valley, PA, USA). Primary secondary amine (PSA) sorbent with 40 µm of particle size was purchased from Agilent Technologies (Santa Clara, CA, USA), as well as nylon filters of 13 mm of diameter and 0.2 µm of

porosity. Ultrapure water was obtained with a Milli-Q Direct UV3® system Millipore (Billerica, MA, USA). Ammonium formate ≥99.0% and formic acid ≥98.0% were acquired from Sigma Aldrich (St. Louis, MO, USA).

Sample preparation

Extraction procedure was performed using modified QuEChERS acetate method [23] that consisted in extracting 10 g of homogenized sample (apple, pear and grape) using 10 mL of acetonitrile containing 1% acetic acid (v/v), and hand shaking. Partition step was performed adding $MgSO_4$ and NaAc with consecutive hand shaking, and centrifugation. Clean-up step was performed by dispersive solid phase extraction using PSA and $MgSO_4$. The cleaned extract was filtered and diluted 2 times with the mixture of mobile phase 1:1 (v/v) before analysis.

LC-QToF/MS conditions

Quantification of the studied pesticides was performed using an LC-QToF/MS instrument from Agilent Technologies (Santa Clara, CA, USA); a LC system model 1260 coupled with the accurate mass QToF/MS model 6530, and a nitrogen generator system Genius NM32LA from Peak Scientific (Billerica, MA, USA). An electrospray ionization source with jet stream technology operated in the positive mode was used. The MS condition were as follow: capillary voltage 3500 V, drying gas (nitrogen) 10 L min^{-1} (300 ºC), sheath gas flow 10 L min^{-1} (350 ºC), nozzle voltage 1000 V, fragmentor 175 V and skimmer 65 V. Reference masses were *m/z* 121.0509 (purine, $C_5H_5N_4$) and 922.0098 (hexakis (1H, 1H, 3H-tetrafluoropropoxy) phosphazine, $C_{18}H_{19}O_6N_3P_3F_{24}$), this solution was used in constant flow rate during the run. Pesticides chosen for this study show monoisotopic mass between 190.0433 and 732.4681, and QToF/MS was operated in 2 GHz mode, extended dynamic range, low mass range (until *m/z* 1700) and acquisition rate of 1 spectra/sec.

Quantitative determination was developed using Quantitative MassHunter B.05.01 software, it was set up a method for 152 compounds with their respective protonated molecular ion $[M+H]^+$, with its respective ammonium $[M+NH_4]^+$, or sodium $[M+Na]^+$ adduct for identification and quantification in the full scan mode, as showed in Table 1.

Chromatographic conditions employed were: column Zorbax Eclipse Plus C_{18} (2.1 x 100 mm; 1.8 µm particle size) maintained at 35 ºC, with injection volume of 5 µL. Mobile phase used was (A) water:methanol 98:2 (v/v) and (B) methanol, both containing 0.1% (v/v) of formic acid and 5 mmoL L^{-1} of ammonium formate. The gradient program started at 20% B (holding 0.25 min), follow by increasing B to 80% in 4 min (holding 5 min) and decreasing to initial condition in 10 min (holding 5 min) at a flow rate of 300 µL min^{-1}, and a run time of 15 min.

Method validation

Method validation was performed according to the European SANCO/12571/2013 guidelines [30]. Before extraction blank samples were spiked at 0.01, 0.04 and 0.100 mg kg^{-1} by adding a mixture of 152 pesticides. Figures of merit evaluated for method validation were linearity of the analytical curve, accuracy (recovery test), precision, limits of detection (LOD) and quantification (LOQ) and matrix effect (ME). Linearity of the analytical curves was evaluated using coefficient of determination (r^2) from matrix-matched curves in six concentration levels between 1-100 µg L^{-1}. Accuracy, in terms of recovery, and precision through the relative standard deviation (RSD) of apple, pear, and grape matrices were measured using 3 replicates of each spiked level. LOQ was determined as the lowest concentration level spiked that presented pre-defined acceptance criteria of recovery from 70 to 120% and RSD <20% [30]. Therewith, LOD was calculated as being the LOQ divided by 3.33. Matrix effect was calculated employing the slope of the analytical curves prepared in the solvent and in the blank of the matrix as: ME%=[(slope of matrix-matched curve/slope of analyte in solvent curve)-1]x100. When the change in the chromatographic response is above 20%, the matrix effect is considered to have influence on the analysis [31].

Results and Discussion

Chromatographic conditions

Information such as compound name, exact mass and elemental composition was inserted in a Microsoft® Excel spreadsheet, then the exact mass of each

compound was calculated and the list of 152 compounds and exact masses (Table 1) was saved as comma separated values file (.csv), which was used by MassHunter software as library. The *m/z* was calculated through the monoisotopic mass of each compound plus the mass of adduct formed (NH_4^+ or Na^+) or protonated molecular ion (H^+) minus the mass of one electron (0.00055 Da).

None of the analyzed pesticides were found in the blank sample extracts after initial screening. After this, the next step was to add a known concentration of pesticide standards to obtain retention time, and to measure the experimental mass. An initial evaluation was performed to observe the compounds that would present signal in the conditions tested.

LC-QToF/MS analyses were performed in a short run of 15 min for each sample and for quantification proposes external calibration using matrix-matched was employed to assure the best reliable results for each sample. The 152 pesticides studied were identified and quantified using full scan mode and positive ionization. This study was focused on quantification of pesticide residues in fruits using full scan mode, and the MS/MS scan mode has not been evaluated. Figure 1 presents a LC-QToF/MS chromatogram of a mixture of all compounds spiked in each matrix extract. The intensity differences among pear, apple and grape were due to matrix effects.

Especial care is required when dealing with complex matrices to avoid misidentification. For example, during method optimization through the monoisotopic mass ion in the library employed for first identification, the same *m/z* was filtered for two different peaks with distinguished retention time but similar mass spectrum, as showed in Figure 1B. It was possible to conclude that the peak in 10.55 min corresponds to oleamide, a common interference in high resolution mass spectrometry. Both compounds dodemorph and oleamide show the same molecular formula $C_{18}H_{35}NO$. Oleamide is a split agent used in polyethylene films and some labware, like polyprolylene tubes [32].

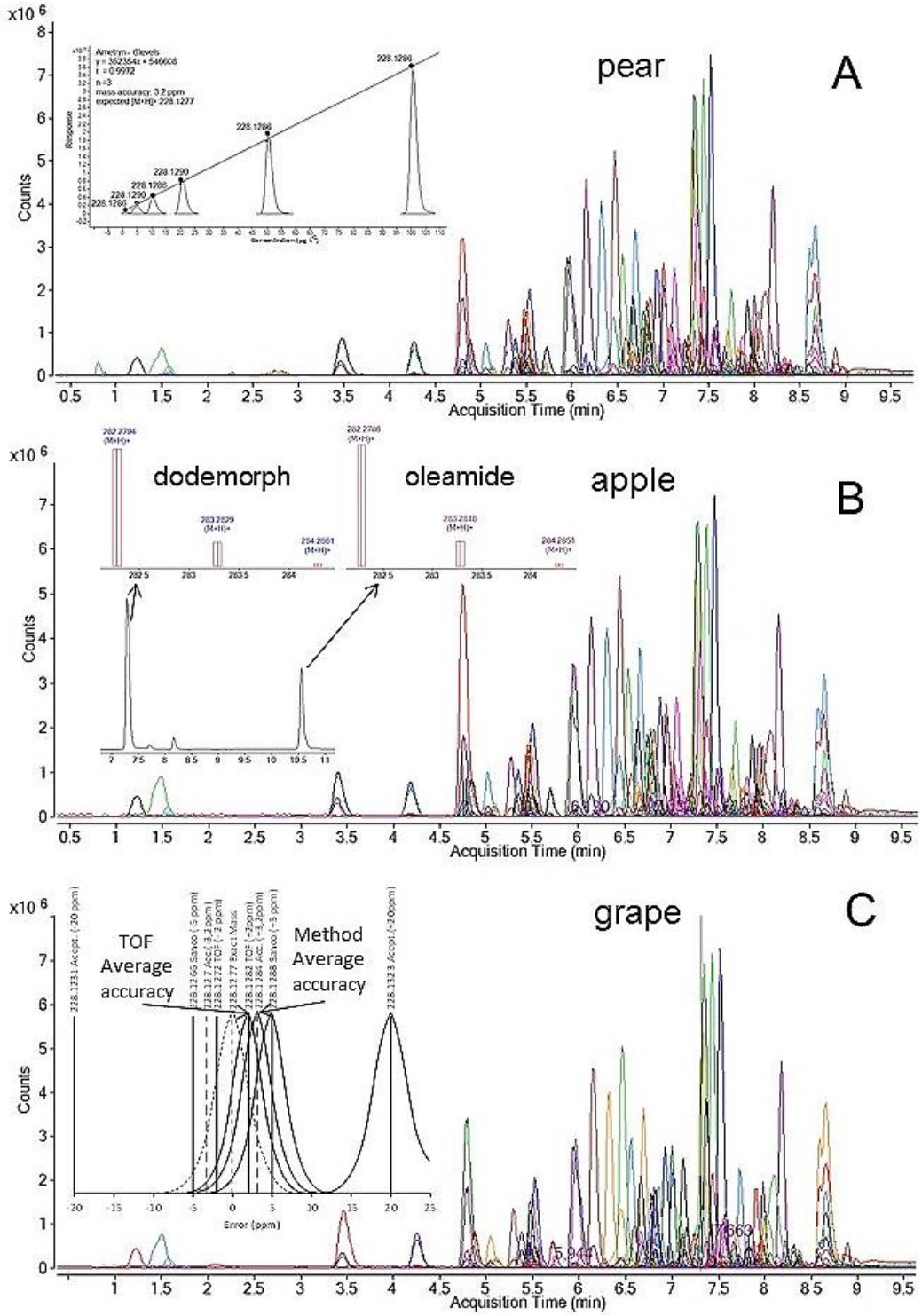

Figure 1. LC-QToF/MS chromatograms from matrix blank extracts spiked with 152 pesticides at 100 µg kg^{-1} for (A) pear, (B) apple and (C) grape. Details in (A) linearity of the response for amethryn, in (B) mass spectrum of dodemorph and its interference with the same molecular formula and similar mass spectrum and in (C) mass accuracy ranges for screening (20 ppm), recommended by SANCO (5 ppm), average for all compounds (3.22 ppm) and for ToF instrument (2 ppm).

Method Validation

The linearity of the analytical curve was studied using matrix-matched standards and the response function was found to be linear with coefficient of determination (r^2) values higher than 0.99. Blank samples were confirmed by their analysis using the current extraction procedure, afterwards these samples were spiked at the concentration levels of 0.01, 0.04 and 0.100 mg kg^{-1} (n= 3) for recovery and precision essays. For some compounds, the higher concentration level was 50 µg L^{-1} due to limited dynamic range of the QToF/MS, it occurs due to the saturation of the detector after a certain quantity of ions is measured, and when it happens a dilution of the sample can be performed to overcome this issue. This effect can occur when many ions arise the detector, especially when using dynamic range of the TDC [33]. An example of the response for amethryn in pear is presented in Figure 1A, which shows the excellent mass accuracy during all the linear range (demonstrated by the numbers presented in the top of the peaks); obtained linearity presented r^2= 0.9972, values of mass accuracy from *m/z* 228.1283 to 228.1290, mass error of 3.2 ppm, and calculated *m/z* 228.1277 $[M+H]^+$.

The linear range in matrix extract, method LOQ, averages of accuracy and precision, and mass accuracy results for each compound are summarized in Table 1 grouped by type of ion formed. Method accuracy and precision were evaluated for 152 pesticides spiked at concentration levels of 0.01, 0.04 and 0.100 mg kg^{-1} in apple, pear and grape. LOQ for apple, pear and grape was 0.01 mg kg^{-1} for 112, 120 and 118 compounds, and 0.04 mg kg^{-1} for 22, 12 and 17 compounds, respectively. Values of LOD were between 0.003 and 0.012 mg kg^{-1}. These limits were appropriate since they attend national [3] and international [4] maximum residues limits (MRLs).

Table 1. Retention time, molecular formula, monoisotopic mass, *m/z* calculated and validation results (linear range in the respective matrix, LOQ, recoveries, precision and mass accuracy) obtained for 152 compounds with the proposed method.

Pesticides	RT (min)	Molecular formula	Monoisotopic mass	*m/z* calculated	Apple				Pear				Grape			
					Linear range µg L^{-1}	LOQ_m mg kg^{-1}	Rec±RSD %	Mass Acc. ppm	Linear range µg L^{-1}	LOQ_m mg kg^{-1}	Rec±RSD %	Mass Acc. ppm	Linear range µg L^{-1}	LOQ_m mg kg^{-1}	Rec±RSD %	Mass Acc. ppm
$[M+H]^+$ / 1-100 µg L^{-1} [a]																
Allethrin	9.0	$C_{19}H_{26}O_3$	302.1882	303.1955	5 - 100	0.01	99 ± 6	3.3	1 - 100	0.01	97 ± 10	2.0	1 - 100	0.01	96 ± 11	0.8
Ametryn	7.4	$C_9H_{17}N_5S$	227.1205	228.1277	2 - 100	0.01	95 ± 1	1.8	1 - 100	0.01	94 ± 3	1.4	1 - 100	0.01	93 ± 3	0.7
Azamethiphos	6.6	$C_9H_{10}N_2O_5PSCl$	323.9737	324.9809	1 - 100	0.01	94 ± 3	1.5	1 - 100	0.01	92 ± 8	3.6	1 - 100	0.01	92 ± 4	1.5
Azimsulfurom	7.3	$C_{13}H_{16}N_{10}O_5S$	424.1026	425.1099	2 - 100	0.04	67 ± 1	0.6	1 - 100	0.01	66 ± 4	3.8	1 - 100	0.01	89 ± 5	6.0
Azinphos ethyl	7.9	$C_{12}H_{16}N_3O_3PS_2$	345.0371	346.0443	5 - 50	0.04	100 ± 3	9.9	5 - 50	0.01	87 ± 4	11.1	1 - 50	0.01	104 ± 7	10.4
Azoxystrobin	7.5	$C_{22}H_{17}N_3O_5$	403.1168	404.1241	1 - 100	0.01	97 ± 2	0.7	1 - 100	0.01	99 ± 3	0.2	1 - 100	0.01	95 ± 4	0.2
Benfuracarb	8.7	$C_{20}H_{30}N_2O_5S$	410.1875	411.1948	1 - 100	0.01	78 ± 2	2.4	1 - 100	0.01	76 ± 3	2.6	1 - 100	0.01	77 ± 13	3.5
Bitertanol	8.3	$C_{20}H_{23}N_3O_2$	337.1790	338.1863	-	-	-	-	1 - 100	-.	-	-	1 - 100	-	-	-
Boscalid	7.7	$C_{18}H_{12}N_2OCl_2$	342.0327	343.0399	2 - 100	0.01	95 ± 2	0.9	1 - 100	0.01	96 ± 5	2.5	1 - 100	0.01	94 ± 4	2.8
Carbofuran	6.8	$C_{12}H_{15}NO_3$	221.1052	222.1125	1 - 100	0.01	101 ± 2	0.3	1 - 100	0.01	96 ± 5	2.8	1 - 100	0.01	92 ± 3	0.4
Carbofuran-3-hidroxy	5.4	$C_{12}H_{15}NO_4$	237.1001	238.1074	1 - 100	0.01	96 ± 6	0.6	1 - 100	0.01	97 ± 11	3.9	1 - 100	0.01	84 ± 3	0.6
Carbophenothion	9.3	$C_{11}H_{16}O_2PS_3Cl$	341.9739	342.9811	1 - 100	0.01	93 ± 4	0.1	1 - 100	0.01	89 ± 14	0.3	1 - 50	0.01	81 ± 6	0.7
Carbosulfan	10.3	$C_{20}H_{32}N_2O_3S$	380.2134	381.2206	1 - 100	.-	-	-	1 - 100	.	-	-	1 - 100	-	-	-
Carboxin	7.0	$C_{12}H_{13}NO_2S$	235.0667	236.0740	1 - 100	0.01	91 ± 2	0.6	1 - 100	0.01	90 ± 2	0.6	1 - 100	0.01	84 ± 6	0.7
Carpropamid	8.2	$C_{15}H_{18}NOCl_3$	333.0454	334.0527	1 - 50	0.01	91 ± 9	12.2	1 - 100	0.01	88 ± 14	9.4	1 - 100	0.04	114 ± 8	8.8
Chlorfenvinphos	8.2	$C_{12}H_{14}O_4PCl_3$	357.9695	358.9768	1 - 100	0.01	96 ± 8	12.2	1 - 100	0.01	97 ± 15	9.2	1 - 100	0.01	105 ± 8	8.6
Clothianidin	5.0	$C_6H_8N_5O_2SCl$	249.0087	250.0160	1 - 100	0.01	97 ± 2	0.3	1 - 100	0.01	99 ± 2	1.5	1 - 100	0.01	98 ± 4	0.6
Cyanazine	6.5	$C_9H_{13}N_6Cl$	240.0890	241.0963	1 - 100	0.01	101 ± 1	1.9	1 - 100	0.01	105 ± 4	1.2	1 - 100	0.01	122 ± 3	1.7
Diazinon	8.3	$C_{12}H_{21}N_2O_3PS$	304.1010	305.1083	2 - 100	0.04	103 ± 11	4.8	2 - 100	0.01	84 ± 14	5.2	1 - 100	0.04	99 ± 9	3.5
Dicrotophos	4.7	$C_8H_{16}NO_5P$	237.0766	238.0839	1 - 100	0.01	93 ± 1	0.7	1 - 100	0.01	78 ± 15	0.3	1 - 100	0.01	94 ± 3	2.3
Difenoconazole	8.4	$C_{19}H_{17}N_3O_3Cl_2$	405.0647	406.0720	1 - 50	0.04	91 ± 8	2.7	1 - 100	0.01	111 ± 5	4.3	1 - 50	0.01	90 ± 7	5.7
Diflubenzuron	8.0	$C_{14}H_9N_2O_2ClF_2$	310.0321	311.0393	10 - 50	0.04	98 ± 21	10.7	5 - 50	0.01	100 ± 5	15.0	1 - 50	0.04	83 ± 7	12.3

Pesticides	RT (min)	Molecular formula	Monoisotopic mass	*m/z* calculated	Apple				Pear				Grape			
					Linear range µg L^{-1}	LOQ_m mg kg^{-1}	Rec±RSD %	Mass Acc. ppm	Linear range µg L^{-1}	LOQ_m mg kg^{-1}	Rec±RSD %	Mass Acc. ppm	Linear range µg L^{-1}	LOQ_m mg kg^{-1}	Rec±RSD %	Mass Acc. ppm
Dodemorph	7.3	$C_{18}H_{35}NO$	281.2719	282.2791	1 - 100	0.01	95 ± 3	0.9	1 - 100	0.01	90 ± 2	0.5	1 - 100	0.01	94 ± 3	0.6
Epoxiconazole	8.0	$C_{17}H_{13}N_3OClF$	329.0731	330.0804	1 - 50	0.01	95 ± 8	2.7	1 - 50	0.01	92 ± 5	2.3	1 - 50	0.01	99 ± 4	2.3
Ethion	9.0	$C_9H_{22}O_4P2S_4$	383.9876	384.9949	1 - 100	0.01	96 ± 3	1.0	1 - 100	0.01	88 ± 4	0.9	1 - 100	0.01	90 ± 5	0.8
Ethoprophos	8.0	$C_8H_{19}O_2PS_2$	242.0564	243.0637	1 - 50	0.01	95 ± 14	10.8	1 - 50	0.01	94 ± 3	12.2	1 - 50	0.01	98 ± 4	11.6
Ethoxysulfuron	7.8	$C_{15}H_{18}N_4O_7S$	398.0896	399.0969	1 - 100	-	-	-	1 - 100	-	-	-	1 - 100	-	-	-
Etrimfos	8.3	$C_{10}H_{17}N_2O_4PS$	292.0647	293.0719	1 - 100	0.01	92 ± 15	13.8	1 - 100	0.04	82 ± 20	7.8	1 - 100	0.04	99 ± 8	8.3
Fempropathrin	9.3	$C_{22}H_{23}NO_3$	349.1678	350.1751	5 - 100	.-	-	-	2 - 100	-	-	-	1 - 100	-	-	-
Fenazaquin	9.9	$C_{20}H_{22}N_2O$	306.1732	307.1805	1 - 100	0.01	91 ± 3	1.3	1 - 100	0.01	88 ± 4	0.3	1 - 100	0.01	88 ± 4	0.1
Fenpropimorph	7.5	$C_{20}H_{33}NO$	303.2562	304.2635	1 - 100	0.01	95 ± 2	0.4	1 - 100	0.01	91 ± 2	0.8	1 - 100	0.01	93 ± 4	0.5
Fenpyroximate (E)	9.5	$C_{24}H_{27}N_3O_4$	421.2002	422.2074	2 - 100	0.01	98 ± 2	4.3	1 - 100	0.01	92 ± 2	3.8	1 - 100	0.01	95 ± 3	3.7
Fenthion	8.2	$C_{10}H_{15}O_3PS_2$	278.0200	279.0273	1 - 100	0.04	97 ± 9	14.1	1 - 100	-	-	-	1 - 100	0.1	98 ± 12	9.6
Fenthion sulfoxide	6.9	$C_{10}H_{15}O_4PS_2$	294.0149	295.0222	1 - 100	0.01	95 ± 1	1.0	1 - 100	0.01	94 ± 2	1.3	1 - 100	0.01	100 ± 2	1.0
Fluazifop-p-buthyl	8.7	$C_{19}H_{20}NO_4F_3$	383.1344	384.1417	1 - 100	0.01	97 ± 5	0.8	1 - 100	0.01	85 ± 4	1.2	1 - 100	0.01	91 ± 5	0.9
Fluroxypyr	6.5	$C_7H_5N_2O_3Cl_2F$	253.9661	254.9734	2 – 100	-	-	-	1 - 100	-	-	-	1 - 100	-	-	-
Flusilazole	8.0	$C_{16}H_{15}N_3F_2Si$	315.1003	316.1076	10 - 50	0.04	98 ± 6	12.0	1 - 50	0.01	101 ± 7	15.0	1 - 50	0.01	84 ± 12	13.9
Flutolanil	7.7	$C_{17}H_{16}NO_2F_3$	323.1133	324.1206	1 - 50	0.01	98 ± 1	1.5	1 - 50	0.01	93 ± 2	1.7	1 - 100	0.01	94 ± 3	1.2
Fosthiazate	7.1	$C_9H_{18}NO_3PS_2$	283.0466	284.0538	1 - 100	0.01	96 ± 1	1.5	1 - 100	0.01	92 ± 1	2.3	1 - 100	0.01	96 ± 4	1.8
Furathiocarb	8.8	$C_{18}H_{26}N_2O_5S$	382.1562	383.1635	1 - 100	0.01	95 ± 3	0.1	1 - 100	0.01	88 ± 3	0.1	1 - 100	0.01	90 ± 3	0.0
Hexythiazox	9.1	$C_{17}H_{21}N_2O_2SCl$	352.1012	353.1085	2 - 100	0.01	93 ± 4	3.3	2 - 100	0.01	94 ± 6	3.9	1 - 100	0.01	91 ± 3	3.3
Imazalil	7.1	$C_{14}H_{14}N_2OCl_2$	296.0483	297.0556	1 - 100	0.01	99 ± 4	0.4	1 - 100	0.01	88 ± 7	0.3	1 - 100	0.01	92 ± 3	0.0
Imazapic	5.9	$C_{14}H_{17}N_3O_3$	275.1270	276.1343	1 - 100	-	-	-	1 - 100	-	-	-	1 - 100	-	-	-
Imazaquin	6.8	$C_{17}H_{17}N_3O_3$	311.1270	312.1343	1 - 100	-	-	-	1 - 100	-	-	-	1 - 100	-	-	-
Imazethapyr	6.5	$C_{15}H_{19}N_3O_3$	289.1426	290.1499	1 - 100	-	-	-	1 - 100	-	-	-	1 - 100	-	-	-
Imidacloprid	5.0	$C_9H_{10}N_5O_2Cl$	255.0523	256.0596	1 - 100	0.01	98 ± 4	0.0	1 - 100	0.01	95 ± 4	0.9	1 - 100	0.01	98 ± 3	0.1
Indoxacarb	8.4	$C_{22}H_{17}N_3O_7ClF_3$	527.0707	528.0780	1 - 100	0.01	99 ± 5	2.8	1 - 100	0.01	95 ± 6	3.5	1 - 100	0.01	99 ± 3	4.1
Iprovalicarb	7.9	$C_{18}H_{28}N_2O_3$	320.2100	321.2173	1 - 100	0.01	96 ± 3	6.4	1 - 100	0.01	95 ± 4	7.1	1 - 100	0.01	94 ± 4	6.1

Pesticides	RT (min)	Molecular formula	Monoisotopic mass	*m/z* calculated	Apple				Pear				Grape			
					Linear range µg L^{-1}	LOQ_m mg kg^{-1}	Rec±RSD %	Mass Acc. ppm	Linear range µg L^{-1}	LOQ_m mg kg^{-1}	Rec±RSD %	Mass Acc. ppm	Linear range µg L^{-1}	LOQ_m mg kg^{-1}	Rec±RSD %	Mass Acc. ppm
Isoxaflutole	7.0	$C_{15}H_{12}NO_4SF_3$	359.0439	360.0512	1 - 100	-	-	-	1 - 100	-	-	-	1 - 100	0.01	107 ±11	3.2
Linuron	7.6	$C_9H_{10}N_2O_2Cl_2$	248.0119	249.0192	1 - 50	0.01	89 ± 10	6.6	1 - 100	0.01	93 ± 13	4.2	1 - 50	0.04	89 ± 11	4.2
Mephosfolan	6.6	$C_8H_{16}NO_3PS_2$	269.0309	270.0382	1 - 100	0.01	93 ± 4	0.9	1 - 100	0.01	83 ± 12	3.5	1 - 100	0.01	88 ± 4	1.1
Metalaxyl	7.3	$C_{15}H_{21}NO_4$	279.1471	280.1543	1 - 50	0.01	97 ± 2	2.7	1 - 50	0.01	96 ± 6	3.2	1 - 100	0.01	97 ± 3	2.9
Metconazole	8.4	$C_{17}H_{22}N_3OCl$	319.1451	320.1524	1 - 50	-	-	-	1 - 100	-	-	-	1 - 100	-	-	-
Methidathion	7.4	$C_6H_{11}N_2O_4PS_3$	301.9619	302.9691	1 - 50	0.01	86 ± 4	2.3	1 - 50	0.01	93 ± 8	1.6	1 - 100	0.01	95 ± 5	1.3
Methiocarb sulfone	4.8	$C_{11}H_{15}NO_4S$	257.0722	258.0795	1 - 100	0.01	94 ± 2	0.2	1 - 100	0.01	90 ± 1	0.4	1 - 100	0.01	94 ± 3	0.5
Methiocarb sulfoxide	5.2	$C_{11}H_{15}NO_3S$	241.0773	242.0845	1 - 100	0.01	93 ± 2	0.6	1 - 100	0.01	91 ± 4	3.5	1 - 100	0.01	94 ± 3	0.4
Metobromuron	7.2	$C_9H_{11}N_2O_2Br$	258.0004	259.0077	1 - 100	0.01	92 ± 3	1.6	1 - 100	0.01	93 ± 6	1.5	1 - 100	0.01	96 ± 4	1.2
Metoxuron	6.3	$C_{10}H_{13}N_2O_2Cl$	228.0666	229.0738	1 - 100	0.01	95 ± 3	2.8	1 - 100	0.01	93 ± 4	3.9	1 - 100	0.01	95 ± 3	1.0
Metsulfuron methyl	6.8	$C_{14}H_{15}N_5O_6S$	381.0743	382.0816	1 - 100	-	-	-	1 - 100	-	-	-	1 - 100	0.01	67 ± 6	0.7
Monensin	10.5	$C_{36}H_{61}O_{11}$	692.4112	693.4184	1 - 100	0.01	93 ± 3	2.5	1 - 100	0.01	90 ± 4	3.3	1 - 100	0.01	91 ± 5	3.2
Monolinuron	7.1	$C_9H_{11}N_2O_2Cl$	214.0509	215.0582	1 - 50	0.01	99 ± 3	1.6	1 - 50	0.01	95 ± 5	1.6	1 - 100	0.01	96 ± 3	1.1
Nuarimol	7.6	$C_{17}H_{12}N_2OClF$	314.0622	315.0695	1 - 100	0.01	85 ± 24	2.4	1 - 100	0.01	91 ± 6	10.5	1 - 100	0.01	99 ± 5	8.9
Omethoate	1.4	$C_5H_{12}NO_4PS$	213.0225	214.0297	1 - 100	0.01	95 ± 3	1.3	1 - 50	-	-	-	1 - 100	0.1	78 ± 3	2.0
Phosalone	8.4	$C_{12}H_{15}NO_4PS_2Cl$	366.9869	367.9941	1 - 100	-	-	-	1 - 100	-	-	-	1 - 50	-	-	-
Phosmet	7.5	$C_{11}H_{12}NO_4PS_2$	316.9945	318.0018	1 - 50	0.01	95 ± 2	2.2	1 - 50	0.01	92 ± 4	3.8	1 - 50	0.01	95 ± 4	3.7
Picoxystrobin	8.0	$C_{18}H_{16}NO_4F_3$	367.1031	368.1104	2 - 50	0.01	95 ± 7	10.3	1 - 50	0.01	90 ± 6	10.0	1 - 50	0.01	92 ± 6	9.6
Piridate	10.2	$C_{19}H_{23}N_2O_2SCl$	378.1169	379.1242	1 - 100	0.01	81 ± 3	1.3	1 - 100	0.01	81 ± 3	0.7	1 - 100	0.01	82 ± 4	0.0
Prochloraz	8.3	$C_{15}H_{16}N_3O_2Cl_3$	375.0308	376.0381	1 - 100	0.01	96 ± 17	11.9	1 - 100	0.01	86 ± 20	8.4	1 - 100	0.04	100 ± 19	7.3
Procymidone	7.9	$C_{13}H_{11}NO_2Cl_2$	283.0167	284.0240	1 - 50	0.04	97 ± 10	8.6	1 - 50	0.01	93 ± 10	8.6	1 - 50	0.01	92 ± 9	7.4
Profenofos	8.8	$C_{11}H_{15}O_3PSClBr$	371.9351	372.9424	1 - 100	0.01	95 ± 2	0.4	1 - 100	0.01	87 ± 2	0.4	1 - 100	0.01	87 ± 4	0.4
Profoxydim	8.6	$C_{24}H_{32}NO_4SCl$	465.1741	466.1813	1 - 100	0.01	95 ± 3	0.2	1 - 100	0.01	87 ± 5	0.3	1 - 100	0.01	87 ± 3	0.4
Propiconazole	8.3	$C_{15}H_{17}N_3O_2Cl_2$	341.0698	342.0771	1 - 100	0.04	98 ± 10	12.0	1 - 100	0.01	88 ± 14	10.7	1 - 100	0.01	103 ±15	10.3
Propyzamide	7.8	$C_{12}H_{11}NOCl_2$	255.0218	256.0290	1 - 50	0.01	98 ± 4	8.3	1 - 50	0.01	100 ± 5	7.6	1 - 50	0.01	94 ± 6	7.7
Prothiofos	9.9	$C_{11}H_{15}O_2PS_2Cl_2$	343.9628	344.9701	1 - 100	0.01	95 ± 9	0.7	1 - 100	0.01	90 ± 12	0.2	1 - 100	0.04	97 ± 5	0.1

Pesticides	RT (min)	Molecular formula	Monoisotopic mass	*m/z* calculated	Apple				Pear				Grape			
					Linear range µg L^{-1}	LOQ_m mg kg^{-1}	Rec±RSD %	Mass Acc. ppm	Linear range µg L^{-1}	LOQ_m mg kg^{-1}	Rec±RSD %	Mass Acc. ppm	Linear range µg L^{-1}	LOQ_m mg kg^{-1}	Rec±RSD %	Mass Acc. ppm
Pyraclostrobin	8.3	$C_{19}H_{18}N_3O_4Cl$	387.0986	388.1059	1 - 100	0.01	107 ±8	6.7	1 - 100	0.01	89 ± 14	5.1	1 - 100	0.01	95 ± 10	5.4
Pyrazosulfuron ethyl	7.7	$C_{14}H_{18}N_6O_7S$	414.0958	415.1030	1 - 100	0.01	66 ± 3	5.9	1 - 100	0.01	66 ± 6	5.3	1 - 100	0.01	71 ± 5	5.2
Pyridaben	9.8	$C_{19}H_{25}N_2OSCl$	364.1376	365.1449	1 - 100	0.01	94 ± 1	0.6	1 - 100	0.01	91 ± 3	0.4	1 - 100	0.01	91 ± 2	0.4
Pyridaphenthion	7.8	$C_{14}H_{17}N_2O_4PS$	340.0647	341.0719	1 - 50	0.01	98 ± 2	3.7	1 - 50	0.01	97 ± 4	3.3	1 - 100	0.01	94 ± 4	2.9
Pyrimethanil	7.5	$C_{12}H_{13}N_3$	199.1109	200.1182	1 - 100	0.01	90 ± 4	0.5	1 - 100	0.01	90 ± 4	3.5	1 - 100	0.01	90 ± 6	3.4
Pyrimiphos ethyl	8.9	$C_{13}H_{24}N_3O_3PS$	333.1276	334.1349	1 - 100	0.01	95 ± 3	0.3	1 - 100	0.01	88 ± 4	0.0	1 - 100	0.01	89 ± 4	0.7
Pyrimiphos methyl	8.3	$C_{11}H_{20}N_3O_3PS$	305.0963	306.1036	1 - 100	0.01	94 ± 8	5.3	1 - 100	0.01	94 ± 3	11.8	1 - 100	0.01	83 ± 7	13.0
Quinclorac	6.1	$C_{10}H_5NO_2Cl_2$	240.9697	241.9770	1 - 100	-	-	-	1 - 100	-	-	-	1 - 100	-	-	-
Quinoxyfen	9.1	$C_{15}H_8NOCl_2F$	306.9967	308.0040	1 - 100	0.01	90 ± 4	1.5	1 - 100	0.01	93 ± 4	1.6	1 - 100	0.01	90 ± 3	1.5
Spinosad	8.2	$C_{41}H_{65}NO_{10}$	731.4608	732.4681	1 - 100	0.01	93 ± 2	0.5	1 - 100	0.01	91 ± 1	0.0	1 - 100	0.01	95 ± 4	0.3
Tebufenpyrad	8.8	$C_{18}H_{24}N_3OCl$	333.1608	334.1681	2 - 100	0.01	99 ± 3	5.9	1 - 100	0.01	93 ± 2	2.9	1 - 100	0.01	94 ± 6	1.5
Terbuthylazine	7.7	$C_9H_{16}N_5Cl$	229.1094	230.1167	1 - 50	0.01	96 ± 3	2.4	1 - 100	0.01	93 ± 3	2.1	1 - 50	0.01	98 ± 3	2.5
Tetraconazole	7.8	$C_{13}H_{11}N_3OCl_2F_4$	371.0215	372.0288	1 - 50	0.01	97 ± 3	7.8	1 - 50	0.01	95 ± 3	7.3	1 - 100	0.01	97 ± 3	6.8
Thiabendazole	4.7	$C_{10}H_7N_3S$	201.0361	202.0433	20 - 100	-	-	-	1 - 100	0.01	80 ± 4	1.4	1 - 100	0.01	86 ± 2	0.7
Thiacloprid	5.9	$C_{10}H_9N_4SCl$	252.0236	253.0309	1 - 100	0.01	96 ± 3	1.5	1 - 100	0.01	96 ± 3	3.2	1 - 100	0.01	99 ± 3	2.1
Thiamethoxam	3.2	$C_8H_{10}N_5O_3SCl$	291.0193	292.0266	1 - 100	0.01	95 ± 2	0.1	1 - 100	0.01	88 ± 7	0.0	1 - 100	0.01	96 ± 4	0.2
Thifensulfuron methyl	6.7	$C_{12}H_{13}N_5O_6S_2$	387.0307	388.0380	1 - 100	-	-	-	1 - 100	-	-	-	1 - 100	-	-	-
Thiodicarb	7.0	$C_{10}H_{18}N_4O_4S_3$	354.0490	355.0563	1 - 100	0.01	96 ± 2	1.8	1 - 100	0.01	94 ± 2	1.8	1 - 100	0.01	96 ± 2	1.7
Tolclofos methyl	8.4	$C_9H_{11}O_3PSCl_2$	299.9544	300.9616	2 - 50	0.04	88 ± 11	7.6	5 - 100	0.04	89 ± 15	12.2	2 - 100	0.04	81 ± 13	14.0
Triasulfuron	6.6	$C_{14}H_{16}N_5O_5SCl$	401.0561	402.0633	1 - 100	0.01	83 ± 4	1.0	1 - 100	0.01	82 ± 7	0.8	1 - 100	0.01	84 ± 7	0.9
Trichlorphon	5.3	$C_4H_8O_4PCl_3$	255.9226	256.9299	1 - 100	0.01	91 ± 3	0.9	1 - 100	0.01	92 ± 5	4.1	1 - 100	0.01	96 ± 3	1.4
Tricyclazole	6.1	$C_9H_7N_3S$	189.0361	190.0433	1 - 100	0.01	93 ± 3	2.9	1 - 100	0.01	86 ± 6	4.2	1 - 100	0.01	90 ± 5	2.4
Triflumuron	8.3	$C_{15}H_{10}N_2O_3ClF_3$	358.0332	359.0405	1 - 100	0.04	102 ± 17	5.3	1 - 50	0.01	88 ± 26	4.9	1 - 100	0.04	119 ± 15	3.6
Vamidothion	5.4	$C_8H_{18}NO_4PS_2$	287.0415	288.0488	1 - 100	0.01	93 ± 2	1.8	1 - 100	0.01	91 ± 2	4.5	1 - 50	0.01	92 ± 2	1.8
Vinclozolin	7.7	$C_{12}H_9NO_3Cl_2$	284.9959	286.0032	1 - 50	0.01	99 ± 7	9.6	1 - 100	0.01	91 ± 7	6.9	1 - 100	0.01	93 ± 8	6.7

$[M+H]^+$ / 1-50 µg L^{-1} [a]

Pesticides	RT (min)	Molecular formula	Monoisotopic mass	*m/z* calculated	Apple				Pear				Grape			
					Linear range µg L^{-1}	LOQ_m mg kg^{-1}	Rec±RSD %	Mass Acc. ppm	Linear range µg L^{-1}	LOQ_m mg kg^{-1}	Rec±RSD %	Mass Acc. ppm	Linear range µg L^{-1}	LOQ_m mg kg^{-1}	Rec±RSD %	Mass Acc. ppm
Azaconazole	7.4	$C_{12}H_{11}N_3O_2Cl_2$	299.0228	300.0301	1 - 50	0.01	96 ± 2	1.9	1 - 50	0.01	98 ± 2	1.3	1 - 50	0.01	107 ± 3	0.9
Bupirimate	8.0	$C_{13}H_{24}N_4O_3S$	316.1569	317.1642	1 - 50	0.01	96 ± 5	8.2	1 - 50	0.01	97 ± 4	10.0	1 - 50	0.01	95 ± 3	9.4
Buprofezin	8.4	$C_{16}H_{23}N_3OS$	305.1562	306.1635	1 - 100	0.01	91 ± 2	0.4	1 - 100	0.01	91 ± 5	0.5	1 - 100	0.01	89 ± 4	0.6
Clomazone	7.5	$C_{12}H_{14}NO_2Cl$	239.0713	240.0786	1 - 50	0.01	96 ± 2	1.1	1 - 100	0.01	97 ± 4	2.8	1 - 50	0.01	93 ± 3	2.1
Diuron	7.4	$C_9H_{10}N_2OCl_2$	232.0170	233.0243	1 - 50	0.01	96 ± 2	0.5	1 - 50	0.01	93 ± 3	0.8	1 - 50	0.01	96 ± 3	1.3
Fenbuconazole	8.0	$C_{19}H_{17}N_4Cl$	336.1142	337.1215	5 - 100	0.01	113 ± 12	13.7	1 - 50	0.04	96 ± 10	8.4	1 - 50	0.04	114 ± 14	15.5
Fenhexamid	7.9	$C_{14}H_{17}NO_2Cl_2$	301.0636	302.0709	1 - 50	0.01	92 ± 2	11.9	1 - 50	0.01	86 ± 3	13.4	1 - 50	0.01	90 ± 5	14.0
Flufenacet	7.9	$C_{14}H_{13}N_3O_2SF_4$	363.0665	364.0737	1 - 50	0.01	99 ± 4	8.6	1 - 50	0.01	91 ± 2	6.8	1 - 50	0.01	96 ± 3	6.6
Isoxathion	8.3	$C_{13}H_{16}NO_4PS$	313.0538	314.0610	1 - 50	0.01	96 ± 8	5.3	1 - 50	0.01	93 ± 13	9.5	1 - 50	0.01	96 ± 9	9.4
Malathion	7.8	$C_{10}H_{19}O_6PS_2$	330.0361	331.0433	1 - 50	0.01	97 ± 3	3.8	1 - 50	0.01	99 ± 10	2.6	1 - 100	0.01	95 ± 5	0.1
Mepronil	7.8	$C_{17}H_{19}NO_2$	269.1416	270.1489	1 - 50	0.01	98 ± 2	7.2	1 - 50	0.01	94 ± 6	6.7	1 - 50	0.01	97 ± 4	6.3
Monocrotophos	4.1	$C_7H_{14}NO_5P$	223.061	224.0682	1 - 50	0.04	114 ± 13	1.2	1 - 50	-	-	-	1 - 100	0.01	92 ± 7	1.9
Oxadixyl	6.4	$C_{14}H_{18}N_2O_4$	278.1267	279.1339	1 - 50	0.01	96 ± 3	2.1	1 - 50	0.01	94 ± 4	3.4	1 - 100	0.01	96 ± 4	3.0
Paraoxon	7.2	$C_{10}H_{14}NO_6P$	275.0559	276.0632	1 - 100	0.01	89 ± 14	2.0	1 - 50	0.01	90 ± 10	2.4	1 - 100	0.01	106 ± 8	2.1
Pencycuron	8.4	$C_{19}H_{21}N_2OCl$	328.1342	329.1415	1 - 50	0.01	92 ± 12	1.5	1 - 100	0.01	110 ± 8	4.6	1 - 50	0.01	86 ± 10	6.0
Propanil	7.6	$C_9H_9NOCl_2$	217.0061	218.0134	1 - 100	0.01	98 ± 10	3.2	1 - 100	0.04	111 ± 7	1.2	1 - 50	0.01	96 ± 10	2.1
Pyrazophos	8.4	$C_{14}H_{20}N_3O_5PS$	373.0861	374.0934	1 - 50	0.01	94 ± 8	0.6	1 - 100	0.01	100 ± 6	1.2	1 - 50	0.01	89 ± 9	2.2
Pyrimicarbe	6.4	$C_{11}H_{18}N_4O_2$	238.143	239.1503	1 - 50	0.01	99 ± 1	2.8	1 - 50	0.01	93 ± 3	5.4	1 - 50	0.01	94 ± 6	2.7
Quinalphos	8.2	$C_{12}H_{15}N_2O_3PS$	298.0541	299.0614	2 - 50	0.01	96 ± 12	11.6	1 - 50	0.01	82 ± 14	14.8	1 - 50	0.01	95 ± 6	15.7
Simazine	6.8	$C_7H_{12}N_5Cl$	201.0781	202.0854	1 - 50	0.01	95 ± 3	0.8	1 - 50	0.01	95 ± 4	2.8	1 - 50	0.01	99 ± 4	1.2
Thiobencarb	8.4	$C_{12}H_{16}NOSCl$	257.0641	258.0714	1 - 50	0.04	92 ± 10	2.0	1 - 100	0.01	111 ± 5	8.5	1 - 50	0.01	78 ± 14	11.3
Thiophanate methyl	6.7	$C_{12}H_{14}N_4O_4S_2$	342.0456	343.0529	1 - 50	0.01	83 ± 5	1.4	1 - 50	0.04	76 ± 2	3.0	1 - 50	-	-	-
Triazophos	7.8	$C_{12}H_{16}N_3O_3PS$	313.0650	314.0723	1 - 50	0.01	97 ± 2	7.5	1 - 50	0.01	95 ± 3	8.3	1 - 50	0.01	93 ± 3	7.4
Tridemorph	8.1	$C_{19}H_{39}NO$	297.3032	298.3104	1 - 50	0.01	113 ± 2	6.2	1 - 50	0.01	86 ± 6	10.4	1 - 50	0.01	87 ± 7	8.3
Trifloxystrobin	8.5	$C_{20}H_{19}N_2O_4F_3$	408.1297	409.1370	1 - 50	0.01	100 ± 10	0.8	1 - 100	0.01	119 ± 12	1.7	1 - 50	0.01	71 ± 28	3.9

$[M+H]^+$ / 2-100 µg L^{-1} [a]

Pesticides	RT (min)	Molecular formula	Monoisotopic mass	*m/z* calculated	Apple				Pear				Grape			
					Linear range µg L^{-1}	LOQ_m mg kg^{-1}	Rec±RSD %	Mass Acc. ppm	Linear range µg L^{-1}	LOQ_m mg kg^{-1}	Rec±RSD %	Mass Acc. ppm	Linear range µg L^{-1}	LOQ_m mg kg^{-1}	Rec±RSD %	Mass Acc. ppm
Carbendazim	3.2	$C_9H_9N_3O_2$	191.0695	192.0768	1 - 100	0.01	97 ± 4	0.8	1 - 50	0.01	106 ± 3	1.0	1 - 100	-	-	-
Diethofencarb	7.5	$C_{14}H_{21}NO_4$	267.1471	268.1543	1 - 50	-	-	-	1 - 50	-	-	-	1 - 100	0.04	102 ± 10	3.8
Methomyl	7.0	$C_5H_{10}N_2O_2S$	162.0463	163.0536	5 - 100	0.04	106 ± 3	2.5	1 - 100	0.01	90 ± 9	5.5	1 - 100	0.01	92 ± 6	6.2
Myclobutanil	7.8	$C_{15}H_{17}N_4Cl$	288.1142	289.1215	2 - 100	0.01	94 ± 4	8.6	1 - 100	0.01	97 ± 9	9.6	2 - 100	0.01	92 ± 6	9.7
Propoxur	6.7	$C_{11}H_{15}NO_3$	209.1052	210.1125	1 - 100	-	-	-	20 - 100	-	-	-	10 - 100	-	-	-
Pymetrozine	1.5	$C_{10}H_{11}N_5O$	217.0964	218.1036	1 - 100	-	-	-	1 - 100	0.01	74 ± 18	18.3	1 - 100	0.01	74 ± 8	18.7
Tebuconazole	8.2	$C_{16}H_{22}N_3OCl$	307.1451	308.1524	2 - 100	0.01	89 ± 8	13.0	2 - 100	0.04	86 ± 20	12.0	5 - 100	0.04	100 ± 14	12.3
Triadimefon	7.8	$C_{14}H_{16}N_3O_2Cl$	293.0931	294.1004	1 - 100	0.01	99 ± 4	12.2	1 - 100	0.01	93 ± 5	14.5	2 - 100	0.04	100 ± 10	19.1
$[M+H]^+$ / 10-100 µg L^{-1} [a]																
Triadimenol	7.8	$C_{14}H_{18}N_3O_2Cl$	295.1088	296.1160	10 - 100	0.04	96 ± 4	2.0	10 - 100	0.04	96 ± 8	12.2	10 - 100	0.04	101 ± 3	12.9
$[M+H]^+$ / 2-50 µg L^{-1} [a]																
Acetamiprid	5.5	$C_{10}H_{11}N_4Cl$	222.0672	223.0745	5 - 100	0.01	96 ± 3	13.8	1 - 100	0.01	98 ± 3	9.7	1 - 100	0.01	95 ± 3	14.2
Atrazine	7.3	$C_8H_{14}N_5Cl$	215.0938	216.1011	2 - 50	0.01	96 ± 2	5.6	2 - 50	0.01	96 ± 4	6.1	2 - 50	0.01	98 ± 2	5.1
Dimoxystrobin	8.1	$C_{19}H_{22}N_2O_3$	326.1630	327.1703	1 - 50	0.01	96 ± 5	10.0	1 - 50	0.01	88 ± 5	14.9	1 - 50	0.01	92 ± 4	14.5
$[M+H]^+$ / 5-100 µg L^{-1} [a]																
Cyproconazole	7.9	$C_{15}H_{18}N_3OCl$	291.1138	292.1211	2 - 100	0.01	110 ± 4	15.5	1 - 100	0.04	90 ± 14	19.1	1 - 100	0.01	108 ± 9	17.4
Mevinphos	6.0	$C_7H_{13}O_6P$	224.045	225.0523	10 - 100	0.04	95 ± 2	5.8	10 - 100	0.04	89 ± 3	4.6	5 - 100	0.01	92 ± 7	7.2
Tebufenozide	8.1	$C_{22}H_{28}N_2O_2$	352.2151	353.2224	1 - 100	0.04	104 ± 3	7.3	1 - 100	0.04	101 ± 3	7.2	1 - 100	0.01	104 ± 8	4.9
$[M+H]^+$ / 5-50 µg L^{-1} [a]																
Oxyfluorfen	8.7	$C_{15}H_{11}NO_4ClF_3$	361.0329	362.0401	20 - 100	0.04	118 ± 8	1.2	2 - 100	0.01	71 ± 12	7.6	20 - 100	0.04	92 ± 15	1.4
$[M+Na]^+$ / 1-100 µg L^{-1} [a]																
Aldicarb	6.2	$C_7H_{14}N_2O_2S$	190.0776	213.0670	1 - 100	0.01	93 ± 2	2.3	1 - 100	0.01	93 ± 4	1.2	2 - 100	0.01	94 ± 5	3.9
Oxamyl	2.1	$C_7H_{13}N_3O_3S$	219.0678	242.0570	1 - 100	0.04	96 ± 3	0.3	1 - 100	0.01	92 ± 6	3.1	1 - 100	0.01	84 ± 7	3.0
Mecarbam	7.9	$C_{10}H_{20}NO_5PS_2$	329.0521	352.0410	1 - 50	0.01	98 ± 6	7.8	1 - 50	0.01	86 ± 5	2.1	1 - 50	0.01	83 ± 7	1.5
$[M+NH_4]^+$ / 1-100 µg L^{-1} [a]																
Acrinathrin	9.6	$C_{26}H_{21}NO_5F_6$	541.1324	559.1660	5 - 100	0.04	98 ± 13	1.7	1 - 50	0.04	72 ± 16	1.5	1 - 50	0.04	104 ± 22	1.3

Pesticides	RT (min)	Molecular formula	Monoisotopic mass	*m/z* calculated	Apple				Pear				Grape			
					Linear range µg L^{-1}	LOQ_m mg kg^{-1}	Rec±RSD %	Mass Acc. ppm	Linear range µg L^{-1}	LOQ_m mg kg^{-1}	Rec±RSD %	Mass Acc. ppm	Linear range µg L^{-1}	LOQ_m mg kg^{-1}	Rec±RSD %	Mass Acc. ppm
Aramite	8.9	$C_{15}H_{23}O_4SCl$	334.1006	352.1340	1 - 100	0.01	95 ± 8	1.9	1 - 100	0.01	92 ± 10	2.6	1 - 100	0.01	90 ± 12	2.3
Deltamethrin	9.5	$C_{22}H_{19}NO_3Br_2$	502.9732	521.0070	1 - 50	0.01	95 ± 17	0.8	1 - 50	-	-	-	1 - 100	0.01	91 ± 19	2.2
Desmedipham	7.4	$C_{16}H_{16}N_2O_4$	300.1110	318.1450	1 - 100	0.01	94 ± 3	2.7	1 - 100	0.01	91 ± 3	0.4	1 - 100	0.01	96 ± 4	1.3
Etofenprox	10.6	$C_{25}H_{28}O_3$	376.2038	394.2380	1 - 100	0.01	91 ± 3	2.4	1 - 100	0.01	91 ± 5	2.5	1 - 100	0.01	89 ± 5	2.5
Piperonylbutoxide	9.0	$C_{19}H_{30}O_5$	338.2093	356.2430	1 - 100	0.01	93 ± 3	0.1	1 - 100	0.01	91 ± 7	0.0	1 - 100	0.01	90 ± 4	0.1
Propargite	9.3	$C_{19}H_{26}O_4S$	350.1552	368.1890	1 - 100	0.01	94 ± 2	1.1	1 - 100	0.01	92 ± 3	.1.0	1 - 100	0.01	89 ± 5	0.1
$[M+NH_4]^+$ / 1-50 µg L^{-1} [a]																
Fenamidone	8.4	$C_{17}H_{17}N_3OS$	311.1092	329.1430	1 - 50	0.04	93 ± 8	2.3	1 - 100	0.01	110 ± 8	1.2	1 - 50	0.01	86 ± 10	3.1
$[M+NH_4]^+$ / 5-100 µg L^{-1} [a]																
Diniconazole	8.7	$C_{15}H_{17}N_3SCl_2$	341.0520	359.0860	10 - 100	0.04	96 ± 10	8.4	5 - 100	0.04	91 ± 6	9.5	5 - 100	0.04	88 ± 15	9.4

[a] Ion selected (adduct) / Linear range in solvent; RT: Retention time; Rec: Recovery; Acc.= Accuracy, n= 3; LOQ_m: method experimental LOQ.

Recovery values obtained for the matrices studied were appropriate for most of the pesticides analyzed, except for bitertanol, carbosulfan, ethoxysulfuron, fempropathrin, fluroxypir, imazapic, imazaquin, imazethapyr, phosalone, metconazole, propoxur, quinclorac, thifensulfuron methyl, which ones did not recovered in all three matrices. Some compounds could not be recovered in just one matrix: carbendazin and thiophanate methyl in grape, deltamethrin, fenthion, monocrotophos and omethoate in pear; pymetrozine and thiabendazole in apple. And in two matrices diethofencarb, isoxaflutole and metsulfuron methyl in apple and pear. Probably, these compounds could not be properly extracted due to the extraction procedure applied, being necessary a specific condition for them. An example of this requirement is the group of herbicides imidazolinones (imazapic, imazaquin and imazethapyr), they are weak acids with dissociation constants “pKa” ranging from 1.9 to 3.8 [34,35]. The stability of some pesticides depends strongly on the pH and the use of solvents systems with pH<pKa is required. Besides this, some compounds showed recoveries values above or below the recommended range established by the international legislation (70 to 120%) as: azimsulfuron in apple (67%) and pear (66%), cyanazine in grape (122%), pyrazosulfuron ethyl in apple (66%) and pear (66%), metsulfuron methyl in grape (67%). According to SANCO [30] recovery values in the range of 60 to 140% may be used in routine multiresidue analyses, especially because the RSD values for all these pesticides were lower than 6%. In this way, considering the compounds that showed recovery between 60 to 140% the proposed method was validated for a total number of 134, 132 and 137 pesticides in apple pear and grape, respectively. Precision, which was evaluated through the RSD value, is adequate when lower than 20% [30]. Only the compounds acrinathrin (22%) and trifloxystrobin (28%) in grape; diflubenzuron (21%) and nuarimol (24%) in apple and triflumuron (26%) in pear showed RSD values higher than this recommended value.

Mass accuracy tolerance accepted for initial identification was 20 ppm considering the combination of factors: retention time and reference standards; being 19.1 ppm the highest value for mass accuracy error. Results for all compounds are listed in Table 1 and summarized in Figure 2A, which shows distribution of compounds related to mass errors ranges. In general, results are in accordance with SANCO regulations [30], due to

mass accuracy error <5 ppm for 91 compounds in apple and grape, and in 87 compounds for pear. Beyond that, around 33% of the compounds showed mass accuracy error higher than 5 ppm. In average, the error for each matrix was 3.02 ppm (apple), 3.12 ppm (pear), and 3.50 ppm (grape), with average among matrices of 3.22 ppm. According to the study developed by Croley et al. [36], co-elution and ion suppression significantly hinder the ability to make an accurate mass measurement and, in some cases, become more important than the instrument resolution and mass measurement accuracy.

It is well known that matrix effects are one of the main drawbacks of liquid chromatography methods, making quantification in real samples difficult in some cases. Co-eluting compounds from the sample matrix can affect the analyte ionization process leading to a signal enhancement or suppression. These undesirable effects typically cause loss of method accuracy, precision and sensitivity leading to incorrect quantification and also to problems for a safe confirmation. Matrix effect can be minimized applying an efficient clean-up, as well using internal standards and matrix-matched standards [37].

Matrix effect was evaluated for all the compounds in all the matrices studied and the obtained results are represented in Figure 2B. Observing the results of matrix effect calculated, most of the compounds do not show neither signal suppression nor signal enhancement considering acceptable values between -20 and 20%. Signal enhancement was found for 8, 11 and 15 pesticides in apple, pear and grape, respectively. Signal suppression in apple, pear and grape was showed for 11, 16 and 12 pesticides, respectively. In this study, for most compounds, the matrix effect was not considered significant because the values were in a range of ± 20% [31]. However, for some pesticides such as tolclofos methyl, the matrix effect showed higher intensity in the presence of the matrix. For this reason, curves were prepared in the matrix extract for the quantification of the analytes.

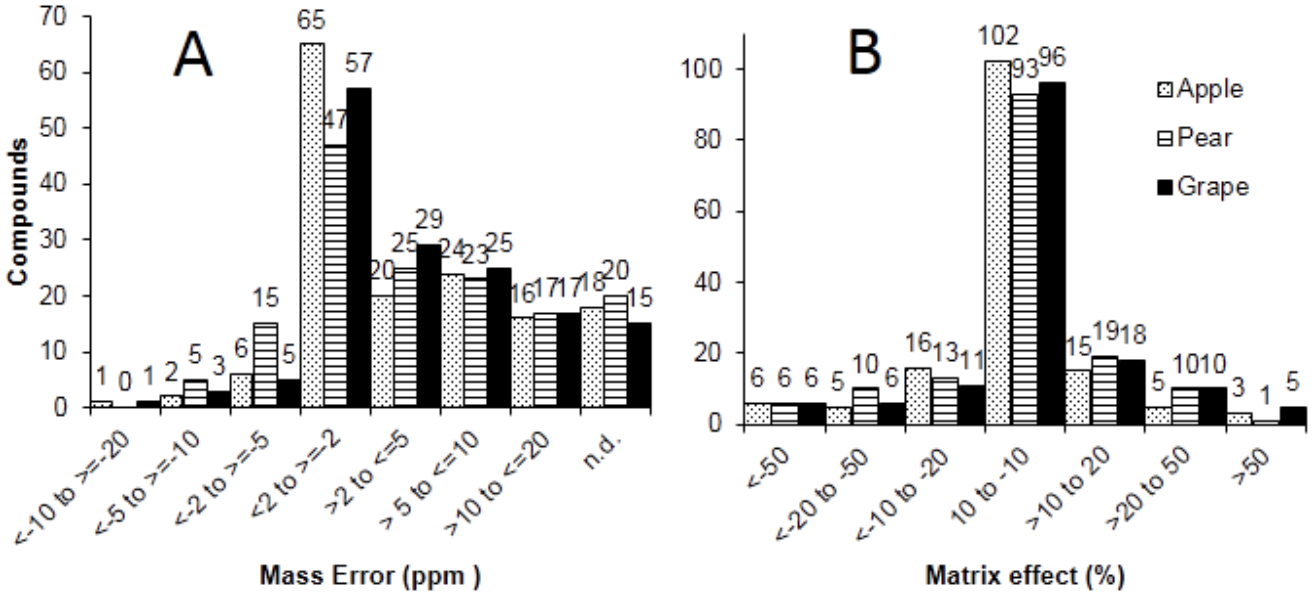

Figure 2. Values of (A) mass error and (B) matrix effect in apple, pear and grape, combining modified QuEChERS method and LC-QToF/MS determination.

Unsatisfactory mass resolution and mass accuracy show an important aspect to be improved since they affect the quality of the results especially for the development of automated data analysis strategies in food samples, which can present wide variations in matrix background. A good chromatographic separation reflects in satisfactory values of mass errors, as demonstrated in the work reported by Croley et al. [36]. Authors evaluated the separation of 6 isobars compounds, in a 30 min gradient run, obtaining chromatographic separation for all them, with two of the compounds displaying partial co-elution. For the compounds that did not co-elute, the observed spectra yielded the expected results with *m/z* values within the expected mass measurement error (<2 ppm). However, when evaluating the co-eluting compounds a large mass error was observed. Besides this, the use of stationary phase with small particle size provides can increase column efficiency with better baseline separation and narrower peaks [38].

Method Applicability

The proposed validated method was applied in 5 samples of each matrix, which were purchased at local supermarkets in Santa Maria, Rio Grande do Sul State (Brazil).

As presented in Table 2, pesticide residues were found in 5 samples of pear and apple, and 4 samples of grape. Comparing the results with the MRL values established by Brazilian legislation [3] and international legislation [4], carbendazim is a fungicide, which is permitted for apple, citrus crops, beans, cotton, soybeans and wheat. Propiconazole is also a fungicide and its use in apple crops is not permitted, as well as thiacloprid for pear. Difenoconazole and imidacloprid are allowed for grape crops and the values found are below the established MRL. Pyraclostrobin is also allowed for apple and grape crops and the residues were below the MRL. Residues of phosmet are below the MRL for apple. Thiophanate methyl was detected only in one sample of pear, but this compound is rapidly converted in carbendazim (its main degradation product) found in apple and pear samples. The analyzed samples showed concentration of pesticides below the European and Brazilian MRL values.

Table 2. Pesticide residues determined in commercial apple, pear and grape samples.

	Sample					LOQ	MRL (mg kg^{-1})	
Pesticides	1	2	3	4	5	(mg kg^{-1})	EU	Brazil
	Apple (mg kg^{-1})							
carbendazim	0.022	<LOQ	<LOQ	<LOQ	0.025	0.01	0.2	5.0
phosmet	<LOQ	n.d.	n.d.	n.d.	n.d.	0.01	0.5	1.0
pyraclostrobin	n.d.	n.d.	n.d.	<LOQ	<LOQ	0.01	0.5	2.0
propiconazole	n.d.	0.027	n.d.	0.038	n.d.	0.04	0.15	n.p.
	Pear (mg kg^{-1})							
carbendazim	0.034	0.032	0.011	<LOQ	<LOQ	0.01	0.2	n.p.
thiabendazole	n.d.	0.074	0.138	0.177	0.158	0.01	5.0	10.0
thiacloprid	<LOQ	n.d.	0.012	0.012	<LOQ	0.01	0.3	n.p.
thiophanate methyl	<LOQ	n.d.	n.d.	n.d.	n.d.	0.04	0.5	n.p.
	Grape (mg kg^{-1})							
difenoconazole	n.d.	<LOQ	<LOQ	<LOQ	n.d.	0.01	0.5	0.2
imidacloprid	n.d.	0.023	n.d.	n.d.	0.044	0.01	1.0	1.0
pyraclostrobin	n.d.	0.021	0.030	n.d.	<LOQ	0.01	2.0	2.0

EU= European Union; n.d.: not detected; n.p.= not permitted.

Conclusion

In this work, a multiclass screening and rapid quantitative analysis combining modified QuEChERS method and LC-QToF/MS in full scan mode for the determination of pesticide residues proved to be effective for more than 130 pesticides in apple, pear and grape samples. Validation study demonstrated good recovery and precision for a wide number of compounds, in the concentration levels of 0.01, 0.040 and 0.100 mg kg^{-1} with recovery values from 66 to 122%, and satisfactory RSD values for almost all compounds validated. Linearity values were adequate with values of r^2 higher than 0.99 in the range of 1 to 100 µg L^{-1}, as well as LOQ from 0.01 to 0.04 mg kg^{-1}, which attends LMR values of national and international legislations. LC-QToF/MS detection using $[M+H]^+$, $[M+NH_4]^+$ or $[M+Na]^+$, as well as, mass accuracy, provided reliability for the proposed method especially for those compounds with mass accuracy error lower than 5 ppm, attending international parameters for pesticide residues determination with high resolution mass spectrometry.

The proposed method after validated was applied to real samples and results demonstrated that in general the MRL values are respected being in according to Brazilian and European Union legislations. Results showed that the proposed method can be applied for routine analyses of pesticide residues in fruit matrices as apple, pear and grape. QuEChERS as a generic sample preparation procedure combined with the analysis by LC-QToF/MS showed great benefits, like good sensibility, mass resolution, and mass accuracy, allowing fast multiresidue determination of a wide scope of pesticides in a short analysis time.

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References

(1) García-Reyes, J.F., Hermano, M.D., Molina-Díaz, A., & Fernández-Alba, A.R. (2007) *TrAC-Trend Anal. Chem.* 26, 828-841.

(2) Frenich, G.A., Liébanas, A.J.F., Mateu-Sánchez, M., & Vidal, J.L.M. (2003) *Talanta* **60**, 765-774.

(3) MAPA, *Ministério da Agricultura, Pecuária e Abastecimento.* http://agrofit.agricultura.gov.br/agrofit_cons/principal_agrofit_cons (accessed November 29, 2015)

(4) *Codex Alimentarius*. http://www.codexalimentarius.org/standards/pesticide-mrls (accessed November 29, 2015)

(5) Ferrer, I., García-Reyes, J.F., & Fernandez-Alba, A. (2005) *TrAC-Trend Anal. Chem.* **24**, 671-682.

(6) Pareja, L., Cesio, V., Heinzen, H., & Fernández-Alba, A. R. (2011) *Talanta* **83**, 1613-1622.

(7) Hanot, V., Goscinny, S., & Deridder, M. (2015) *J. Chromatogr. A* **1384**, 53-66.

(8) Bakırcı, G.T., Acay, D.B.Y., Bakırcı, F., Ötleş, S. (2014) *Food Chem.* **160**, 379-392.

(9) Golge, O., Kabak, B. (2015) *J. Food Compost. Anal.* **41**, 86-97.

(10) Malato, O., Lozano, A., Mezcua, M., Agüera, A., & Fernandez-Alba, A.R. (2011) *J. Chromatogr. A* 1218, 7615-7626.

(11) Jardim, A.N.O., & Caldas, E.D. (2012) *Food Control* **25**, 607-616.

(12) Núñez, O., Gallart-Ayala, H., Ferrer, I., Moyano, E., & Galceran, M.T. (2012) *J. Chromatogr. A* **1249**, 164-180.

(13) Gómez-Ramos, M.M., Ferrer, C., Malato, O., Aguera, A., & Fernandez-Alba, A.R. (2013) *J. Chromatogr. A* **1287**, 24-37.

(14) Picó, Y., Barceló, D. (2008) *TrAC Trends Anal. Chem.* **27**, 821-835.

(15) Dominicis, E. de, Commissati, I., & Suman, M. (2012) *J. Mass Spectrom.* **47**, 1232-1241.

(16) Ferrer, I., Thurman, E.M., & Zweigenbaum, J.A. (2007) *Rapid Commun. Mass Spectrom.* **21**, 3869-3882.

(17) Gómez, M.J., Gómez-Romero, M.M., Malato, O., Mezcua, M., & Fernandez-Alba, A.R. (2010) *J. Chromatogr. A* **1217**, 7038-7058.

(18) Rodrigues, S.A., Caldas, S.S., Kurz, M.H.S., Cabrera, L.C., Duarte, F.A., Zanella, R., & Primel E.G. (2012) *Anal. Methods* **4**, 1820-1824.

(19) Soler, C., Mañes, J., & Picó, Y. (2005) *J. Chromatogr. A* **1067**, 115-125.

(20) Farré, M., Picó, Y., & Barceló, D. (2013) *Anal. Chem.* **85**, 2638-2644.

(21) Sismoto, M., Paschoal, J.A.R., Teles, J.A., Estaiano, R.A.R., & Reyes, F.G.R.A. (2014) *J. Food Composit. Anal.* **34**, 153-162.

(22) Anastassiades, M., Lehotay, S.J., Štajnbaher, D., & Schenck, F.J. (2003) *J. AOAC Int.* **86**, 412-431.

(23) Lehotay, S.J., Maòtovská, K., & Lightfield, A.R. (2005) *J. AOAC Int.* **88**, 615-629.

(24) Boix, C., Ibáñez, M., Sancho, J.V., Léon, N., Yusá, V., & Hernández, F. (2014) *Food Chem.* **160**, 313-320.

(25) Ferrer, I., García-Reyes, J.F., Mezcua, M., Thurman, E.M., & Fernández-Alba, A.R. (2005) *J. Chromatogr. A* **1082**, 81-90.

(26) Grimalt, S., Sancho, J.V., Pozo, O.J., & Hernández, F. (2010) *J. Mass Spectrom.* **45**, 421-436.

(27) Lehotay, S.J., Maštovská, K., & Yun, S.J. (2005) *J. AOAC Int.* **88**, 630-638.

(28) He, Q., Huang, J., Yang, X., Yan, X., He, J., Li, S., & Jiang, J. (2016) *Food Control* **64**, 70-76.

(29) Grimalt, S., Dehouck, P. (2016) *J. Chromatogr. A* **1433**, 1-23.

(30) Directorate General for Health and Consumer Affairs, DG SANCO (2013) *Guidance Document on Analytical Quality Control and Validation Procedures for Pesticide Residues Analysis in Food and Feed*, Document SANCO 12571/12013, European Union, Brussels, Belgium.

(31) Facco, J.F., Martins, M.L., Bernardi, G., Prestes, O.D., Adaime, M.B., & Zanella, R. (2015) *Anal. Methods* **7**, 359-365.

(32) McDonald, G.R., Hudson, A.L., Dunn, S.M.J., You, H., Baker, G.B., Whittal, R.M., Martin, J.W., Jha, A., Edmondson, D.E., & Holt, A. (2008) Science **322**, 917-917.

(33) Chernushevich, I.V., Loboda, A.V., & Thomson, B.A. (2001) *J. Mass Spectrom*. **36**, 849-865.

(34) Martins, G.L., Friggi, C.A., Prestes, O.D., Vicari, M.C., Friggi, D.A., Adaime, M.B., & Zanella, R. (2014) *Clean Soil Air Water* **42**, 1441-1449.

(35) Croley, T.R., White, K.D., Callahan, J.H., & Musser, S.M. (2012) *J. Am. Mass Spectrom.* **23**, 1569-1578.

(36) Kemmerich, M., Bernardi, G., Adaime, M.B., Zanella, R., Prestes, O.D. (2015) *J. Chromatogr. A* **1412**, 82-89.

(37) Marín, J.M., Gracia-Lor, E., Sancho, J.V., López, F.J., & Hernández, F. (2009) *J. Chromatogr. A* **1216**, 1410-1420.

(38) Kmellár, B., Pareja, L., Ferrer, C., Fodor, P., & Fernández-Alba, A.R. (2011) *Talanta* **84**, 262-273.

6 ARTIGO 3 – TRANSFORMATION OF IONOPHORE ANTIMICROBIALS IN POULTRY LITTER DURING PILOT-SCALE COMPOSTING

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Abstract

Ionophores are the second top selling class of antimicrobials used in food-producing animals in the United States. In chickens, ionophores are used as feed additives to control coccidiosis; up to 80% of administered ionophores are excreted in the litter. Because poultry litter is commonly used to fertilize agricultural fields, ionophore residues are contaminants of concern in the environment. This study aims to develop a liquid chromatography with tandem mass spectrometry (LC-MS/MS) method to quantify ionophores, and identify their transformation products (TPs) in poultry litter after on-farm pilot-scale composting. The validation parameters of the optimized method showed good accuracy, ranging from 71 to 119% recovery and relative standard deviation (precision) of ≤19% at three different spiked concentration levels (10, 50 and 100 µg/kg). Monensin, salinomycin and narasin, were detected in the poultry litter samples prior to composting at 290.0±40, 426±46, and 3113±318 µg kg^{-1}, respectively. This study also aims to investigate the effect of different composting conditions on the removal of ionophores, such as the effect of turning or aeration. Results revealed a 13-68% reduction in ionophore concentrations after 150 d of composting, depending on whether

the compost was aerated, turned, or subjected to a combination of both aeration and turning). Three transformation products and one metabolite of ionophores were identified in the composted litter using high-resolution liquid chromatography with quadrupole time-of-flight mass spectrometry (LC-QToF/MS).

Capsule

Degradation of ionophores in poultry litter using different composting conditions is reported, and transformation products were identified using high resolution mass spectrometry.

Keywords

Antibiotics; broiler chickens; monensin; biodegradation, QuEChERS

Introduction

Antimicrobials have been widely used in animal production as feed additives for the treatment and prevention of infectious diseases. In 2012, ionophores comprised 31% of the 4.6 million kg of antimicrobials sold in the United States (U.S.) for food-producing animals (FDA, 2014). Ionophore antimicrobials (IPAs) are widely used in poultry production to control coccidiosis (Bak et al., 2013; Chapman, 1984) that is caused by intestinal parasites predominantly affecting broiler (Dorne et al., 2013). Broiler chickens production in the U.S. continues to increase, with over 43 billion pounds of poultry meat produced annually (USDA, 2012). Therefore, it can be expected that the use of IPAs in the poultry industry will rise concomitantly.

Ionophores are polyether carboxylic acids that have the ability to complex with metal cations through the multiple oxygen atoms on the cyclic ether moieties, and the carboxylic and alcoholic end groups that interact to form a pseudocyclic conformation (Martínez-Villalba et al., 2009; Sun et al., 2014b). Monensin (MON), lasalocid (LAS), salinomycin (SAL), narasin (NAR) and maduramicin (MAD) are the most widely used IPAs in poultry production. The IPAs are poorly absorbed in the bird's gut, hence up to 80% are excreted intact in urine and feces (Hansen et al., 2009; Sun et al., 2014a). Due to the considerable quantities of waste generated in the poultry industry and the high concentrations of nutrients (nitrogen (N), phosphorus (P) and potassium (K)) in poultry litter, land application is the best solution to manage poultry waste (Abraham and Kepford, 2000; Biswas et al., 2012). Ionophores could persist in aged poultry litter even after three years of unattended and unmanaged storage on farms, therefore land application of poultry litter can be a significant source of IPA contaminantion in the environment (Biswas et al., 2012).

Composting results in a waste product with lower density and volume, while also decreasing noxious odors and toxins from poultry litter. Composted litter has a lower rate of N mineralization and thus, lowers the loss of ammonia (NH_3) through volatilization. Hence, land application of composted litter poses a lower risk of polluting groundwater by nitrate compared to the application of fresh poultry litter (Abraham and Kepford, 2000). Composting can be performed using commercial in-vessel methods that rely on a

variety of forced aeration and mechanical turning techniques to accelerate the degradation process by controlling moisture content, temperature, and oxygen levels (Misra et al., 2003).

Composting has additionally been identified as a viable means of reducing the environmental impacts of manure-borne antimicrobials, including ionophores. Ramaswamy et al. (2010) studied the degradation of SAL in manure during a 38 d composting and reported that the concentration of SAL decreased by 99.8%. The biodegradation of SAL was also investigated in lab-scale bioreactors under aerobic and anaerobic conditions with a mixed culture of soil bacteria. Biodegradation occurred under aerobic conditions with only trace concentrations of SAL remaining after 200 h; however, SAL remained persistent under anaerobic conditions (Hansen et al., 2012). In a separate study, LAS degradation was evaluated in poultry manure under different storage conditions (aging in a pile versus composting), and in soil that had been fertilized with LAS-containing manure. It was found that the half-life of LAS was shortest in manure-fertilized soil, followed by composted manure; and as expected, the half-life of LAS was longest in untreated manure (Žižek et al., 2014).

Limited information is available in the literature regarding the transformation of IPAs during composting of poultry litter. Therefore, a study on the influence of different composting conditions on the fate of IPAs, and on the formation of their transformation products (TPs) is warranted. Sun et al. (2014) evaluated the degradation of MON, NAR and SAL in poultry litter and in soil microcosms, with varying temperatures and water content under laboratory and field conditions. The study concluded that water and temperature played an important role in the degradation of SAL and NAR, although MON remained stable under all conditions. Water content and temperature are critical parameters that affect the microbial activity in soil, influencing the degradation of organic compounds (Sun et al., 2014a; Tiquia & Tam, 2002). The structures of ionophore TPs were postulated based on the mass spectral fragmentation patterns of the newly observed chromatographic peaks (Sun et al., 2014a). The photodegradation rates of these three IPAs were also assessed under UV and solar irradiation (Sun et al., 2014b). MON showed resistance to direct photolysis, whereas SAL and NAR were degraded via direct absorption of UV or sunlight.

In order to study the fate of IPAs during composting of poultry litter, a robust sample preparation and analytical method is needed. Trace analysis of IPAs can be achieved using liquid chromatography with tandem mass spectrometry (LC-MS/MS) because IPAs are not amenable to gas chromatography. In addition, detection by MS/MS offers the highest selectivity and sensitivity for quantifying chemical contaminants in complex matrices such as poultry litter. Chromatographic separation of IPAs has been achieved with either a C_{18} column (Biswas et al., 2012; Hansen et al., 2009) or a reversed-phase amide column (Sun et al., 2013). Different sample preparation procedures have been reported in the literature to extract IPAs from environmental matrices. Pressurized liquid extraction followed by solid phase extraction (SPE) for clean-up has been described for the determination of IPAs in soil, sediment, and poultry litter, with high recoveries (71-133%) and good precision (≤16% relative standard deviation or RSD) achieved (Bak et al., 2013). Solid-liquid extraction followed by SPE clean-up using hydrophilic-lipophilic balanced cartridges for MON, NAR, SAL, LAS and nigericin (NIG) in aged poultry litterhas also been reported with recoveries between 92-104.4% (Biswas et al., 2012). However, the above-mentioned techniques are time-consuming. A sample preparation technique using QuEChERS (**Qu**ick, **E**asy, **Ch**eap, **E**ffective, **R**ugged, and **S**afe) that involves an extraction step with acetonitrile (MeCN) and a liquid-liquid partitioning facilitated by addition of anhydrous magnesium sulfate ($MgSO_4$) and sodium chloride (NaCl) offers a faster alternative. A modified QuEChERS method uses a dispersive solid phase extraction (d-SPE) step that involves adding the extract into a tube containing sorbents and salts eliminates the need for evaporation and results into a shorter sample preparation time (Anastassiades et al., 2003).

Therefore, the first objective of this study was to develop a robust sample preparation method using QuEChERS for the LC-MS/MS analysis of IPAs in poultry litter. The second objective of this study was to quantify the IPAs present in the poultry litter before and after three different pilot-scale composting processes: with aeration, turning, and combined aeration and turning. Finally, this study also aimed to identify stable TPs formed during poultry litter composting using liquid chromatography with quadrupole time of flight mass spectrometry (LC-QToF/MS). Results from this study will

provide insights on the composting conditions that favor the degradation of IPAs, and the identities of stables TPs formed during composting of poultry litter.

Material and Methods

Chemicals

MON (86.8%), LAS (100 mg L^{-1}), MAD (97.9%), NAR (≥98%), and the surrogate standard (SS) NIG (≥98%) were purchased from Sigma Aldrich (St. Louis, MO). SAL (95%) was obtained from VWR International (Radnor, PA). The internal standard (IS) triphenyl phosphate (TPP, ≥99%), typically used in sample preparation following QuEChERS was also purchased from Sigma Aldrich. Barnstead NANOpure^{TM} Diamond purification system (Waltham, MA) was used to obtain 18.2 MΩ water. LC/MS grade methanol and acetonitrile were purchased from EMD Millipore (Billerica, MA). Formic acid (88%) used for LC separations was purchased from Fisher Scientific (Pittsburgh, PA). Sample preparation based on QuEChERS was performed using anhydrous magnesium sulfate ($MgSO_4$) from J. T. Baker (Center Valley, PA) and sodium chloride (NaCl) from VWR International, as well as Florisil sorbent from Restek (Bellefont, PA). Polytetrafluoroethylene (PTFE) syringe filters of 13 mm and 0.2 µm of porosity were purchased from VWR International.

Composting procedures

The dynamics of composting are scale-dependent, and are influenced by vessel shape and size; so they are difficult to model at the laboratory bench-top scale. For this project, composting bins of approximately two tons capacity (1.5 m^3) were constructed using pressure-treated lumber (Anexo 1). Bins were intended to replicate, at a pilot-scale, the several aspects of commercial in-vessel, aerated, turned compost operations designed for management of large quantities of organic waste (Misra et al., 2003). Each bin was constructed with swinging doors to allow removal of material during turning, and a metal lid to cover media after reloading. Lids aid in controlling composting parameters by moderating convective heat loss, reducing evaporative water loss, and preventing unintentional water introduction via precipitation. Bins requiring aeration were fitted with

positive pressure systems forcing atmospheric air through a bottom plenum to maintain oxygen levels for optimal microbial activity.

On arrival (12/11/2014) poultry litter was divided into the four treatment categories: aerated (A), turned (T), turned and aerated (T/A), and piled (P; not composted). During the first 14 d post-arrival, the A and T/A compost bins were aerated using a “2-min ON:18-min OFF” cycle. The T and T/A bins were mechanically turned by dumping, mixing, and re-loading 5 d per week, for the first two weeks. After the 2-week turning period, aeration was discontinued for the remainder of the composting period. All four treatments (3 composts, 1 pile) were maintained until 5/7/2015 (approximately 150 d) when the pile was used in field application, at which point materials were collected for post-compost analysis.

Sample information

A single 20,000 kg batch of poultry litter, obtained from a whole-house cleanout of a conventional broiler operation, was delivered to the University of Maryland - Wye Research and Education Center, Queenstown, MD, USA. On delivery, the entire batch was dumped into a common pile and mixed using a front-end loader. Three 2000 kg aliquots were apportioned into compost bins with the remaining 14,000 kg left in an uncovered pile, which is the normal practice after a whole-house cleanout. All samples for analysis of IPAs were collected in triplicate. Approximately five 2-kg sub-samples were collected as material was introduced into each compost bin. The three resulting 10-kg batches were individually homogenized to generate three unique 100-g wet weight “pre-compost” samples, collectively representative of the entire pile and individually representative of initial conditions in compost bins. Upon completion of composting, media from each bin was dumped, multiple spatially-distinct 2-kg sub-samples were homogenized, and triplicate 100-g wet weight “post-compost” samples were collected for analysis. Media from the pile was also sampled at this time by collecting triplicate 100-g wet weight aliquots from a homogenized 10-kg batch of material obtained following appropriate sampling procedures (UME 2013). Briefly, to ensure a representative sample a composite was made from 10 sub-samples taken from various locations and depths of the pile. All pre- and post-compost samples were placed in 250-mL pre-

cleaned amber wide-mouth glass jars and immediately stored at -20°C before overnight delivery on dry ice to the University at Buffalo. Additional pre- and post-compost samples were collected for nutrient analysis in satisfaction of nutrient management requirements prior to field application of poultry litter. Individual 500-g wet weight samples were taken from the pile on delivery and from bins and the pile on conclusion of composting. Sample collection followed protocols to select “representative” amounts of material reflecting the overall batch (UME 2013). Samples were collected from multiple locations within the pile and within compost bins but may not proportionally represent mean nutrient concentrations within each source. Since the pile was well-mixed on delivery, the initial nutrient sample should be satisfactory. Bins were mixed via dumping and reloading one or more times including at the end of composting. The nutrient sample from the pile at the end of the compost period included representative surface and interior material, but, because the pile was not re-mixed, it may not reflect the average moisture content and/or nutrient concentration of the pile as a whole

Sample extraction and clean-up

Following the original QuEChERS method (Anastassiades et al., 2003), 5 g aliquots of each of the freeze-dried poultry litter samples were weighed in 50-mL polypropylene tubes. Due to the lack of isotopically labelled standards, NIG was used as a surrogate standard, such that the final concentration of NIG in the extract to be injected is expected to be 50 µg L^{-1}; Nanopure^{TM} water (10 mL) was added into each of the tubes containing the litter samples, and then vortexed for 1 min. The extraction was performed by adding 10 mL acetonitrile, followed by vortexing for 1 min and ultrasonication for 15 min. Partitioning of the IPAs into the organic phase was achieved by adding 4.0 g $MgSO_4$ and 1 g NaCl to the tube. Samples were vortexed for 1 min and centrifuged at 3400 rpm for 10 min. Sample clean-up by d-SPE was performed by transferring 1 mL of the upper organic layer to a 15-mL tube containing 150 mg $MgSO_4$ and 25 mg Florisil sorbent. The mixture was vortexed for 1 min and centrifuged at 3400 rpm for 10 min. The final extracts were filtered and 200 µL of the extract was diluted with 800 µL of 50:50 (v/v) methanol:acetonitrile solution. The internal standard, 10 µL of TPP, was added into the extract such that its final concentration is 10 µg L^{-1}.

Analysis by LC-MS/MS and LC-QToF/MS - instrument conditions

For quantification of IPAs, samples were analyzed using a Surveyor HPLC coupled to a TSQ Quantum Ultra^{TM} triple quadrupole mass spectrometer (LC-MS/MS system) (Thermo Scientific, Waltham, MA). The optimized MS conditions were: spray voltage of 4000 V, vaporizer temperature of 258 ºC, capillary temperature of 214 ºC, sheath gas pressure of 60 psi, and auxiliary gas pressure of 5 psi. Nitrogen was used as vaporizer and argon as collision gas. Due to the high affinity of ionophores to complex with monovalent metal cations, sodium adducts $[M+Na]^+$ were used as precursor ions to quantify IPAs under positive electrospray ionization (ESI+). Analyses were performed using the selected reaction monitoring (SRM) mode for quantification, with the transitions listed in Table 1. Chromatographic separation was achieved on a C_{18} BetaBasic column (100 x 2.1 mm; 3 µm) from Thermo Scientific (Waltham, MA) using a gradient elution, with mobile phase A (0.1% formic acid in water) and mobile phase B (50:50 (v/v) methanol:acetonitrile). The program started with a 2-min isocratic elution with 15% mobile phase B, then increasing B to 80% within 4 min, and holding B at this condition for 1 min. Then, the mobile phase was changed to 95% B within 4 min, and returned back to 15% B within 2 min. The solvent was held at 15% B for an additional 2 min to allow equilibration to initial conditions. A 5-µL sample was injected. The total LC-MS/MS run time was 15 min at a flow rate of 300 µL min^{-1}.

The identification of the TPs was performed using an Agilent 1260 LC system coupled to an Agilent 6530 Accurate Mass QToF/MS (Santa Clara, CA). The same reversed-phase column used in the quantification of ionophores by LC-MS/MS was used in the identification of TPs by LC-QToF/MS. Column temperature was kept at 25 °C. A 20-µL sample was injected. The mobile phase elution program started with 2 min isocratic elution with 10% B, followed by increasing mobile phase B to 95% within 10 min, and holding B at this condition for 9 min. Then, the mobile phase returned back to 10% B within 1 min, where it was held for an additional 8 min to allow equilibration at the initial condition. The total run time was 30-min at a flow rate of 200 µL min^{-1}. Analysis using LC-QToF/MS was performed under full scan mode with positive ESI+. The instrument was calibrated prior to each run with maximum error residual of <0.1 ppm during calibration, using a low flow of calibration solution containing the internal

reference masses *m/z* 121.0509 (purine, $C_5H_5N_4$) and 922.0098 (hexakis (1H, 1H, 3H-tetrafluoropropoxy) phosphazine, $C_{18}H_{19}O_6N_3P_3F_{24}$). The MS parameters were as follows: gas and sheath temperatures of 300ºC, drying gas flow of 6 L min^{-1}, sheath gas flow of 11 L min^{-1}, Vcap of 3500 V, nozzle voltage of 1000 V, fragmentor voltage of 175 V, skimmer voltage of 65 V, and OCT 1RF Vpp of 750 V. The acquisition was performed at 1 spectra/s collecting data over 50-1700 *m/z*. MS/MS studies for the TPs were performed using the same conditions described above during analysis in the full scan mode and at 15, 35, and 55 eV collision energies.

Sample quantification

Sample quantification during method validation was performed using matrix-matched calibration curves. The curves were prepared using extracts from an organic poultry litter sample spiked with known amounts of IPAs ranging from 0.5 to 200 µg L^{-1}. Real poultry litter samples were quantified using a three-point standard addition method due to the complexity of the samples; with this approach it was possible to minimize quantification errors resulting from the co-extracted matrix components. The area of the TPP signal was used to normalize the analyte signal to correct for run-to-run variations in sample volume, injection volume, and drifts in instrument sensitivity.

Table 1. Optimized LC-MS/MS parameters for the quantification of ionophores.

Compound	Exact Mass (theoretical)	Retention time (min)	Precursor ion $[M+Na]^+$	Quantification ion, *m/z* (CE)	Confirmation ion, *m/z* (CE)
Lasalocid – LAS	590.3819	12.4	613.4	377.0 (31)	359.0 (33)
Monensin – MON	670.4292	11.5	693.5	461.4 (57)	675.7 (20)
Nigericin – NIG (SS)	724.4762	12.9	747.5	236.9 (53)	703.9 (48)
Salinomycin – SAL	750.4918	11.8	773.6	431.3 (48)	531.4 (46)
Narasin – NAR	764.5075	12.4	787.6	431.0 (43)	279.0 (52)
Maduramycin – MAD	916.5395	12.2	939.6	877.6 (24)	859.7 (43)
Triphenyl phosphate – TPP (IS)	326.0707	8.7	327.1*	151.8 (40)	215.0 (33)

*[M+H]+; CE: collision energy; exact mass of the empirical formula of each ionophore; SS=surrogate standard; IS=internal standard

Results and Discussion

Extraction and clean-up with QuEChERS

After chromatographic separation was achieved in a short run time of 15 min using authentic standards, the optimization of a sample extraction procedure for the target IPAs was performed. The extraction procedure was based on the original QuEChERS method, with some minor modifications in order to obtain good percent recoveries for all target IPAs from the poultry litter. To minimize signal suppression due to co-extracted matrix components, the amount of freeze-dried poultry litter was limited to ~5 g. The sample extraction included an ultrasonication step to ensure effective extraction of IPAs because they can be strongly adsorbed to the solid matrix (Hansen et al., 2009). The clean-up step was modified by using Florisil sorbent instead of the primary secondary amine (PSA) and C_{18} sorbents that are typically used in QuEChERS. This modification resulted in cleaner extracts and improved extraction recoveries for all the IPAs.

For method validation, organic poultry litter (from chickens that did not receive IPAs in their feeds) was spiked at three different levels (n=6), 10, 50 and 100 µg kg^{-1}, to evaluate accuracy (recovery test), precision, and limit of quantification (LOQ). Extraction recoveries between 71-120% (Fig. 1) and RSDs lower than 19% were obtained. The overall sample preparation and LC-MS/MS method provided the needed limits og quantification (LOQs ~10 µg kg^{-1}) for this study. The calibration curve constructed using 0.5 to 200 µg L^{-1} gave a coefficient of determination (r^2) of ≥0.99. All the concentrations reported in this study are based on dry-litter.

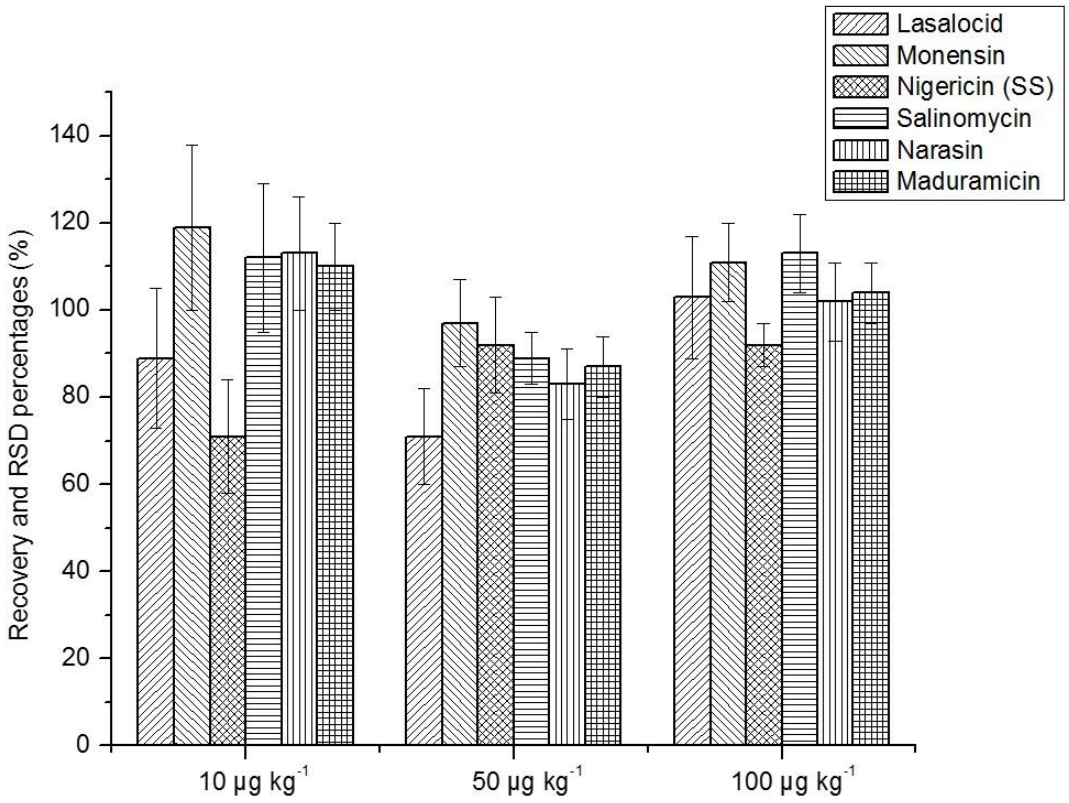

Figure 1. Method validation results showing % extraction recoveries and relative standard deviation (RSD) from poultry litter spiked at three different concentrations (10, 50, and 100 µg kg^{-1}) of ionophores (n=6).

Poultry litter characteristics before and after composting

Poultry litter contains macro- and micronutrients essential for soil fertility and plant growth. Because characterization of poultry litter samples is important to understand its benefit for soil conditioning and ultimately crop yield, total N, P, and K (Anexo 2) were analyzed by sending the litter samples, taken before and after composting, to the AgroLab (101 Clukey Dr. Harrington, DE 19952). Total N concentrations were largely unchanged during composting despite a significant loss (≥20%) of total material volume, suggesting that carbon conversion approximately matched ammonia volatilization, or that reduction in volume due to loss of water or simply rearrangement of material did not change N concentration. This is beneficial since N is an essential nutrient, and literature generally reports a reduction of total N during composting (Dolliver et al., 2008; Ramaswamy et al., 2010). Similarly, total K was unchanged in the composted and piled

poultry litter. Total P remained constant in the compost bins (losses/increases <10%), but increased (>60%) in the piled litter. It has been reported earlier that composting can lead to an increase in total P (Ramaswamy et al., 2010). The increase in total P observed in the piled poultry litter may be attributable to the maintenance of a higher average temperature during the whole period of storage (Anexo 3).

The pH values of poultry litter were generally neutral (range 7.3 to 8.1) and attributed to the naturally occurring composting bacteria. Moisture is important in enhancing chemical reactions and nutrient transport required for rapid microbial growth. It has been shown that microbial activity in animal wastes is strongly correlated with the amount of moisture content (Sun et al., 2014a; Tiquia & Tam, 2002). A balance of moisture is necessary; if there is too little water the compost process will slow down and stop, and if too much water is present the supply of oxygen becomes limited. In the current study, the moisture level of the poultry litter upon arrival was 44%. Aerated compost bins (A and T/A) showed a modest loss of moisture (33.1 and 40.1%, respectively) through evaporation, while the non-aerated bin (T) remained relatively constant at 46%. The piled poultry litter was left uncovered, so it received additional moisture in the form of precipitation explaining the increase to 60% moisture.

Temperature is also important in assessing the effectiveness of composting. This presented a challenge considering that the compost bins and the piled poultry litter were maintained outside, and the investigation was performed during the winter. The recorded temperature profiles for the composted and piled poultry litter over the turning and aeration period of 40 d is presented in Anexo 3. On arrival the poultry litter had a temperature of 26.7°C. The temperature increased to a maximum of 37.2°C within the pile (measured at a depth of approximately 50 cm) over approximately one week before slowly dropping to 25.6°C for over a period of approximately one month, and remaining stable. Compost bins warmed rapidly when aeration was initiated. The A bin exceeded 53.3°C within 3 d, reached a peak temperature of 75.0°C at 7 d, temperatures ≥50°C for the remainder of the initial 2-week period were observed. The T/A bin reached 55.6°C at 3 d and a maximum temperature of 67.8°C at 5 d. The temperature of both aerated bins dropped significantly over the next 25 d to less than 7.5°C. Turning the contents in the bins was found to cause a reduction in temperature. Measurement before and after the

process of dumping, mixing and reloading bins indicated an average temperature loss of 6.5°C from the central region of the bins for each turning event. The heat loss during turning and the lack of aeration led to a slower temperature increase in the T bin relative to the increase in temperature observed in the A and T/A bins; the peak temperature in T bin was only 52.3°C and was reached at 14 d. The T bin, however, was able to maintain temperatures higher than 20°C for a longer period until the last measurement made at 40 d. On the other hand, the temperatures of the A and T/A bins dropped to less than 15°C when measured after 4 weeks, and was below 10°C during the last measurement was made at 40 d. Overall, the recorded temperatures indicate an incomplete composting process with microbial activity peaking and dissipating quickly in the two aerated treatments and more slowly in the turned treatment.

Changes in ionophore concentrations during composting

Poultry litter samples were analyzed in triplicate before and after the three different composting processes. In addition, samples from the piled litter were also analyzed. Three (MON, SAL and NAR) of the five IPAs analyzed were present at high concentrations in the poultry litter samples (Table 2). Before composting, the average concentrations were 290±40; 426±46 and 3113±318 μg kg^{-1} for MON, SAL and NAR, respectively. NIG (SS) was not detected in the unspiked samples because it has not been used as feed addictive in the U.S. (Biswas et al., 2012). Similarly, MAD and LAS were also not detected in any of the poultry litter samples. The concentrations of MON decreased depending on the composting process: for turned and piled the concentrations of MON were reduced by less than 20%, while aerated and turned/aerated composting showed reductions by 35.6% and 39.9%, respectively. After composting, SAL and NAR showed similar percent reductions in the aerated and piled samples. The similarity in the degradation behavior between NAR and SAL is not surprising considering that their structures are very similar, with the difference of only one more methyl group on the butanoic acid-conjugated oxane ring in NAR compared to SAL (Fig. 3).

During turned and turned/aerated composting, SAL was reduced more substantially (67.6 and 49.4%, respectively) than NAR (39.5 and 12.9%, respectively).

Sun et al. (2014a) concluded that the effect of poultry litter water content on the degradation of IPAs depends on the temperature, and vice versa. Attributing the changes on IPA concentrations with one parameter (e.g. moisture) is not straight-forward because the changes in the moisture, temperature, oxygen content, and microbial composition can all play important roles in the degradation of IPAs, and these parameters need to be evaluated separately. Higher moisture content and temperature promoted the reduction of SAL and NAR concentrations during turned composting and piled storage; the percent reductions in SAL and NAR were higher when higher temperatures existed for a longer period relative to the other composting procedures. In general, an increase of temperature leads to a higher microbial growth rate, which in turn results in higher biodegradation rates of organic compounds.

The behavior of MON degradation is more difficult to explain; the higher temperatures in A and T/A bins during the first week could have facilitated the degradation of MON during the beginning of the composting period, despite the significant temperature drop to less than 7.5ºC after the third week. The moisture contents in A and T/A bins were also slightly lower relative to the other bins, but yet MON was transformed at a higher rate with ~36% in the A bin and ~40% in the T/A bin, while the %transformation was only ~16% in T and ~20% in piled litter. A shorter field study with a total duration of 57 days showed that poultry litter stacking do not present significant degradation for MON and SAL; in this case water content started in 33%, and reduced to 29% by day 57, and the temperature ranged from 30 to 50 °C (Sun et al., 2014a).

Data were examined using ANOVA to determine if statistically significant differentces ($p<0.05$) between concentrations of IPAs in each litter treatment compared to their initial concentrations can be observed (Table 2). Concentrations of MON in A and T/A bins were significantly lower from the initial MON concentration prior to composting; however MON concentrations in T bin and in piled litter did not show a significant differences compared to the initial concentration. Except for the A bin ($p=0.199$), the concentrations of SAL in the T bin, T/A bin, and piled poultry litter were significantly lower from its initial concentration in the litter. For NAR, significant decreases in concentrations were observed in the T bin and in the piled litter compared

to its initial concentration. Due to these varying results, making a generalization as to which composting process is most effective in degrading IPAs is not straightforward. However, one can infer that turning the compost (in either T or T/A bins) increased the degradation of SAL, NAR, and MON significantly, suggesting that aerobic microbes may play an important role in IPA degradation because turning introduces oxygen into the litter. In addition, where there is significant differences in concentrations observed, the extent of transformation is higher when the temperature is higher. For example, concentration of SAL was significantly decreased in both T and T/A bins; however, 68% transformation was observed in T bin (higher temperature during all monitoring period) and only 49% transformation was observed in T/A bin (temperature peaked to ~70 °C during first week, but dropped down to less than 30 °C during the rest of the composting period). SAL is generally more biodegradable in the T bin than in the A bin. It appears that the %transformation of SAL was decreased when air was increased (A bin). MON on the other hand showed higher % transformation during composting with aeration (both in A and T/A), suggesting that forced aeration favors the reduction of MON in the litter. For SAL and NAR, the percentage of reduction in concentrations in the piled litter is either as high or higher than any of the composting procedures. These findings are important because most of the large poultry operations store their litter by piling. Hence, SAL and NAR may be effectively reduced by simply piling the litter for a longer period prior to land application.

Table 2: Concentration, percent (%) transformation, p-value and ratios of transformation product (TP) or metabolite (M1) to parent ionophore in poultry litter (PL) (n=3*).

	PL-before composting	PL-aerated	PL-turned	PL-turned/aerated	PL-pile
MONENSIN					
Conc. (µg kg^{-1})	290±40	184±34	240±22	172±26	229±2
%Transformation	-	35.6	15.9	39.9	19.8
p-value	-	0.028	0.162	0.014	0.073
TP3:MON	1.28	1.79	2.89	3.18	0.84
M1:MON	1.09	0.75	0.20	0.55	0.54
SALINOMYCIN					
Conc. (µg kg^{-1})	426±46	358±61	138±45	215±23	147±10
%Transformation	-	15.9	67.6	49.4	65.3
p-value	-	0.199	0.001	0.002	0.001
TP1:SAL	0.51	0.57	2.70	0.96	2.15
NARASIN					
Conc. (µg kg^{-1})	3113±318	2402±451	1885±214	2712±123	1409±61
%Transformation	-	22.8	39.5	12.9	54.7
p-value	-	0.089	0.005	0.112	0.001
TP1:NAR	0.12	0.14	0.34	0.15	0.38
TP2:NAR	0.02	0.11	0.05	0.03	0.10

*All samples were collected and analyzed in triplicate; post-composting samples were collected in 04/07/2015. Conc.: concentration; %transformation: percentage of transformation; Confidence level $p<0.05$.

Identification of transformation products by LC-QToF/MS

Three TPs (by-product of degradation in poultry litter after excretion) and one metabolite (formed through metabolism of ionophore by chicken before excretion) were identified using high-resolution mass spectrometry and accurate mass measurements with LC-QToF/MS. First, a search for previously reported ionophore TPs was performed by extracting the known ions from the mass spectra acquired by LC-QToF/MS. For example, *m/z* 531 corresponding to TP1 that has already been reported in the literature was found in the mass spectra of all poultry litter extracts. But previous studies speculated the structure of TP1 based only on low resolution mass spectrometry (Hansen et al., 2012; Sun et al., 2014a, 2014b). The observed accurate mass of *m/z* 531.3295 is the sodium adduct $[M+Na]^+$ of TP1, which was used as precursor ion for MS/MS characterization (Fig. 2) due to its high signal intensity. The proposed empirical formula for TP1 was confirmed using high accurate mass measurements achieved by LC-QToF/MS. The results obtained (Table 3) showed mass error of less than 1 ppm between the theoretical and the observed sodium adduct of the TP1 molecular ion.

Mass error of ≤5 ppm is an accepted limit for QToF/MS instruments when used to acquire accurate mass measurements for predicting empirical formulas (Bristow and Webb, 2003).

TP1 is a common transformation product shared by SAL and NAR due to the similarity in their chemical structures, as shown in the pathway depicted in Fig. 3. TP1 is formed via C-C bond cleavage between the 3-hydroxy and the 4-methyl positions situated in the 3-hydroxy-4-methyl-5-oxooctan-2-yl]-5-methyloxan-2-yl]butanoic acid group of SAL. The only difference between SAL and NAR is the presence of two methyl groups in the oxane ring in NAR, instead of one methyl group in SAL.

The other approach that was performed to search for ionophore TPs was to extract for molecular ions corresponding to the loss of H_2O, since dehydration is a common transformation reaction of organic compounds in the environment. This approach revealed a signal corresponding to the sodium adduct $[M+Na-H_2O]^+$ from NAR (*m/z* 769.4880), which is designated as TP2 in Fig. 3. This is the first report of TP2 being formed after composting of NAR containing animal wastes. While it is possible that SAL may also degrade by losing water due to its similarity in structure with NAR, an analogous TP corresponding to $[M+Na-H_2O]^+$ from SAL was not detected, possibly because of the lower concentrations of SAL in the poultry litter. The observed monoisotopic mass for the sodium adduct of TP2 has less than 2 ppm error with respect to the theoretical accurate mass of the proposed empirical formula (Table 3). Finally, the MS/MS fragmentation of TP2 (Fig. 2) produced major fragment ions, *m/z* 531.3279 and *m/z* 431.2395, that are similar to the molecular ion and fragment ion of TP1, respectively, indicating that TP2 is derived from NAR (Fig. 3).

Similarly, *m/z* 805 was extracted from the LC-QToF/MS data because a TP with *m/z* 805 has been reported previously (Sun et al., 2014b) corresponding to the sodium adduct of SAL after addition of two oxygen atoms. However, based on the MS/MS fragmentation pattern shown in Fig. 2 it can be inferred that *m/z* 805 is not related to SAL. Instead, the fragmentation pattern of *m/z* 805 is similar to MON. Another ion with m/z 547 was observed and has a fragmentation pattern similar to MON; this was designated as TP3 (Fig. 3) and is formed through the cleavage of the C-C bond connecting the 3-methoxy-2-methylpentanoic acid group and the oxane (ring A) in MON.

The error between the observed accurate mass of TP3 (*m/z* 547.3237) and the theoretical accurate mass of the proposed empirical formula is less than 2 ppm (Table 3). Due to the higher mass of the observed ion at *m/z* 805, it was postulated that this compound is a conjugated metabolite of MON, and was therefore designated as M1 (*m/z* 805.4729). The metabolite M1 has a fragmentation pattern that is characteristic of TP3; the major fragment ions observed from M1 (*m/z* 547.3232, 447.2347, 361.1984, and 265.1411) are the same as the fragment ions observed from TP3 (*m/z* 547.3232, 447.2349, 361.1975, and 265.1402), and differ only from each other by less than 5 ppm.

Because of the higher molecular mass of M1 and the similarity in its MS/MS fragmentation pattern with TP3, we speculated that M1 is a conjugated metabolite of MON that is excreted by the chicken, and is later transformed to TP3 during composting. Unfortunately, due to the low concentration of M1 we were unable to isolate this metabolite for further characterization and identification. It is important to emphasize that all three TPs and M1 were detected in the poultry litter prior to composting or piled storage. Therefore, in order to assess whether these compounds were excreted as metabolic products of ionophores or formed as degradation products of composting, the area ratios of the signal intensities of each TP and metabolite relative to the parent ionophore were compared before and after composting or storage (Table 2). Most notably, the ratios of TP3:MON and M1:MON before composting were 1.28 and 1.09, respectively. The TP3:MON ratio increased after composting, which means that TP3 is being formed as MON is being degraded. However, M1:MON ratio decreased after composting or storage, suggesting that M1 was generated prior to composting and was transformed during composting or piled storage. When comparing the changes in the signal intensities of M1 before and after composting or storage with the changes in the signal intensities of TP3, especially in T bin, it is notable that M1 decreased while TP3 increased, suggesting that M1 is being converted to TP3 as depicted in Fig. 3. This observation is another evidence that supports our hypothesis that M1 is a metabolite of MON that is excreted by the chickens, and is later transformed to TP3 during composting of the litter. The lack of MON biodegradation during poultry litter storage has been reported (Sun et al., 2014a); the study showed that after poultry litter application in soil, MON was biodegraded, which means that MON degraders may be widely present

in soil but not in the litter. Therefore, TP3 in the poultry litter may be attributed mainly from the degradation of M1.

Table 3. Transformation products and metabolite of ionophores in poultry litter identified by accurate mass measurements and fragmentation pattern using LC-QToF/MS.

Identified Transformation Products and Metabolite	Empirical Formula $[M+Na]^+$	Theoretical Accurate Mass *m/z*	Observed Accurate Mass *m/z*	Error (ppm)	Retention Time t_R (min)
TP1	$C_{29}H_{48}NaO_7$	531.3298	531.3295	-0.56	14.4
TP2	$C_{43}H_{70}NaO_{10}$	769.4867	769.4880	1.69	16.2
TP3	$C_{29}H_{48}NaO_8$	547.3247	547.3237	-1.83	11.4
M1	-	-	805.4729	-	11.3

Note: The empirical formula and accurate mass listed are for the sodium adducts of each ionophore.

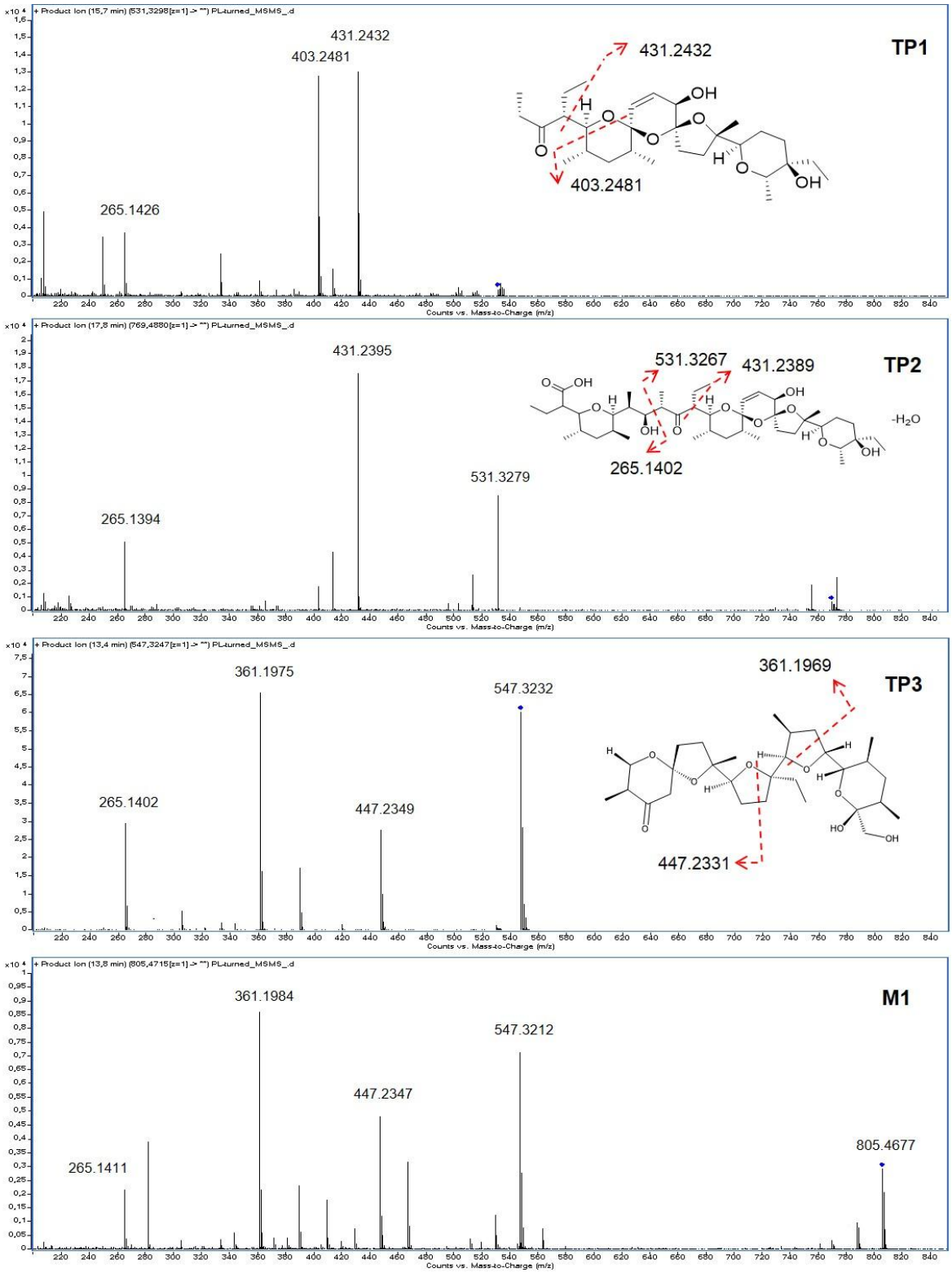

Figure 2: MS/MS spectrum of the transformation products and metabolites identified in poultry litter.

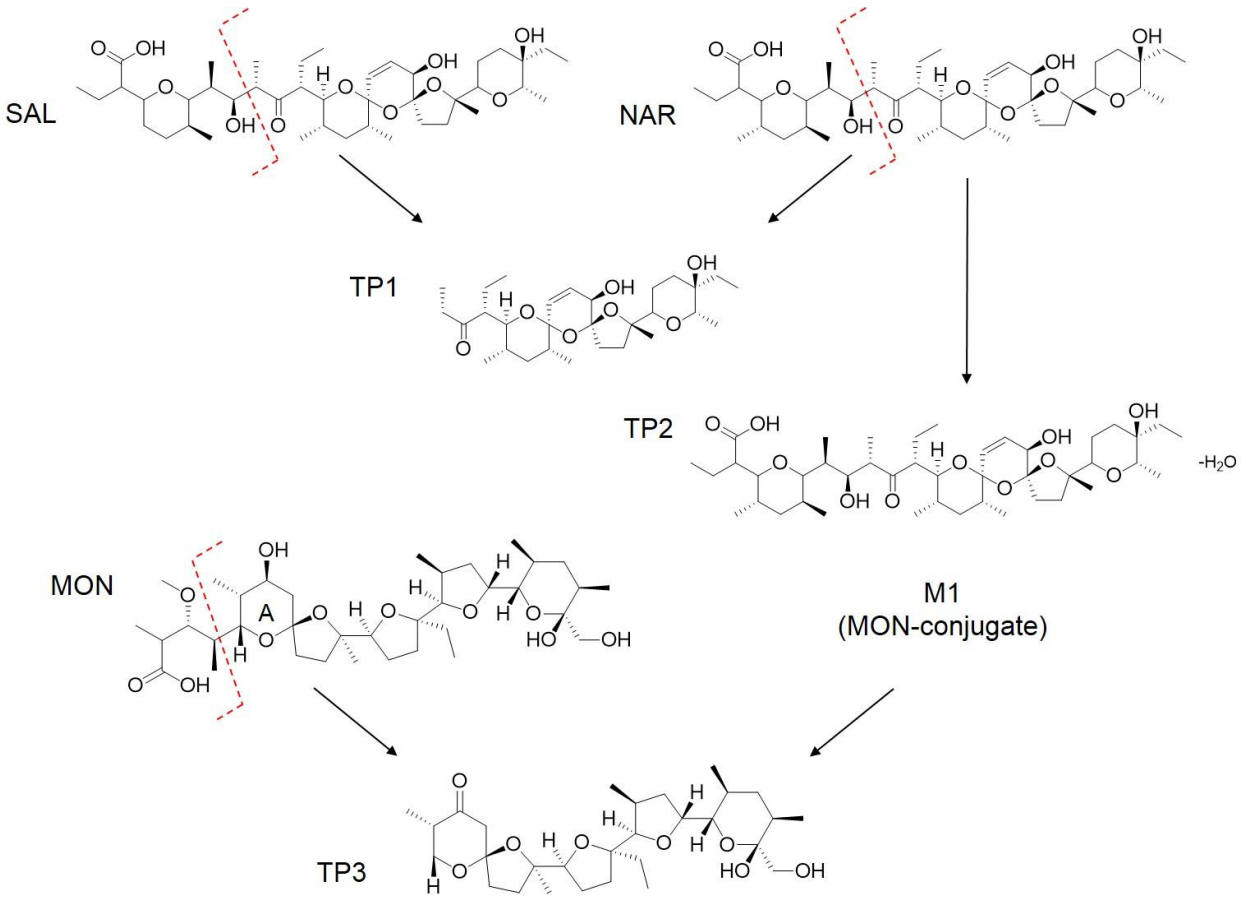

Figure 3. Proposed degradation pathway for NAR, SAL, MON and postulated MON-conjugate in poultry litter.

Conclusions

In this study a sample preparation procedure using QuEChERS method was optimized for the determination of IPAs in poultry litter using LC-MS/MS. The results demonstrated high overall recoveries ranging from 71 to 120%, and good precision with RSD of ≤19%. These results are satisfactory considering that poultry litter is a highly complex matrix with many potential interferents that can suppress signal intensities and result in erroneous quantification. Of all IPAs detected in the poultry litter NAR was the highest in initial concentration, followed by SAL and MON. On-farm composting showed to be most effective in decreasing IPAs using the T bin for NAR (~40% transformation) and SAL (~70% transformation). However, piled storage was surprisingly as effective as turned composting in decreasing the concentrations of NAR (~55% transformation) and SAL (~65% transformation). On the contrary, MON behaved differently than NAR and SAL in that MON was only ~15% transformed in the T bin and ~20% transformed in the piled litter. These results suggest that the degradation of IPAs in poultry litter is complex and different variables can affect the rate of transformation of each IPA. Temperature appeared to be an important factor for the degradation of IPAs during turned composting and piled storage. However, moisture and temperature are both important factors affecting biodegradation because microbial growth, composition, and activity in the poultry litter will be directly affected by these parameters. Therefore, the relative importance of moisture and temperature on the transformation of IPAs in poultry litter during composting needs to be evaluated under more controlled conditions.

The structures of TPs were proposed based on the MS/MS fragmentation pattern of the observed sodium adducts of the molecular ions $[M+Na]^+$. The structures of TP1 and TP2 were proposed based on their characteristic MS fragmentation pattern, both showing the fragment *m/z* 431. TP2 fragments showed *m/z* 531, which correspond to the main TP of NAR. The MS fragmentation of TP3 reflects the cleavage of the aliphatic chain from the ring A of MON, and the oxidation of the hydroxyl group in the ring. The MS/MS fragments *m/z* 447, 361, and 265 are consistent with the proposed structure for TP3 and its formation from the degradation of MON. The MS/MS fragmentation pattern of M1 showing *m/z* 547, 447 and 361 that are the same fragments observed for TP3 are

in accordance to the postulated MON-conjugate being an excreted metabolite of MON in the litter that is subsequently transformed to TP3. Based on the changes in the ratios of M1:MON and TP3:MON, it is reasonable to assume that TP3 is being generated both from the degradation of MON and the MON-conjugate (M1).

Identification of TPs of IPAs is a challenging task particularly in a complex matrix such as poultry litter; however advanced techniques such as LC-QToF/MS can facilitate identification of TPs. While three TPs were detected in this study, it should be noted that due to the sample preparation procedure used, which includes dilution, it is possible that minor TPs formed at low concentrations could have been missed because they are below the detection limits of LC-QToF/MS. Therefore, future work will focus on the identification of minor TPs that are stable in the poultry litter using bench-scale composting with spiked IPAs at higher concentrations to facilitate detection.

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References

1. Abraham, J.; Kepford, R. CEEOT-LP Application to environmental issues in poultry production. *Livestock and the Environment* 2000, 1-22.

2. Anastassiades, M., Lehotay, S.J., Stajnbaher, D., Schenck, F.J. 2003. Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. *J AOAC Int*, 86(2), 412-31.

3. Bak, S.A., Hansen, M., Pedersen, K.M., Halling-Sørensen, B., Björklund, E. 2013. Quantification of four ionophores in soil, sediment and manure using pressurised liquid extraction. *J Chromatogr A*, 1307(0), 27-33.

4. Biswas, S., McGrath, J.M., Sapkota, A. 2012. Quantification of ionophores in aged poultry litter using liquid chromatography tandem mass spectrometry. *J Environ Sci Health B*, 47(10), 959-966.

5. Bristow, A.W., Webb, K.S. 2003. Intercomparison study on accurate mass measurement of small molecules in mass spectrometry. *J Am Soc Mass Spectrom*, 14(10), 1086-98.

6. Chapman, H.D. 1984. Drug resistance in avian coccidia (a review). *Vet Parasitol*, 15(1), 11-27.

7. Dolliver, H., Gupta, S., Noll, S. 2008. Antibiotic degradation during manure composting. *J Environ Qual*, 37(3), 1245-53.

8. Dorne, J.L., Fernandez-Cruz, M.L., Bertelsen, U., Renshaw, D.W., Peltonen, K., Anadon, A., Feil, A., Sanders, P., Wester, P., Fink-Gremmels, J. 2013. Risk assessment of coccidostatics during feed cross-contamination: animal and human health aspects. *Toxicol Appl Pharmacol*, 270(3), 196-208.

9. FDA. 2014. U.S. Food and Drug Administration (U.S. FDA). Summary Report on Antimicrobials Sold or Distributed for Use in Food-Producing Animals; U.S. FDA: Silver Spring, MD, 2014.

10. Hansen, M., Bjorklund, E., Krogh, K.A., Brandt, A., Halling-Sorensen, B. 2012. Biotic transformation of anticoccidials in soil using a lab-scale bio-reactor as a precursor-tool. *Chemosphere*, 86(2), 212-5.

11. Hansen, M., Björklund, E., Krogh, K.A., Halling-Sørensen, B. 2009. Analytical strategies for assessing ionophores in the environment. *TrAC-Trend Anal Chem*, 28(5), 521-533.

12. Martínez-Villalba, A., Moyano, E., Galceran, M.T. 2009. Fast liquid chromatography/multiple-stage mass spectrometry of coccidiostats. *Rapid Commun Mass Sp*, 23(9), 1255-1263.

13. Misra, R.V., Roy, R.N., Hiraoka, H. 2003. On-farm composting methods. *FAO, Food and Agriculture Organization of the United Nations*.

14. Ramaswamy, J., Prasher, S.O., Patel, R.M., Hussain, S.A., Barrington, S.F. 2010. The effect of composting on the degradation of a veterinary pharmaceutical. *Bioresour Technol*, 101(7), 2294-9.

15. UME. University of Maryland Extension, 2013. Sampling manure for nutrient content. Factsheet NM-6. https://extension.umd.edu/sites/default/files/_docs/articles /NM-6_Revised_0.pdf. Accessed November, 2015.

16. Sun, P., Barmaz, D., Cabrera, M.L., Pavlostathis, S.G., Huang, C.-H. 2013. Detection and quantification of ionophore antibiotics in runoff, soil and poultry litter. *J Chromatogr A*, 1312(0), 10-17.

17. Sun, P., Cabrera, M.L., Huang, C.-H., Pavlostathis, S.G. 2014a. Biodegradation of Veterinary Ionophore Antibiotics in Broiler Litter and Soil Microcosms. *Environ. Sci. Technol.*, 48(5), 2724-2731.

18. Sun, P., Pavlostathis, S.G., Huang, C.H. 2014b. Photodegradation of veterinary ionophore antibiotics under UV and solar irradiation. *Environ Sci Technol*, 48(22), 13188-96.

19. Tiquia, S.M., Tam, N.F.Y. 2002. Characterization and composting of poultry litter in forced-aeration piles. *Process Biochem*, 37(8), 869-880.

20. USDA, U.S. Department of Agriculture, 2012. http://www.ers.usda.gov/topics/animal-products/poultry-eggs/background.aspx Accessed: September, 2015.

21. Žižek, S., Dobeic, M., Pintarič, Š., Zidar, P., Kobal, S., Vidrih, M. 2014. Degradation and dissipation of the veterinary ionophore lasalocid in manure and soil. *Chemosphere*, 14(0).

7 CONSIDERAÇÕES FINAIS

O emprego da cromatografia líquida acoplada a espectrometria de massas de alta resolução demonstrou ser uma ferramenta analítica bastante eficiente por combinar tanto os benefícios de identificação e quantificação de contaminantes orgânicos alvo, quanto a identificação e confirmação de produtos de transformação e metabólitos em matrizes complexas utilizando uma análise cromatográfica rápida. Porém, isso não diminui os cuidados necessários com o preparo de amostra, muito pelo contrário, com o emprego de HRMS faz-se tão ou mais importante o preparo de amostra, a fim de minimizar o efeito de matriz devido a presença de interferentes, em especial de componentes isobáricos, os quais podem ser identificados equivocadamente como sendo o analito de interesse.

Tendo em vista isso, o uso do método QuEChERS para a extração de contaminantes orgânicos mostrou-se eficiente para todas as matrizes trabalhadas, filé de peixe, frutas (maçã, pera e uva) e cama de frango, porém deve-se salientar a importância da otimização dos procedimentos para cada uma das matrizes, pois devido a complexidade dessas, as mesmas apresentam comportamentos diferentes frente as variações realizadas em cada uma das otimizações, como por exemplo sais/sorventes de partição e de limpeza utilizados.

Sendo assim, a combinação de moderna instrumentação analítica, preparo de amostra eficiente e minucioso tratamento de dados proporciona a identificação e quantificação em nível de traços de contaminantes orgânicos em matrizes complexas.

REFERÊNCIAS BIBLIOGRÁFICAS

ABRAHAM, J.; KEPFORD, R. CEEOT-LP Application to environmental issues in poultry production, 2000. <http://tiaer.tarleton.edu/pdf/PR0001.pdf> Acesso em janeiro de 2016.

ANASTASSIADES, M. et al. Fast and easy multiresidue method employing acetonitrile extraction/partitioning and “dispersive solid-phase extraction” for the determination of pesticide residues in produce. **J AOAC Int**, 86 (2003) 412.

ANVISA (AGÊNCIA NACIONAL DE VIGILÂNCIA SANITÁRIA), 2010. <http://www.anvisa. gov.br> Acesso em janeiro de 2016.

ANVISA (AGÊNCIA NACIONAL DE VIGILÂNCIA SANITÁRIA); *Guia para Validação de Métodos Analíticos e Bioanalíticos*, RE nº 899, de 29/05/2003.

BAIRD, C. **Química Ambiental**, 2ª ed., Bookman: Porto Alegre, 2002.

BARBOSA, L. C. A. **Os pesticidas, o homem e o meio ambiente.** Viçosa: UFV, 2004.

BIRKETT, J. W.; LESTER, J. N. **Endocrine Disrupters in wastewater and sludge treatment processes.** 1ª ed., IWA Publishing CRC Press LLC: USA, 2003.

BISWAS, S.; MCGRATH, J. M.; SAPKOTA, A. Quantification of ionophores in aged poultry litter using liquid chromatography tandem mass spectrometry. **J Environ Sci Health B**, 47 (2012) 959.

BOTITSI, H. V. et al. Current mass spectrometry strategies for the analysis of pesticides and their metabolites in food and water matrices. **Mass Spectrometry Reviews**, 30 (2011) 907.

BRACK, W. et al. Effect-directed analysis supporting monitoring of aquatic environments - An in-depth overview. **Sci Total Environ**, 544 (2016) 1073.

BRASIL, Decreto nº 4.074, de 04 de janeiro de 2002. Regulamenta a Lei nº 7.802, de 11 de julho de 1989, que dispõe sobre a pesquisa, a experimentação, a produção, a embalagem e rotulagem, o transporte, o armazenamento, a comercialização, a propaganda comercial, a utilização, a importação, a exportação, o destino final dos resíduos e embalagens, o registro, a classificação, o controle, a inspeção e a fiscalização de agrotóxicos, seus componentes e afins, e dá outras providências. <http://www.mprs.mp.br/ambiente/legislacao/id501.htm> Acesso em janeiro de 2016.

BRAZILIAN CHICKEN (UNIÃO BRASILEIRA DE AVICULTURA E ASSOCIAÇÃO BRASILEIRA DOS PRODUTORES E EXPORTADORES DE FRANGOS), 2012 <http://abpa-br.com.br/files/publicacoes/41c30a0f46702351b561675f70fae077.pdf> Acesso em janiero de 2016.

BU, Q. et al. Pharmaceuticals and personal care products in the aquatic environment in China: A review. **J Hazard Mater**, 262 (2013) 189.

CHEN, W. et al. Fates and transport of PPCPs in soil receiving reclaimed water irrigation. **Chemosphere**, 93 (2013) 2621.

CHIARADIA, M. C.; COLLINS, C. H.; JARDIM, I. C. S. F. O estado da arte da cromatografia associada à espectrometria de massas acoplada à espectrometria de massas na análise de compostos tóxicos em alimentos. **Quim Nova***,* 31 (2008) 623.

CRIPPEN, T. L. et al. Poultry litter and the environment: Physiochemical properties of litter and soil during successive flock rotations and after remote site deposition. **Sci Total Environ**, 553 (2016) 650-661.

DEBLONDEA, T.; COSSU-LEGUILLEB, C.; HARTEMANN, P. Emerging pollutants in wastewater: A review of the literature. **Int J Hyg Environ Health***,* 214 (2011) 442.

DEME, P. & UPAGHYAYULA, V. V. R. Ultra performance liquid chromatography atmospheric pressure photoionization high resolution mass spectrometric method for determination of multiclass pesticide residues in grape and mango juices. **Food Chem**, 1142 (2015) 1142.

DENOBILE, M.; NASCIMENTO, E. S. Validação de método para determinação de resíduos dos antibióticos oxitetraciclina, tetraciclina, clortetraciclina e doxicilina, em leite, por cromatografia líquida de alta eficiência. **Rev Bras Ciên Farm**, 40 (2004) 1.

EFSA (AUTHORITY EUROPEAN FOOD SAFETY), 2015. The 2013 European Union report on pesticide residues in food. **EFSA Journal**, 13 (2015) 169.

EUROPEAN UNION, 2009a. Regulamento (CE) nº 470/2009 do Parlamento Europeu e do Conselho, de 6 de maio de 2009.

EUROPEAN UNION, 2009b. Regulation (EC) nº 1107/2009 of the European Parliament and of the Council, de 21 de outubro de 2009.

EUROPEAN UNION, 2010. <http://europa.eu/legislation_summaries/food_safety/contamination_environmental_factors/l21113_pt.htm> Acesso em janeiro de 2016.

FACCO, J. F. et al. Optimization and validation of a multiresidue method for pesticide determination in maize using gas chromatography coupled to tandem mass spectrometry. **Anal Methods**, 7 (2015) 359.

FAIRBAIRN, D. J. et al. Sediment-water distribution of contaminants of emerging concern in a mixed use watershed. **Sci Total Environ**, 505 (2015) 896-904.

FAO (FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS), 2013a. <http://faostat.fao.org/site/379/DesktopDefault.aspx?PageID=379> Acesso em janeiro de 2016.

FAO (FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS), 2013b <https://www.fao.org.br/iccirppcplcfALC.asp> Acesso em janeiro de 2016.

FENIK, J.; TANKIEWICZ, M.; BIZIUK, M. Properties and determination of pesticides in fruits and vegetables. **Trends Analyt Chem**, 30 (2011) 814.

FERRER, I. et al. Multi-residue pesticide analysis in fruits and vegetables by liquid chromatography-time-of-flight mass spectrometry. **J Chromatogr A**, 1082 (2005) 81-90.

FERRER, C. et al. Overcoming matrix effects using the dilution approach in multiresidue methods for fruits and vegetables. **J Chromatogr A**, 1218 (2011) 7634.

FERRER, I.; GARCÍA-REYES, J. F.; FERNANDEZ-ALBA, A. F. Identification and quantification of pesticides in vegetables by liquid chromatography time-of-flight mass spectrometry. **Trends Analyt Chem**, 24 (2005) 671.

FERRER, I.; THURMAN, E. M. **Liquid chromatography time-of-flight mass spectrometry. Principles, tools, and applications for accurate mass analysis.** Ed. Wiley, 2009.

FERRER, I.; THURMAN, E. M.; ZWEIGENBAUM, J. LC/TOF-MS analysis of pesticides in fruits and vegetables: the emerging role of accurate mass in the unambiguous identification of pesticides in food. In: ZWEIGENBAUM, J. **Mass spectrometry in food safety: methods and protocols.** Springer, 2011. p. 193-218.

FIORAVANÇO, J. C. & OLIVEIRA, P. R. D. de. Produção e importação brasileira de pera no período de 2001 a 2012. **Informações Econômicas**, 44, (2014) 16-22.

FURTULA, V.; HUANG, L.; CHAMBERS, P. A. Determination of veterinary pharmaceuticals in poultry litter and soil by methanol extraction and liquid chromatography-tandem mass spectrometry. **J Environ Sci Health** B, 44 (2009) 717.

GARCÍA, S. O. de et al. Ranking of concern, based on environmental indexes, for pharmaceutical and personal care products: An application to the Spanish case. **J Environ Manage**, 129 (2013) 384.

GARCÍA-REYES, J. F. et al. Comprehensive screening of target, non-target and unknown pesticides in food by LC-TOF-MS. **Trends Analyt Chem**, 26 (2007) 828.

GARIBOTT, V. Os agrotóxicos e o direito de escolha dos cidadãos. **Bol Epidemiológico**, 14 (2012) 1.

GLISHA, G. L.; BURINSKY, D. J. Hybrid Mass Spectrometers for Tandem Mass Spectrometry. **J Am Soc Mass Spectrom**, 19 (2008) 161.

GÓMEZ, M. J. et al. Rapid automated screening, identification and quantification of organic micro-contaminants and their main transformation products in wastewater and river Waters using liquid chromatography-quadrupole-time-of-flight mass spectrometry with an accurate-mass database. **J Chromatogr A**, 1217 (2010) 7038.

GOSETTI, F. et al. Signal suppression/enhancement in high-performance liquid chromatography tandem mass spectrometry. **J Chromatogr A**, 1217 (2010) 3929.

GRACIA-LOR, E. et al. Multi-class determination of personal care products and pharmaceuticals in environmental and waste water samples by ultra-high performance liquid-chromatography-tandem mass spectrometry. **Talanta** 99 (2012) 1011.

GRIMALT, S. et al. Quantification, confirmation and screening capability of UHPLC coupled to triple quadrupole and hybrid quadrupole time-of-flight mass spectrometry in pesticide residue analysis. **J Mass Spectrom**, 45 (2010) 421-436.

GROSS, J. H. **Mass spectrometry. A textbook.** 1ª ed. Springer, Alemanha, 2004.

GUTIÉRREZ, C. et al. Effect of soil properties, heavy metals and emerging contaminants in the soil nematodes diversity. **Environ Pollut**, 213 (2016) 184-194.

HANOT, V.; GOSCINNY, S.; DERIDDER, M. A simple multi-residue method for the determination of pesticides in fruits and vegetables using a methanolic extraction and ultra-high-performance liquid chromatography-tandem mass spectrometry: Optimization and extension of scope. **J Chromatogr A**, 1384 (2015) 53.

HARRIS, D. C. **Análise Química Quantitativa.** 7ª ed. LTC, Rio de Janeiro, 2008, cap. 25.

HERRERO-HERNÁNDEZ, E. et al. Occurrence of pesticides and some of their degradation products in waters in a Spanish wine region. **J Hydrol**, 486 (2013) 234.

HILDEBRANDT, A.; LACORTE, S.; BARCELÓ, D. Assessment of priority pesticides, degradation products, and pesticide adjuvants in groundwaters and top soils from agricultural areas of the Ebro river basin. **Anal Bioanal Chem**, 387 (2007) 1459.

HOFFMANN, E. de; STROOBANT, V. **Mass Spectrometry. Principles and Applications.** 3ª ed. Wiley, Inglaterra, 2007.

ICH (INTERNATIONAL CONFERENCE ON HARMONISATION OF TECHNICAL REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE), 2005. <http://www.ich.org/>. Acesso em janeiro de 2016.

INMETRO (INSTITUTO NACIONAL DE METROLOGIA, NORMALIZACAO E QUALIDADE INDUSTRIAL); Orientações sobre Validação de Métodos de Ensaios Químicos, DOQ-CGCRE-008, ver. 4, 2011.

JAKIMSKA, A. et al. Development of a liquid chromatography–tandem mass spectrometry procedure for determination of endocrine disrupting compounds in fish from Mediterranean rivers. **J Chromatogr A**, 1306 (2013) 44.

JARDIM, A. N. O., & CALDAS, E. D. Brazilian monitoring programs for pesticide residues in food – Results from 2001 to 2010. **Food Control**, 25 (2012) 607.

JOKANOVIĆ, M. Biotransformation of organophosphorus compounds. **Toxicology**, 166 (2001) 139.

KALACHOVA, K. et al. Gas chromatography-triple quadrupole tandem mass spectrometry: a powerful tool for the (ultra)trace analysis of multiclass environmental contaminants in fish and fish feed. **Anal Bioanal Chem**, 405 (2013) 7803.

KALYONCU, L.; AGCA, I.; AKTUMSEK, A. Some organochlorine pesticide residues in fish species in Konya, Turkey. **Chemosphere**, 74 (2009) 885.

KAUFMANN, A. et al. Development of an improved high resolution mass spectrometry based multi-residue method for veterinary drugs in various food matrices. **Anal Chim Acta**, 700 (2011) 86.

KLEYWEGT, S. et al. Pharmaceuticals, hormones and bisphenol A in untreated source and finished drinking water in Ontario, Canada - Occurrence and treatment efficiency. **Sci Total Environ**, 409 (2011) 1481.

KRIS-ETHERTON, P. M.; HARRIS, W. S.; APPEL, L. J. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. **Arterioscler Thromb Vasc Biol**, 23 *(*2003) 20.

KWONA, H.; LEHOTAY, S. J.; GEIS-ASTEGGIANTE, L. Variability of matrix effects in liquid and gas chromatography-mass spectrometry analysis of pesticide residues after QuEChERS sample preparation of different food crops. **J Chromatogr A**, 1270 (2012) 235.

LANÇAS, F. M. A Cromatografia Líquida Moderna e a Espectrometria de Massas: finalmente “compatíveis”? **Scientia Chromatographica**, 1 (2009) 35.

LAZARTIGUES, A. et al. Multiresidue method for the determination of 13 pesticides in three environmental matrices: water, sediments and fish muscle. **Talanta**, 85 (2011a) 1500.

LAZARTIGUES, A. et al. Multiresidue method to quantify pesticides in fish muscle by QuEChERS-based extraction and LC-MS/MS. **Anal Bioanal Chem**, 400 (2011b) 2185.

LeDOUX, M. Analytical methods applied to the determination of pesticide residues in foods of animal origin. A review of the past two decades. **J Chromatogr A**, 1218 (2011) 1021.

LI, C. et al. Occurrence of antibiotics in soils and manures from greenhouse vegetable production bases of Beijing, China and an associated risk assessment. **Sci Total Environ,** 521 (2015) 101.

LIU, J.; WONG, M. Pharmaceuticals and personal care products (PPCPs): A review on environmental contamination in China. **Environ Int**, 59 (2013) 208.

MALATO, O. et al. Benefits and pitfalls of the application of screening methods for the analysis of pesticide residues in fruits and vegetables. **J Chromatogr A**, 1218 (2011) 7615.

MAPA (MINISTÉRIO DA AGRICULTURA, PECUÁRIA E ABASTECIMENTO), 2010. <http://www.agricultura.gov.br> Acesso em janeiro de 2016.

MAPA (MINISTÉRIO DA AGRICULTURA, PECUÁRIA E ABASTECIMENTO*), Secretaria de Política Agrícola*, Informativo n°54, vol. 54, 2013.

MAPA (MINISTÉRIO DA AGRICULTURA, PECUÁRIA E ABASTECIMENTO), 2016 <http://www.agricultura.gov.br/vegetal/culturas/uva> Acesso em fevereiro, 2016.

MARÍN, J. M. et al. Application of ultra-high-pressure liquid chromatography-tandem mass spectrometry to the determination of multi-class pesticides in environmental and wastewater samples. Study of matrix effects. **J Chromatogr A**, 1216 (2009) 1410.

MARSHALL, A. G.; HENDRICKSON, C. L. High-resolution mass spectrometers. **Annu Rev Anal Chem**, 1 (2008) 579.

MASIA, A. et al. Combined use of liquid chromatography triple quadrupole mass spectrometry and liquid chromatography quadrupole time-of-flight mass spectrometry in systematic screening of pesticides and other contaminants in water samples. **Anal Chim Acta**, 761 (2013) 117.

MELLO, L. M. R. de. Produção e mercado da pera de 2001 a 2010: panorama nacional e mundial. **Comunicado Técnico**, 133 (2013a) 1.

MELLO, L. M. R. de. Vitivinicultura brasileira: panorama 2012. **Comunicado Técnico**, 137 (2013b), 1.

MOTTALEB, M. A. et al. Gas chromatography-mass spectrometry screening methods for select UV filters, synthetic musks, alkylphenols, an antimicrobial agent, and an insect repellent in fish. **J Chromatogr A**, 1216 (2009) 815.

MPA (MINISTÉRIO DA PESCA E AQUICULTURA), 2010. <http://www.mpa.gov.br/> Acesso em fevereiro de 2014.

MPA (MINISTÉRIO DA PESCA E AQUICULTURA), 2011. <http://www.mpa.gov.br/files/d ocs/Boletim_MPA_2011_pub.pdf> Acesso em janeiro de 2016.

MUNARETTO, J. S. et al. Development of a multiresidue method for the determination of endocrine disrupters in fish fillet using gas chromatography-triple quadrupole tandem mass spectrometry. **Talanta**, 116 (2013) 827.

NÁCHER-MESTRE, J. et al. Qualitative screening of undesirable compounds from feeds to fish by liquid chromatography coupled to mass spectrometry. **J Agric Food Chem**, 61 (2013) 2077.

NASSEF, M. et al. Acute effects of triclosan, diclofenac and carbamazepine on feeding performance of Japanese medaka fish (*Oryzias latipes*). **Chemosphere**, 80 (2010) 1095.

NORLI, H. R.; CHRISTIANSEN, A.; DERIBE, E. Application of QuEChERS method for extraction of selected persistent organic pollutants in fish tissue and analysis by gas chromatography mass spectrometry. **J Chromatogr A**, 1218 (2011) 7234.

NÚÑEZ, O. et al. Strategies for the multi-residue analysis of 100 pesticides by liquid chromatography-triple quadrupole mass spectrometry. **J Chromatogh A**, 1249 (2012) 164-180.

OLDAL, B. et al. Pesticide residues in Hungarian soils. **Geoderma**, 135 (2006) 163.

ORRICO JÚNIOR, M. A. P.; ORRICO, A. C. A.; LUCAS JÚNIOR, J. de. Compostagem dos resíduos da produção avícola: cama de frangos e carcaças de aves. **Eng Agríc**, 30 (2010) 538.

PACHECO-SILVA, E; SOUZA, J. R. DE; CALDAS, E. D. Veterinary drug residues in milk and eggs. **Quim Nova**, 37 (2014) 111.

PARRILLA VÁZQUEZ, P. et al. A sensitive and efficient method for routine pesticide multiresidue analysis in bee pollen samples using gas and liquid chromatography coupled to tandem mass spectrometry. **J Chromatogr A**, 1426 (2015) 161.

PETERS, R. J. B. et al. Multi-residue screening of veterinary drugs in egg, fish and meat using high-resolution liquid chromatography accurate mass time-of-flight mass spectrometry. **J Chromatogr A**, 1216 (2009) 8206.

PINHO, G. P. et al. Efeito de matriz na quantificação de agrotóxicos por cromatografia gasosa. **Quim Nova**, 32 (2009) 987.

PIZZUTTI, I. R. et al. Method validation for the analysis of 169 pesticides in soya grain, without clean up, by liquid chromatography-tandem mass spectrometry using positive and negative electrospray ionization. **J Chromatogr A**, 1142 (2007) 123.

PRESTES, O. D. et al. QuEChERS – Um método moderno de preparo de preparo de amostra para determinação multirresíduo de pesticidas em alimentos por métodos cromatográficos acoplados à espectrometria de massas. **Quim Nova**, 32 (2009)1620.

PRESTES, O. D. et al. O estado da arte na determinação de resíduos de medicamentos veterinários em alimentos de origem animal empregando técnicas cromatográficas acopladas à espectrometria de massas. **Quim Nova**, 36 (2013) 697.

PRiF (PESTICIDE RESIDUE IN FOOD), 2015. Expert Committee on Pesticide Residues in Food. <https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/498714/pesticide-residues-school-fruit-veg-spring-term-2015.pdf> Acessado em fevereiro de 2016.

RAMIREZ, A. J. et al. Analysis of pharmaceuticals in fish using liquid chromatography-tandem mass spectrometry. **Anal Chem**, 79 (2007) 3155.

REZAEI, F.; HOSSEINI, M. M. New method based on combining ultrasonic assisted miniaturized matrix solid-phase dispersion and homogeneous liquid–liquid extraction for the determination of some organochlorinated pesticides in fish. **Anal Chim Acta**, 702 (2011) 274.

RIBANI, M. et al. Validação em métodos cromatográficos e eletroforéticos. **Quim Nova**, 27 (2004) 771.

RIDGWAY, K.; LALLJIE, S. P. D.; SMITH, R. M. Sample preparation techniques for the determination of trace residues and contaminants in foods. **J Chromatogr A**, 1153 (2007) 36.

SANCO, COMMISSION OF THE EUROPEAN COMMUNITIES (2013). **Document nº SANCO/12571/2013.** *Method validation and quality control procedures for pesticide residues analysis in food and feed.*

SAPOZHNIKOVA, Y.; LEHOTAY, S. J. Multi-class, multi-residue analysis of pesticides, polychlorinated biphenyls, polycyclic aromatic hydrocarbons, polybrominated diphenyl ethers and novel flame retardants in fish using fast, low-pressure gas chromatography-tandem mass spectrometry. **Anal Chim Acta**, 758 (2013) 80.

SCHNELL, S. et al. Single and combined toxicity of pharmaceuticals and personal care products (PPCPs) on the rainbow trout liver cell line RTL-W1. **Aquat Toxicol**, 93 (2009) 244.

SEMINÁRIO MERCADO DE AGROTÓXICOS E REGULAÇÃO, 2., 2012, Brasília, DF. Sala de imprensa. Brasília, DF: ANVISA, 2012. <http://portal.anvisa.gov.br/wps/content/anvisa+portal/anvisa/sala+de+imprensa/menu+-+noticias+anos/2012+noticias/seminario+volta+a+discutir+mercado+de+agrotoxicos+em+2012> Acesso em janeiro de 2016.

SOUSA, F. A. de. et al. Influence of pH and matrix components in the chromatographic response of pesticides. **Chromatographia**, 76 (2013) 67.

STASHENKO, E. E.; MARTÍNEZ, J. R. GC-MS: Más de un Analizador de Masas, ¿Para qué? **Scientia Chromatographica**, 2 (2010) 25.

STUART, M. et al. Review of risk from potential emerging contaminants in UK groundwater. **Sci Total Environ**, 416 (2012) 1.

SUÁREZ-MAHECHA, H. et al. Importância de ácidos graxos poliinsaturados presentes em peixes de cultivo e de ambiente natural para a nutrição humana. **Bol Inst Pesca**, 28 (2002) 101.

SUN, P. et al. Biodegradation of veterinary ionophore antibiotics in broiler litter and soil microcosms. **Environ Sci Technol**, 48 (2014a) 2724.

SUN, P. et al. Detection and quantification of ionophore antibiotics in runoff, soil and poultry litter. **J Chromatogr A**, 1312 (2013) 10.

SUN, P. et al. Photodegradation of veterinary ionophore antibiotics under UV and solar irradiation. **Environ Sci Technol**, 48 (2014b) 13188.

TERRY Jr., A. V. Functional consequences of repeated organophosphate exposure: Potential non-cholinergic mechanisms. **Pharmacol Ther**, 134 (2012) 355.

THOMPSON, M.; ELLISON, S. L. R.; WOOD, R. Harmonized guidelines for single laboratory validation of methods of analysis. (IUPAC Technical Report). **Pure Appl Chem**, 74 (2002) 835.

USDA (U.S. DEPARTMENT OF AGRICULTURE), 2012a <http://www.ers.usda.gov/topics/animal-products/poultry-eggs/background.aspx> Acesso em janeiro de 2016.

USDA (U.S. DEPARTMENT OF AGRICULTURE), 2012b <http://usda.mannlib.cornell.ed u/MannUsda/viewDocumentInfo.do?documentID=1825> Acesso em fevereiro de 2016.

VIRTUOSO, M. C. da S. et al. Reutilização da cama de frango. **Nutritime**, 12 (2015) 3964.

WANG, J.; GARDINALI, P. R. Analysis of selected pharmaceuticals in fish and the fresh water bodies directly affected by reclaimed water using liquid chromatography-tandem mass spectrometry. **Anal Bioanal Chem**, 404 (2012) 2711.

WHO (WORLD HEALTH ORGANIZATION), 2009. <http://www.who.int/ipcs/publications/pesticides_hazard_2009.pdf?ua=1> Acesso em janeiro de 2016.

WHO (WORLD HEALTH ORGANIZATION), 2015. <http://www.who.int/features/qa/87/en/> Acessado em fevereiro de 2016.

WILKOWSKA, A.; BIZIUK, M. Determination of pesticide residues in food matrices using the QuEChERS methodology. **Food Chem**, 125 (2011) 803.

WILLE, K. et al. Coupled chromatographic and mass-spectrometric techniques for the analysis of emerging pollutants in the aquatic environment. **Trends Analyt Chem**, 35 (2012) 87.

WOUDNEHA, M. B.; OROS, D. R. Pyrethroids, pyrethrins, and piperonyl butoxide in sediments by high-resolution gas chromatography/high-resolution mass spectrometry. **J Chromatog A**, 1135 (2006) 71.

WÜST, B.; GLAUNER, T. Rapid pesticide screening and identification using the high resolution all ions MS/MS technique. **Agilent Technologies**, (2013) 1.

YONKOS, L. T. et al. Poultry litter-induced endocrine disruption in fathead minnow, sheepshead minnow, and mummichog laboratory exposures. **Environ Toxicol Chem**, 29 (2010) 2328.

ANEXOS

ARTIGO 2 – TRANSFORMATION OF IONOPHORE ANTIMICROBIALS IN POULTRY LITTER DURING PILOT-SCALE COMPOSTING

ANEXO 1: Poultry litter piled on arrival (A), in a pilot-scale turned composting bin (B) and a picture of the compost being turned (C, D).

ANEXO 2: Total nitrogen, phosphorus, potassium, pH and percent moisture content of poultry litter samples before and after composting.

	Total N (%)	Total P (%)	Total K (%)	pH	Moisture (%)
Before composting	3.70	4.80	4.37	7.7	44.3
Aerated (A bin)	3.80	4.70	4.30	7.3	33.1
Turned (T bin)	3.50	5.30	4.80	7.7	46.1
Turned/Aerated (T/A bin)	3.83	5.04	4.75	7.4	40.1
Piled	3.95	7.94	4.13	8.1	59.9

ANEXO 3: Temperature profiles of poultry litter in compost bins and the pile over the 40 d period from arrival on December 11, 2014 to January 20, 2015.