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Leticia
Pérez Mayán

PhD Thesis

Analytical approaches for the determination of plant protection products used in viticulture

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DOCTORAL THESIS

Analytical approaches for the determination of plant protection products used in viticulture

Leticia Pérez Mayán

INTERNATIONAL PHD SCHOOL OF THE UNIVERSITY OF SANTIAGO DE COMPOSTELA

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Nosce te ipsum (γνωθι σεαυτόν)

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INDEX

ABBREVIATIONS AND ACRONYMS

A	
ACE	Acetamiprid
ACN	Acetonitrile
AIF	All-Ion Fragmentation
AME	Ametoctradin
AMPA	(Aminomethyl)phosphonic acid
APCI	Atmospheric-pressure chemical ionization source
API	Atmospheric-pressure ionization source
APPI	Atmospheric-pressure photoionization source
ASE	Accelerated solvent extraction
AZO	Azoxystrobin

B	
BEN	Benalaxyl
BIT	Bitertanol
BOSC	Boscalid
BPR	Backpressure regulator

C	
CAP-triol	Caprolactone-triol
CAR	Carbendazim
CAR-PDMS	Carboxen-polydimethyl siloxane
CGA 108906	Metalaxyl CGA 108906
CGA 62826	Metalaxyl CGA 62826
CE	Collision energy
CHLOR	Chlorpyrifos
CHLORA	Chlorantraniliprole
CHLORM	Chlorpyrifos methyl
CID	Collision-induced dissociation
CLOF	Clofentezine
CLOT	Clothianidin
CSP	Chiral stationary phase
CW20M	Carbowax 20M
CYF	Cyflufenamide
CYM	Cymoxanil
CYP	Cyprodinil

CYP-4OH	Cyprodinil-4OH

D

DAD	Diode-Array Detection
DC	Direct current voltage
DCM	Dichloromethane
DDA	Data dependent acquisition mode
DEA	Diethylamine
DIA	Data independent acquisition mode
DIF	Difenoconazole
DIM	Dimethomorph
DINI	Diniconazole
DINO	Dinotefuran
DLLME	Dispersive liquid-liquid microextraction
dSPE	Dispersive solid-phase extraction
DVB-PDMS	Divinylbenzene-polydimethylsiloxane

E

ECD	Electron capture detector
EE	Extraction efficiency
EF	Enantiomeric fraction
EI	Electron ionization
EMRL	Maximum residue levels for substances banned or in disuse
EPA	Environmental Protection Agency
ESI	Electrospray ionization source
EU	European Union

F

FA	Formic acid
FEN	Fenpropidin
FENA	Fenamidone
FENH	Fenhexamide
FID	Flame ionization detector
FLUD	Fludioxonil
FLUF	Flufenoxuron
FLUO	Fluopicolide
FLUS	Flusilazole
FMOC-Cl	9-fuorenylmethylchloroformate
FOS	Fosetyl
FPSE	Fabric phase sorptive extraction

G

GAC	Green Analytical Chemistry
GC	Gas chromatography
GLY	Glyphosate
GUS	Groundwater ubiquity score

H

HCl	Hydrochloric acid
HILIC	Hydrophilic interaction liquid chromatography
HPLC	High-performance liquid chromatography
HQ	Hazard quotient
HR	High-resolution (analyzer)
HRMS	High resolution mass spectrometry

I

IEC	Ion-exchange chromatography
IGR	Insect growth regulators
IMA	Imazalil
IMI	Imidacloprid
IMI-OLE	Imidacloprid-olefin
IPROV	Iprovalicarb
IS	Internal Standard
IUPAC	International Union of Pure and Applied Chemistry

J

JRC	Joint Research Centre

L

LC	Liquid chromatography
LD50	Lethal dose
LOD	Limit of detection
Log D	Partition coefficient octanol/water
Log Kow	Logarithm (octanol-water partition coefficient)
LOQ	Limit of quantification

M

MAE	Microwave-assisted extraction
MAN	Mandipropamid
ME	Matrix effect
MEC	Measured environmental concentration
MeOH	Methanol
MET	Metalaxyl

METHI	Methiocarb
METR	Metrafenone
MIP	Molecularly Imprinted Polymer
MRL	Maximum residue levels
MRM	Multiple reaction monitoring
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
MSPD	Matrix solid-phase dispersion
MSPE	Magnetic solid-phase extraction
MYC	Myclobutanil

N

NaCl	Sodium chloride
NITE	Nitenpyram
NH_4Ac	Ammonium acetate

O

OC	Organic carbon

P

P. I-VIII	Publication I-VIII
PA	Polyacrylate
PCAP-PDMS-PCAP	Polycaprolactone-poly-dimethylsiloxane-polycaprolactone
PDA	Photodiode-Array Detection
PDMS	Poly(dimethylsiloxane)
PEG	Polyethylene glycol
PEG-PPG-PEG	Poly(ethyleneglycol)-poly(propyleneglycol)-poly(ethyleneglycol)
PEN	Penconazole
PFs	Processing factors
PLE	Pressurized liquid extraction
PNEC	Predicted non-effect concentration
ppb	Parts per billion
PPDB	EU Pesticides Database
PPPs	Plant protection products
PROC	Prochloraz
PROP	Propiconazole
PS-DVB	Polystyrene-divinyl benzene
PTFE	Polytetrafluoroethylene
PTHF	Poly(tetrahydrofuran)
PYRI	Pyrimethanil
PYR-4OH	Pyrimethanil-4OH
PYRA	Pyraclostrobin

Q	
Q	Quadrupole
QqQ	Triple quadrupole
QRai	Quadrupole resolved all ions
QSAR	Quantitative structure-activity relationship
QTOF	Quadrupole-time-of-flight
QuEChERS	Quick, easy, cheap, effective, rugged and safe
QUIN	Quinoxyfen

R	
RAM	Restricted access materials
RF	Radio frequency
RPLC	Reversed-phase liquid chromatography
RQ	Risk quotient

S	
SAX	Strong anionic exchanger
SCX	Strong cationic exchanger
SFC	Supercritical fluid chromatography
SFE	Supercritical fluid extraction
SIM	Selected ion monitoring
SLE	Solid-liquid extraction
SPE	Solid-phase extraction
SPME	Solid-phase microextraction
SWATH	Sequential window acquisition of all theoretical fragment ion spectra

T	
TEBF	Tebufenozide
TEBU	Tebuconazole
THC	Thiacloprid
THIAB	Thiabendazole
THIOM	Thiophanate methyl
THM	Thiamethoxam
TOF	Time-of-flight
TP	Transformation product
TRIAF	Triadimefon
TRIAL	Triadimenol
TRIF	Trifloxystrobin

U	
UAE	Ultrasonic assisted extraction
UHPLC	Ultra-high-performance liquid chromatography
UPLC	Ultra-performance liquid chromatography
USE	Ultrasonic solvent extraction
UV	Ultraviolet

W	
WAX	Weak anionic exchanger
WCX	Weak cationic exchanger
WHO	World Health Organization

Z	
ZOX	Zoxamide

ABSTRACT

Plant protection products have been employed during last decades to ensure the product quality and the productivity in the agricultural sector. Characteristics of the components of applied formulations are variable as function of the crop and the target disease. Furthermore, those sprayed compounds can remain on the crop, be transferred to the soil, be leached to groundwater, or even reach the transformation products derived from the agricultural production. In particular, this Thesis is focused on the study of the presence of pesticides in vineyard soil and wines to draw some conclusions regarding pesticide application, processing factors, degradation and potential pollution of the soil compartment.

Regarding the analytical determination of pesticide residues, laboratories face different challenges, as for example: 1) the complexity of the environmental samples, 2) the need to cover the maximum number of analytes in one method, and 3) the lack of an universal methodology able to determine the wide variety of organic pesticides used during decades and those new active ingredients. Furthermore, analytical approaches are required to evaluate the presence of metabolites and their potential toxicity compared to the parent pesticides. Hence, different analytical methodologies are developed along the present Thesis in order to cover the maximum number of compounds and different chemical families, as so as transformation products, achieving sensitive and reliable detection of pesticide residues in wine and soil samples.

To this aim, research included in this Thesis was divided into four chapters following the four main objectives proposed. The topics developed in each chapter are described below.

Chapter 1. Development of multianalyte methodologies for the determination of pesticides in wine samples by LC-MS. Four publications were developed with this aim, resulting in the development of three multianalyte approaches in wine and the simultaneous determination of two fungicides and their 4-hydroxyanilino derivatives, as so as the evaluation of other hydroxylated and glycosylated metabolites in wine and grape samples.

Chapter 2. Assessment of new strategies for the determination of polar compounds. Following this topic, one publication including two methodologies for the accurate determination of glyphosate, (aminomethyl)phosphonic acid and Fosetyl in wine samples was elaborated.

Chapter 3. Optimization of extraction and determination of pesticide residues in vineyard soil. A multianalyte method was optimized and validated in order to detect the presence of pesticide residues in this matrix and their time-course control.

Chapter 4. Evaluation of SFC as a green alternative to detect the presence of PPPs in environmental samples. Finally, two methodologies were developed with SFC as separation technique. Firstly, seven neonicotinoids were determined in wine samples. The second approach was focused on the determination of five chiral fungicides and on the evaluation of their enantiomeric distribution in wine and soil samples.

RESUMEN

El uso de productos fitosanitarios en agricultura ha experimentado un incremento notable en las últimas décadas. Este hecho, ha despertado el interés de los organismos públicos y la población en general en cuanto a sus posibles efectos adversos a corto, medio y largo plazo, tanto para el medioambiente como para diferentes animales y para los propios humanos. Una vez realizada la aplicación de los compuestos comerciales, estos pueden sufrir acumulación o transferirse a diferentes compartimentos medioambientales. En el caso del vino, los pesticidas pueden llegar a este producto en función de sus factores de transferencia (PFs). Por otro lado, los pesticidas que alcancen el suelo pueden sufrir lixiviación, evaporación o acumularse en esta matriz en función de su estructura química, de las características del suelo y del clima.

La creciente preocupación por el efecto y posible toxicidad de los pesticidas en organismos no diana ha suscitado interés en el control de los productos comercializados, pero también de diferentes matrices medioambientales que puedan ayudar a comprender mejor su comportamiento. Otra consecuencia ha sido el crecimiento exponencial de la agricultura ecológica, lo que, por otro lado, requiere un control mucho más exhaustivo y métodos analíticos más sensibles que permitan asegurar la ausencia de pesticidas en productos derivados de este tipo de producción agrícola.

En este sentido, durante la realización de la presente Tesis se han desarrollado diferentes metodologías analíticas centradas en el uso de la espectrometría de masas, de baja y alta resolución, para la determinación de compuestos fitosanitarios y sus productos de transformación (TPs) en vinos y suelos de viñedo. Además, la publicación III incluye el uso de uvas, con fines semicuantitativos, en el estudio de productos de transformación.

Para condensar de forma sistemática todos los aspectos importantes desarrollados en el presente trabajo, la memoria de Tesis doctoral se ha dividido en seis secciones principales:

La S**ección I** incluye una amplia introducción sobre los diferentes tipos de pesticidas, su presencia en el medioambiente y la legislación aplicable a estos compuestos. También se describen todas las etapas en el proceso de análisis de productos fitosanitarios, teniendo en cuenta su estado sólido o líquido. Para ello, se exponen en profundidad las bases de la extracción en fase sólida (SPE) y la FPSE (*Fabric phase sorptive extraction*) en el caso de vinos; y la extracción con líquidos presurizados (PLE), en el caso de las muestras de suelo. Dentro de la etapa de determinación, se profundiza en la separación de los analitos mediante cromatografía líquida (LC), o cromatografía de fluidos supercríticos (SFC), así como la detección de los compuestos empleando espectrómetros de masas de baja o alta resolución, con capacidad para medir relaciones *m/z* exactas, como son el triple cuadrupolo (QqQ) y el cuadrupolo-tiempo de vuelo (QTOF), respectivamente. Por último, se presentan los compuestos seleccionados en el estudio, sus características, las diferentes familias a las que pertenecen y su uso regulado en uvas de vinificación.

En la **Sección II** se lleva a cabo la justificación de los diferentes trabajos incluidos en la Tesis, poniendo en contexto la importancia de su control en las matrices seleccionadas. Asimismo, se presentan los objetivos principales propuestos para la realización de la Tesis, que son los siguientes:

Objetivo 1. Desarrollo de metodologías multianalito para la determinación de residuos de productos fitosanitarios en muestras de vino. La cromatografía líquida de ultra-alta resolución (UHPLC) combinada con espectrometría de masas en tándem (MS/MS) se utilizará para llevar a cabo la cuantificación de los analitos. Los métodos optimizados deberán permitir la determinación de un gran número de pesticidas con los menores efectos de matriz posibles en concentraciones entre los sub ppb y 100-200 ppb. Adicionalmente, se incluirá la determinación de productos de transformación y la búsqueda de derivados glicosilados mediante LC-QTOF-MS.

Objetivo 2. Desarrollo de estrategias de separación alternativas a la cromatografía líquida en fase reversa (RPLC) para la determinación de pesticidas polares como el glyphosate (GLY), sus productos de transformación y compuestos análogos.

Objetivo 3. Desarrollo de métodos de extracción para la determinación de pesticidas en suelos de viñedo. Los procedimientos de determinación desarrollados en el objetivo 1 se aplicarán al análisis de los extractos de suelo.

Objetivo 4. Evaluación de la cromatografía de fluidos supercríticos (SFC) como una alternativa a la cromatografía líquida (LC), y de gases (GC), para la determinación de pesticidas en vino. Asimismo, SFC se utilizará en la determinación de fungicidas quirales y en el control de su distribución enantiomérica en muestras de vino y suelos de viñedo.

En la **Sección III** se hace una descripción de los procedimientos metodológicos empleados en los trabajos que se incluyen en la Tesis.

Las metodologías analíticas desarrolladas se aplicaron principalmente a la determinación de productos fitosanitarios en muestras de vino y suelos de viñedo. Puntualmente, se llevó a cabo el análisis de muestras de uva de vinificación con fines semicuantitativos.

Por un lado, las muestras líquidas se procesaron mediante SPE, FPSE o mediante derivatización, así como inyección directa, que se empleó en alguna aplicación. Por otro lado, las muestras de suelo se procesaron mediante PLE y las uvas se analizaron mediante extracción sólido-líquido (SLE).

Por último, la separación analítica y la determinación de los residuos de pesticidas se llevó a cabo empleando la cromatografía líquida en fase reversa (RPLC) para la mayoría de los compuestos considerados. También se evaluó la cromatografía líquida de interacción hidrofílica (HILIC) en el caso de algunos compuestos polares y, por otro lado, la SFC se empleó en dos aplicaciones como alternativa a las anteriormente citadas. Los instrumentos combinados con las técnicas anteriores fueron el QqQ y el QTOF. De esta forma, los resultados se procesaron empleando los softwares MassLynx® o MassHunter® en función del equipo de Waters® o Agilent® en cuestión, respectivamente.

La **Sección IV** incluye brevemente los resultados obtenidos durante el trabajo realizado. Los estudios llevados a cabo durante el desarrollo de la Tesis doctoral han dado lugar a ocho publicaciones, que se han distribuido en cuatro capítulos atendiendo a su similitud en cuanto a la matriz analizada, las características de los compuestos, o la técnica analítica empleada.

Capítulo 1. Desarrollo de metodologías multianalito para la determinación de pesticidas en muestras de vino mediante cromatografía líquida acoplada a espectrometría de masas (LC-MS).

En este capítulo se incluyen cuatro publicaciones en las que se desarrollan diferentes métodos de extracción para la matriz de vino. La primera de ellas recoge la determinación de 50 compuestos mediante SPE. El segundo de los trabajos se centra en la extracción de 21 pesticidas mediante FPSE. La cuarta publicación desarrolla la extracción y determinación de 48 compuestos mediante SPE *on-line*. Además, el tercer trabajo se centra en la detección de derivados hidroxilados y glicosilados de dos fungicidas (cyprodinil, CYP y pyrimethanil, PYRI) en vino y uvas.

Estas metodologías han sido optimizadas y validadas para, posteriormente, ser aplicadas a muestras de vino comercial. La determinación de los compuestos se realizó mediante UPLC o LC-QqQ-MS, excepto en el caso de la tercera publicación, en la que se empleó un sistema QTOF para la búsqueda e identificación de nuevos metabolitos de interés.

Capítulo 2. Evaluación de nuevas estrategias para la determinación de compuestos polares.

Este capítulo incluye una publicación en la que se desarrollan dos metodologías para la determinación de glyphosate (GLY) y su principal producto de transformación, (aminomethyl)phosphonic acid (AMPA), en vino. Por un lado, un método de extracción mediante un polímero impreso molecularmente (MIP) y posterior derivatización con 9-fluorenylmethylchloroformate (FMOC-Cl) permitió la detección mediante RPLC de los compuestos a niveles de concentración muy bajos. Por otro lado, se optimizó un método directo para la detección de GLY, AMPA y Fosetyl (no compatible con la derivatización) utilizando una columna de intercambio aniónico fuerte. Ambos métodos permitieron el estudio de la posible presencia de los analitos en muestras reales de vino.

Capítulo 3. Optimización de la extracción y determinación de residuos de pesticidas en suelos de viñedo.

Dentro de este capítulo se incluye una publicación centrada en la optimización de un método de extracción que permite la determinación de una gran variedad de pesticidas en suelos. Con ese objetivo, se ha optimizado la extracción de los compuestos mediante PLE y su determinación se ha llevado a cabo empleando el método analítico desarrollado en la publicación I. Esta metodología optimizada se aplicó a muestras de suelo recogidas en Galicia durante un período de dos años para controlar la presencia de residuos de fungicidas e insecticidas, así como para determinar su velocidad de disipación y/o acumulación en esta matriz.

Capítulo 4. Evaluación de SFC como una alternativa "verde" para la determinación de productos fitosanitarios en muestras medioambientales.

En este capítulo se incluyen dos publicaciones en las que el método de separación de los analitos es la SFC. La primera publicación se centra en la determinación de siete insecticidas neonicotinoides por SFC y su comparación con los resultados obtenidos mediante UHPLC. Los datos obtenidos sitúan la SFC como la mejor técnica para la separación y detección de estos compuestos en muestras de vino. La metodología optimizada se aplicó a muestras de vino comercial de la campaña 2018, cuantificando imidacloprid (IMI) en la mitad de las muestras procesadas.

El segundo trabajo se ha centrado en la optimización de la determinación de cinco fungicidas quirales, dos acilalaninas y tres triazoles. Además, se ha puesto especial interés en el control de su perfil enantiomérico, ya que se siguen utilizando formulaciones de compuestos racémicos a pesar de que, en ocasiones, uno de los enantiómeros no sea activo. Para ello, se utilizaron los métodos de extracción SPE (publicación I) y PLE (publicación VI) para recuperar los fungicidas de las muestras de vino y suelo, respectivamente. Para la separación se utilizaron dos columnas con una fase estacionaria quiral basada en polisacáridos. La metodología optimizada se aplicó a muestra reales para obtener las fracciones enantioméricas de cada compuesto y se pudo comprobar como el metalaxyl (MET) se aplica en ocasiones de forma racémica. Además, este compuesto muestra una degradación enantioselectiva y dependiente de la muestra.

En la **Sección V** se incluye la discusión general de los trabajos incluidos en la Tesis, profundizando en el desarrollo metodológico de cada trabajo y comentando los resultados obtenidos en cada uno de ellos. Además, se muestra la clara correlación que existen entre ellos. Por ejemplo, el método de extracción desarrollado en la publicación I se utiliza en la publicación VII. Otro ejemplo podría ser el uso del método analítico elaborado en la publicación I, que se ha aplicado en las publicaciones II, III y VII.

Por último, la **Sección VI** describe las conclusiones obtenidas en los trabajos presentados a lo largo de la Tesis doctoral, que se citan a continuación:

Capítulo 1. Desarrollo de metodologías multianalito para la determinación de productos fitosanitarios en muestras de vino.

- Se determinaron cincuenta compuestos mediante SPE y UPLC-ESI-QqQ-MS, mejorando los métodos previamente presentados en cuanto a tiempo de análisis, precisión y límites de cuantificación (LOQs). El análisis de muestras reales reveló la presencia de pesticidas, aunque se encontraban en pequeñas concentraciones. Asimismo, se detectaron residuos de pesticidas en vinos con la etiqueta de producción ecológica. Por todo ello, se espera que la metodología desarrollada sea de utilidad para el control de pesticidas en vinos comerciales de producción tradicional y ecológica.
- Se ha desarrollado, y aplicado por primera vez a vinos, un método basado en FPSE en combinación con UPLC-ESI-MS/MS para la determinación de fungicidas (19 especies) e insecticidas (3 compuestos) con el mínimo uso de disolventes orgánicos. Se han optimizado diferentes parámetros relacionados con el rendimiento de la extracción, alcanzando LOQs por debajo de 1 ng/mL, incluso sin llegar a las condiciones de equilibrio en la etapa de microextracción. Se llevó a cabo el análisis de un conjunto de muestras de vino comercial, hallándose residuos de pesticidas en concentraciones dentro del rango entre 0.2 ng/mL y 130 ng/mL.
- Se ha llevado a cabo la determinación simultánea de PYRI, CYP y sus derivados 4-hidroxianilina en muestras de vino. Se analizó una gran cantidad de muestras de vino comercial, con una confirmación previa de la presencia de los compuestos de interés, y se observó que los "ratios" entre los derivados y los fungicidas variaba entre vinos tintos y blancos, siendo mayores en el caso de los vinos tintos. Además, se comprobó la coexistencia de derivados hidroxilados, en diferentes posiciones del anillo pirimidina, en vino, así como en uvas tratadas previamente con una formulación conteniendo CYP. También se detectaron derivados glicosilados en las

muestras de vino. Sin embargo, se requiere un estudio más detallado de los derivados y su potencial toxicidad comparada con los fungicidas originales.

- La monitorización automática de 48 pesticidas se optimizó con el uso de SPE *on-line* y LC-MS/MS. Se estableció una metodología con buena sensibilidad y selectividad, que cuenta con ventajas como la reducción en la manipulación de la muestra, menor coste y una reducción en la generación de residuos sólidos. Los límites de cuantificación se situaron por debajo de 1 ng/mL utilizando 3 µL de muestra e incluso valores diez veces inferiores si se incrementa el volumen hasta los 25 µL. Se analizaron muestras de vino comercial, confirmando y cuantificando la presencia de un 25% de los compuestos considerados a niveles por encima de 10 ng/mL, en al menos una de las muestras. Por otro lado, solo una de las muestras con la etiqueta de producción ecológica se encontraba totalmente libre de residuos de pesticidas. Teniendo en cuenta los LOQs del método, las concentraciones de residuos de fitosanitarios en las muestras restantes con distintivo ecológico se situaron entre 0.1 ng/mL y 6 ng/mL.

Capítulo 2. Desarrollo de metodologías para la determinación de pesticidas aniónicos en muestras de vino.

- Se aplicaron dos aproximaciones diferentes para la determinación de pesticidas aniónicos en muestras de vino.
- Una combinación de un MIP seguido de una derivatización con FMOC-Cl permitió la extracción y determinación efectiva de GLY y AMPA en muestras de vino mediante UPLC-MS/MS. Los LOQs se situaron en 0.5 ng/mL para GLY y 1 ng/mL para AMPA.
- La determinación directa de GLY, AMPA y Fosetyl se llevó a cabo en vino con el uso de una columna de intercambio aniónico fuerte en combinación con un instrumento QqQ. Los LOQs alcanzados fueron de 0.2 ng/mL para Fosetyl, 1 ng/mL para GLY y 8.3 ng/mL para AMPA. A pesar de que AMPA muestra un límite más alto que en el caso del protocolo con derivatización, este valor es aceptable para asegurar un control riguroso en muestras de vino.
- Se analizó un conjunto de muestras de vino comercial y se detectaron residuos de GLY y Fosetyl en un 70% de las muestras aproximadamente, al contrario que el AMPA, que solo se detectó en una muestra por debajo de su LOQ. Las concentraciones de Fosetyl detectadas se situaron entre 0.5 ng/mL y 63.8 ng/mL, mientras que para GLY se mantuvieron entre 1.4 ng/mL y 31.4 ng/mL.

Capítulo 3. Desarrollo de métodos de extracción para la determinación de pesticidas en muestras de suelo.

- Se desarrolló un método multiresiduo para la determinación de pesticidas en muestras de suelo de viñedo. Se obtuvo una metodología con buena sensibilidad y selectividad para un conjunto de 44 pesticidas, utilizando PLE en combinación con UPLC-ESI-MS/MS.
- El método validado se aplicó a muestras reales de suelo recolectadas en Galicia (España) al principio de la primavera. Los resultados mostraron la presencia de residuos de doce fungicidas y un insecticida (IMI) en concentraciones superiores a 10 ng/g. Las concentraciones más altas detectadas se correspondieron con dimethomorph (DIM), carbendazim (CAR), myclobutanil (MYC), pyraclostrobin (PYRA) y fluopicolide (FLUO).

- Los "ratios" de disipación desde el final del otoño hasta la primavera se situaron por debajo de 50% para la mitad de los analitos, lo que implica que esos compuestos son propensos a sufrir acumulación en el suelo si se aplican en campañas de producción consecutivas.
- El análisis de la evolución temporal de los compuestos ha arrojado datos sobre los niveles de contaminación de los suelos de viñedo en la zona de estudio. En este caso, se han identificado compuestos que permanecen en el suelo en concentraciones superiores a 100 ng/g en años consecutivos.

Capítulo 4. Evaluación de SFC como una alternativa para la detección de insecticidas de polaridad media y alta, y de fungicidas quirales en muestras relacionadas con el sector vitivinícola.

- Se desarrolló y optimizó un método que incluye SPE combinada con SFC-ESI-MS/MS para la determinación de siete insecticidas neonicotinoides en vino. Los LOQs se situaron en el rango entre 1 ng/mL y 11 ng/mL. Las pruebas realizadas mediante SFC sitúan esta técnica con una mejor separación y forma de pico, menor supresión de señal y, por lo tanto, una técnica más conveniente para el análisis de neonicotinoides en vino en comparación con UPLC en fase reversa.
- Se analizaron veinticinco muestras de vino blanco producidas en Galicia, siguiendo la metodología optimizada. Acetamiprid (ACE) se detectó en dos muestras por debajo de su LOQ. Por el contrario, el IMI se cuantificó en la mitad de las muestras procesadas en un rango de concentraciones entre 1.3 ng/mL y 33 ng/mL.
- Adicionalmente, el empleo de dos métodos cromatográficos de SFC permitió la determinación de cinco fungicidas quirales (acilalaninas y triazoles). Los LOQs se situaron entre 0.5 ng/mL y 2.5 ng/mL para vino, y entre 1.3 ng/g y 6.5 ng/g para suelos.
- Se analizó un conjunto de diecisiete vinos y se detectaron tebuconazole (TEBU) y MYC en algunos de ellos sin mostrar variaciones en sus fracciones enantioméricas, con relación a los racematos empleados en las formulaciones comerciales. Por el contrario, el MET se detectó en todas las muestras con variaciones en su fracción enantiomérica (EF entre 0.05 y 0.57), lo que implica el uso de la mezcla enantiomérica o la aplicación de la forma activa (metalaxyl-M, MET-M) en algunos casos. Sería necesario contar con más información sobre los tratamientos aplicados para poder extraer conclusiones sobre posibles cambios en su fracción enantiomérica durante el proceso de vinificación.
- También se procesaron siete muestras de suelo y todos los compuestos fueron detectados en al menos una de las muestras. No se detectaron diferencias para MYC, TEBU y benalaxyl (BEN) en cuanto a sus fracciones enantioméricas. Por el contrario, el MET mostró diferencias en los "ratios" de disipación para sus isómeros en función de la muestra de suelo y el punto de muestreo, produciéndose así una degradación enantioselectiva.

RESUMO

O uso de produtos fitosanitarios en agricultura experimentou un notable incremento nas últimas décadas. Este feito espertou o interese dos organismos públicos e a poboación en xeral en canto ós seus posibles efectos adversos a curto, medio e longo prazo, tanto para o medioambiente como para diferentes animais e os propios humanos. Logo de realizar a aplicación das formulacións comerciais ós cultivos, estes produtos poden sufrir acumulación ou transferirse a diferentes compartimentos medioambientais. No caso do viño, os pesticidas poden chegar a estar presentes neste produto final en función dos seus factores de transferencia (PFs) durante o proceso de vinificación. Por outro lado, os pesticidas que acaden o solo poden sufrir procesos de lixiviación, evaporación ou acumulación nesta matriz en función da súa estrutura química, das características do solo e do clima da zona xeográfica na que se atope.

A crecente preocupación polo efecto e posible toxicidade dos produtos fitosanitarios en organismos non diana tamén espertou o interese no control dos produtos comercializados que derivan da produción agrícola, mais tamén de diferentes matrices medioambientais que poden axudar a unha mellor comprensión do comportamento destes compostos. Outra consecuencia foi o crecemento exponencial da agricultura ecolóxica, o que, por outra banda, require un control moito máis exhaustivo e métodos analíticos máis sensibles, que permitan asegurar a ausencia de residuos de pesticidas en produtos derivados deste tipo de produción agrícola.

Neste sentido, durante a realización da presente Tese desenvolvéronse diferentes metodoloxías analíticas centradas no uso da espectrometría de masas (MS), de baixa e alta resolución, para a determinación de compostos fitosanitarios e os seus produtos de transformación (TPs) en viños e solos de viñedo. A maiores, a publicación III inclúe o uso de uvas de vinificación, con fins semicuantitativos, no estudo de produtos de transformación de dous funxicidas amplamente utilizados (cyprodinil, CYP e pyrimethanil, PYR).

Para abranguer de forma sistemática tódolos aspectos importantes desenvoltos no presente traballo, a memoria de Tese doutoral dividiuse en seis seccións principais.

A **Sección I** inclúe unha ampla introdución sobre os diferentes tipos de produtos fitosanitarios, a súa presenza no medioambiente e a lexislación aplicable a estes compostos na actualidade. Tamén se describen tódalas etapas dentro do proceso de análise de pesticidas, tendo en conta o seu estado sólido ou líquido. Con ese fin, móstranse en profundidade as bases da extracción en fase sólida (SPE) e a FPSE (*fabric phase sorptive extraction*) no caso dos viños; e a extracción con líquidos presurizados (PLE), no caso das mostras de solo. Dentro da etapa de determinación, profundábase na separación dos analitos mediante cromatografía líquida (LC), ou cromatografía de fluídos supercríticos (SFC), así como na detección dos compostos empregando espectrómetros de masas, de baixa ou alta resolución, con capacidade para medir relacións *m/z* exactas, como son o triplo cuadrupolo (QqQ) e o cuadrupolo-tempo de voo (QTOF), respectivamente. Por último, preséntanse os compostos

seleccionados neste estudo, as súas características, as diferentes familias ás que pertencen e o seu uso regulado en uvas de vinificación.

Na **Sección II** lévase a cabo a xustificación dos diferentes traballos incluídos na Tese, poñendo en contexto a importancia do control dos produtos fitosanitarios nas matrices seleccionadas. Tamén se presentan os obxectivos principais planeados para a elaboración da Tese, que son os seguintes:

Obxectivo 1. Desenvolvemento de metodoloxías multianalito para a determinación de residuos de produtos fitosanitarios en mostras de viño. A cromatografía líquida de ultra-alta resolución (UHPLC) combinada con espectrometría de masas en tándem utilízase para levar a cabo a cuantificación dos analitos seleccionados. Os métodos optimizados deberán permitir a determinación dun gran número de residuos de pesticidas, con baixos efectos de matriz, en niveis de concentración entre os sub ppb e os 100-200 ppb. De xeito adicional, incluirase a determinación de produtos de transformación e a busca de derivados glicosilados dalgúns pesticidas de interese mediante LC-QTOF-MS.

Obxectivo 2. Desenvolvemento de estratexias de separación alternativas á cromatografía líquida en fase reversa (RPLC) para a determinación de residuos de pesticidas polares como o glyphosate (GLY), os seus produtos de transformación e compostos análogos.

Obxectivo 3. Desenvolvemento de métodos de extracción para a determinación de produtos fitosanitarios en solos de viñedo. Os procedementos de determinación optimizados no obxectivo 1 aplicaranse á análise dos extractos de solo.

Obxectivo 4. Avaliación da cromatografía de fluídos supercríticos como unha alternativa á cromatografía líquida (LC) e de gases (GC), para a determinación de residuos de pesticidas con polaridade media e alta en mostras de viño. Do mesmo xeito, a SFC empregarase na determinación de funxicidas quirais e no control da súa distribución enantiomérica en mostras de viño e solos de viñedo.

Na **Sección III** faise unha descrición dos procedementos metodolóxicos empregados nos traballos que se inclúen na Tese.

As metodoloxías analíticas desenvoltas aplicáronse principalmente á determinación de produtos fitosanitarios en mostras de viño e solos de viñedo. Puntualmente, levouse acabo a análise de mostras de uva de vinificación con fins semicuantitativos.

As mostras líquidas procesáronse mediante SPE, FPSE ou mediante derivatización, así como a inxección directa, que se empregou nalgunha aplicación. Por outra banda, as mostras de solo procesáronse con PLE e as uvas analizáronse mediante extracción sólido-líquido (SLE).

Por último, a separación analítica e determinación leváronse a cabo empregando cromatografía líquida en fase reversa (RPLC) para a maioría dos compostos considerados. Tamén se evaluou a cromatografía líquida de interacción hidrofílica (HILIC), no caso dalgúns compostos polares e, por último, a SFC empregouse en dúas aplicacións como alternativa ás anteriores. Os instrumentos combinados coas técnicas anteriores foron o QqQ e o QTOF. Deste xeito, os resultados procesáronse empregando os software MassLynx® ou MassHunter® en función do equipo de Waters® ou Agilent® empregado, respectivamente.

A **Sección IV** comprende unha breve compilación dos resultados obtidos co traballo realizado. Os estudos levados a cabo durante o desenvolvemento da Tese doutoral deron lugar

a oito publicacións, que se distribúen en catro capítulos atendendo á súa similitude en canto á matriz analizada, ás características dos compostos, ou á técnica analítica empregada.

Capítulo 1. Desenvolvemento de metodoloxías multianalito para a determinación de produtos fitosanitarios en mostras de viño mediante cromatografía líquida acoplada a espectrometría de masas (LC-MS).

Este capítulo abrangue catro publicacións nas que se desenvolven diferentes métodos de extracción para a matriz de viño. A primeira delas recolle a determinación de cincuenta compostos mediante SPE. O segundo dos traballos céntrase na extracción de vinte e un pesticidas mediante FPSE. A cuarta publicación desenvolve a extracción e determinación de corenta e oito compostos mediante SPE *on-line*. Ademais, o terceiro traballo incluído neste capítulo céntrase na detección de derivados hidroxilados e glicosilados de dous funxicidas (cyprodinil, CYP e pyrimethanil, PYRI) en viño e uvas de vinificación.

Estas metodoloxías foron optimizadas e validadas para, posteriormente, aplicalas a mostras de viño comercial. A determinación dos compostos levouse a cabo mediante UPLC ou LC-QqQ-MS, excepto no caso da terceira publicación, na que se empregou un sistema QTOF para a busca e identificación de novos metabolitos de interese.

Capítulo 2. Avaliación de novas estratexias para a determinación de compostos polares.

Este capítulo inclúe unha publicación na que se desenvolven dúas metodoloxías para a determinación de GLY e o seu principal produto de transformación, (aminomethyl)phosphonic acid (AMPA), en mostras de viño. Por un lado, un método de extracción mediante o uso dun polímero impreso molecularmente (MIP) e a posterior derivatización empregando 9-fluorenylmethylchloroformate (FMOC-Cl) permitiu a determinación mediante RPLC dos compostos en niveis de concentración moi baixos. Por outro lado, optimizouse un método directo para a determinación de GLY, AMPA e Fosetyl (non compatible co proceso de derivatización), empregando unha columna de intercambio aniónico forte. Ámbolos dous métodos permitiron o estudo da posible presenza dos analitos en mostras de viño comercial.

Capítulo 3. Optimización da extracción e determinación de residuos de pesticidas en solos de viñedo.

Dentro deste capítulo inclúese unha publicación centrada na optimización dun método de extracción que permita a determinación dunha gran variedade de produtos fitosanitarios en solos de viñedo. Con ese obxectivo, optimizouse o proceso de extracción dos compostos mediante PLE e a súa determinación levouse a cabo empregando o método analítico desenvolto na publicación I. Diferentes mostras de solo recollidas en Galicia (España) durante un período de dous anos foron analizadas para controlar a posible presenza de residuos de funxicidas e insecticidas, así como para determinar a velocidade de disipación e/ou acumulación destes compostos na matriz en cuestión.

Capítulo 4. Avaliación da cromatografía de fluídos supercríticos (SFC) como unha alternativa "verde" para a determinación de produtos fitosanitarios en mostras medioambientais.

Este capítulo abrangue dúas publicacións nas que o método de separación dos analitos é a SFC. O primeiro traballo céntrase na determinación de sete insecticidas neonicotinoides mediante SFC e a súa comparación cos resultados obtidos para estes mesmos compostos empregando UHPLC. Os datos obtidos sitúan a SFC como a mellor técnica para a separación

e determinación destes compostos en mostras de viño. A metodoloxía optimizada aplicouse a mostras de viño comercial da campaña 2018, cuantificando imidacloprid (IMI) na metade das mostras procesadas.

O segundo traballo incluído neste capítulo centrouse na optimización da determinación de cinco funxicidas, dúas acilalaninas e tres triazoles. A maiores, centrouse o interese no control do seu perfil enantiomérico, xa que se seguen empregando formulacións de compostos racémicos a pesar de que, en ocasións, un dos enantiómeros non sexa activo. Para iso, empregáronse os métodos de extracción SPE (publicación I) e PLE (publicación VI) para recuperar os funxicidas das mostras de viño e solo, respectivamente. A separación cromatográfica levouse a cabo empregando dúas columnas diferentes cunha fase estacionaria quiral baseada en polisacáridos. Esta metodoloxía optimizada aplicouse á análise de mostras reais para obter as fraccións enantioméricas de cada composto e púidose comprobar como o metalaxyl (MET) se aplica en ocasións na súa forma racémica. Ademais, este composto mostra unha degradación enantioselectiva e dependente da mostra analizada.

Na **Sección V** desenvólvese a discusión xeral dos traballos incluídos na Tese, profundando no desenvolvemento metodolóxico de cada traballo e comentando os resultados obtidos en cada un deles. Asemade, móstrase a correlación que existe entre as diferentes publicacións. Por exemplo, o método de extracción desenvolto na publicación I é o mesmo empregado na publicación VII. Outro exemplo podería ser o uso do método analítico elaborado na publicación I, que se aplicou posteriormente nos traballos das publicacións II, III e VII.

Por último, a **Sección VI** describe as conclusións obtidas nos traballos presentados ao longo da presente Tese doutoral, que se citan a continuación:

Capítulo 1. Desenvolvemento de metodoloxías multianalito para a determinación de produtos fitosanitarios en mostras de viño.

- Levouse a cabo a determinación de cincuenta compostos empregando SPE en combinación con UPLC-ESI-QqQ-MS, mellorando os métodos previamente presentados en canto a tempo de análise, precisión e límites de cuantificación (LOQs). A análise de mostras reais revelou a presenza de residuos de pesticidas, inda que se atopaban en pequenas concentracións. Do mesmo xeito, detectáronse residuos de pesticidas en viños coa etiqueta de produción ecolóxica. En base a todo isto, agárdase que a metodoloxía desenvolta sexa de utilidade para o control de residuos de pesticidas en viños comerciais, tanto de produción tradicional como ecolóxica.
- Desenvolveuse un método baseado na extracción mediante FPSE e determinación con UPLC-ESI-MS/MS para a determinación de funxicidas (19 compostos) e insecticidas (3 especies) co mínimo uso de disolventes orgánicos, ademais de aplicarse por primeira vez a mostras de viño. Optimizáronse diferentes parámetros relacionados co rendemento da extracción, acadando LOQs por debaixo de 1 ng/mL, incluso sen chegar ás condicións de equilibrio na etapa de microextracción. Tamén se levou a cabo unha análise dun conxunto de mostras de viño comercial, cuantificando residuos de pesticidas en concentracións entre 0.2 ng/mL e 130 ng/mL.
- Determináronse de xeito simultáneo os funxicidas PYRI e CYP, e os seus derivados 4-hidroxianilina en mostras de viño. A maiores, analizouse unha grande cantidade de mostras de viño comerciais (60 mostras), cunha confirmación previa da

presenza dos compostos de interese, e observouse que os "ratios" entre os derivados e os funxicidas eran variables comparando viños tintos e brancos, sendo maiores no caso dos viños tintos. Tamén se comprobou a coexistencia de derivados hidroxilados en diferentes posicións do anelo pirimidina en viño, así como en uvas previamente tratadas cunha formulación que contiña CYP. Por outro lado, detectáronse derivados glicosilados en mostras de viño. En base ós resultados, sería preciso realizar un estudo máis exhaustivo dos derivados dos funxicidas e a súa potencial toxicidade comparada cos funxicidas de partida.

- Optimizouse a monitorización automática de corenta e oito pesticidas mediante SPE *on-line* en combinación con LC-MS/MS. Deste xeito, estableceuse unha metodoloxía cunha boa sensibilidade e selectividade, ademais de contar con diferentes vantaxes como son a redución na manipulación das mostras, un menor custo de análise e a redución na xeración de residuos sólidos. Os límites de cuantificación situáronse por debaixo de 1 ng/mL empregando 3 µL de mostra e incluso se chegaron a valores dez veces menores incrementando o volume de mostra ata os 25 µL. A continuación, levouse a cabo a análise de mostras de viño comercial, confirmando e cuantificando a presenza dun 25% dos compostos estudados en niveis de concentración por enriba de 10 ng/mL, cando menos nunha das mostras procesadas. Por outra banda, só unha das mostras coa etiqueta de produción ecolóxica analizadas estaba totalmente libre de residuos de pesticidas. Tendo en conta os LOQs do método, as concentracións de residuos de fitosanitarios nas mostras restantes co distintivo de produción ecolóxica situáronse en valores ente 0.1 ng/mL e 6 ng/mL.

Capítulo 2. Desenvolvemento de metodoloxías analíticas para a determinación de pesticidas aniónicos en mostras de viño.

- Determináronse residuos de pesticidas aniónicos en mostras de viño, aplicando dúas metodoloxías analíticas previamente optimizadas e validadas.
- A extracción e determinación de GLY e AMPA en mostras de viño levouse a cabo combinando o uso dun MIPLOQs seguido dunha derivatización con FMOC-Cl e posterior análise mediante UPLC-MS/MS. Este protocolo permitiu acadar LOQs da orde de 0.5 ng/mL para GLY e 1 ng/mL para AMPA.
- Por outro lado, levouse a cabo a determinación directa de GLY, AMPA e Fosetyl en mostras de viño, empregando unha columna de intercambio aniónico forte en combinación cun detector QqQ. Os LOQs acadados situáronse en 0.2 ng/mL para Fosetyl, 1 ng/mL para GLY e 8.3 ng/mL para AMPA. Pese a que AMPA mostra un límite de cuantificación máis alto que no caso do protocolo coa derivatización, este valor é aceptable para asegurar un control rigoroso deste composto en mostras de viño.
- Analizouse un conxunto de mostras de viño comercial e detectáronse residuos de GLY e Fosetyl aproximadamente nun 70 % das mostras procesadas. Pola contra, o composto AMPA só se detectou nunha mostra, por debaixo do seu LOQ. As concentracións de Fosetyl nas mostras analizadas situáronse entre 0.5 ng/mL e 63.8 ng/mL, mentres que os niveis de GLY se mantiveron entre 1.4 ng/mL e 31.4 ng/mL.

Capítulo 3. Desenvolvemento de métodos de extracción para a determinación de pesticidas en mostras de solo.

- Levouse a cabo o desenvolvemento dun método multiresiduo para a determinación de residuos de fitosanitarios en mostras de solo de viñedo. Deste xeito, obtívose unha metodoloxía cunha boa sensibilidade e selectividade para un conxunto de corenta e catro pesticidas, empregando PLE en combinación con UPLC-ESI-MS/MS.
- Este método validouse e, posteriormente, empregouse para a análise de mostras reais de solo recollidas en Galicia (España) ó comezo da primavera. Os resultados obtidos mostraron a presenza de residuos de doce funxicidas e un insecticida (IMI) en niveis de concentración superiores a 10 ng/g. As concentracións máis altas detectadas correspóndense cos compostos dimethomorph (DIM), carbendazim (CAR), myclobutanil (MYC), pyraclostrobin (PYRA) e fluopicolide (FLUO).
- Os "ratios" de disipación comparando os datos obtidos a finais do outono e a comezos da primavera situáronse por debaixo do 50% para a metade dos analitos, o que implica que estes compostos son propensos a sufrir procesos de acumulación no solo de viñedo no caso de aplicarse en campañas de produción consecutivas.
- A análise da evolución temporal dos compostos detectados en concentracións significativas permitiu levar a cabo unha estimación dos niveis de contaminación dos solos de viñedo na zona de estudo. En concreto, identificáronse compostos que permanecen no solo en niveis de concentración superiores a 100 ng/g en anos consecutivos.

Capítulo 4. Avaliación da SFC como unha alternativa para a detección de insecticidas de polaridade media e alta, e de funxicidas quirais en mostras relacionadas co sector vitivinícola.

- Levouse a cabo o desenvolvemento e optimización dun método que emprega SPE combinada con SFC-ESI-MS/MS para a determinación de sete insecticidas neonicotinoides en viño. Os LOQs situáronse no rango entre 1 ng/mL e 11 ng/mL. As probas realizadas mediante SFC sitúan esta técnica cromatográfica como a máis idónea en termos de separación e forma de pico, menor supresión de sinal na fonte de ionización e, polo tanto, unha técnica máis convinte para a análise dos neonicotinoides estudados en mostras de viño, en comparación coa técnica de UPLC en fase reversa.
- Analizáronse vinte e cinco mostras de viño branco producidas en Galicia, seguindo o protocolo previamente optimizado. Por un lado, detectouse o composto acetamiprid (ACE) por debaixo do seu LOQ en dúas mostras. Pola contra, o IMI foi cuantificado na metade das mostras procesadas nun intervalo de concentracións entre 1.3 ng/mL e 33 ng/mL.
- Por outro lado, o emprego de dous métodos cromatográficos de SFC permitiu a determinación de cinco funxicidas quirais (acilalaninas e triazoles). Os LOQs acadados situáronse entre 0.5 ng/mL e 2.5 ng/mL no caso de mostras de viño e entre 1.3 ng/g e 6.5 ng/g para mostras de solo de viñedo.
- Levouse a cabo a análise dun conxunto de dezasete mostras de viño. Os compostos tebuconazole (TEBU) e myclobutanil (MYC) detectáronse nalgúns dos viños procesados sen mostrar variacións nas súas fraccións enantioméricas, con relación aos racematos empregados nas formulacións comerciais. Pola contra, o MET detectouse en tódalas mostras procesadas con variacións na súa fracción enantiomérica (EF entre 0.05 e 0.57), o que implica o uso da mestura enantiomérica ou a aplicación da forma activa (metalaxyl-M, MET-M) nalgúns casos. Sería preciso contar con máis información dos tratamentos aplicados para poder extraer conclusións sobre posibles cambios na fracción enantiomérica do MET durante o proceso de vinificación.

- Tamén se levou a cabo a análise de sete mostras de solo de viñedo e detectáronse tódolos compostos, cando menos nunha das mostras. Neste caso non se detectaron diferenzas para as fraccións enantioméricas de MYC, TEBU e benalaxyl (BEN). Pola contra, o MET mostrou diferenzas nos "ratios" de disipación para os seus isómeros en función da mostras de solo e o punto de mostraxe, producíndose deste xeito unha degradación enantioselectiva.

I. INTRODUCTION

I.1. PESTICIDES: DEFINITION AND CLASSIFICATION

The term pesticide involves any chemical or biological product used to control, repel, or attack undesirable organisms interfering with the correct development of plants or animals.

Pesticides are classified taking into consideration various criteria related to their use, mode of action, chemical structure, or toxicity. Categorization regarding pesticides use is the most extended, and includes groups such as acaricides, biocides, fungicides, herbicides, insecticides, nematicides and rodenticides. Once applied to plants, pesticides have different modes to display their action on the targeted organisms. For instance, they can be classified as contact or systemic pesticides, whether they only persist on the surface or reach the internal tissues.

Nevertheless, the World Health Organization (WHO) recommends the use of pesticides acute toxicity for their classification. According to this criterium, pesticides are classified using their estimated lethal dose LD50 (dose required to kill half of the tested animals when entering the body). Following that categorization, pesticides are distributed in five levels:

Ia – Extremely hazardous
Ib – Highly hazardous
II – Moderately hazardous
III – Slightly hazardous
U – Unlikely to present acute hazard

Moreover, those substances can be distributed considering their chemical family, as for example acidics, azoles, carbamates, neonicotinoids, organochlorines, organophosphates, triazines, ureas, etc. This classification is crucial from an analytical point of view, since compounds with similar structures are prone to show analogous behaviour regarding their extraction and determination conditions [1] .

Pesticides play a key role in the sustainability of the modern agriculture production. Conversely, since pesticide industry was established almost one century ago, synthetic compounds replaced natural substances employed to combat pests. The population growth and the rise in the food demand forced the spread of pesticides use, including also industrial and urban applications. Nowadays, the development strategy for creating new pesticides is focused on 1) reducing dosage, 2) increasing degradability, and 3) achieving a better selectivity (reducing toxicity for non-target organisms) [2]. Currently, 455 active substances are authorized in the EU [3]. Specifically, Spain is the European country with the largest consumption of pesticides according to Eurostat statistics, accounting 75,397 Ton in 2019. Furthermore, Spain (941,154 Ha), together with France (802,896 Ha) and Italy (650,698 Ha), is the European country with the largest agricultural area dedicated to wine production [4].

I.1.1. FUNGICIDES

Fungicides are used as antimycotic agents to control fungal infections in crops. Specifically, vineyards are affected by different fungi, which might compromise wine production and quality. The most common fungal diseases affecting vines are downy mildew (*Plasmopara viticola*), powdery mildew (*Uncinula necator*) and grey mold (*Botrytis cinerea*)

[5]. Geography and weather conditions are decisive factors for the development of diverse fungi variants, which force farmers to spray specific fungicide formulations.

Fungicides can either be systemic, contact, or translaminar. Systemic fungicides are taken up and distributed through the entire plant, while translaminar fungicides act in the surface and inside the plant, but only in the sprayed area. Finally, contact fungicides protect the surface where the formulation is deposited [6]. Furthermore, fungicides can be classified as regards to their chemical structure in groups such as amides, benzimidazoles, carbamates, dicarboximides, dithiocarbamates, imidazoles, morpholines, pyridines, pyrimidines, quinolines, strobilurins, thiazoles, triazoles, and ureas [7].

Since the former inorganic fungicidal treatments used during the 1940s, fungicide formulations were constantly evolving to achieve a better balance between effectiveness and toxicity. For instance, azolic fungicides are one of the most efficient fungicides to treat grey mold, yet their toxicity is proved on non-target species such as fish [8]. Therefore, research is moving towards new safer and eco-friendly formulations [9].

I.1.2. HERBICIDES

Herbicides are substances used to prevent and/or control the growth of unwanted plants. These chemicals can be applied in pre-harvest or post-harvest and classified as selective or non-selective, as function of their specificity for weeds over crops. For instance, as glyphosate (GLY) is a non-specific herbicide, genetic modification of crops is mandatory to reduce target site sensitivity and enhance detoxification [10].

A wide range of chemical groups are available: amides, acetamides, benzoic acids, bipyridyl derivatives, dinitro compounds, dinitraniline, imidazolidones, methyl uracil, nitriles, phenoxy acid derivatives, polycyclic alkanoic, triazines and triazolopyrimidines [11]. Every structure set different characteristics regarding their mode of action when applied and, also, their effects on plants, animals and humans. The majority of herbicides are very toxic to animals, such as fish, and humans either by skin contact, inhalation or ingestion [12]. Thus, their application should be controlled to avoid environmental damages. In Spain, GLY is the dominant herbicide used in vineyards, representing nearly 89% of the global market of herbicides sprayed on vineyards [13].

I.1.3. INSECTICIDES

Insecticides are pesticides employed to control the presence of hazardous insects. Their main target is the nervous system, although other organs can be affected. The most dangerous insects for vineyards are the grape moth (*Lobesia botrana*), vine mealybug (*Planococcus ficus*), and the citrus mealybug (*Planococcus citri*) [5]. Insecticides can be classified according to their chemical family as follows: organochlorines, organophosphates, carbamates, pyrethroids, formamidines, avermectins, neonicotinoids, spinosyns, and insect growth regulators (IGR) [11].

Neonicotinoids are synthetic insecticides derived from nicotine and broadly used since 1991. The trend of insecticide development has changed from organophosphorus, carbamate, and synthetic pyrethroids to nicotinic and diamide insecticides [2]. However, neonicotinoids showed potential toxicity to honeybees and, thus, the EU banned the outdoor uses of imidacloprid (IMI), clothianidin (CLOT) and thiamethoxam (THM) in 2018, and their renewal expired on 2019-2020. Thus, acetamiprid (ACE)is the only neonicotinoid approved for its use at this moment (2022). Therefore, a new generation of neonicotinoids is being developed based on their low toxicity for honeybees (i.e. flupyradifurone, flupyrimin and triflumezopyrim).

I.2. PESTICIDES IN THE VINEYARD ENVIRONMENT: SOIL AND WINE SAMPLES

Formulations sprayed to crops only reach their target in a 0.1% extent [9]. After application, pesticides can suffer different chemical or physical transformations as function of their chemical nature, volatility and solubility (Figure 1). Abiotic (hydrolysis, photolysis, oxidation) and biotic (biodegradation, metabolism) transformations can control the disappearance rate of pesticides [14].

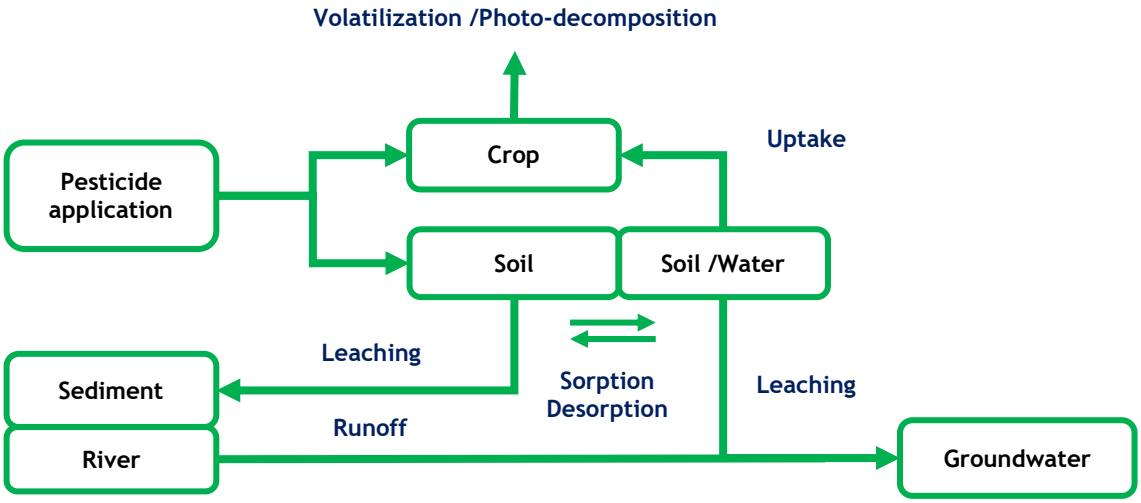


Figure 1. Pathways of applied pesticides in the environment compartments.

Soil characteristics also contribute to the adsorption/mobility of compounds, i.e., soil texture, organic matter, and pH, as so as climate conditions (wind, temperature, humidity and rainfall). All the previous factors contribute to the diffuse chemical pollution [15]. Thus, pesticides can be present not only in the target crop and the surrounding area, but also in other environmental compartments. For instance, formulations applied in a specific agricultural area could reach groundwater and, even, rivers and the sea. Favourable mobility rate and chemical characteristics could lead to water and organisms' pollution and accumulation. Bhagat et al. [8] have proven the negative effect of azole compounds in the aquatic organisms. Azoles and analogues caused health problems related to growth, reproduction, immunology and behaviour [8].

Vineyards are permanent crops receiving a large number of fungicides and insecticides to ensure a good quality production. Firstly, grapes are processed to obtain wine and, therefore, some pesticide residues could remain during the vinification process, reaching the final product. Processing factors (PFs), the ratio between the residue in the processed commodity and the initial fruit, are compound dependent. It has been demonstrated that pesticides water solubility and log K_{ow} are the main factors controlling the presence/absence of residues in wine. Pesticides with a high log K_{ow} show low PFs, meanwhile more hydrophilic compounds are transferred in a bigger extent to wine [16]. In this vein, Table 1 shows PFs for a selection of the analytes consulted in the European database of PFs [17] and different research conducted in wine [16,18]. Undoubtedly, the PF of a given compound is also affected by the vinification technique and, particularly, by the contact time between grape peels and must during fermentation operations.

Table 1. Processing factors (PFs) calculated for pesticides belonging to different chemical families.

Active substance	Processing factor	Ref.	Active substance	Processing factor	Ref.
ACE	0.43	[16]	IMA	0.055	[16]
AME	0.03	[17]	IMI	0.38	[16]
AZO	0.22	[16]	IPROV	0.65	[17]
BOSC	0.03	[18]	MAN	0.013	[18]
CAR	0.38	[16]	MYCL	0.12	[17]
CHLORA	0.23	[17]	PEN	0.06	[16]
CHLOR	0.06	[17]	PEN	0.13	[17]
CHLORM	0.10	[17]	PYRAN	0.05	[17]
CYP	0.0057	[16]	TEBU	0.11	[17]
DIM	0.2	[16]	TEBF	0.31	[17]
FLUD	0.0092	[18]	Tetraconazole	0.44	[17]
FLUD	0.08	[17]	THIAB	0.16	[16]
FLUS	0.09	[17]	THIOM	0.79	[17]

Recent research has proved the presence of fungicides and insecticides in grapes and wine samples [19,20]. Additionally, exhaustive research has been done to group the latest works regarding the determination of pesticides in wine matrix. Table 2 shows the notable works regarding the determination of plant protection products (PPPs) in wines, including the set of pesticides studied, the extraction, separation and detection methods used, and their limits of quantification (LOQs).

Table 2. Summary of the outstanding research developed from 2010 to 2021 regarding the determination of pesticide residues in wine samples.

Matrix	Pesticides	Extraction method	Separation/detection method	LOQs (µg/L)	Ref.
Wine	187	QuEChERS	UPLC-ESI-QqQ-MS	5	[21]
Wine	173	QuEChERS	UHPLC-QTOF and GC-QqQ	2.60 - 21.39 (µg/kg)	[19]
Wine	49	QuEChERS	UHPLC-MS/MS	0.1 – 20 (LOD)	[22]
Wine	50	SPE	UHPLC-ESI-QqQ-MS	0.1-15	[23]
Wine	185	Direct injection	UHPLC-ESI-QqQ-MS	10-20 (µg/kg)	[24]
Wine	15	Direct injection	HPLC-ESI-QqQ-MS	1	[25]
Wine	90	QuEChERS	UHPLC-QqQ-MS	10-20	[26]
Wine	25	SPE	GC-EI-MS/MS	0.01-50	[27]
Wine/grape	406	QuEChERS	UHPLC-ESI-QqQ-MS	1-10 (µg/kg)	[20]
Wine	22	FPSE	UHPLC-ESI-QqQ-MS	0.03-0.3	[28]
Wine/must	15	QuEChERS	UPLC-ESI-QqQ-MS	10-125	[29]
Wine	48	SPE online	UPLC-ESI-QqQ-MS	0.1-2.5	[30]
Wine	160	dSPE	GC-EI-QqQ-MS	0.01-0.05 (mg/kg)	[31]
Wine	27	DLLME	GC-EI-Q-MS	0.025 – 0.77 (LOD)	[32]
Wine	27	SPE- DLLME	GC–QTOF-MS	0.2-1.4	[33]
Wine	5	SPE	UPLC-ESI-QqQ-MS	0.2-0.8	[34]

Lately, concern on soil pollution has raised abruptly. EU is conducting an exhaustive survey with regard to land use and land cover in order to obtain an overview of socioenvironmental characteristics of the EU territory (biodiversity, soil degradation, climate change effects, etc) [35]. In this vein, pesticide research can be focused on those regions with a high agricultural use. Silva et al. [36] evaluated the presence of pesticide residues in European agricultural soils in order to draw some conclusions regarding pesticide use and persistence in this compartment. Furthermore, there are some recent data from different production areas in Spain reflecting the presence of pesticide residues in vineyard soils [29,37]. The following table (Table 3) shows the characteristics of the research carried out in the last decade for the determination of pesticides in soil and related solid samples.

Table 3. Summary of the research in the last decade regarding the determination of pesticides in solid samples (soil and sediment).

Matrix	Pesticides	Extraction method	Separation/detection method	LOQs (µg/kg)	Ref.
Soil	49	PLE	UHPLC-QqQ	0.2-13	[38]
Soil	17	SLE	UPLC-ESI-Q	0.22-0.65	[39]
Soil	21	Soxhlet	GC-ECD	0.002 – 0.21	[40]
Soil	76	QuEChERS	UPLC-QqQ and GC-EI-Q-Orbitrap	10-50 (LC) 5 (GC)	[36]
Soil	15	SLE	UPLC-ESI-QqQ	10-250	[29]
Soil	7	PLE	GC-MS	LOD 1.4 – 4.6	[41]
Soil	6	SLE	GC-MSD	4-6	[42]
Soil and plants	42	UAE and PLE	UHPLC-Orbitrap	10-50	[43]
Soil	3	SLE	HPLC-MS/MS	50	[44]
Soil, sediment	18	SLE + MSPE	Chiral LC-ESI-QqQ-MS	0.5	[45]
Soil, sediment	7	UAE	GC-MS	300-500 µg/ml	[46]
Soil, sediment	7	SFE – DLLME	GC-FID	1-9	[47]
Soil	32	UAE	HPLC-ESI-Q-MS	1.5-5	[48]

As concern about the extended use of PPPs has risen, different risk assessment approaches have been adopted by institutions to estimate the possible adverse consequences of these chemicals. Those approaches calculate indexes using scores given to different physicochemical and toxicological properties of the substances under study. Scientists have adopted the hazard quotient (HQ) and risk quotient (RQ) methods, recommended by the Environmental Protection Agency (EPA), to stablish different levels of toxicity for specific organisms regarding health or ecological risk assessment, respectively. The RQ compares the measured environmental concentration (MEC) of the substance and its predicted non-effect concentration (PNEC) as follows: RQ = MEC / PNEC. The PNEC can be calculated by experimental assays or using a model to estimate the effect on the organism. For instance, quantitative structure-activity relationship (QSAR) models are recognized as alternatives to animal testing. Vašíčková et al. characterized the environmental risk for agroecosystems using the RQ and considering in-soil invertebrates and microorganisms [49].

I.3. LEGISLATION

Legislation related to pesticides use is continuously updating. Those rules include an strict protocol for approval of new phytosanitary compounds, setting limits to the presence of those chemicals in food commodities and monitoring pesticides in foodstuff and in the environment [7].

In the EU, the first Directive was established in the 1970s, limiting the use of some compounds proved to be toxic for the aquatic organisms and the environment [50]. Since then, the European Commission has been revising the authorized pesticides to ensure safety conditions in agriculture, industry and domestic activities. Currently, pesticides approved to be employed in the EU are embodied in the EU Pesticides Database (PPDB) [3].

Not only is the control of pesticide residues necessary in environmental matrices, but also it should be regulated in foodstuff. For this purpose, maximum residue levels (MRLs) were established for food and feed to reduce the human exposure to these chemicals. MRL is defined by the IUPAC (International Union of Pure and Applied Chemistry) as the maximum concentration of a residue that is legally permitted or recognized as acceptable in, or on, a food, agricultural commodity, or animal feedstuff, as set by Codex or a national regulatory authority. It is normally expressed as mg/kg of fresh weight [51] and it is important to note that MRLs are based on good agricultural practices, not assumed as maximum toxicological limits. The EMRL is a version of the previous index, to stablish the maximum concentrations for substances in disuse or that have been banned but they can be still present in food and

feed. If a pesticide is not specifically enlisted, a general default MRL of 0.01 mg/kg is set. Although those limits are established for a wide variety of food commodities, there are some processed foods not regulated yet, as it is the case of wine. Some countries have established their own regulation regarding this matrix, but there is no international consensus.

Monitorization of pesticide residues is carried out by authorized institutions to draw some conclusions regarding their presence and persistence in foodstuff. For instance, the Joint Research Centre (JRC) has published in 2020 the third Watch List, a report about the concerning substances in water in order to consider them as priority substances to be monitored [52]. The azole compounds (imazalil (IMA), ipconazole, metconazole, penconazole (PEN), prochloraz (PROC), tebuconazole (TEBU) are proposed to be included in the next Watch List in inland surface waters. In addition, 1,2,4-triazole is presented as a key compound to monitor the presence of azole substances in the environment.

Document Nº SANTE/12682/2019 includes the analytical quality control and method validation procedures for pesticide residues analysis in food and feed. It is the latest version of the required methodologies to quantify the presence of phytosanitary products in foodstuff [53].

I.4. ANALYSIS OF PESTICIDES

In general, any analytical procedure to measure environmental pollutants involves four steps: sample collection and pre-treatment, extraction and purification, separation and quantification (Figure 2) [54].

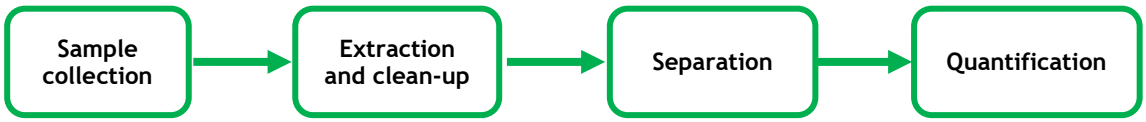


Figure 2. Steps for the determination of pesticides in environmental samples.

I.4.1. SAMPLING AND PRE-TREATMENT

Sample collection is the critical step to ensure the representativeness of the characteristics of the population. A robust sampling design should be established in order to collect an appropriate number of samples and enough sample volume to achieve accurate results.

There are different types of samples regarding the sampling protocol applied. A grab sample is collected at a specific location and time. Thus, this sampling approach can be only used in case of low variability (the system to be studied presents a high homogeneity), or when the degradation rate is extremely high. On the contrary, composite samples consist of various grab samples taken over a period or at different locations in an area. Sampling locations for soil and sediment can be set as function of longitudes and latitudes whilst air and water need to specify the time as they considerably vary with time [54]. A major advantage of wine samples is their representativeness of the whole container (usually, stainless containers at caves have capacities in the range from 10.000 L to 50.000 L).

Once sample is collected in an appropriate vessel, it should be transported to the laboratory as soon as possible, keeping it away from direct sunlight and avoiding high temperatures. Soil samples are stripped of course materials such as stones, grass, and pieces of vine canes. Then, samples are homogenized, freeze-dried for 48 hours, and sieved, usually under 2 mm. Before extraction, samples are stored either at room temperature or refrigerated in amber glass vessels. On the other hand, wine samples are acquired from local

supermarkets, or directly from wine producers, keeping them at room temperature in a dark room before opening and extraction.

I.4.2. EXTRACTION OF ANALYTES

Lately, research was focused on the development of more environmentally friendly extraction protocols. Green analytical chemistry (GAC) addresses a reduction in the amount of sample, volume of reagents (organic solvents), operator exposure, waste generation and energy saving through miniaturization and automation [55]. In any case, the selection of a suitable extraction technique depends on the physical form of the sample.

I.4.2.1. Aqueous samples: wines

Despite the increasing use of microextraction techniques, solid-phase extraction (SPE) and QuEChERS (quick, easy, cheap, effective, rugged and safe) remain as the most used methodologies to extract and/or concentrate pesticides from wine (Table 2). Both techniques are widely accepted in control laboratories and their effectiveness can be tuned by appropriate selection of extraction sorbents and solvents. Moreover, they offer the possibility to process several samples in parallel.

I.4.2.1.1. Solid-phase extraction (SPE)

SPE comprises a liquid phase (sample matrix or solvent with analytes) which is in contact with a solid phase (sorbent). The first application was described in 1950 by Braus et al. [56]. Since then, this technique has evolved to be considered one of the most versatile extraction methods nowadays. Typically, the sample with the target compounds flows over the solid sorbent, which retains the compounds by their favorable interactions whilst the interferences flow out. The target compounds are recovered by solvent displacement for analysis [57]. SPE sorbents are available in disk or cartridge format (more common).

SPE extraction compiles different steps (Figure 3):

❖ Sorbent conditioning: the first step consists of the solvation of the functional groups from the sorbent to improve later interactions with analytes. Usually, the first solvent used in the conditioning step is the same considered for the elution of compounds.

❖ Sample loading: sample passes slowly through the sorbent and analytes are retained.

❖ Washing: interferences are eliminated with an adequate solvent, at the same time as sorbent is cleaned.

❖ Dryness: using a nitrogen flow or vacuum, sorbent is completely dried.

❖ Analyte elution: analytes break their interaction with sorbent and are eluted with an adequate solvent, usually compatible with the determination technique. Thus, a purified (clean-up) and concentrated (trace enrichment) extract is obtained.

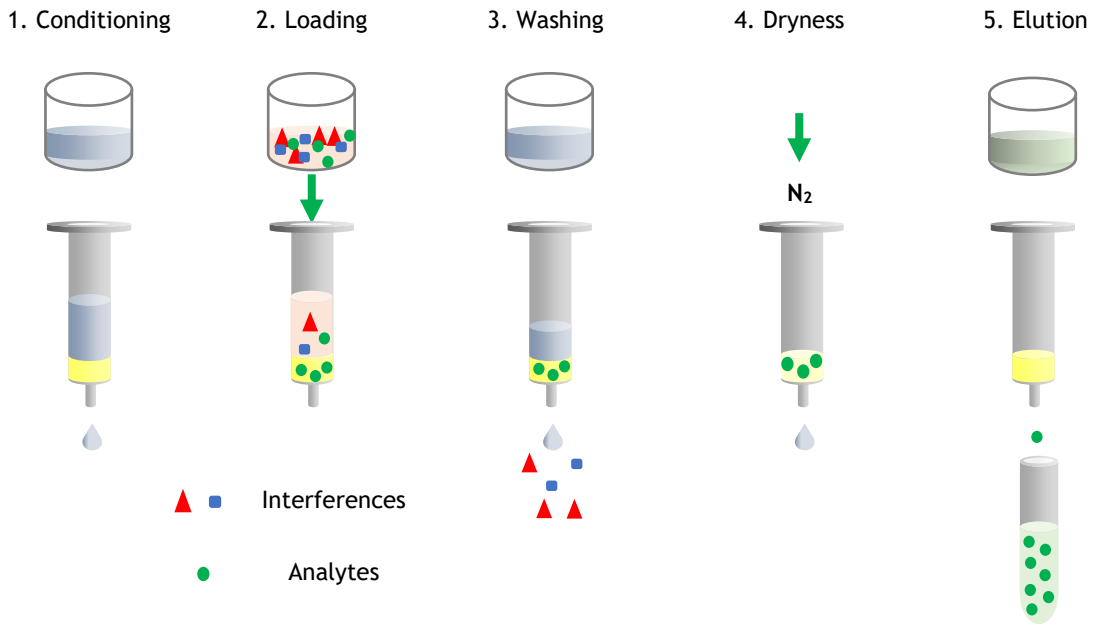


Figure 3. SPE steps

SPE (offline mode) has been evolving to a semi-automated and automated protocol in order to increase assay reproducibility and throughput, reducing also overall costs. Thus, the 12-position manifold initially used has been replaced by a 96-well SPE plate vacuum manifold or positive pressure SPE processor, significantly reducing operator manipulation. Nevertheless, uncapping/recapping and evaporation/reconstitution are manual interventions intended to be eliminated [58].

The online SPE approach was developed to avoid manipulation variability, increase sample throughput, and to reduce the investment on the expensive 96-well format workstations. A preanalytical column (SPE cartridge) is used for the sample extraction and the eluate is entirely transferred to the separation column. This approach requires an exhaustive method development and a trustworthy software to control the process. Usually, key parameters during optimization of SPE online combined with liquid chromatography (LC) are those related to the compatibility between the elution solvent and the chromatographic separation step. The final aim is to do not disturb the performance of the LC separation step when combined online with SPE.

Extraction efficiency is affected by different parameters, such as the type of matrix, sorbent, solvents, and analytes. Furthermore, pH and ionic strength should also be taken into consideration.

Sorbent materials for SPE can be classified in three main groups [57]:

a) *Inorganic oxides*

Silica gel, alumina (Al_2O_3), Florisil (a synthetic hydrated magnesium silicate) and diatomaceous earth are the most important sorbents. Functional groups present in their surface are responsible of polar, ion-exchange, and Lewis acid/base interactions. Nonpolar compounds (aromatic and alkenes with polarizable functional groups) are poorly retained by these sorbents, whilst polar compounds with hydrogen bonding functional groups (sulphonic acid, carboxylic acid, phenol and hydroxyl) are highly retained, and those with a dipole character (nitro, ester and ketone) show a medium retention rate [57]. Applications of inorganic oxides include the extraction of polar pesticides from oils and fats; however, they are of limited usefulness in case of aqueous matrices.

b) *Low-specificity sorbents*

Chemically bonded silica, carbon polymers and porous polymers are the main types included in this group. Their retention efficiency is controlled by dispersive and polar intermolecular interactions.

Silica-based sorbents: they are generally synthesized by reaction of monofunctional or trifunctional silanes with silica gel, followed by end-capping with small silanol groups. Isolation of low-mass compounds from aqueous solutions is usually performed by long alkyl chains, whilst short alkyl chains are used for macromolecules. Monofunctional reagents result in monomeric surface coverage, while trifunctional reagents can react both with the silica surface and hydrolyzed reagent forming extended polymeric layers of higher carbon loading and greater pH stability [57].

*Carbon-based sorbents***:** the main forms of carbon sorbents are activated carbon, graphitized carbon black, carbon nanotubes, and porous graphitic carbon. They show specificity for planar molecules containing polar functional groups and for systems rich in polarizable electrons [59].

Porous polymer sorbents: they are generally copolymers of styrene and divinylbenzene. The sulphonated polymers and vinylpyrrolidone copolymer are fully water wettable, which means that sorbent can get dried during the extraction process with no negative effects in compounds retention.

c) *High-specificity sorbents:*

A wide range of selective sorbents based on ion exchange, bio-affinity, molecular recognition, and restricted access materials have been developed to improve selectivity.

Mixed-mode sorbents are created as an alternative to improve the selectivity of reversed-phase sorbents during SPE. Thus, the addition of ionic groups provides two types of interactions: ion-exchange (specific interactions) and reversed-phase (non-specific interactions). A wide variety of sorbents have been commercialized. There are categorized in four subgroups based on their functional group and strength: Strong ion-exchange materials remain charged regardless of the sample pH, whereas weak ion-exchange materials may be fully or partially charged, or neutral depending on the pH. Strong cationic exchangers (SCX) are functionalized with sulphonic acid and weak cationic exchangers (WCX) usually have a carboxylic acid. Quaternary amine groups are used for strong anionic exchangers (SAX), whereas ternary, secondary and primary amines are usually employed for weak anionic exchangers (WAX) (Figure 4) [60].

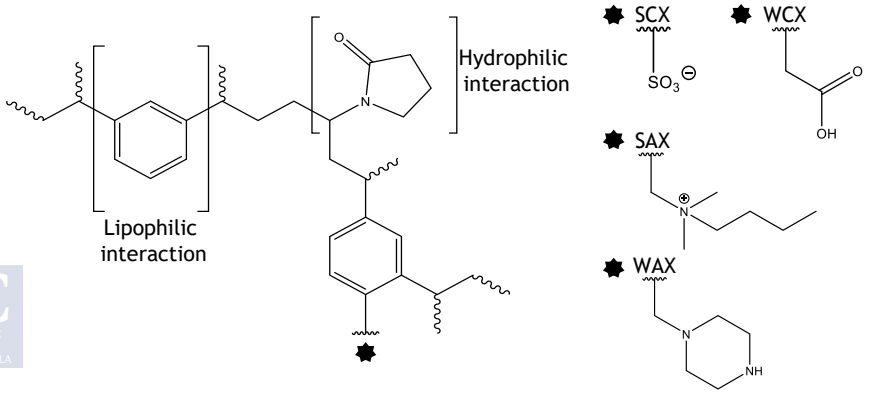


Figure 4. Examples of chemical structures for mixed-mode sorbents. SCX (strong cationic exchanger), WCX (weak cationic exchanger), SAC (strong anionic exchanger) and WAX (weak anionic exchanger).

Immunosorbents: their synthesis is based on the covalent binding of a suitable antibody to an appropriate sorbent. The interaction antibody-antigen (target analyte) provide high selectivity for the extraction of complex matrices. Not only are compound-specific sorbents available, but also there are class-specific immunosorbents for mycotoxins, phenylurea herbicides, and polycyclic aromatic hydrocarbons [61].

Molecularly Imprinted Polymers (MIP) are easier and less expensive to prepare than immunosorbents. They are usually formed by polymerization of monomers in the presence of a template (target analyte), which results in a specific imprint with chemical and physical recognition of the analyte molecule. The monomers are chosen regarding their interaction with functional groups of the template molecule. Once the process is completed, the template molecule is extracted, and the resulting MIPs are stable, robust and resistant. MIPs research is expected to rise in the near future in order to obtain more commercially available sorbents [62].

Restricted access materials (RAM): the sorbent is configured to allow the interaction of the specific compound with the functionalized areas, while the non-compatible compounds elute directly. Initially, RAM were developed for the isolation of low-molecular-mass drugs from biological fluids and, currently, there are some applications for the determination of herbicides in water [63].

SPE has been applied, mainly the offline mode, to the determination of pesticides in matrices such as water [63, 64] and wine [27, 65, 66]. Online SPE was also proposed for the determination of pesticides residues in water samples [67, 68] and, as far as could be traced, the first application of online SPE for the determination of pesticides in wine was developed as part of the present doctoral Thesis [30].

I.4.2.1.2. Fabric phase sorptive extraction (FPSE)

To overcome some limitations of SPE, solid-phase microextraction (SPME) was introduced by Pawliszyn et al. in 1987 [70] as a sample preparation technique with minimum manipulation and solvent-free. Most common SPME sorbents are poly(dimethylsiloxane) (PDMS), polyacrylate (PA), polyethylene glycol (PEG), carboxen-polydimethyl siloxane (CAR-PDMS), divinylbenzene-polydimethylsiloxane (DVB-PDMS), and carbopack Z/PDMS.

However, this technique also shows some drawbacks compensated with the introduction of the fabric-phase sorptive extraction (FPSE) by Kabir and Furton in 2016 [71]. FPSE uses a fabric (cellulose, polyester or fiberglass) as substrate to chemically bond a sol-gel sorbent (covalent bond), which overcomes the problems derived from the physical adhesion used in other solid-phase microextraction techniques.

FPSE membranes are composed by the following building blocks [72]:

❖ A fabric substrate such as cellulose, polyester, and fiberglass fabrics, with sol-gel active functional groups.

❖ Sol-gel inorganic precursor/organically modified inorganic precursor used to link through a covalent bond the substrate and the sorbent polymer. This layer plays an important role in the selectivity and polarity of the FPSE membrane.

❖ A sol-gel active inorganic polymer such as PDMS, poly(-dimethyldiphenylsiloxane); or an organic polymer such as PEG, poly(tetrahydrofuran) (PTHF). Polymer structure is crucial to provide specific intermolecular interactions.

The FPSE protocol can be divided into four steps. Firstly, FPSE membranes need to be cleaned with an adequate organic solvent and water. Then, membranes are directly introduced in a vial with the aqueous sample and a magnetic stir bar for a period of time. In the next step,

the FPSE membrane is removed from the vial, rinsed with water and dried with a lint-free tissue. Finally, coated fabrics are transferred to a vial, and analytes are desorbed with a small volume of solvent, compatible with the determination technique.

Over the last years, FPSE has been extensively applied to the determination of different chemical compounds in water [73], urine [74], nutritional supplements [75] and milk [76]. However, FPSE has been barely applied to matrices such as wine [28].

I.4.2.2. Solid samples: soil

The most traditional methodology for the extraction of pesticides from environmental solid samples, such as soil, was solid-liquid extraction (SLE), including Soxhlet extraction. However, that technique implies the use of large volumes of solvent, and it is time-consuming. Thus, new extraction techniques have been developed for those type of matrices. Those extractions are microwave-assisted extraction (MAE), matrix solid-phase dispersion (MSPD), pressurized liquid extraction (PLE), QuEChERS extraction and ultrasonic solvent extraction (USE). Among them, most employed methodologies for solid samples are QuEChERS and PLE.

I.4.2.2.1. Pressurized liquid extraction (PLE)

Pressurized liquid extraction (PLE), also known as accelerated solvent extraction (ASE), was first reported by Richter et al. in 1996 [77]. This technique combines elevated temperatures and pressures with solvents, or mixture of solvents, to extract compounds from solid samples. Extraction efficiency is influenced by physical properties and the polarity of the solvent, or the mixture of solvents. On one hand, high temperatures increase the analytes solubility, breaking matrix-analyte interactions, and improving the diffusion rate, which can be translated into a better desorption. On the other hand, high pressure maintains the solvent under its boiling point. Both factors decrease the surface tension and viscosity of the solvent, thus, extraction of analytes in the pores of the matrix is carried out more effectively and using a fewer amount of solvent [77].

There are two possible modes to perform the PLE process: static and dynamic. In the dynamic mode, solvent is continuously pumped through the extraction cell, accelerating the diffusion rate, but consuming a large amount of solvent and diluting the final extract. Static mode is usually employed in the commercial instruments. In this mode, the extraction cell is filled with solvent and maintained at a pre-selected temperature, for a period of time, before introducing a fresh volume of solvent (flush volume) to repeat the extraction. Static mode allows to concentrate the sample extract and a lower use of solvent [78]. A scheme of the PLE equipment is represented in Figure 5, and PLE procedure (static mode) is described below:

❖ Cell preparation: sample (previously lyophilized or dried) is weighed and loaded into the stainless-steel cell. The extra space, below and above the sample, is filled with an inert material and both sides are closed with frits to avoid sample losses.

❖ Loading the cell: automatic arms pick up the cell and move it into the oven; then, pressure (1500-3000 psi) is applied to seal the cell.

❖ Filling with solvent and heating the cell: solvent is pumped into the cell (cell size is recognized by the system) until cell is full and heat is applied for a period of time to ensure the sample reaches the predefined temperature (50-200^{o}C).

❖ Flushing with fresh solvent: static valve opens and extraction solvent flows into the collection vial. Then, fresh solvent is pumped through the cell to perform various extraction cycles (if required).

❖ Purging: the remaining solvent is displaced with purge gas, so collection vial contains all the solvent and analytes from the sample.

❖ End relief and unloading the cell: residual pressure is released from the cell and the system is vented. The cell is unloaded from the oven and returned to the tray.

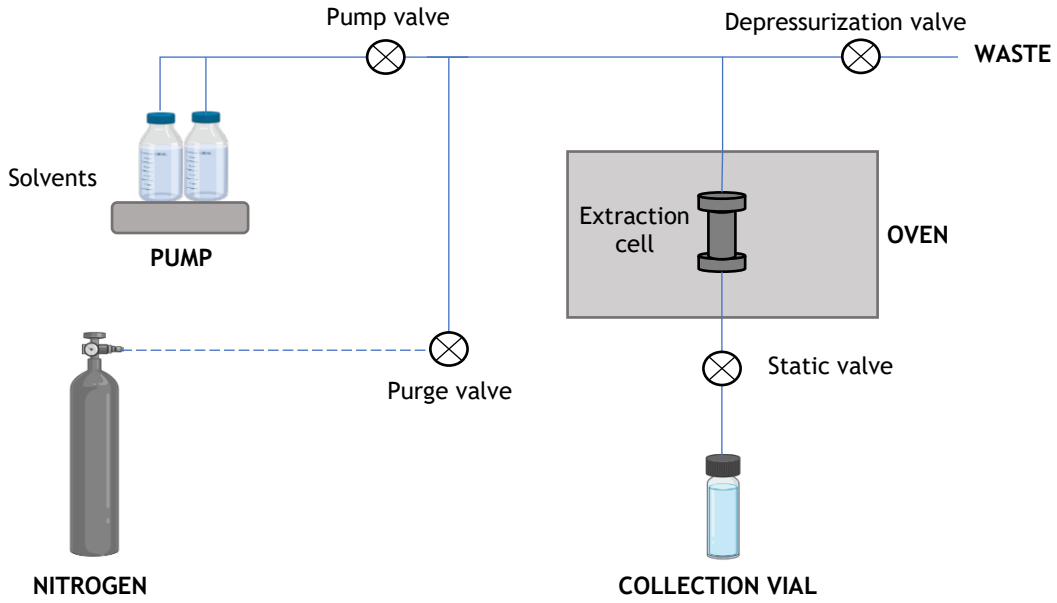


Figure 5. Parts of the pressurized liquid extraction (PLE) equipment. Created with BioRender.com®

It can clearly be seen that the majority of the procedure is carried out by the equipment. Thus, only sample preparation and loading cells in the tray is performed by the operator, while extraction of 24 samples can be performed automatically. Nevertheless, it is necessary to previously optimize some extraction parameters such as temperature, pressure, extraction time, flush volume, number of extraction cycles and type of solvent or mixture of solvents.

I.4.3. ANALYSIS

The analysis of organic compounds in environmental samples has been challenging. The variable structure of pesticides, from nonpolar to medium-high polarity, is decisive in the selection of the separation and determination technique. Historically, the most used chromatographic techniques have been gas chromatography (GC) and LC, usually combined with mass spectrometry (MS). Nevertheless, in the last decade, supercritical fluid chromatography (SFC) has appeared as an alternative to both techniques.

I.4.3.1. Separation

The early pesticides were nonpolar and thermally stable, thus, the preferred technique for their environmental analysis was the GC. However, GC requires the previous derivatization for the new formulations to ensure their volatility and thermal stability. For this reason, LC is the most widely used analytical separation technique nowadays.

I.4.3.1.1. Liquid Chromatography

LC is based on the partition of the analytes between a solid stationary phase and a liquid mobile phase. It is widely used for the determination of non-volatile, polar, or thermally unstable compounds due to its versatility and sensitivity. Traditional LC has evolved to high-performance liquid chromatography (HPLC), using a smaller particle size (2-5 µm); and ultra-high-performance liquid chromatography (UHPLC) (< 2µm). UHPLC allows to work at very high pressures, which provides a shorter analysis time, better peak shape, and higher resolution. Figure 6 shows the scheme of the main parts of a LC system.

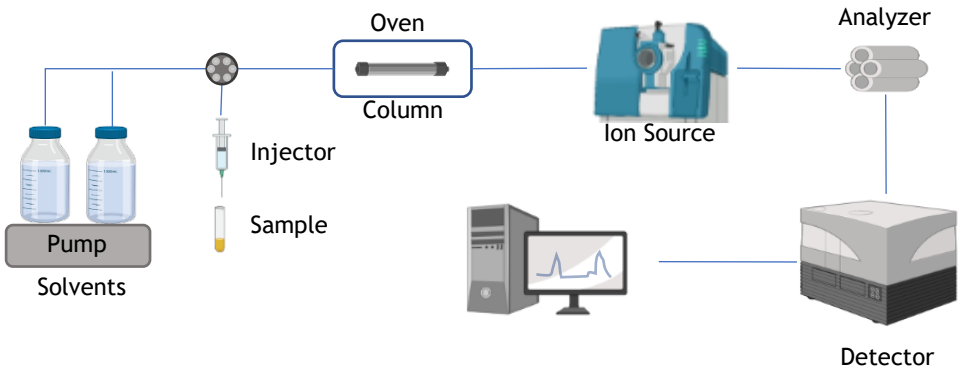


Figure 6. Components of a liquid chromatography system. Created with BioRender.com®

There are different types of LC as function of the separation mechanism, such as adsorption, partition, ion exchange, molecular exclusion, affinity, and chiral chromatography [79].

Partition chromatography is the most commonly applied to the determination of organic compounds in environmental samples. There are two types based on the polarity of the stationary phase: normal-phase (polar: nitro, diol, amino, nitrile) and reversed-phase (nonpolar: C_8, C_{18}, cyclohexyl, phenyl), being the latest one the most used. The majority of LC-based applications for environmental samples employ C_{18} reversed-phase columns. Reversed-phase LC (RPLC) permits the strong retention of nonpolar compounds, using a weak (water) or strong (acetonitrile, ACN), methanol (MeOH)) mobile phase for elution.

However, the analysis of highly polar pesticides is a complex task using normal-phase UHPLC, since polar compounds are hardly separated using a polar stationary phase. For this reason, hydrophilic interaction liquid chromatography (HILIC) has been proposed as an alternative. HILIC is a variant of the normal-phase LC, but with a more complex separation mechanism. HILIC employs traditional polar stationary phases such as amino, diol, cyano or silica, but the mobile phase used is similar to those employed in the RPLC mode; and also allows the analysis of charged compounds [69]. The typical stationary phase consists of silica or silica gel functionalized with several polar groups. Therefore, HILIC is a good choice in the separation of uncharged highly hydrophilic and amphiphilic compounds that are too polar for RPLC, but have insufficient charge to allow effective electrostatic retention in ion-exchange chromatography (IEC).

In this vein, IEC is used to separate compounds by their adsorption into the stationary phase containing fixed charges. There are two modes of action: cation exchangers and anion exchangers. The cation exchangers contain negatively charged groups and they are used to separate positive ions; whilst anion exchangers contain positively charged groups and they are used to separate negative ions. For instance, sulphonic acid and carboxylic acid are used as cation exchangers, while amines are employed as anion exchangers. Elution of analytes is

usually carried out employing a high concentration of competing ions in the mobile phase [70].

Finally, there has been a noticeable development regarding chiral separations using LC. Thus, the aim is to separate enantiomeric molecules using chiral stationary phases (CSPs) with a liquid mobile phase. For this purpose, the stationary phase should contain anisotropic selectors that interact in a different extent with each enantiomer. Lately, stationary phases have been classified into coated and immobilized phases, based on the crosslinking or covalent bond established, respectively, between the support particle and the chiral selector [80]. Generally, coated phases are simple to be prepared and show higher selectivity than their immobilized analogues, yet they have limited solvent compatibilities [71].

I.4.3.1.2. Supercritical fluid chromatography

Supercritical fluid chromatography (SFC) is a chromatographic technique similar to HPLC, which includes the replacement of the majority of the liquid mobile phase with a dense compressed gas. CO_2 is the preferred mobile phase used, combined with co-solvents, to improve the separation of the analytes. A backpressure regulator (BPR) is required on the system outlet to ensure the mobile phase remains as single dense phase throughout the chromatographic system (column and connection with detection technique). Major advantages of this technique are high flowrates, which comprises faster separations due to the higher diffusivity and low viscosity of supercritical CO_2, and lower organic solvent usage [72]. SFC also improves efficiency and shows shorter equilibration times than those required for chiral or achiral separations carried out with classical LC eluents. The main parts of the SFC equipment from Agilent® are represented in the Figure 7.

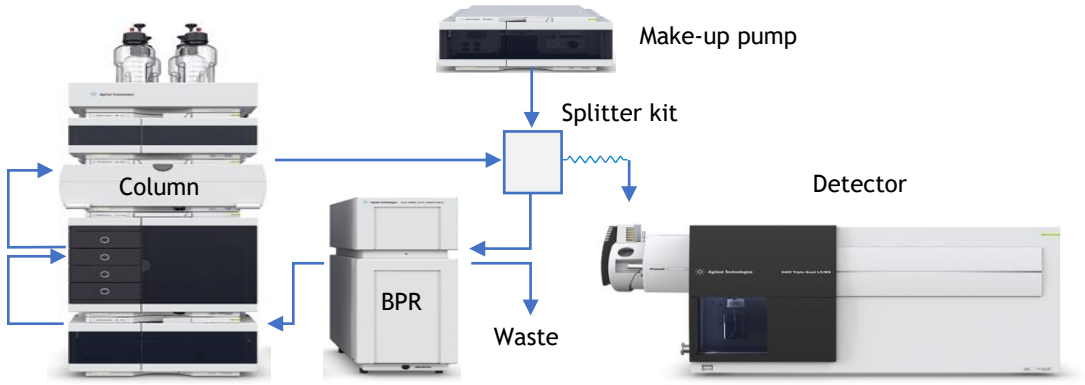


Figure 7. Components of supercritical fluid chromatography (SFC) equipment from Agilent®.

SFC is considered a green analytical technique and has become the method of choice for the pharmaceutical industry, specifically for the separation of enantiomers and other isomers over the last decades. Despite this advantage, when combined with MS through an electrospray ionization (ESI) source, a make-up auxiliary solution is required to improve the efficiency of compounds ionization. The main CSPs are derivatives of cellulose and amylose (coated or immobilized to silica), brush-type (Pirkle-type) CSPs, native and derivatized cyclodextrins and cyclofructans, quinine-based and macrocyclic antibiotics-based CSPs. Different derivatives (esters or carbamates) of cellulose and amylose show good selectivity when used as selectors in silica based CSPs and they are commercially available nowadays by different vendors. The outstanding of SFC applications for the determination of pesticides are shown in Table 4. It must be noticed that the majority of chiral SFC applications are applied to a single or a couple of compounds.

Table 4. Summary of SFC applications to the determination of chiral pesticides in foodstuff and vegetables.

Matrix	Pesticides	Extraction method	Separation/detection method	LOQs (µg/kg)	Ref.
Soil / Tobacco	Metalaxyl	QuEChERS	Chiral SFC-ESI-QqQ	17-20	[81]
Tea, grape apple	Diniconazole	QuEChERS	Chiral SFC-QTOF	5-10	[82]
Vegetables	Triticonazole	QuEChERS	Chiral SFC-UV	2.5-4 mg/kg	[83]
Fruits, vegetables, cereals and soil	Fenbuconazole + TPs	QuEChERS	Chiral SFC-ESI-QqQ	0.13-3.31	[84]
Cucumber, potato, soil	Pyrisoxazole	QuEChERS	Chiral SFC-ESI-QqQ	0.15-0.48	[85]
Lettuce	10 pesticides	QuEChERS	Chiral SFC-UV	200-600	[86]
Appel and soil	Triadimefon	QuEChERS	Chiral SFC-DAD	2000	[87]
Malt and beer	Triadimefon and triadimenol	QuEChERS	Chiral SFC-ESI-QqQ	2	[88]
Grape and soil	Pydiflumetofen	QuEChERS	Chiral SFC-ESI-QqQ	5	[89]
Strawberry wine	Tetraconazole	QuEChERS	Chiral SFC-ESI-QqQ	5 µg/L	[90]
Papaya and avocado	1 organophosphate, 2 carbamates, 2 triazine	QuEChERS	SFC- PDA	220-800	[91]
Dried species	162 pesticides	QuEChERS	SFC-ESI-QqQ	50-200	[92]
Wine	7 neonicotinoids + 1TP	SPE	SFC-ESI-QTOF	1-11 ng/mL	[93]

I.4.3.2. Determination

Mass spectrometry (MS) is an analytical methodology used to provide both quantitative and qualitative information of molecules previously transformed into ions. A mass spectrometer includes: an ionization source, a mass analyzer, and a detector. Introduction of samples from LC, or SFC, to the ionization source requires the vaporization of the sample into ions in gas phase (positive or negative charges). Then, ions are accelerated by an electrical field and separated according to their mass-to-charge ratio, *m/z*. Ions reach the detector and signals are generated and recorded to display the relative abundance according to their *m/z* ratio [79].

I.4.3.2.1. Ionization source

The major challenge was the combination of LC/SFC with the high vacuum region of the mass spectrometer through the ionization source. The dominant interfaces are atmospheric-pressure ionization (API) sources, for example atmospheric-pressure chemical ionization (APCI), atmospheric-pressure photoionization (APPI) and ESI. Clearly, it can be seen in Tables 1–3 that the predominant ionization source is the ESI. Those ionization sources are considered soft techniques that preserve the molecular ion, in 99% of the cases.

The transfer of ionic species from solution into the gas phase by ESI involves three steps (Figure 8): (1) dispersal of a fine spray of charge droplets, followed by (2) solvent evaporation and (3) ion ejection from the highly charged droplets. The ions are then accelerated into the mass analyzer for analysis of their *m/z* ratios and measurement of ion intensity [94].

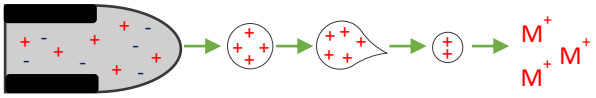


Figure 8. Mechanism of electrospray ionization (ESI).

GC commonly employs electron ionization (EI), where sample is bombarded with a beam of electrons and produces a radical cation. This technique is applicable for volatile and semi-volatile compounds with a nonpolar character. It is classified as a hard ionization source that produces reproducible MS spectra, involving considerable molecular fragmentation.

Nowadays, EI and ESI are recognized as complementary ionization sources to cover multiresidue methods using GC-MS and LC-MS type techniques.

I.4.3.2.2. Analyzer and detector: tandem mass spectrometry (MS/MS)

Analysis of ions generated in the ionization source is a critical step to obtain accurate information about the molecules of interest. Low-resolution analyzers, such as quadrupole (Q) and linear ion traps, and high-resolution (HR) analyzers, like time-of-flight (TOF) and Orbitrap, can be used to perform the analysis of pesticides in environmental samples.

The most popular analyzer in environmental analysis is the Q. The system is composed by four parallel and equidistant metal rods. Each pair of opposite rods is connected electrically to equal, but opposite, direct current (DC) voltage combined with a radio frequency (RF) (Figure 9). Thus, ions travel as function of their *m/z* ratio to the detector and their movement is controlled by changes in the voltages applied. Meanwhile, undesirable ions get neutralized and fail to reach the detector. Q analyzer is robust, economic and easily interfaced with LC, SFC or GC systems, what situated Q as the most popular one for routine analysis and research of pre-defined compounds [95].

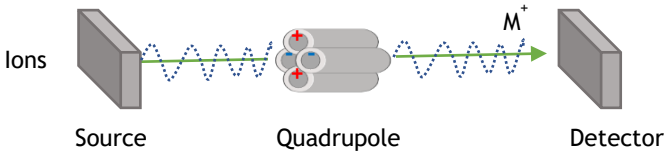


Figure 9. Operation mode of a quadrupole (Q) analyzer.

The TOF analyzer is another system commonly applied nowadays (Figure 10). In this case, a voltage (significantly higher than in case of Q-based instruments) is applied to accelerate ions and introduce them into the drift region, with no more electric or magnetic field. Supposedly, all ions have the same kinetics energy at the beginning of the tube, thus lighter ions are expected to reach faster the detector [87]. Modern TOF instruments provide accurate mass measurements with high sensitivity for an unlimited *m/z* ratio range.

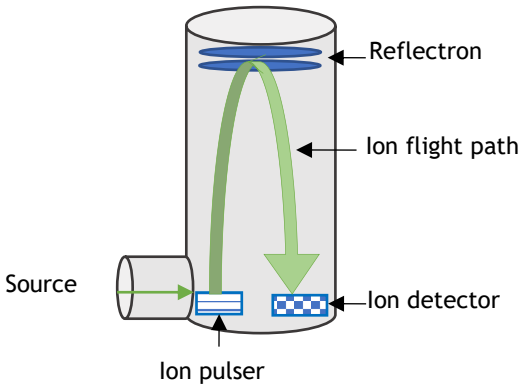


Figure 10. Scheme of a time-of-flight (TOF) analyzer.

Analyzers described above are commonly combined to create hybrid instruments, the so-called tandem mass spectrometry (MS/MS). In this doctoral Thesis, triple quadrupole (QqQ) and quadrupole-time-of-flight (QTOF) instruments were used.

a) *Triple quadrupole (QqQ)*

The QqQ is based on two in line quadrupoles (Q1, Q3) separated by a collision cell (q2). Precursor ions can be filtered in Q1 and product ions are filtered in Q3, whilst q2 produces fragmentation by collision-induced dissociation (CID).

There are different modes of operation as function of the analytical objective (Figure 11) [95].

❖ Product ion scan: Q1 (SIM, Selected ion Monitoring) selects an ion with a specific *m/z* ratio, which is fragmented in q2 and Q3 scans to generate a full-product ion spectrum.

❖ Precursor ion scan: Q3 (SIM) selects a product ion with a constant *m/z* ratio and Q1 scan the possible precursor ions.

❖ Neutral loss scan: Q1 and Q3 operate in scan mode. This mode of operation is equivalent to a single quadrupole, but with decreased intensity because of the transmission losses.

❖ Multiple reaction monitoring (MRM): a precursor ion is selected in Q1 (SIM), and 2-3 product ions are monitored in Q3 (SIM) after fragmentation in q2. This mode is the preferred one to perform multianalyte quantitative analysis due to its selectivity and sensitivity.

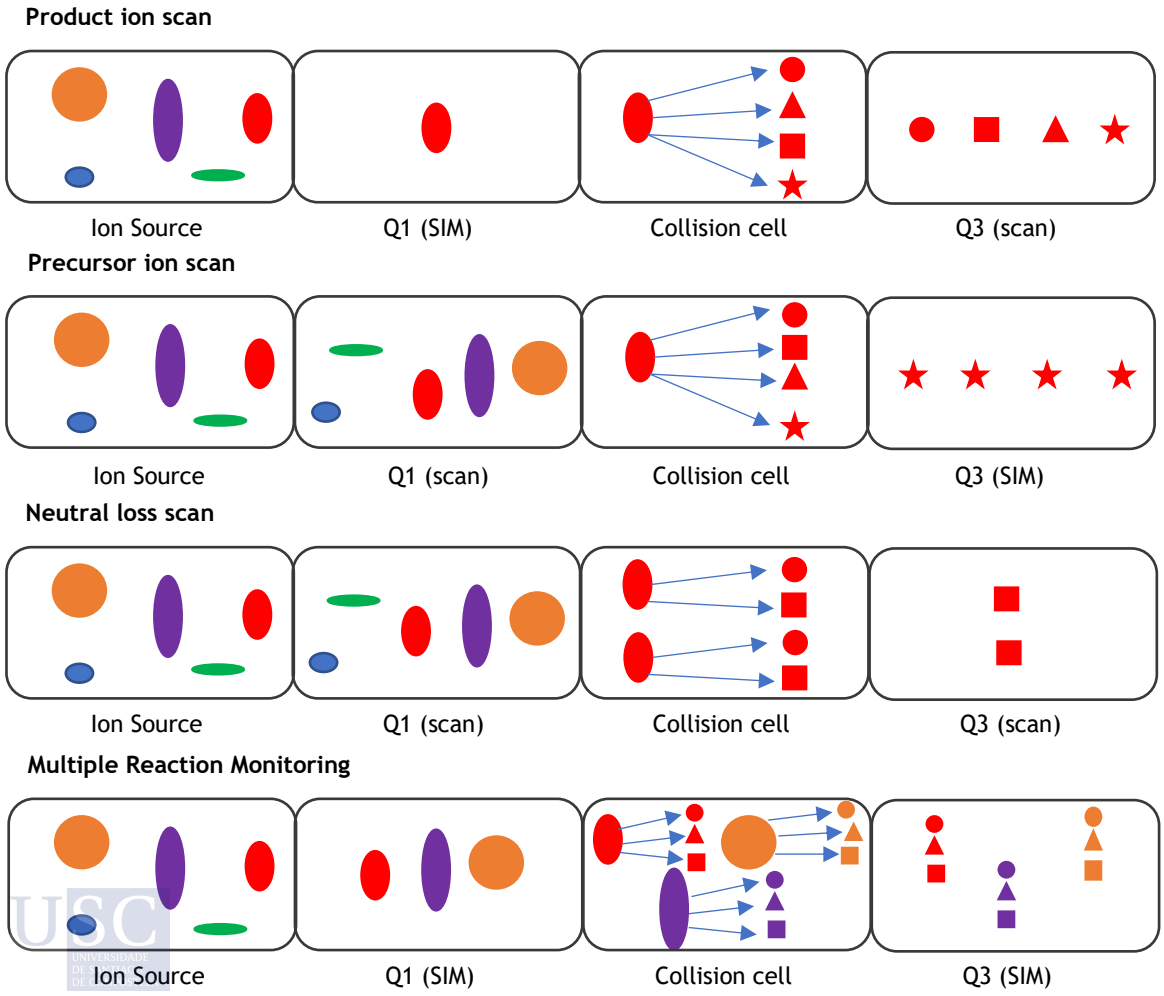


Figure 11. Triple quadrupole operation modes.

b) *Quadrupole-time-of-flight (QTOF)*

High resolution mass spectrometry (HRMS) can be obtained with the combination of a Q and TOF detectors, QTOF instrument. Despite this denomination, a more appropriate term is accurate MS (mass resolution of TOF instruments varies depending on the model, acquisition frequency and *m/z* values, although is rarely above 50000). In this case, the MS1 is a transmission quadrupole and MS2 is an orthogonal-injection reflectron TOF mass spectrometer (Figure 12).

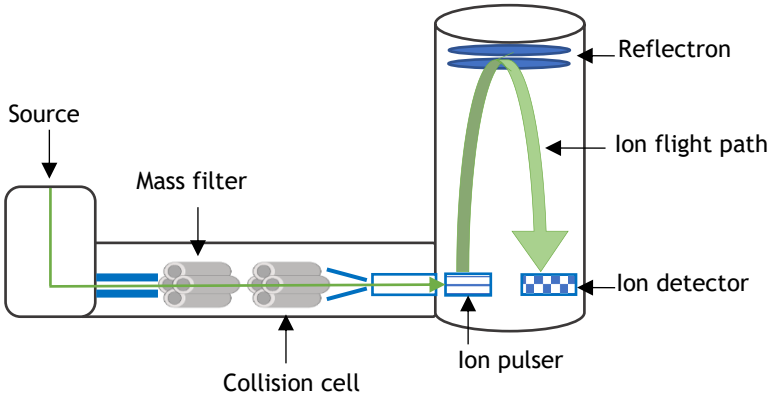


Figure 12. Scheme of a quadrupole-time-of-flight (QTOF) mass spectrometer. Adapted from Agilent®.

Both qualitative and quantitative information can be obtained from a single injection with high resolution and accuracy [96]. QTOF system has been extensively used for the screening (non-target) of transformation products (TPs) in combination with ESI source in LC, as so as with qualitative purposes regarding fragmentation patterns of target analytes. Thus, analysis of target, suspected or non-target compounds can be carried out as function of the acquisition mode predefined in the instrument. In this vein, two different acquisition modes can be configured (Figure 13).

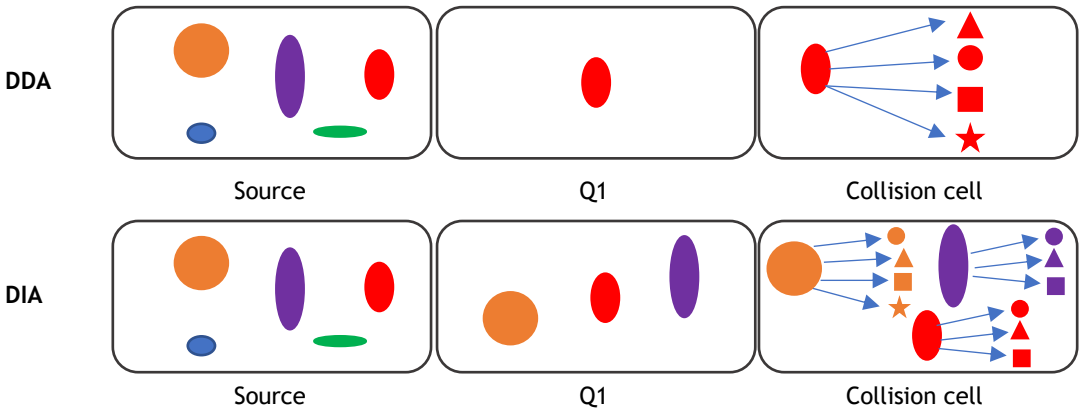


Figure 13. Operational procedure of data dependent acquisition (DDA) and data independent acquisition (DIA) modes.

❖ *Data independent acquisition mode* (DIA) involves the record of data with any preselection in the Q analyzer (scan mode). Different collision energies (CE) can be applied in a single run to obtain as much information as possible about the pseudo-MS/MS spectra of the compounds present in the sample injected. This mode of acquisition adopts different

nomenclature depending on the manufacturer: all ions (Agilent), MS^E mode (Waters) and AIF (All-Ion Fragmentation, Thermo) [97]. Data acquired could be equivalent to a MS/MS spectrum if there was a simple mixture of standards, which are separated by LC. Nevertheless, interferences are the main drawback of DIA, even though its MS/MS acquisition rate. To overcome this difficulty, an innovative mode has been developed, the quadrupole-resolved all ions (Q-Rai), so called SWATH (sequential window acquisition of all theoretical fragment ion spectra) [98]. This approach creates different *m/z* windows, sequentially filtered by the Q analyzer, in order to reduce interferences in the chromatograms. DIA combined with a database of accurate product ion spectra is a valuable approximation to detect and identify non-target compounds present in complex sample extracts (provided that they are recorded in the database).

❖ *Data dependent acquisition mode* (DDA) is recommended in suspected or target analysis. Different ions are preselected in a first MS scan to generate their fragmentation in the collision cell. Both MS and MS/MS spectra can be recorded in a single run, automatically switched, and controlled by different criteria (intensity, charge state, isotopic pattern). Two strategies can be adopted in this acquisition mode:

- Target MS/MS experiment: a list of precursor ions (*m/z* ratio and retention time) is established. The instrument performs MS/MS of the predefined *m/z* values in the retention time window set.
- Auto MS/MS experiment: instrument automatically selects precursor ions based on predefined criteria (intensity, isotopic pattern, charge); moreover, a list of preferred/excluded ions can be set.

Both strategies provide MS and MS/MS data in a single run at the expense of information loss. As previously commented, in the DIA mode, the obtained product ion spectrum might be generated by more than one precursor. On the other hand, when the system is operating in the DDA, precursor ions with intensities below the defined threshold in the MS mode will never reach the collision cell, as so as the limited number of precursors per cycle for compounds with a close retention time. For this reason, in some cases it is necessary an additional target MS/MS for confirmation.

Several LC-HRMS databases (MassBank [99], *m/z* cloud [100] and METLIN [101]) are available for structure confirmation purposes in both target and non-target analysis. Nevertheless, their variability among different instruments is a shortcoming and limits their applicability [102].

I.5. TARGET COMPOUNDS

The pesticides included in this Thesis belong to a wide variety of chemical families. Most of the analytes are fungicides, mainly composed by triazoles, strobilurins, benzimidazoles, carbamates, imidazoles and pyrimidines. As regards the insecticide compounds, they are classified into organophosphates, neonicotinoids, diamines, tetrazines and hydrazines. Lastly, herbicides selected for their study are included in the phosphonic acid group.

Selection has been made based on their environmental relevance in terms of priority substances, their occurrence in previous research studies and their feasibility for LC and possible SFC applications. Furthermore, a special interest has been taken to those substances commonly applied in Spain and, in particular, in the region of Galicia, where a systematic study about their presumably presence in soil was conducted. In this vein, Table 5 shows the maximum concentrations found for pesticide residues as a result of different determinations in soil and wine samples collected in Spain (Iberian Peninsula and Canary Islands). In the case of soils, compounds remained below 50 ng/g, except for metalaxyl (MET, 160 ng/g).

Regarding wines, boscalid (BOSC) is the only pesticide above 100 ng/mL (160 ng/mL), whilst the majority of compounds detected are situated between 10 ng/mL - 85 ng/mL. Data will be compared with the doctoral research conducted in **Section V (General discussion)**.

Table 5. Summary of pesticide residues found in soil and wine samples through research conducted in Spain.

Compound	Year	Region	Soil (ng/g)	Wine (ng/mL) *	Ref.
Boscalid	2015-2016	Basque Country	25		[103]
	2009	Galicia		160 (W)	[18]
	2012-2017	Canary Islands		14 (R)	[19]
Carbendazim	2012-2017	Canary Islands		76 (R)	[19]
Cyprodinil	2004-2006	Galicia	40		[42]
	2009	Galicia		4.2 (W)	[18]
Dimethomorph	2015-2016	Basque Country	25		[103]
	2012-2017	Canary Islands		3 (R)	[19]
Fenhexamide	2011-2014	-		35 (R) 64 (W)	[33]
Fludioxonil	2009	Galicia		17 (W)	[18]
Iprodione	2012-2017	Canary Islands		61 (R)	[19]
	2011-2014	-		52 (R) 73 (W)	[33]
Mandipropamid	2009	Galicia		31 (W)	[18]
Metalaxyl	2004-2006	Galicia	160		[42]
	2012	Salamanca	12		[37]
	2012-2017	Canary Islands		47 (R)	[19]
Metalaxyl CGA 62826	2012	Salamanca	16		[37]
Metrafenone	2015-2016	Basque Country	11		[103]
Penconazole	2004-2006	Galicia	30		[42]
Pyrimethanil	2012-2017	Canary Islands		44 (R)	[19]
	2011-2014	-		82 (R)	[33]
	2012	Salamanca	1		[37]
Tebuconazole	2015-2016	Basque Country	10		[103]
	2012-2017	Canary Islands		14 (R)	[19]
Thiophanate-methyl	2012-2017	Canary Islands		56 (R)	[19]
Triadimenol	2012	Salamanca	26		[37]

*W: white wine; R: red wine.

Tables 6-8 provide a list of the target compounds whose determination was considered during my Thesis work, their physicochemical properties, and their legal status. Moreover, Figures 14-15 show pesticide structures classified regarding their chemical family.

Table 6. List of target fungicides and TPs studied, with details of their CAS number, classification, molecular weight, pKa and regulated use (EU).

Analyte	CAS	Chemical group	Formula	Molecular weight	pKa [α]	Status [β]	MRL wine grapes (mg/kg) [γ]
Ametoctradin	865318-97-4	Triazole	$C_{15}H_{25}N_5$	275.4	0.1	√	6
Azoxystrobin	131860-33-8	Strobilurin	$C_{22}H_{17}N_3O_5$	403.4	0.94	√	3
Benalaxyl	71626-11-4	Acylalanine	$C_{20}H_{23}NO_3$	325.4	1.5	√ Ben-M / X Ben(2021)	0.3
Bitertanol	55179-31-2	Triazole	$C_{20}H_{23}N_3O_2$	337.4	1.97	X (2015)	0.01
Boscalid (nicobifen)	188425-85-6	Nicotinamide derivative	$C_{18}H_{12}Cl_2N_2O$	343.2	-0.11	√	5
Carbendazim	10605-21-7	Benzimidazole	$C_9H_9N_3O_2$	191.2	4.28	X (2014)	0.5
Cyflufenamid	180409-60-3	Benzoacetamide	$C_{20}H_{17}F_5N_2O_2$	412.4	12.05	√	0.2
Cymoxanil	57966-95-7	Urea	$C_7H_{10}N_4O_3$	198.2	10.56	√	0.3
Cyprodinil	121552-61-2	Aminopyrimidine	$C_{14}H_{15}N_3$	225.3	3.10	√	3
Cyprodinil-4OH	195157-66-5	-	C14H15N3O	241.3	-	-	-
Difenoconazole	119446-68-3	Triazole	$C_{19}H_{17}Cl_2N_3O_3$	406.3	1.95	√	3
Dimethomorph	110488-70-5	Morpholine	$C_{19}H_{17}Cl_2N_3O_3$	387.9	-0.46	√	3
Diniconazole	70217-36-6	Triazole	$C_{15}H_{17}Cl_2N_3O$	326.2	1.58	X (2011)	0.01
Fenamidone	161326-34-7	Carbamate	$C_{17}H_{17}N_3OS$	311.4	0.98	X (2018)	0.01
Fenhexamide	126833-17-8	Hydroxyanilide	$C_{14}H_{17}Cl_2NO_2$	302.2	7.4	√	15
Fenpropidin	67306-00-7	Piperidine	$C_{19}H_{31}N$	273.5	9.7	√	0.01
Fludioxonil	131341-86-1	Phenylpyrrole	$C_{12}H_6F_2N_2O_2$	248.2	14.7	√	4
Fluopicolide	239110-15-7	Benzamide	$C_{14}H_8Cl_3F_3N_2O$	383.6	1.32	√	2
Flusilazole	85509-19-9	Triazole	$C_{16}H_{15}F_2N_3Si$	315.4	2.32	X (2008)	0.01
Imazalil	35554-44-0	Imidazole	$C_{14}H_{14}Cl_2N_2O$	297.2	6.48	√	0.01
Iprovalicarb	140923-17-7	Carbamate	$C_{18}H_{28}N_2O_3$	320.4	13.67	√	2

Table 6. (cont.) List of target fungicides and TPs studied, with details of their CAS number, classification, molecular weight, pKa and regulated use (EU).

Analyte	CAS	Chemical group	Formula	Molecular weight	pKa α	Status β	MRL wine grapes (mg/kg) γ
Mandipropamid	374726-62-2	Benzoacetamide	$C_{23}H_{22}ClNO_4$	411.9	14.47	√	2
Metalaxyl CGA 108906	104390-56-9	-	$C_{14}H_{17}NO_6$	295.3	3.25	-	-
Metalaxyl	70630-17-0	Acylalanine	$C_{15}H_{21}NO_4$	279.3	15.80	√ Met √ Met-M	1
Metalaxyl CGA 62826	87764-37-2	-	$C_{14}H_{19}NO_4$	265.3	4.20	-	-
Metrafenone	220899-03-6	Benzophenone	$C_{19}H_{21}BrO_5$	409.3	1.30	√	7
Myclobutanil	88671-89-0	Triazole	$C_{15}H_{17}ClN_4$	288.8	2.27	X (2021)	1.5
Penconazole	66246-88-6	Triazole	$C_{13}H_{15}Cl_2N_3$	284.2	2.06	√	0.5
Prochloraz	67747-09-5	Imidazole	$C_{15}H_{16}Cl_3N_3O_2$	376.7	2.55	√	0.03
Propiconazole	60207-90-1	Triazole	$C_{15}H_{17}Cl_2N_3O_2$	342.2	1.95	X (2018)	0.01
Pyraclostrobin	175013-18-0	Strobilurin	$C_{19}H_{18}ClN_3O_4$	387.8	0.11	√	2
Pyrimethanil	53112-28-0	Aminopyrimidine	$C_{12}H_{13}N_3$	199.3	3.44	√	5
Pyrimethanil 4-OH	81261-84-9	-	C12 H13 N3 O	215.2	-	-	-
Quinoxyfen	124495-18-7	Quinoline	$C_{15}H_8Cl_2FNO$	308.1	3.93	X (2018)	1
Tebuconazole	107534-96-3	Triazole	$C_{16}H_{22}ClN_3O$	307.8	2.01	√	1
Thiabendazole	148-79-8	Benzimidazole	$C_{10}H_7N_3S$	201.3	4.08	√	0.01
Thiophanate methyl	23564-05-8	Carbamate	$C_{12}H_{14}N_4O_4S_2$	342.4	7.51	X (2020)	3
Triadimefon	43121-43-3	Triazole	$C_{14}H_{16}ClN_3O_2$	293.8	1.93	X (2009)	0.01
Triadimenol	55219-65-3	Triazole	$C_{14}H_{18}ClN_3O_2$	295.8	1.97	X (2019)	0.3
Trifloxystrobin	141517-21-7	Strobilurin	$C_{20}H_{19}F_3N_2O_4$	408.4	2.37	√	3
Zoxamide	156052-68-5	Benzamide	$C_{14}H_{16}Cl_3NO_2$	336.6	-1.09	√	5

Information extracted from PubChem Substances and Compound database (https://pubchem.ncbi.nlm.nih.gov/). α: pKa (dissociation constant at 25ºC) calculated with ChemAxon software. β: Status (√ Approved for its use; X Not approved for its use (expiration date)). EU Pesticides Database – Active substances, safeners and synergists (https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/active-substances/?event=search.as). γ: MRL (Maximum Residue Limits) in wine grapes (mg/kg) (EU Pesticides Database – Search Pesticide Residues (https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/mrls/?event=search.pr).

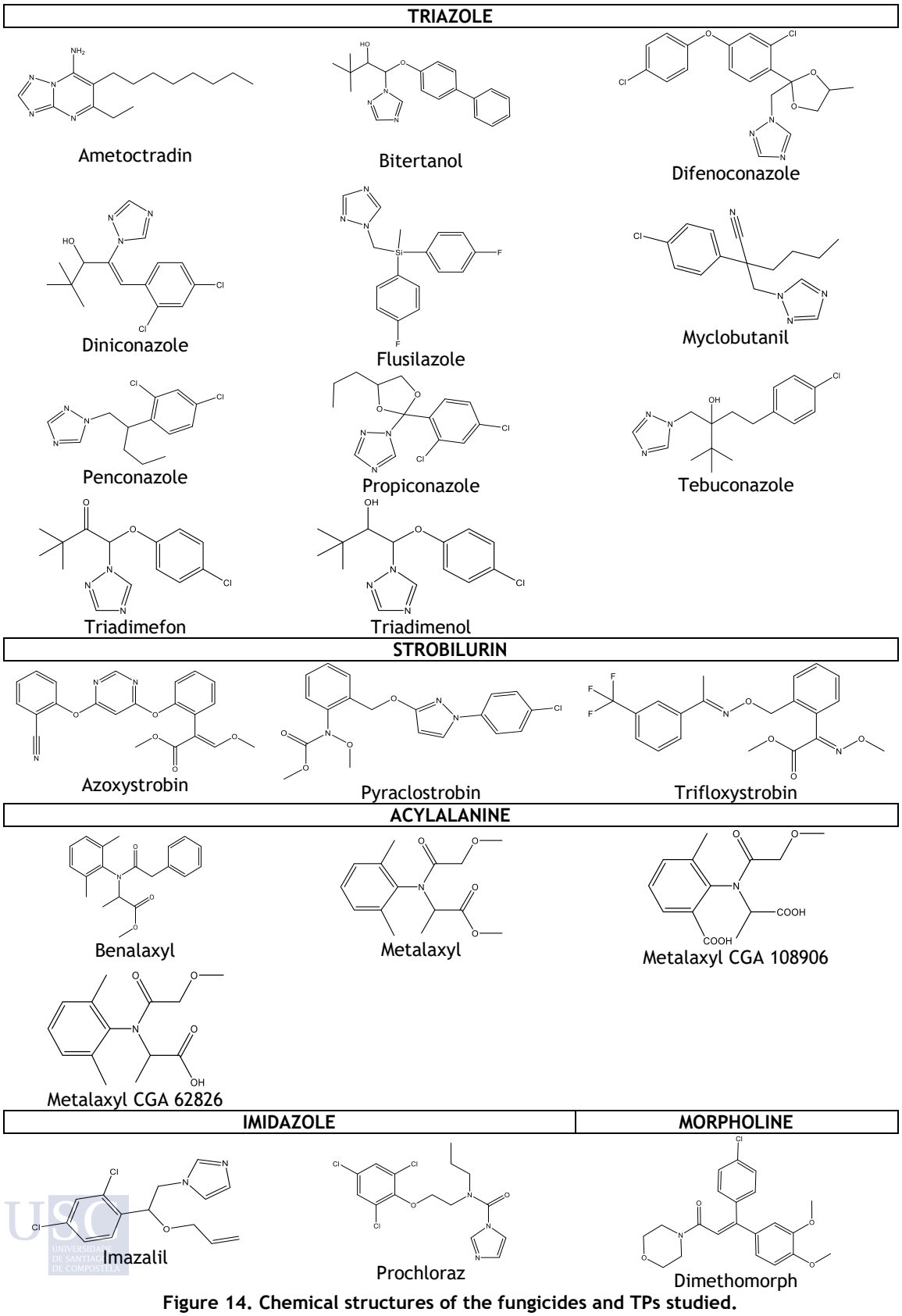
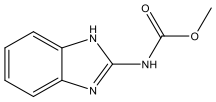
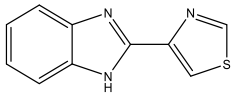


Figure 14. Chemical structures of the fungicides and TPs studied.

BENZIMIDAZOLE

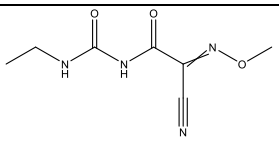


Carbendazim



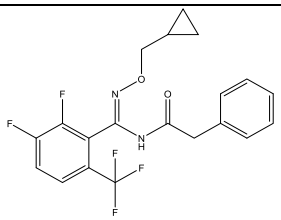
Thiabendazole

UREA

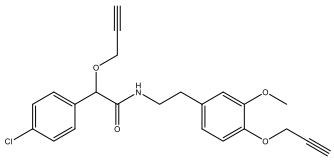


Cymoxanil

BENZOACETAMIDE

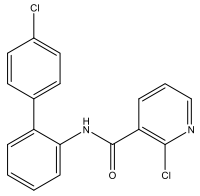


Cyflufenamide



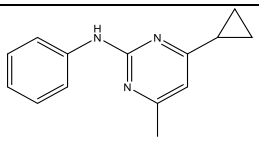
Mandipropamid

NICONTINAMIDE DERIVATIVE

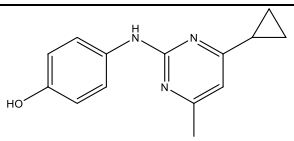


Boscalid

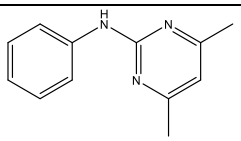
AMINOPYRIMIDINE



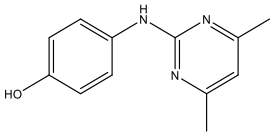
Cyprodinil



Cyprodinil-4OH

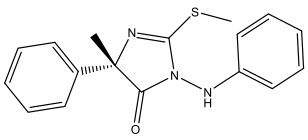


Pyrimethanil

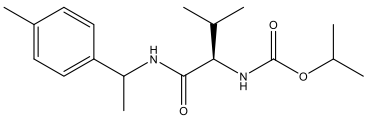


Pyrimethanil-4OH

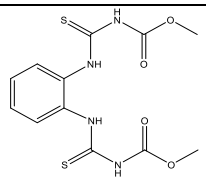
CARBAMATE



Fenamidone

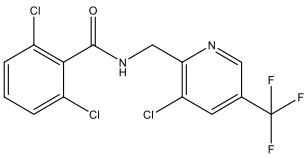


Iprovalicarb

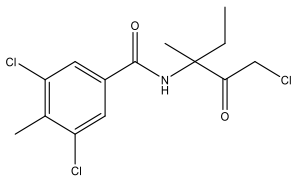


Thiophanate methyl

BENZAMIDE

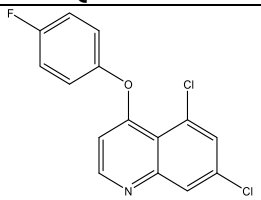


Fluopicolide



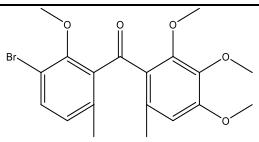
Zoxamide

QUINOLINE



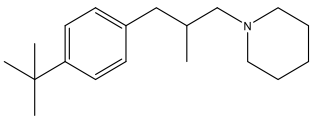
Quinoxyfen

BENZOPHENONE



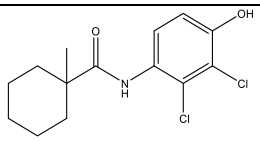
Metrafenone

PIPERIDINE



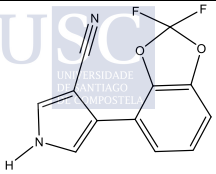
Fenpropidin

HYDROXYANILIDE



Fenhexamide

PHENYLPYRROLE



Fludioxonil

Figure 14. (cont.) Chemical structures of the fungicides and TPs studied.

Table 7. List of insecticides studied, with details of their CAS number, classification, molecular weight, pKa and regulated use (EU).

Analyte	CAS	Chemical group	Formula	Molecular weight	pKa α	Status β	MRL wine grapes (mg/kg) γ
Acetamiprid	135410-20-7	Neonicotinoid	$C_{10}H_{11}ClN_4$	222.6	4.16	√	0.5
Chlorpyrifos	2921-88-2	Organothiophosphate	$C_9H_{11}Cl_3NO_3PS$	350.5	-	X (2020)	0.01
Chlorpyrifos - methyl	5598-13-0	Organothiophosphate	$C_7H_7Cl_3NO_3PS$	322.5	3.71	X (2020)	0.01
Chlorantraniliprole	500008-45-7	Anthranilic diamine	$C_{18}H_{14}BrCl_2N_5O_2$	483.1	-0.73	√	1
Clofentezine	74115-24-5	Tetrazine	$C_{14}H_8Cl_2N_4$	303.1	-	√	1
Clothianidin	210880-92-5	Neonicotinoid	$C_6H_8ClN_5O_2S$	249.6	12.29	X (2019)	0.7
Dinotefuran	165252-70-0	Neonicotinoid	$C_7H_{14}N_4O_3$	202.2		X (2009)	0.9
Flufenoxuron	101463-69-8	Benzoylurea	$C_{21}H_{11}ClF_6N_2O_3$	488.7	8.99	X (2009)	0.01
Imidacloprid	138261-41-3	Neonicotinoid	$C_9H_{10}ClN_5O_2$	255.6	12.35	X (2020)	1 (0.7 from May 2022)
Imidacloprid-olefin	115086-54-9	Neonicotinoid	C9H8ClN5O2	253.6		-	-
Methiocarb	2032-65-7	Carbamate	$C_{11}H_{15}NO_2S$	225.3	14.77	X (2019)	0.03
Nitenpyram	150824-47-8	Neonicotinoid	$C_{11}H_{15}ClN_4O_2$	270.7		X (2009)	0.01
Tebufenozide	112410-23-8	Benzohydrazine	$C_{22}H_{28}N_2O_2$	352.4	10.13	√	4
Thiacloprid	111988-49-9	Neonicotinoid	$C_{10}H_9ClN_4S$	252.7	1.62 (Basic)	X (2020)	0.01
Thiamethoxam	153719-23-4	Neonicotinoid	$C_8H_{10}ClN_5O_3S$	291.7	0.40	X (2019)	0.4

Information extracted from PubChem Substances and Compound database (https://pubchem.ncbi.nlm.nih.gov/). α: pKa (dissociation constant at 25ºC) calculated with ChemAxon software. β: Status (√ Approved for its use; X Not approved for its use (expiration date)). EU Pesticides Database – Active substances, safeners and synergists (https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/active-substances/?event=search.as). γ: MRL (Maximum Residue Limits) in wine grapes (mg/kg) (EU Pesticides Database – Search Pesticide Residues (https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/mrls/?event=search.pr).

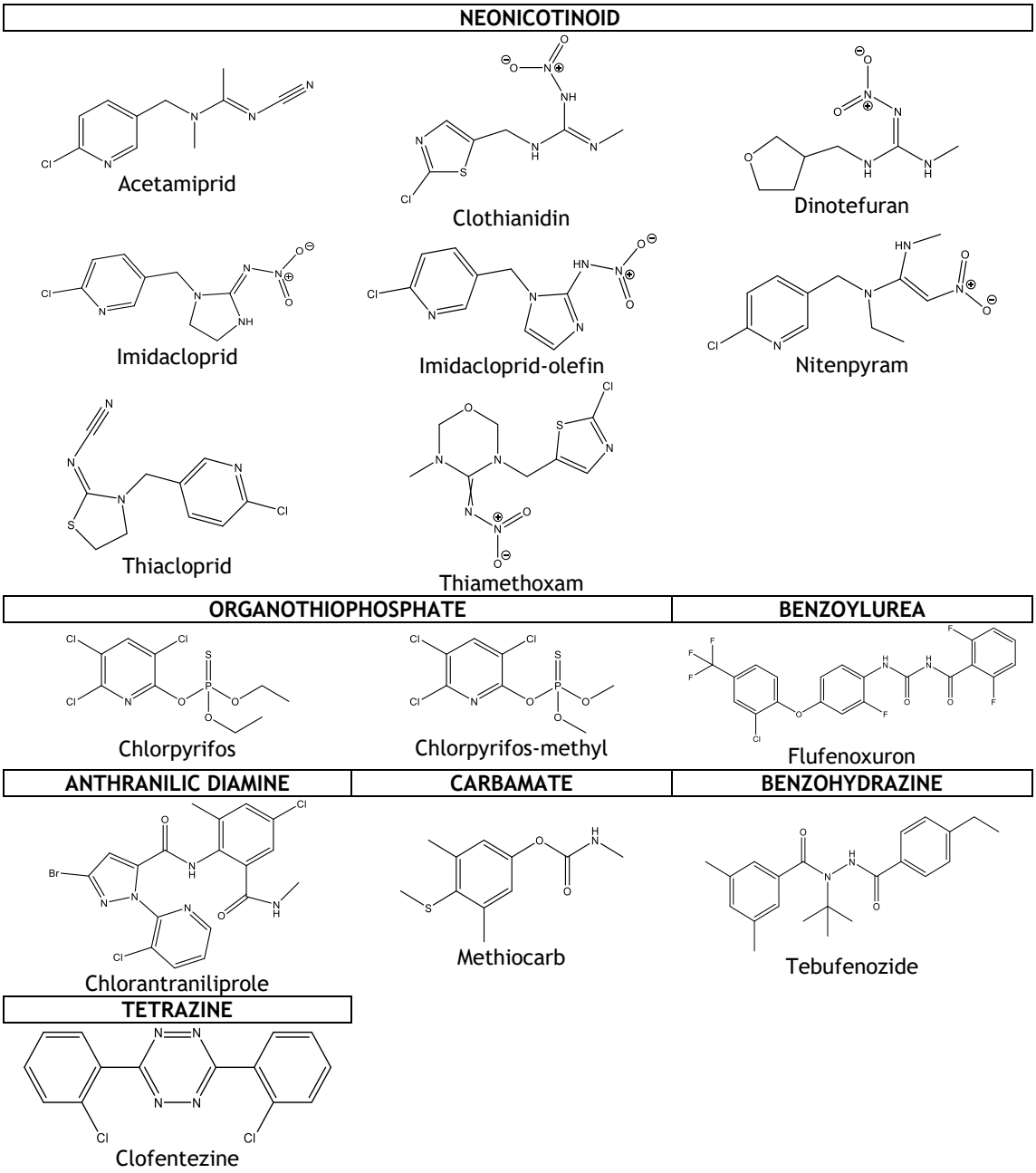
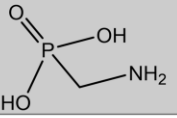
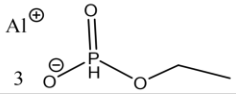
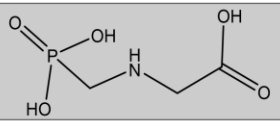


Figure 15. Chemical structures of insecticides studied.

Table 8. List of anionic pesticides and TPs studied, with details of their CAS number, classification, molecular weight, pKa and regulated use (EU).

Analyte	Chemical Structure	CAS	Chemical group	Formula	Molecular weight	pKa [α]	Status [β]	MRL wine grapes (mg/kg) [γ]
AMPA (Aminomethyl)phosphonic acid		1066-51-9	Phosphonic acid	CH_6NO_3P	111.04	-	-	-
Fosetyl (Aluminium tris(ethyl phosphonate)		39148-24-8	Ethyl phosphonate	$C_6H_{18}AlO_9P_3$ ($C_2H_7PO_3$)	354.10 (109.04)	-	√	200
Glyphosate (N-(Phosphonomethyl)glycine)		1071-83-6	Phosphonic acid	$C_3H_8NO_5P$	169.07	-	√	0.5

Information extracted from PubChem Substances and Compound database (https://pubchem.ncbi.nlm.nih.gov/). α: pKa (dissociation constant at 25ºC) calculated with ChemAxon software. β: Status (√ Approved for its use; X Not approved for its use (expiration date)). EU Pesticides Database – Active substances, safeners and synergists (https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/active-substances/?event=search.as). γ: MRL (Maximum Residue Limits) in wine grapes (mg/kg) (EU Pesticides Database – Search Pesticide Residues (https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/mrls/?event=search.pr).

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II. JUSTIFICATION AND OBJECTIVES

Pesticides have been used to control different pests in the agricultural sector. Thus, vineyards receive considerable amounts of plant protection products (PPPs) to ensure productivity and high-quality fruits, not affected by microbiological problems. This practice also involves the possible transfer of the sprayed compounds to different environmental compartments. In the case of wine, pesticides are transferred to the final product as function of their processing factors (PFs), which provides a real challenge to set the legal limits of those compounds in wine. Regarding soil, PPPs will be leached, evaporated, or accumulated in concordance with their chemical structure, biodegradability, soil characteristics and climate conditions.

Nowadays, pesticides are a concerning issue due to their potential toxicity to non-target species. Moreover, their possible (and in some cases proved) presence in water, has highlighted the importance of controlling PPPs not only in the final product, but also in the environment. In addition, ecological production has risen abruptly in recent years, which requires an exhaustive control to ensure the absence of PPPs in products with the ecological label. Finally, official organisms are working to ensure sustainability of agriculture reducing the use of hazardous compounds.

In this vein, selective and sensitive analytical methods are required to detect and to quantify low doses of PPPs present in environmental matrices. In this line, this Thesis includes the use of mass spectrometry, with low- and high-resolution mass analyzers, for the determination of PPPs and their transformation products in wines and vineyard soils (wine grapes are included in **Publication III** for semi-quantitative purposes).

Therefore, research activities developed during this Thesis have been focused on the following aims:

Objective 1. Development of multianalyte methodologies for the determination of PPPs in wine samples. Ultra-high-resolution-liquid- chromatography (UHPLC) combined with low-resolution mass spectrometry, in tandem (MS/MS), will be employed for quantitative purposes. The optimized methodology should allow the determination of a wide range of pesticides, with low or negligible matrix effects, from sub- ppb to 100-200 ppb concentrations. This objective includes the determination of transformation products and the screening of glycosylated forms of certain pesticides by LC-QTOF-MS. Works developed are included in **Publications I-IV**.

Objective 2. Development of separation strategies, alternative to RPLC for the determination of polar PPPs, e.g., glyphosate, TPs and analogue compounds. Data are collected in **Publication V**.

Objective 3. Development of extraction methods for the determination of pesticides in soil matrices. Determination methods optimized in objective 1 will be used and detailed in **Publication VI**.

Objective 4. Assessment of SFC, as an alternative determination technique to LC and GC, for the determination of medium to high polarity PPPs in wine. Likewise, SFC will be evaluated for the determination of chiral PPPs and their enantiomeric distribution in viticulture-related samples. Results are compiled in **Publications VII – VIII**.

III. METHODOLOGY

Analytical methodologies developed and applied in this Thesis have been mainly focussed on the determination of a suite of compounds in wine and vineyard soil samples. Moreover, within **Publication III**, it was considered the use of grape samples to investigate the presence of some pesticide metabolites previously noticed in wine samples. In this particular case, methodologies applied to grapes were only considered for semi-quantitative purposes.

Liquid samples were processed using solid-phase extraction (SPE) either in the offline or in the automated online combination with the determination technique, fabric-phase sorptive extraction (FPSE), and a combination of derivatization reactions followed by SPE and additional clean-up steps. Furthermore, direct analysis of liquid (diluted wine) samples was also employed in some applications. Vineyard soils were extracted employing pressurized liquid extraction (PLE), a combination of high temperature and pression to carry out a version of solid-liquid extraction (SLE). SLE was used to recover fungicides from grapes with semi-quantitative purposes.

Regarding analytical separation of analytes, reversed-phase liquid chromatography (RPLC) was the mainly used for the determination of most of the PPPs considered through this Thesis. Nevertheless, pesticides with a high polar character are not suitable to be separated with those columns. In this case, mixed-mode and strong anionic exchange columns were tested for the determination of potential glyphosate residues existing in wine. In addition, supercritical fluid chromatography (SFC) has been proved to be a more ecofriendly alternative in the determination of some pesticides from environmental samples. In this research, SFC was validated as alternative to LC for the determination of neonicotinoid insecticides. Also, SFC combined with MS was used for the chiral determination of fungicides, in order to evaluate their enantiomeric fractions (EFs) in soil and wine samples.

Finally, triple quadrupole (QqQ) and quadrupole-time-of-flight (QTOF) instruments were employed for the selective determination of target compounds. The latter system worked on MS and target MSMS modes. Results were processed with MassLynx® or MassHunter® software according to the instrument from Waters® or Agilent® employed, respectively.

In addition to data and results compiled in this memory, I have also gained some experience in the use of gas chromatography (GC) combined with time-of-flight mass spectrometry during processing of some pesticides, not amenable to ESI ionization, within the tasks associated to VINOVERT project. Other on-going studies, not completely finished, involved the evaluation of SFC-ESI-MS to the determination of polar, labile pesticides, as it is the case of ethylene and propylene urea fungicides; and the assessment of combined uses of GC-TOF-MS and LC-QTOF-MS for the non-target screening of pesticide residues in wines. Although these activities are not reflected in the scientific publications attached to this memory, they served to complete my formation in the use of above cited techniques, understanding their possibilities and limitations, such as the negative impact of simultaneous MS/MS experiments in the sensitivity of TOF-MS instruments, and the high number of false

positive identifications derived from the most common acquisition modes used in accurate MS screening: *auto MS/MS* and *All ions*.

IV. RESULTS

CHAPTER 1. DEVELOPMENT OF MULTIANALYTE METHODOLOGIES FOR THE DETERMINATION OF PESTICIDES IN WINE SAMPLES BY LC-MS

Vineyards receive considerable amounts of pesticides to ensure the microbiological quality and productivity of vines. These conventional management practices have pointed out the need of a good analytical control to ensure that the final product reaches the consumers in the best conditions regarding PPPs absence. Consensus as regards MRLs in wine is not still set, what force laboratories to improve their methods in terms of sensitivity and selectivity to guarantee the detection of very low residue levels. Thus, analytical methodologies are expected to be useful in the control of conventional and, also, ecological wines. In brief, analytical methodologies tend to include a great number of compounds (likely from different chemical families), with the less manipulation, analysis time as short as possible and a low consumption of organic solvent.

In this chapter, different extraction methodologies were applied to the multiresidue determination of PPP residues in white and red wine wines. Firstly, 50 compounds were extracted using 2 mL of sample volume by SPE. A second approach was focused on the extraction of 21 analytes by FPSE using 10 mL of wine. Finally, 48 PPPs were extracted by online SPE. Those works intended to include the greatest number of pesticides (fungicides and insecticides) from different chemical families with the lower use of organic solvents and sample manipulation. Compounds selection was made using the experience of the research group in this field, and the interaction with the productive sector through participation in the VINOVERT project and further collaborations with EVEGA (Estación de Viticultura y Enología de Galicia), funded by the local administration (Xunta de Galicia, Consellería de Medio Rural).

In addition, hydroxylated and glycosylated derivatives of two frequently applied anilinopyrimide fungicides were investigated in wine and grape samples. The hydroxylated species of anylinopyrimide species involved in this research (PYRI and CYP) have been previously considered as animal metabolites of exposure to these pollutants; however, they have not been previously reported in vegetable origin samples, as it is the case of wine.

Previously described methodologies were exhaustively optimized and validated, and then applied to commercial wine samples. Obtained results have permitted us to identify those compounds more often present in this matrix, which will serve as departure point of further studies aiming the identification of some of them plant transformation products. Moreover, they are expected to serve to farmers and winemakers to assess the impact of their agronomic practices in the quality of the elaborated wines. Determination was performed using QqQ

instruments, except in the case of the hydroxylated and glycosylated metabolites, where a QTOF system was employed to carry out a search of new metabolites of interest.

Publications of those four works are compiled next.

PUBLICATION I

Multianalyte, high-throughput liquid chromatography tandem mass spectrometry method for the sensitive determination of fungicides and insecticides in wine.

Gabriela Castro, Leticia Pérez-Mayán, Tamara Rodríguez-Cabo, María Ramil, Isaac Rodríguez, Rafael Cela

Analytical and Bioanalytical Chemistry, 410, 1139–1150 (2018)

DOI: 10.1007/s00216-017-0724-9

Electronic ISSN: 1618-2650 (Springer)

PUBLICATION II

Fabric phase sorptive extraction followed by ultra-performance liquid chromatography-tandem mass spectrometry for the determination of fungicides and insecticides in wine.

Leticia Pérez-Mayán, Isaac Rodríguez, María Ramil, Abuzar Kabir, Kenneth G. Furton, Rafael Cela

Journal of Chromatography A 1584, 13-23 (2019)

DOI: 10.1016/j.chroma.2018.11.025

ISSN: 0021-9673 (Elsevier)

PUBLICATION III

Residues of anilinopyrimidine fungicides and suspected metabolites in wine samples.

Gabriela Castro, Leticia Pérez-Mayán, Inma Carpinteiro, María Ramil, Rafael Cela, Isaac Rodríguez

Journal of Chromatography A, 1622, 461104 (2020)

DOI: 10.1016/j.chroma.2020.461104

ISSN: 0021-9673 (Elsevier)

PUBLICATION IV

Determination of pesticide residues in wine by solid-phase extraction on-line combined with liquid chromatography tandem mass spectrometry.

Leticia Pérez-Mayán, María Ramil, Rafael Cela, Isaac Rodríguez

Journal of Food Composition and Analysis, 104, 104184 (2021)

DOI: 10.1016/j.jfca.2021.104184

ISSN: 0889-1575 (Elsevier)

CHAPTER 2. ASSESSMENT OF NEW STRATEGIES FOR THE DETERMINATION OF POLAR COMPOUNDS

Glyphosate (GLY) is a well-known, world-wide used herbicide. Despite it is still authorized, concern regarding its toxicity have focused analytical efforts to determine human and animal exposure in order to draw some conclusions regarding its effects and presence in foodstuff samples. (Aminomethyl)phosphonic acid (AMPA) is the main metabolite of GLY, and analytical approaches often include both compounds on account of their common characteristics, such as high polarity, low molecular weight, and ligand properties. In the context of vineyards, GLY is the most popular herbicide to control weeds and soil vegetation competing with vines by hydric resources. Today, GLY treatment remain as the most popular management practice in those areas where mechanic soil treatments are difficult (i.e. vineyards located in hilly areas), or where water scarcity might affect the productivity of vineyards. Traditionally, their determination using LC has been performed as their FMOC-Cl derivatives. Despite this, wine presents more setbacks regarding pH adjustment and precipitation, as so as the lower presumably residue levels than in other foodstuff samples. This statement is made considering the high susceptibility of vines to GLY and the fact that the herbicide is expected to reach vinification grapes either due to drifts during application, and/or uptake from soil through the roots of vines.

In this chapter, two approaches were developed and compared for the determination of GLY and AMPA in wine samples. Firstly, a selective extraction of analytes from wine using a MIP sorbent was combined with FMOC-Cl derivatization and determination by RPLC, with good detection levels and ionization efficiency. Secondly, a direct analysis of wine samples was optimized using mixed-mode and strong anionic exchanger columns. The latter approach included the fungicide Fosetyl, not amenable to FMOC derivatization, and also difficult to be included in multiresidue RPLC methods due to its anionic characteristics. In this vein, both methods were applied to the analysis of commercial wines in order to study the possible presence of these anionic pesticides in wine.

The published study is shown below.

PUBLICATION V

Approaches to liquid chromatography tandem mass spectrometry assessment of glyphosate residues in wine.

Leticia Pérez-Mayán, Gabriela Castro, María Ramil, Rafael Cela, Isaac Rodríguez

Analytical and bioanalytical chemistry, 414, 1445-1455 (2022)

DOI: 10.1007/s00216-021-03775-w

Electronic ISSN: 1618-2650 (Springer)

CHAPTER 3. OPTIMIZATION OF EXTRACTION AND DETERMINATION OF PESTICIDE RESIDUES IN VINEYARD SOIL

Figure 1 (section I.2, Introduction) shows the different possible pathways of PPPs applied to crops and the soil is the second compartment receiving those compounds. Nevertheless, studies are focused on the final products from crops, but few studies have been performed to control the persistence of PPPs in the soil. Vineyards receive a large amount of PPPs every campaign and pesticides can be accumulated in soil, as function of its characteristics and the climatology, in a greater or lesser extent. Also, they can leach to groundwater, or contaminate surface water reservoirs during run-off events, through water erosion of soil.

In this chapter, a multiresidue methodology has been optimized to determine fungicides and insecticides in vineyard soil samples collected in the Northwest of Spain during a 2-year period (Figure 16). Firstly, the analytical procedure was optimized using PLE combined with LC-MS/MS (QqQ instrument). Then, this optimized method was applied to samples collected at the beginning of agriculture campaigns to understand compounds´ behaviour in this matrix. Furthermore, an exhaustive monitoring was performed with more sample collection during the campaigns to draw some conclusions of the stability and presence of pesticides in each season. Some PPPs found in the analyzed soil samples showed potential to be accumulated if applied in consecutive campaigns. As commented in other parts of this memory, identification of heavily polluted vineyard soils and identification of compounds persisting in this matrix represents a first step to further assess the interaction of these compounds with the vineyard environment, including their potential input in the food-web through accumulation in micro-invertebrates, which are the prey of birds and small mammals.

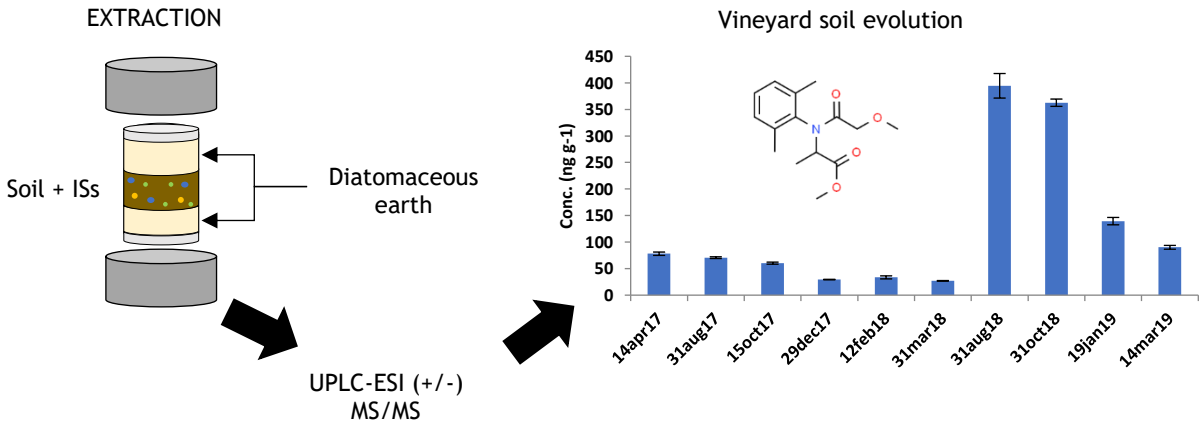

Figure 16. Synopsis of the procedure employed in Publication VI.

This published work is displayed hereunder.

PUBLICATION VI

Multiresidue procedure to assess the occurrence and dissipation of fungicides and insecticides in vineyard soils from Northwest Spain.

Leticia Pérez-Mayán, María Ramil, Rafael Cela, Isaac Rodríguez

Chemosphere, 261, 127696 (2020)

DOI: 10.1016/j.chemosphere.2020.127696

ISNN: 0045-6535 (Elsevier)

CHAPTER 4. EVALUATION OF SFC AS A GREEN ALTERNATIVE TO DETECT THE PRESENCE OF PPPs IN ENVIRONMENTAL SAMPLES

Supercritical fluid chromatography (SFC) has emerged as a sustainable alternative to conventional LC, or GC techniques. CO_2 replaced organic solvents employed in traditional LC applications due to its higher diffusivity and low viscosity under supercritical conditions. Thus, SFC has been employed as separation technique in two works dealing with the determination of neonicotinoid insecticides and the enantiomeric separation of a selection of chiral fungicides, widely employed in vineyards and previously found in the analysis of commercial wine samples.

Firstly, seven commercial insecticides were selected to optimize their determination by SFC-QTOF-MS/MS. Only one out of the seven compounds studied (acetamiprid, ACE) is currently authorized for its use in open agriculture practices (Table 7), because of their association with the disappearance of bees. However, previously processed wine samples highlighted the often occurrence of imidacloprid (IMI) in this matrix, whose use was confirmed during analysis of soil samples. In this work, SPE was employed as extraction technique to recover compounds from wine samples and analytes were separated using UPLC and SFC. SFC conditions were optimized regarding column, CO_2 modifier, back-flush pressure, make-up composition and flowrate, etc; and the final method was applied to 25 commercial wine samples from the 2018 campaign.

Furthermore, five chiral fungicides from two chemical families (acylalanine and triazole) were selected to optimize their determination by SFC-MS/MS and to control their enantiomeric profiles in wine and soil samples. Although the fungicidal activity of one enantiomer is usually much higher than the other, in case of acylalanine fungicides, racemic formulations are still commercialized and applied to crops in competence with other formulations containing only the active enantiomer. In case of azolic fungicides, despite their different activities, they are commercialized just as racemates. Thus, SPE and PLE were used as extraction methods to recover fungicides from wine and soil samples, respectively. SFC separations were carried out using two different polysaccharide based CSPs, optimizing parameters such us the mobile phase modifier and the composition of the make-up solution. The final methods were applied to commercial wine and soil samples.

The published works are compiled in the next page.

PUBLICATION VII

Evaluation of supercritical fluid chromatography accurate mass spectrometry for neonicotinoid compounds determination in wine samples.

Leticia Pérez-Mayán, Miguel Cobo-Golpe, María Ramil, Rafael Cela, Isaac Rodríguez

Journal of Chromatography A, 1620, 460963 (2020)

DOI: 10.1016/j.chroma.2020.460963

ISSN: 0021-9673 (Elsevier)

PUBLICATION VIII

Supercritical fluid chromatography-mass spectrometric determination of chiral fungicides in viticulture-related samples.

Leticia Pérez-Mayán, María Ramil, Rafael Cela, Isaac Rodríguez

Journal of Chromatography A, 1644, 462124 (2021)

DOI: 10.1016/j.chroma.2021.462124

ISSN: 0021-9673 (Elsevier)

V. GENERAL DISCUSSION

Studies carried out in this Thesis are divided into four chapters as function of the main objectives described in **Section II**. Wines and vineyard soils were the samples employed to optimize, validate and apply the different methodologies described.

Compounds selection was based on their occurrence in previous studies, their recent inclusion on the list of banned pesticides, environmental concern and evidence of their systematic use in the investigated farming region. Half of the publications resulted from this Thesis work describe different multiresidue approaches, including from 22 to 50 pesticides, to control the presence of pre-selected compounds in wine and/or soil samples. On the other hand, some methodologies were focused on a specific chemical family, or groups of interest, according to the goal set.

Analysis of complex samples commonly requires a previous sample preparation step to remove unwanted components, to concentrate analytes and to make the final extract compatible with the chromatographic technique. A good selection of the extraction steps and conditions is crucial to attain reliable results. As regards extraction methods, there is a trend to move towards Green Analytical Chemistry (GAC), which fundamentally implies the reduction of organic solvents and wastes. In this vein, SPE, FPSE or even direct injection were selected for liquid samples (wine), and PLE was the chosen one for solid samples (soil).

Analytes selected in the Thesis are organic compounds from different chemical families. The majority of PPPs studied can be separated using RPLC, so a Zorbax Eclipse Plus C18 (50mm x 2.1 mm, 1.8 µm) and an Acquity BEH C18 (50 mm × 2.1 mm, 1.7 µm) were used for this purpose. Nevertheless, highly polar compounds, such as GLY, AMPA and Fosetyl, require the use of mixed-mode columns. For those pesticides, a mixed-mode (HILIC and weak anionic exchange) Torus DEA column (100 mm × 2.1 mm, 1.7 µm) and a strong anionic exchange-type Metrosep A Supp column (150 mm × 2.1 mm, 5 µm) were employed. SFC applications included the use of different columns, such as Viridis, amylose-1 and cellulose-5. Mobile phases, column temperature and flows set for each application are described in Table 9.

Techniques based on mass spectrometry (MS) are the preferred ones for multiresidue control of pesticide residues in environmental samples. In this Thesis, separation of analytes of interest was achieved in 10 min for most applications, except for the SPE online application and the use of the strong anionic exchange column. Chromatographic separations were performed by UHPLC, LC or SFC. Then, analytes were ionized using an ESI source connected to a QqQ or QTOF instruments, where MS or MS/MS acquisition modes were set.

Following these procedures, it was possible to attain LOQs as low as 0.01 µg/L for some analytes in wine samples, being situated in the range from 0.01 µg/L - 11 µg/L as function of the specific methodology employed. In the case of soil samples, LOQs were between 0.2 ng/g and 13 ng/g. Those limits permitted the sensitive detection and quantification of PPPs in

environmental samples, below limits established following the recommendations adapted from MRLs in wines.

Finally, optimized methodologies were applied to a significant set of commercial wines (red and white varieties, conventional and ecological label), mostly produced in the Northwest of Spain, although considering also samples elaborated in different geographic areas, and/or to different soil samples collected in the Northwest of Spain (Galicia). Compounds detection will be deeply discussed hereunder.

Table 9. Summary of the research included in this Thesis.

Matrix	Pesticides	Extraction method (sorbent)	Sample intake	Extract volume	Column	Mobile phases ; column T (°C) ; flow (mL/min)	Analysis time (min)	Separation/detection method	LOQs (µg/L)	Real samples*	Ref.
Wine	50	SPE (Oasis HLB 200mg)	2 mL	2 mL	Zorbax Eclipse Plus C18	H_2O /ACN (0.1% FA) ; 40 ; 0.4	10	UHPLC-ESI-QqQ-MS	< 1 (THIOM, FLUD 15)	20 conv., 5 eco. (2014-2016)	P I
Wine	22	FPSE (Sol-gel CW20M)	10 mL	0.3 mL	Zorbax Eclipse Plus C18	H_2O /ACN (0.1% FA) ; 40 ; 0.4	10	UHPLC-ESI-QqQ-MS	0.03-0.3	9 (5 WW, 4 RW)	P II
Wine / grapes	2 CYP/PYRI + 2 TPs	SPE (Oasis HLB 200mg)	10 mL	2 mL	Zorbax Eclipse Plus C18	H_2O /ACN (0.1% FA) ; 40 ; 0.4	10	UHPLC-ESI-QqQ-MS UHPLC-ESI-QTOF-MS	0.1-0.2	28 WW, 32 RW (2014-2018)	P III
	2 CYP/PYRI + TPs	SLE	2 g	10 mL						Red variety	
Wine	48	SPE online (PS-DVB)	3-25 µL	-	Zorbax Eclipse XDB-C18	H_2O /ACN (0.1% FA) ; 35 ; 0.3	30	LC-ESI-QqQ-MS	0.1-2.5 (3 µL) 0.01-0.5 (25 µL)	25 conv., 5 eco. (2019-2020)	P IV
Wine	GLY, AMPA, FOS	MIP + deriv. FMOC + SPE (Oasis HLB 200mg)	2 mL	0.2 mL	Acquity BEH C18	H_2O /MeOH (0.15% FA) ; 40 ; 0.4	14	UHPLC-ESI-QqQ-MS	0.5-1	17 (2018-2020)	P V
		Direct injection	1 mL	-	Strong anionic exchange-type Metrosep A Supp	H_2O: ACN (1:1), 45 mM NH_4CO_3H / H_2O, 50 mM NH_4CO_3H ; 30 ; 0.3	23	LC-ESI-QqQ-MS	0.4-8.3	44 (2018-2020)	
Soil	49	PLE	2 g	5 mL	Zorbax Eclipse Plus C18	H_2O /ACN (0.15% FA) ; 40 ; 0.4	10	UHPLC-ESI-QqQ-MS	0.2-13 (ng/g)	9 (2017-2019)	P VI
Wine	7	SPE (Oasis HLB 200mg)	2 mL	2 mL	Viridis	CO_2/ MeOH 5 mM NH_4Ac; 45 ; 1.5	10	SFC-ESI-QTOF-MS	1-11	25 (2018)	P VII
Wine	5	SPE (Oasis HLB 200mg)	2 mL	2 mL	Amylose-1 // Cellulose-5	CO_2/ MeOH 5 mM NH_4Ac // CO_2/ ACN 5 mM NH_4Ac; 45 ; 1.5	10	SFC-ESI-QTOF-MS	0.5-2.5	17	P VIII
Soil		PLE	2 g	5 mL					1.3-6.3 (ng/g)	7	

*Conv. (conventional); Eco. (Ecologic label); WW (white wine), RW (red wine)

<u>CHAPTER 1</u>: Development of multianalyte methodologies for the determination of pesticides in wine samples by LC-MS.

Methodologies optimized along this chapter were focused on the detection of PPPs in wine samples. To this aim, different approaches were evaluated in order to achieve the best multianalyte method possible.

Bibliography pointed out to SPE and QuEChERS as the preferred extraction methods for liquid samples (Table 2). So, the first research (**Publication I, P. I**) was focused on the optimization of a SPE extraction to determine a set of 50 pesticides. Optimization of the extraction was conducted employing two different sorbents (Oasis HLB and Oasis MAX) combined with two elution solvents (MeOH and ACN). Although mixed-mode cartridges retained some of the polyphenols existing in the wine matrix, they showed breakthrough problems for some compounds as: carbendazim (CAR), thiabendazole (THIAB) and fenhexamide (FENH); so, Oasis HLB was selected for extraction. Furthermore, a mixture of ACN and MeOH was used as elution solvent to reduce the elution volume needed and to limit the presence of co-extracted pigments in the final extract, which made it incompatible with the determination technique as it was obtained using MeOH for red wines. Recoveries of the SPE procedure and matrix effects (MEs), during the ESI ionization, were optimized for different wine sample volumes, obtaining 2 mL as the suitable amount of sample. In these conditions, linearity, accuracy, repeatability and reproducibility were assessed with acceptable results for the majority of compounds. Regarding procedural LOQs, fludioxonil (FLUD) and thiophanate methyl (THIOM) showed the highest value (15 ng/mL). FLUD could be determined only in the ESI (-) mode with limited sensitivity, whilst the poor LOQ of THIOM might be due to limited stability during sample preparation. Anyway, the LOQs for both compounds in wine were two orders of magnitude below their MRLs in vinification grapes (4 µg/g and 3 µg/g, respectively). The rest of analytes attained LOQs below or equal 1 ng/mL, suitable for their determination at levels far below their regulated MRLs in vinification grapes.

The main advantage of this method was the use of solvent-based standards for the accurate determination of the 90% of compounds included, avoiding the use of different calibration curves for white and red wines. Moreover, no sample concentration is required to quantify very low PPPs concentration with the consequent time and solvent saving.

The application of this methodology to real wine samples (produced between 2014-2016) from a variety of wine producing countries, revealed some outstanding findings. For instance, 46% of compounds (23 analytes) remained undetected. The other compounds showed low detection frequencies (< 20%), except three anti-botrytis (cyprodinil (CYP), FENH and pyrimethanil (PYRI)) and two anti-mildium fungicides (dimethomorph (DIM) and metalaxyl (MET)) detected in more than 50% of the analyzed samples (Figure 17).

Metalaxyl (MET) was detected in 20 samples (80%) with a maximum concentration of 56 ng/mL, but none of its metabolites (CGA 108906 and CGA 62826) were found. Maximum concentrations for the rest of compounds were situated under 100 ng/mL with the exception of PYRI (104.8 ng/mL), FENH (142.6 ng/mL) and iprovalicarb (IPROV, 203.1 ng/mL). IPROV was the only pesticide found in wine at a concentration above 10% of its MRL in grapes (2000 ng/g).

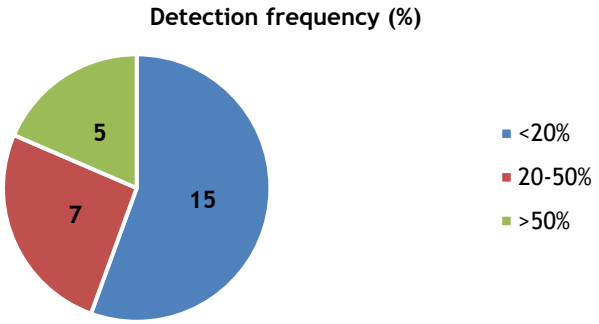

Figure 17. Detection frequencies (%) of compounds studied in wine samples (P. I).

Regarding the total concentration of PPPs in each sample, 44% of samples contained pesticide residues above 50 ng/mL, and three of them showed total concentrations above 200 ng/mL (Figure 18).

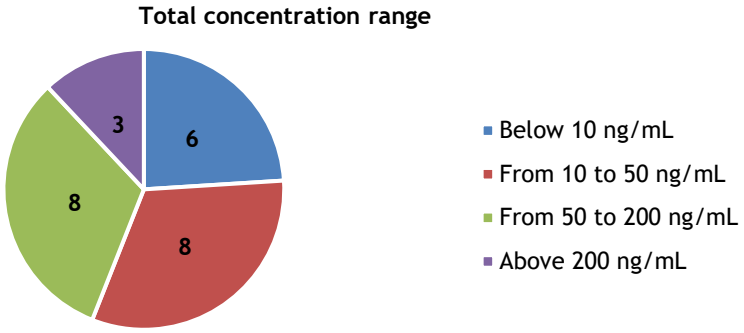

Figure 18. Total concentration range (ng/mL) of PPPs found in the analyzed wine samples (P. I).

Finally, only one out of the five wines with the stamp of ecological production was free of pesticide residues. Nevertheless, compounds´ concentrations stayed below 10 ng/mL, except PYRI in sample E2 (15.3 ng/mL) (Table 10). These findings highlight the importance of the development of sensitive and accurate methodologies to the exhaustive control of wines from ecological production.

Table 10. Concentration of PPPs (ng/mL) found in wines with the ecological label (P. I).

Compound	E1	E2	E3	E4	E5
CAR	4.3	n.d.	n.d.	n.d.	n.d.
CYP	3.4	n.d.	n.d.	n.d.	n.d.
DIM	n.d.	6.1	n.d.	n.d.	n.d.
FENH	n.d.	8.3	n.d.	n.d.	n.d.
IPROV	n.d.	3.5	n.d.	n.d.	n.d.
MET	5.9	4.8	1.2	0.9	n.d.
MYC	n.d.	0.7	n.d.	n.d.	n.d.
PYRI	n.d.	15.3	n.d.	n.d.	n.d.
TEBU	1.9	n.d.	n.d.	n.d.	n.d.
TRIAL	n.d.	0.5	n.d.	n.d.	n.d.
Total Concentration (ng/mL)	**15.5**	**39.2**	**1.2**	**0.9**	**-**

n.d.: non detected.

Microextraction techniques, such as DLLME, have risen over the last decades to overcome some limitations of conventional extractions, e.g., SPE and QuEChERS, moving towards Green Analytical Chemistry (GAC). In this vein, FPSE has been developed as a selective and easily customized alternative based on sol-gel coating technology. **Publication II (P. II)** describes the optimization of FPSE for the determination of 21 PPPs in wine samples.

Firstly, sol-gel coated media was created using an appropriate fabric substrate, an organic polymer, and a linker (sol-gel inorganic/organically modified inorganic polymer). Hence, hydrophilic cotton fabric (100% cellulose) was selected as substrate on the strength of pesticide and matrix characteristics. Five organic polymers with different polarities were used, resulting in five sol-gel coatings: sol-gel CW20M (highly polar), CAP-triol (highly polar), PCAP-PDMS-PCAP (medium polar), PTHF (medium polar) and sol-gel PEG-PPG-PEG (moderately polar).

A first approach to perform the extraction used a 1x1cm square pieces of sorbent freely floating and moving in the diluted wine, but poor reproducibility was obtained. Thus, sol-gel coated sheets were cut into 4 mm diameter discs, attached to a stainless-steel pin, and various series of experiments were carried to optimize the extraction conditions. Under final conditions, 3 discs (4 mm diameter) were attached to a stainless-steel pin in a diluted wine solution (1:1) using a PTFE coated magnetic stir bar to agitate the sample overnight (Figure 19). Then, they were desorbed together using 0.3 mL of ACN: MeOH (80:20) solution (previously used in **P. I**).

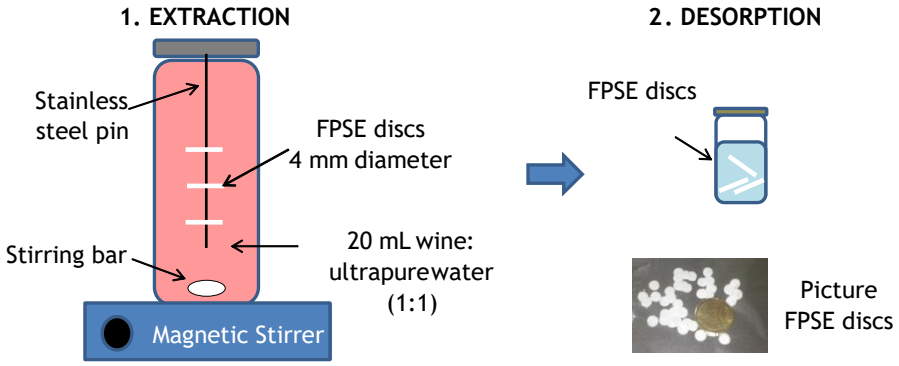

Figure 19. FPSE protocol.

Selection of the FPSE media was performed based on relative extraction efficiencies. The sol-gel CW20M coating attained the highest responses for most PPPs, followed by so-gel PEG-PPG-PEG coating. Thus, sol-gel CW20M coating was selected as FPSE media.

The effect of ionic strength and the extraction kinetics were evaluated. On one hand, no NaCl addition was established as it had almost negligible effects on compounds extraction. On the other hand, kinetics test showed a noticeable improvement in short extraction times, but a slight change until 15 hours, and no variation from that point onwards. Those results are consistent with the complexity of the wine sample and demonstrate the control performed by the diffusion ratio of analytes from the diluted wine to the FPSE media. Extraction efficiencies (EEs) assessment showed higher values to those compounds with larger log D, yet no correlation was proved. A test using 6 fabrics was carried out, attaining best recoveries for those compounds with less affinity to the FPSE coating, but 3 discs and 0.3 mL of desorption solvent were established as they allow to extract analytes with the minimum solvent use.

LOQs were established from 0.03 ng/mL for penconazole (PEN), flusilazole (FLUS), benalaxyl (BEN) and trifloxystrobin (TRIF) to 0.33 ng/mL for chlorpyrifos methyl (CHLORM). Those values are situated two or three orders of magnitude below the MRLs for vinification grapes. Comparing those values with the results obtained in **P. I** (LOQs 0.1 ng/mL - 15 ng/mL), they are deeply lower, at the expense of a much longer sample preparation step.

Under equilibrium conditions (overnight), method performance was evaluated as regards linearity and accuracy. Thus, parameters were acceptable considering the complexity of the wine matrix and two calibration curves were established for the quantification of red and white wine samples, respectively.

This validated methodology was applied to four red wines and five white ones. Table 11 shows the concentrations above LOQs found for analytes on those samples. In general, concentrations stayed below 10 ng/mL for the majority of compounds. Moderately low levels were found for ametoctradin (AME, 12.6 ng/mL), FENH (15.6 ng/mL), boscalid (BOSC, 18.2 ng/mL) and tebuconazole (TEBU, 28 ng/mL). Concentrations above 50 ng/mL were found in one white wine for IPROV (130 ng/mL), still below its limit of 10% of MRL in vinification grapes (2000 ng/g). Comparing those results with concentrations found in **P. I**, IPROV was found in a similar concentration (203.1 ng/mL in **P. I**), while FENH was found in a hundred times smaller level than the previous work (142.6 ng/mL in **P. I**). A limitation of the described methodology was the lower concentration factor provided by all the tested FPSE sorbents for the extraction of most polar compounds, i.e. the neonicotinoid insecticides, from the wine matrix.

Table 11. Concentration of PPPs (ng/mL) found in commercial wine samples by FPSE and LC-MS/MS (P. II).

Compound	WW 1	WW 2	WW 3	WW 4	WW 5	RW 1	RW 2	RW 3	RW 4
PYRI	n.d.	1.2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.6
CYP	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.3
AME	n.d.	4.6	n.d.	12.6	n.d.	n.d.	n.d.	n.d.	n.d.
MET	1	n.d.	1.3	7.5	0.7	5.1	2.4	n.d.	n.d.
PEN	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.2
MYC	0.9	0.49	n.d.	n.d.	0.6	n.d.	n.d.	1	0.6
TRIAL	n.d.	n.d.	n.d.	n.d.	1.6	1.1	n.d.	n.d.	n.d.
FENH	n.d.	16	15.3	n.d.	n.d.	n.d.	n.d.	n.d.	1
TEBU	7.4	n.d.	n.d.	n.d.	1.7	2.2	1.3	7.1	28.1
IPROV	n.d.	n.d.	130	5.3	n.d.	n.d.	n.d.	n.d.	n.d.
BEN	0.4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
BOSC	9	18.2	n.d.	3.2	3.8	1.4	2.2	n.d.	1.1
DIM	n.d.	3	n.d.	4	n.d.	n.d.	n.d.	n.d.	n.d.
AZO	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.5
DIF	n.d.	n.d.	n.d.	n.d.	0.2	n.d.	n.d.	n.d.	0.2
Total concentration (ng/mL)	**18.7**	**43.49**	**146.6**	**32.6**	**8.6**	**9.8**	**5.9**	**8.1**	**34.6**

WW (white wine), RW (red wine), n.d. (non detected)

Regarding the total concentrations of PPPs in each sample, 44% of the samples contained pesticide residues below 10 ng/mL and just one of them showed values above 100 ng/mL (WW4, 146.6 ng/mL).

Although the presence of fungicides in wine has been evaluated, little is known about the coexistence of those compounds and their suspected metabolites in this matrix. For instance, in the previous works, metabolites of MET have been included in the multiresidue methodologies, yet they were not detected in this kind of samples. Thus, **Publication III** (**P.III**) involves the determination of two anilinopyrimidine fungicides (PYRI and CYP) and their suspected metabolites. A first approach was carried out using a QqQ detector to control the possible existence of PYRI and CYP with their 4-hydroxylated metabolites (PYR-4OH and CYP-4OH) in wine samples. Furthermore, retrospective search was performed by a QTOF-MS instrument to evaluate the presence of additional hydroxylated or glycosylated TPs.

Firstly, the sample preparation technique employed was the SPE. The protocol established in **P. I** was tested using ACN and ACN:MeOH (80:20) as elution solvents. ACN allowed to recover analytes and a free-pigment extract, but using a high elution volume (10 mL). Thus, ACN:MeOH (80:20) was selected to elute compounds using 2 mL. The chromatographic method is the same used in the previous publications (Table 9). The method was re-evaluated in terms of recoveries, MEs, linearity and accuracy, with acceptable results. LOQs were established as 0.1 ng/mL for parent compounds (PYRI and CYP) and 0.2 ng/mL for metabolites (PYR-4OH and CYP-4OH).

This optimized methodology was applied to a set of 60 samples (32 white wines and 28 red wines) produced in the interval from 2014 to 2018 (Table 12). All the selected samples contained either PYRI and/or CYP above their LOQs. Taking into account that the MRLs for PYRI and CYP are situated in 6000 and 3000 ng/g, respectively; maximum concentrations found in samples are situated below 2% of this limit. The 4-hydroxyanilino derivatives (PYR-4OH and CYP-4OH) were found nearly in all the wines containing the parent forms.

Table 12. Summary of concentrations (ng/mL) in commercial wine samples processed (P. III).

Compound	Average	Median	Maximum	Positive samples
PYRI	18.7	8.0	99.6	54
CYP	7.9	7.6	31.9	50
PYR-4OH	11.4	4.6	58.0	54
CYP-4OH	2.7	1.7	17.3	45

Then, correlation between parent and hydroxylated forms was investigated. In white wines, metabolites represented the 20% and 12% of the concentration for the parent fungicides (PYRI and CYP, respectively), showing a poor correlation. In the case of red wines, CYP-4OH represented the 50% of CYP concentration, while PYR-4OH concentrations were twice as those for PYRI.

Additional hydroxylated and/or glycosylated forms were investigated using a LC-QTOF-MS system. To this aim, MS spectra and product ion spectra were recorded in positive mode (no relevant signal in the negative mode). Thus, Table 13 shows a list of the 4-hydroxylated metabolites and additional TPs found. PYRI has two additional hydroxylated forms (PYR-TP216A and PYR-TP216B), and one glycosylated derivative (PYR-TP378), apart from PYR-4OH. In the case of CYP, three hydroxylated forms were identified (CYP-TP242A, CYP-TP242B and CYP-TP242C), as well as four glycosylated derivatives (CYP-TP404A to CYP-TP404D). Mass errors for all the TPs identified were situated below 8 ppm.

Table 13. Summary of potential TPs of PYRI and CYP identified by LC-QTOF-MS (P. III).

Parent fungicide	TP	Formula	$[M+H]^+$ Calculated mass (Da)	$[M+H]^+$ Experimental Mass (Da)	Mass error (ppm)
PYRI	PYR-4OH	$C_{12}H_{13}N_3O$	216.1131	216.1128	-1.4
	PYR-TP216A	$C_{12}H_{13}N_3O$	216.1131	216.1122	-4.2
	PYR-TP216B	$C_{12}H_{13}N_3O$	216.1131	216.1117	-2.8
	PYR-TP378	$C_{18}H_{23}N_3O_6$	378.1660	378.1654	-1.6
CYP	CYP-4OH	$C_{14}H_{15}N_3O$	242.1288	242.1287	-0.4
	CYP-TP242A	$C_{14}H_{15}N_3O$	242.1288	242.1292	1.7
	CYP-TP242B	$C_{14}H_{15}N_3O$	242.1288	242.1270	-7.4
	CYP-TP242C	$C_{14}H_{15}N_3O$	242.1288	242.1290	0.8
	CYP-TP404A	$C_{20}H_{25}N_3O_6$	404.1816	404.1814	-0.5
	CYP-TP404B	$C_{20}H_{25}N_3O_6$	404.1816	404.1815	-0.2
	CYP-TP404C	$C_{20}H_{25}N_3O_6$	404.1816	404.1820	1.0
	CYP-TP404D	$C_{20}H_{25}N_3O_6$	404.1816	404.1808	-2.0

Product ion spectra of TPs allowed to infer their structures comparing fragment ions with those obtained for the parent fungicides and the 4-hydroxylated metabolites. For instance, Figure 20 shows the corresponding fragments of the glycosylated forms (PYR-TP378 and CYP-TP404A) with removal of the sugar moiety, leading to the hydroxylated forms. MS3 spectra would be necessary to confirm the identification of the 4-hydroxylated forms as the fragment present on both spectra.

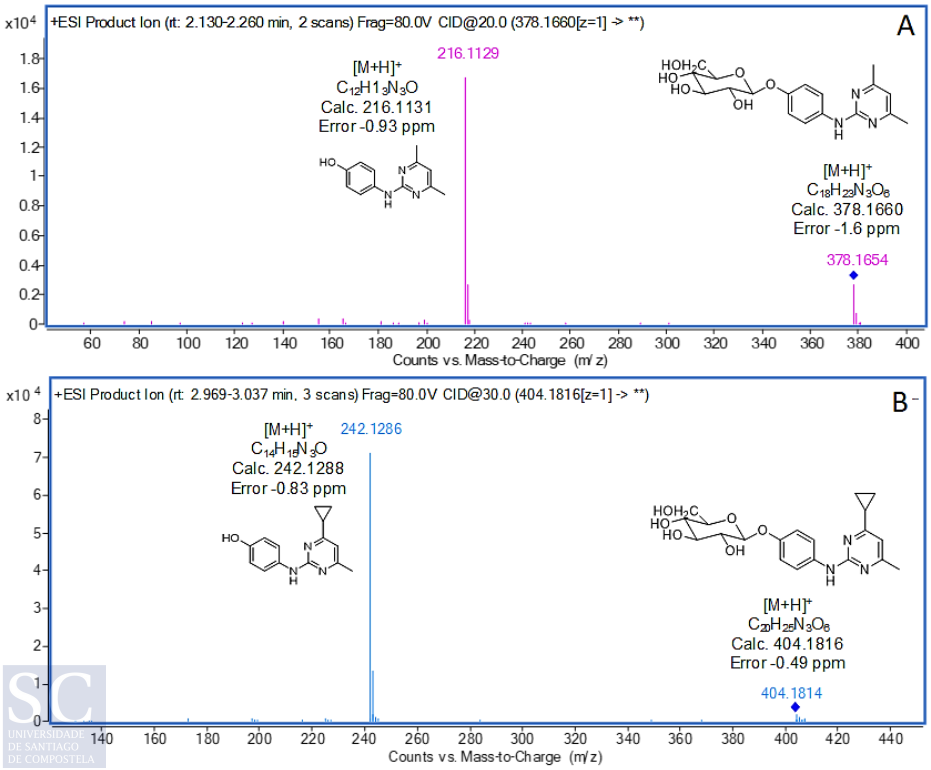

Figure 20. Product ion spectra of PYR-TP378 (A) and CYP-TP404A (B) (P. III).

Fragmentation routes for the rest of the hydroxylated metabolites detected were inferred based on the precursor ones. Hydroxylation positions of CYP were expected to be equivalent

to those reported for PYRI, whilst product ion scan spectra of CYP derivatives were more complex than those of PYRI, with many product ions of low intensity. Thus, the tentative identification of the exact positions of hydroxyl groups was possible just in case of CYP-TP242B, comparing fragmentations with those for CYP-4OH (Figure 21).

Once tentative identifications were established, the origin of the metabolites found was further investigated. For instance, the concentration ratio of PYR-4OH and PYRI in red wines were higher than those for CYP-4OH/CYP, and the PF of PYRI was established in 0.91. Thus, both compounds are necessarily present in grapes before fermentation, so hydroxylation starts at vines. In case of CYP, it was possible to perform an extraction of grapes treated with a commercial formulation containing CYP using SLE. In this case, semi-quantitative results showed a concentration of 1898 ng/g for CYP and 2.3 ng/g for CYP-4OH. Thus, it is proved that hydroxylation started in vines.

Differences between concentration ratios of 4-hydroxylated metabolites and parent fungicides in red and white wines can be explained considering different fermentation and wine elaboration conditions.

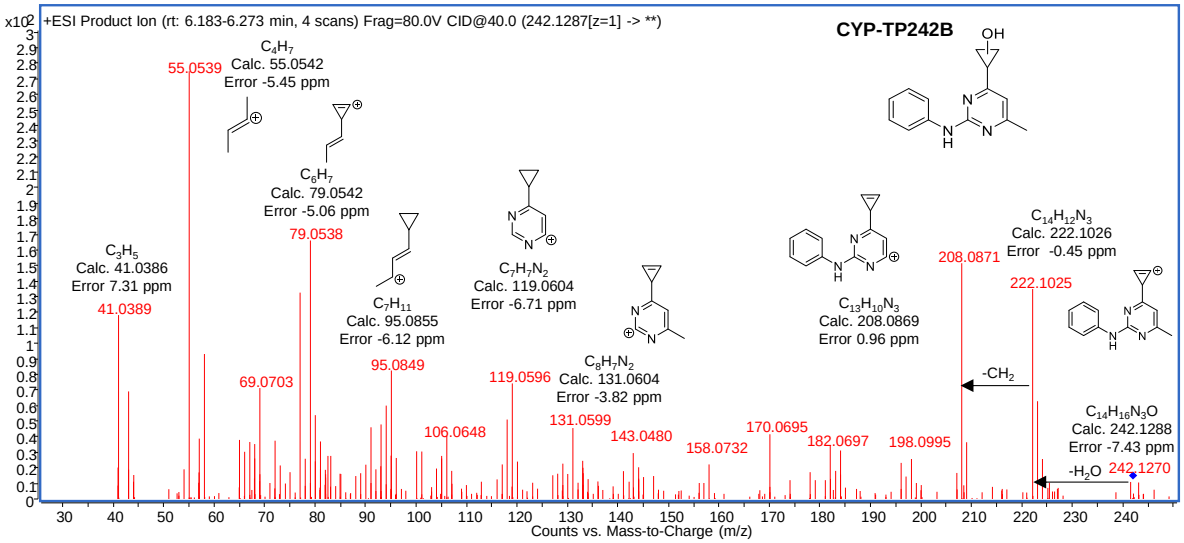

Figure 21. Product ion spectra of CYP-TP242B (P. III).

The last publication included in this chapter is based on the optimization of an automated multiresidue method for the determination of pesticides in wine using online SPE (**Publication IV**, **P. IV**). This online methodology is expected to reduce solvent consumption, improve precision, and increase sample workflow. To this aim, polystyrene-divinyl benzene (PS-DVB) was the sorbent selected to evaluate different parameters affecting the sensitivity, selectivity, and robustness of the proposed method.

Sample preparation was reduced to filtration and dilution (1:1) with water before entering the system. The performance of the online SPE was evaluated in terms of sample volume loaded, washing time, potential cross contamination, and cost per extraction.

Regarding injection volume, addition to white and red wine signal was compared to that for an ethanolic: water solution, obtaining different behaviours as function of the compound. Thus, a sample volume of 25 µL was selected to continue the optimization process, although 3µL were enough to attain levels needed for wines from a traditional production. Then, sorbent washing time was assessed, also evaluating the absence of analytes losses during this step. In this case, compounds poorly retained (e.g. CAR) showed an increase in their retention times and peak widths, whereas strong retained compounds (e.g. zoxamide, ZOX) were not

affected (Figure 22). So, 2 min were established as washing time with 1 mL/min of flowrate. Carry over effects were negligible, so the washing protocol was effective to ensure the repeatability of the online SPE process. Notwithstanding the cost of the PS-DVB cartridges is 10-times that of the Oasis HLB 200 mg (offline SPE, **P. I**), the performance of the online process was maintained during 150 extraction-desorption cycles, achieving a lower cost per extraction employing the reusable cartridge.

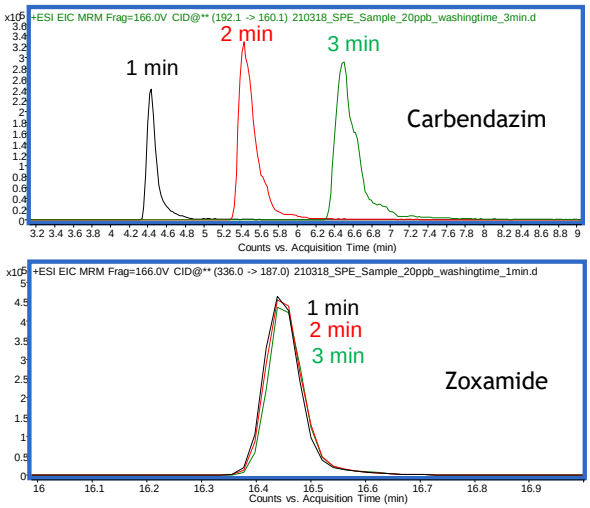

Figure 22. Effect of washing time on peak shape and retention times of carbendazim and zoxamide (P. IV).

The following step was to validate the method in terms of linearity, accuracy, MEs and LOQs for 3 and 25 µL of sample loaded. MEs were evaluated for white and red wine and compared to those data obtained with offline SPE (**P.I**). Table 14 shows the absence of an important effect on the efficiency of the ESI ionization for both methods.

Table 14. MEs for online SPE and offline SPE on the extraction of PPPs from white and red wine (P. IV).

MEs range	White wine		Red wine	
	Online SPE	Offline SPE	Online SPE	Offline SPE
< 70 %	1	1	0	3
70-130 %	46	45	46	44
> 130 %	1	2	2	1

Accuracy was acceptable considering the complexity of the matrix, achieving recoveries between 70% -130% for 94% of the compounds. Linearity was also admissible with the distinction of the use of spiked ethanol: water (12:80) calibration solutions when 3 µL of wine were loaded, whereas matrix-matched calibration solutions (white and red wines) were employed for 25 µL. In both cases, a linear model was achieved in the range of concentrations from 1 ng/mL to 200 ng/mL (3 µL) and from 0.1 ng/mL to 25 ng/mL (25 µL). LOQs were evaluated for 3 µL and 25 µL, rendering, at least, levels three orders of magnitude below MRLs, achieving values between 0.1ng/mL - 1 ng/mL using 3 µL (except CHLORM: 2.5 ng/mL) and 0.01ng/mL - 0.5 ng/mL using 25 µL. Finally, an extraction with 3 µL of sample loaded was established to control pesticide residues in commercial samples with the use of the solvent-based standards for quantification, and a protocol using 25µL was applied to samples with the label of ecological production. However, the online combination of SPE with LC has a major drawback. SPE sorbents are designed to work at a lower pressure than those typically employed in UPLC columns. Thus, given that a 3.5 µm particle size column was used in this

application, the separation step was longer than in case of the previously reported off-line approaches.

The application of the optimized methodology was performed to a set of 25 commercial wines (traditional production) and 5 ecological ones. Whenever possible, the same commercial brands as those used in **P. I** were acquired to compare the evolution of PPPs from 2014-2016 and 2019-2020. Data obtained for the application of the online SPE combined with LC-ESI-MS protocol reflected concentration values below 10 % of MRLs established for vinification grapes. Furthermore, fourteen analytes (30%) were not found in any sample, and the seventeen additional compounds (35%) accounted concentrations below 5 ng/mL.

The seventeen remaining compounds are displayed in Figure 23 in terms of detection frequency (%). A similar trend compared to that obtained in **P.I** is shown, keeping in mind that Figure 23 only includes compounds above 5 ng/mL, whereas data in Figure 17 exhibits compounds above LOQs.

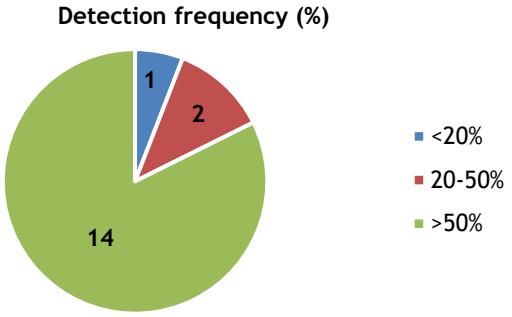

Figure 23. Detection frequency of compounds detected in wine samples above 5 ng/mL (P. IV).

Regarding maximum concentrations, IPROV residues remained in the same range of concentration as in **P. I** (210.09 ng/mL), whilst PYRI and BOSC showed a 50% reduction (66.01 and 30.34 ng/mL, respectively). Total concentrations are summarized in Figure 24, with almost 50% of samples situated between 10 and 50 ng/mL.

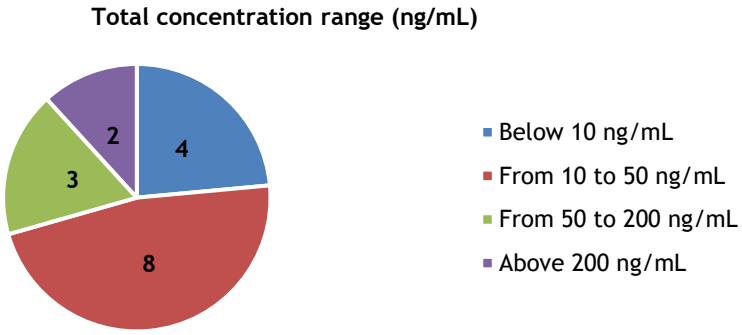

Figure 24. Total concentration range (ng/mL) of pesticides found in the analyzed samples (P. IV).

Finally, four out of the five wines with the label of ecological production contained pesticide residues above LOQs. Table 15 displays individual concentrations in the range from 0.1 to 6.03 ng/mL. In the case of sample 29, concentrations around 6 ng/mL were found for MET and BOSC. In summary, data obtained for this group of wine samples highlighted the need to define a concentration threshold low enough to guarantee the quality standard of

wines commercialized with the ECO stamp, but above LOQs of LC-MS/MS to avoid conflicts derived from drifts during fumigation of nearby vineyards.

Table 15. Concentrations (ng/mL) of pesticides found in wines with the ecological stamp (P. IV).

Compound	RW27	RW28	RW29	RW30	WW12
AME			0.14		
BEN			0.28		
BOSC			6.03	0.26	
CAR		0.45	2.5	0.64	
CYP			2.23	0.15	
DIM			1.75		
FENH			0.86		
FLUD			1.23		
FLUO			0.32		
IMI			0.72		
IPROV			0.1		
MAN			0.12		
MET		0.1	5.75	0.56	
METR			0.43		
MYC		0.2	0.28	0.21	0.2
PYRI			1		
TEBU			1	0.1	
TEBF			0.13		
THIOM			0.82	0.16	
TRIAL			0.16		
Total concentration (ng/mL)	**0**	**0.75**	**25.85**	**2.08**	**0.2**

*RW (red wine), WW (white wine)

<u>CHAPTER 2</u>: Assessment of new strategies for the determination of polar compounds

Glyphosate (GLY) is a worldwide employed herbicide. Despite it is authorized by the EU, concern about its environmental and toxicological effects, and also those for its main metabolite (aminomethyl)phosphonic acid (AMPA), is rising abruptly. Nevertheless, their analytical control is limited due to their high polarity, low molecular weight, zwitterionic character and ligand properties. Thus, in **Publication V** (**P. V**) two approaches were assessed for the determination of potential residues of GLY and AMPA in wine samples. On one hand, derivatization was completed with a previous pre-treatment using MIP sorbent and analysis by RPLC. On the other hand, a direct analysis method for GLY, AMPA and Fosetyl is proposed employing mixed-mode and strong anionic exchange columns to attain an effective chromatographic separation.

The first approach included in this work was focused on the determination of GLY and AMPA as FMOC derivatives, since this derivatization strategy does not work with Fosetyl. Conditions previously employed were not compatible with the wine matrix due to precipitation events when sample pH was adjusted to the range of values required for the FMOC derivatization reaction. To solve this shortcoming, we investigate the suitability of MIP cartridges to isolate both compounds from wine samples. The MIP sorbent showed a high affinity for GLY considering a loading volume of 10 mL; however, AMPA could be retained only after increasing the pH of the diluted wine solution to 7 units. Higher pH values were not tested to prevent precipitation of the wine matrix. In these conditions, Fosetyl was not retained by the MIP sorbent, so it could not be included in this approach. Regarding the elution step, compounds were recovered using 10 mL of an aqueous solution of hydrochloric acid (HCl, 0.1 M). Derivatization conditions were assessed considering different concentrations of FMOC-Cl prepared in ACN and different reaction times. A 1:10 ratio between the FMOC-Cl solution in acetonitrile and the MIP extract was maintained in all the assays and a concentration of 6.5 mM of FMOC-Cl was established. As regards the derivatization time, no significant signal improvement was reached for reaction times over 2 hours, so that was the time set. After stopping the FMOC derivatization reaction, a clean-up step was performed with an Oasis HLB cartridge and dichloromethane (DCM) as washing solution. Elution was carried out with 2 mL of MeOH, and extract was concentrated without losses (Figure 25). Furthermore, effect of the MIP pre-extraction step was assessed comparing the protocol with and without MIP pre-treatment. AMPA showed responses twice as those obtained without the use of MIP, while for GLY this difference was 20-times higher.

The LC-MS determination of derivatized AMPA and GLY was performed in ESI positive mode, discarding the product ion at *m/z* 179.0 (fluorenyl methyl moiety) as it showed a low S/N ratio. MEs were assessed using a commercially available standard of GLY-FMOC added to final extracts (MIP protocol) and a solvent-based solution, where 15% of signal suppression was noticed. Linearity was investigated in the range from 1-100 ng/mL for both compounds using matrix-matched standards and results were acceptable (R^2 > 0.998). Accuracy of the method was investigated with four samples and two different concentration levels, achieving global recoveries in the range 99% - 117%, and 91% - 107% for GLY and AMPA, respectively. LOQs were situated in 0.5 n/mL for GLY and 1 ng/mL for AMPA.

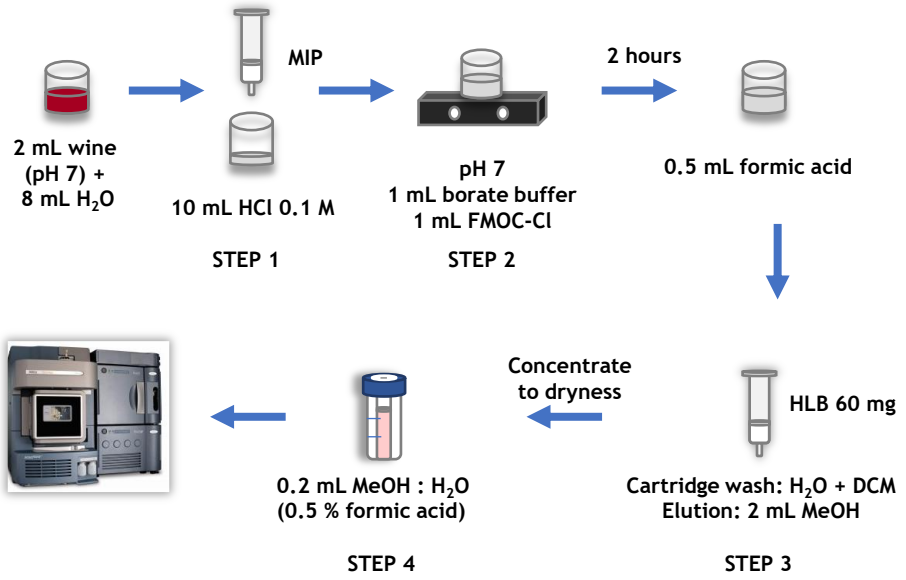

Figure 25. Scheme of the extraction procedure for the derivatization approach (P. V).

The second approach was the direct analysis of GLY, AMPA and Fosetyl. Their simultaneous determination was reached due to a good chromatographic separation (AMPA and Fosetyl) and an acceptable efficiency in the ESI ionization. Two columns were tested: a mixed-mode (Torus DEA) and an anionic exchange (Metrosep). As better conditions (peak shape and S/N ratio) were achieved with the Metrosep column, it was selected testing two different gradients (Figure 26).

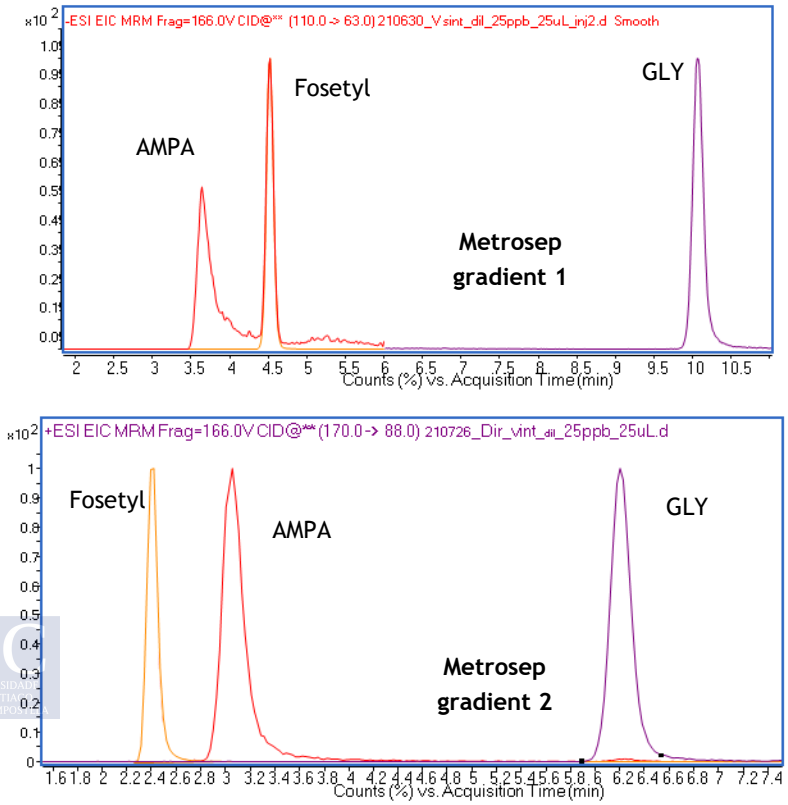

Figure 26. Chromatographic separation of GLY, AMPA and Fosetyl with the Metrosep column and two different gradients (P.V).

MEs were evaluated with both gradients, with a clear trend to higher signal suppression for shorter retention times. Thus, as retention times for AMPA and Fosetyl are inverted as function of the gradient employed, higher signal suppression was noticed for AMPA with gradient 1 and for Fosetyl with gradient 2. GLY suffered moderate signal suppression with gradient 2. Linearity, accuracy and LOQs were evaluated also for both gradients (injection volume =25 µL). Table 16 shows results of linearity and LOQs, with R^2 values above 0.996. Lower procedural LOQs for AMPA were attained with gradient 2, whilst Fosetyl and GLY showed better limits using gradient 1 (selected).

In comparison with the derivatization approach, similar LOQs were achieved for GLY and Fosetyl using the direct injection. In contrast, AMPA has higher procedural LOQs employing the direct injection than those values obtained with FMOC derivatization. The accuracy of direct determination was evaluated with spiked fractions of two different wines (red and white) and two addition levels, achieving recoveries between 90 and 122 %.

Table 16. Linearity and LOQs for GLY, AMPA and Fosetyl with the Metrosep column and gradient 1 (P. V).

Compounds	R^2 (0.5-100 ng/mL)		Instrumental LOQs (ng/mL)		Procedural LOQs (ng/mL)	
	Gradient 1	Gradient 2	Gradient 1	Gradient 2	Gradient 1	Gradient 2
AMPA	0.9961	0.9998	1.5	0.8	8.3	2.8
Fosetyl	0.9998	0.9998	0.2	0.2	0.4	3.3
GLY	0.9985	0.9994	1	1	1.0	1.4

Finally, optimized methodologies were applied to a set of 44 commercial wines from 2018 to 2020 campaigns (10 white and 34 red wines). The FMOC derivatization approach was applied to 27 samples and the rest were analyzed by direct injection. Quantification was performed employing matrix-matched standards in the case of the derivatization protocol, and solvent-based standards for the direct methodology. AMPA was detected below its LOQs in only one sample. Conversely, GLY was detected in 70% of the processed samples in the range from 1.4 ng/mL to 31.4 ng/mL (Figure 27), although GLY concentrations above 10 ng/mL were found just in two samples (W4, 13.2 ng/mL and R8, 31.4 ng/mL). In addition, the residues of GLY were situated far below 10% of the MRL established for vinification grapes in the EU (0.5 mg/kg, Table 8). However, obtained data confirmed the presence of detectable concentrations of the parent herbicide in a significant percentage of wine samples.

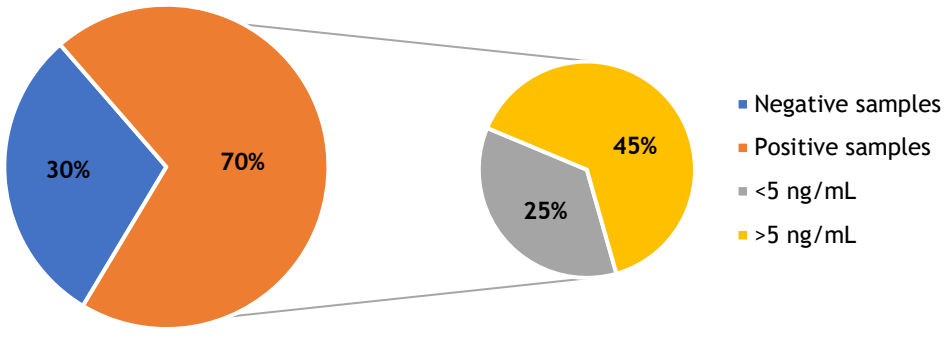

Figure 27. Wine samples with detection of GLY residues above LOQs (P. V).

Concentration values of Fosetyl were found in the range from 0.5 ng/mL to 63.8 ng/mL, with 74% of positive samples, out of the 27 wines processed using direct injection method, with residues detected above its LOQ (Figure 28). Regarding legal limits allowed for Fosetyl

in vinification grapes, even the maximum concentration found in W10 (63.8 ng/mL) is situated below 0.05% of its MRL (200 mg/kg). Further research is required to quantify simultaneously the residues of Fosetyl and its transformation product, phosphonic acid, in the wine matrix.

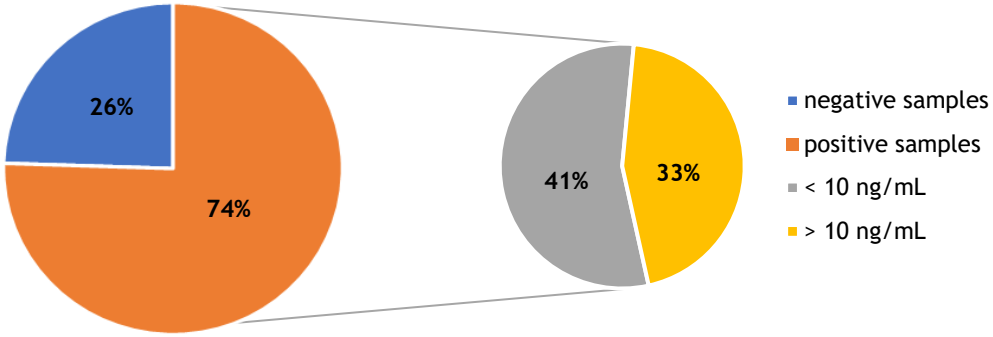

Figure 28. Wine samples with detection of Fosetyl residues above LOQs (P. V).

<u>CHAPTER 3</u>: Optimization of extraction and determination of pesticide residues in vineyard soil

As explained in section I.2. (Figure 1), vineyards are cops receiving a large amount of PPPs to ensure productivity. The type of treatments, doses and frequencies are controlled by environmental conditions, which determine the pressure of pests affecting vineyards from different geographic areas. Thus, presence of pesticide residues in wine has been largely investigated (Table 2), in contrast to the scant research focused on the control of those compounds in soil. Currently, a number of studies are investigating the residues of pesticides existing in agriculture soils and their potential correlation with the microbiota of soil, the development of resistant fungi strains and medium-term productivity of agriculture soils. Soil contamination with pesticides is particularly relevant in case of permanent crops, set during years in the same field, as it is the case of vineyards. Also, understanding the persistence of pesticide residues in soil is a relevant issue to obtain objective information regarding the possibility to transform conventional vineyards to ecologic production ones. In this vein, research included in **Publication VI** (**P. VI**) was conducted to develop a multiresidue technique best able to detect pesticides and control their behaviour in consecutive campaigns.

Firstly, PLE parameters were evaluated and optimized to obtain the best EEs possible for the majority of compounds. In this vein, a Box-Behnken factorial design was performed including temperature (80, 110 and 140 ºC), extraction solvent (MeOH, with the following percentages of ACN: 10, 30, 50 %) and number of static extraction cycles (1, 2, 3), prefixing the rest of parameters involved in the extraction process. Different trends were obtained, although in most cases only the quadratic term associated to temperature was statistically significant (95% confidence level). For instance, MET showed the highest extraction yield at the intermediate temperature (Figure 29A), whilst neonicotinoid insecticides, ZOX and chlorantraniliprole (CHLORA) displayed a negative effect of the temperature (Figure 29B). THIAB was the only analyte with a positive influence of the temperature on its extraction (Figure 29C). Solvent composition and number of cycles did not show a clear effect on extraction, except in the case of CGA62826, obtaining best extraction efficiency with lower percentage of ACN (Figure 29D). Considering that four compounds were not recovered (THIOM, methiocarb (METHI), cymoxanil (CYM) and CGA 108906), lower extraction temperatures were tested. In the case of METHI, an improvement was noticed, so it is presumably degraded at high temperatures. METHI, CYM and THIOM were identified as very labile species, prone to degradation during sample preparation. The metabolite of MET, CGA 108906 showed better recoveries when adding 5% of formic acid (FA) to the extraction solvent, as so as CYM, METHI and CGA 62826.

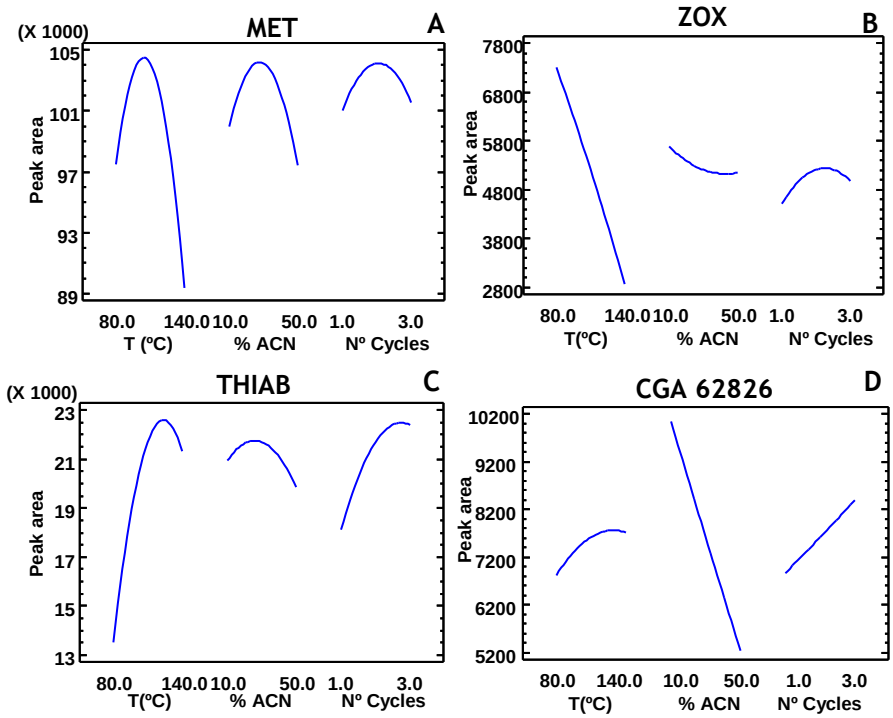

Figure 29. Main effect plots with different trends as function of the factors included in the Box-Behnken design. Metalaxyl (A), zoxamide (B), thiabendazole (C) and CGA 62826 (D) (P. VI).

As a compromise, to achieve the simultaneous extraction of most of the considered compounds, two extractions of each PLE cell were performed as follows:

- First extraction: 80 ºC, 1 cycle of 5 min, MeOH:ACN (70:30)
- Second extraction: 120 ºC, 2 cycles of 5 min, MeOH:ACN:FA (65:30:5)

This extraction methodology allowed to recover 95% of the majority of compounds in the first extraction, while 10% of CAR, PYRI and PEN was noticed in the second extraction. THIAB and CGA 62826 were equally distributed and CGA 108906 was recovered in the second fraction. A scheme of the global sample preparation procedure is presented in Figure 30.

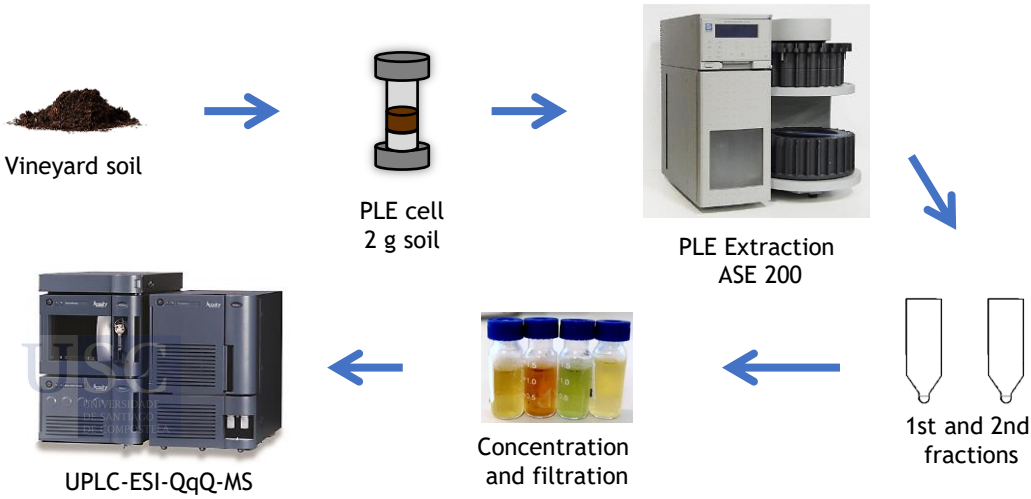

Figure 30. Scheme of the methodology for the determination of PPPs in vineyard soil samples by PLE combined with LC-MS/MS (P. VI).

Once extraction parameters were optimized, this multiresidue methodology was validated in terms of linearity, MEs, EEs and LOQs. LC-MS/MS determination was performed using the analytical method developed for the determination of pesticides in wine in **P. I**. MEs and EEs were assessed in two soils with different organic carbon content (OC 1.5 % and 8.3 %). THIOM was not included in validation tests to avoid overestimation of CAR.

Regarding EEs, results were acceptable, except for THIAB and METHI, and OC content of soil samples presented a negligible effect in the yield of the extraction. On the contrary, MEs were affected as function of the compound. For instance, neonicotinoids were attenuated in a large (clothianidin (CLOT), imidacloprid (IMI) and thiamethoxam (THM)) or moderate (thiacloprid (THC) and acetamiprid (ACE)) extension as they showed ME values below 60% or in the range 60%-80 %, respectively (Figure 31). The majority of analytes showed values in the ranges between 80%-120%; that is, they exhibited no significant signal enhancement, or attenuation. Nevertheless, the sample with high OC content displayed more compounds in the medium attenuation range region (60%-80% of normalized responses to those observed for solvent-based standards) than the sample with low OC content.

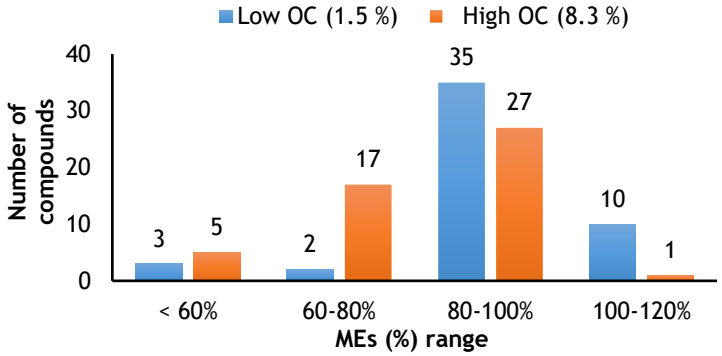

Figure 31. MEs distributed in different ranges as function of the OC content (P.VI).

Accuracy was evaluated for two vineyard soils and two addition levels in triplicate. Recoveries were calculated against solvent-based standards using a limited number of ISs (data for METHI not included) and accuracy results (49 compounds) were admissible. In the case of global recoveries, just four compounds stayed below 70 % (THIAB, CYM, THC and CGA 108906) and Mandipropamid (MAN) above 130 %, so, 90% of compounds displayed overall recoveries in the range from 70 % to 130 %. Linearity was evaluated and acceptable up to 500 ng/g, achieving LOQs in the range between 0.2 ng/g and 13 ng/g.

The optimized and validated methodology was finally applied to vineyard soil samples from Galicia (Spain) in order to evaluate the presence/absence of pesticide residues and their persistence in this matrix in consecutive agriculture campaigns. Nine different sites were analyzed at the start of the vegetative cycle of vines (end of March – beginning of April) during consecutive harvest campaigns and pesticide residues were present in every sample processed. Dilution was required in some samples to adjust compounds within the linear range established. In general, PPPs detected were fungicides with the exception of the insecticides chlorpyrifos (CHLOR) and IMI. As it can be seen in the chart (Figure 32), the compounds with the highest concentration found were fungicides: two anti-mildew agents (DIM and fluopicolide (FLUO)), two anti-powdery mildew compounds (myclobutanil (MYC) and pyraclostrobin (PYRA)) and CAR (TP of THIOM, used to treat mildew). Total concentration of PPPs per sample was between 60 ng/g for sample site 7 and 2800 ng/g for sample site 6, collected in 2019.

Data obtained from other studies performed in some regions from Spain (Table 5) showed values slightly below those detected in the present research. In particular, MET displayed concentrations similar to those obtained by Bermúdez-Couso et al. in samples from 2004-2006 [42].

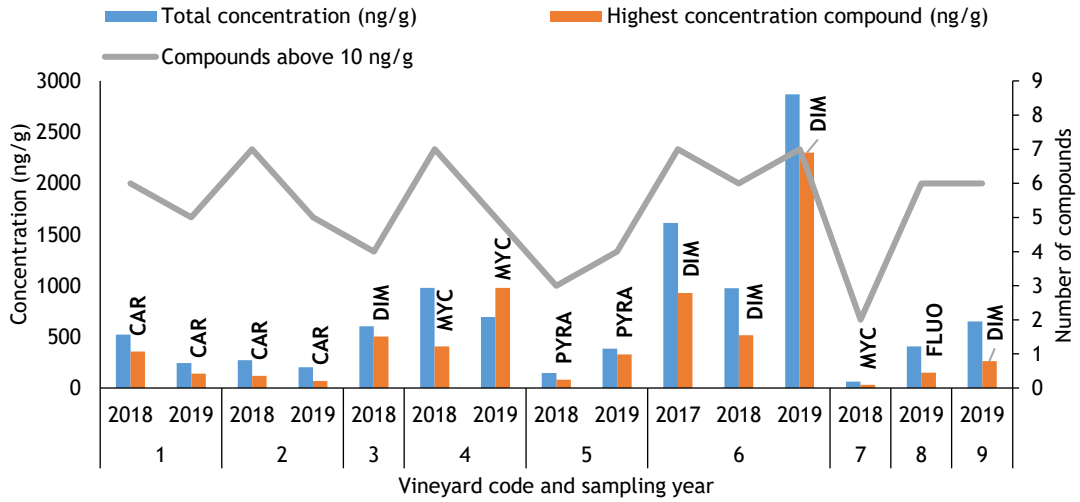

Figure 32. Total pesticide residues, compounds with the highest concentration and number of compounds above 10 ng/mL in samples collected in spring (P. VI).

Furthermore, comparison of total PPP concentrations from sampling campaigns carried out in spring and October from the previous year was studied. As a general trend, total residues found in spring (March- April) were lower than those from the previous year except in the case of sample 1 (2017-2018), where different mechanical processes could have modified the upper layer of soil. Moreover, average dissipation percentages were calculated for those compounds found with appreciable concentrations. Figure 33 shows different results for 9 fungicides, two TPs (CAR and CGA 62826) and one insecticide (IMI), with average dissipation rates situated between 12% (DIM) and 75 % (BEN). For instance, the acylalanine fungicides MET and BEN, and the TP CGA 62826 showed high removal ratios during the wintertime. On the contrary, PYRA, DIM and BOSC had dissipation rates below 20%. In the case of the insecticide IMI, it showed a relative low disappearance (30%), although it has been forbidden since 2018, so no further applications are expected. Those results highlight the potential of some PPPs to be accumulated if applied in consecutive years.

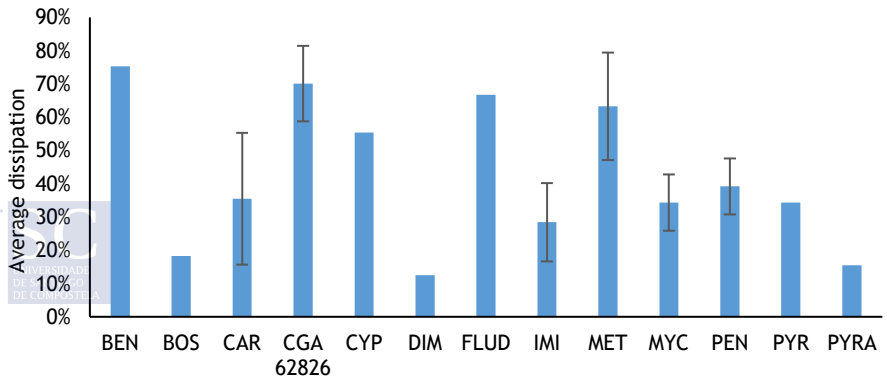

Figure 33. Average dissipation for compounds displaying high concentrations in samples collected in March and October of the previous year (P. VI).

In contrast, CYM was found to be easily degraded when applied to crops, as it was only detected when the sampling was carried out just a few days after application. A possible pathway of dissipation can be the formation of TPs, as it is the case of MET. Temporary control was done for this fungicide and its metabolite CGA 62826, highlighting the clear reduction with time, whilst the TP increased and remained more or less stable (Figure 34).

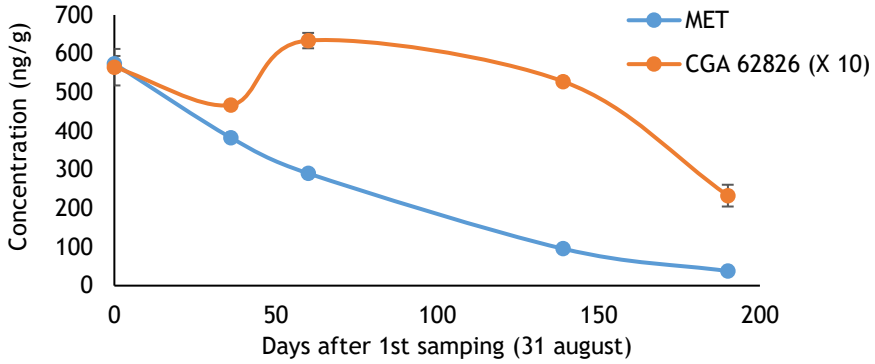

Figure 34. Time-course evolution of MET and CGA62826 in vineyard soil code 5 (P. VI).

Finally, different trends were displayed for compounds present in the samples with higher PPP concentrations. For instance, a clear application was detected for MET, BEN and BOSC in vineyard code 6 at pre-harvest time in 2018 (Figure 35). Nevertheless, MET and BEN residues decrease during wintertime, whilst BOSC increased between October 2018 until January 2019. Washout from vine leaves and leaves incorporation to soil at the end of autumn might lead to an increase in soil residues of poorly biodegradable compounds.

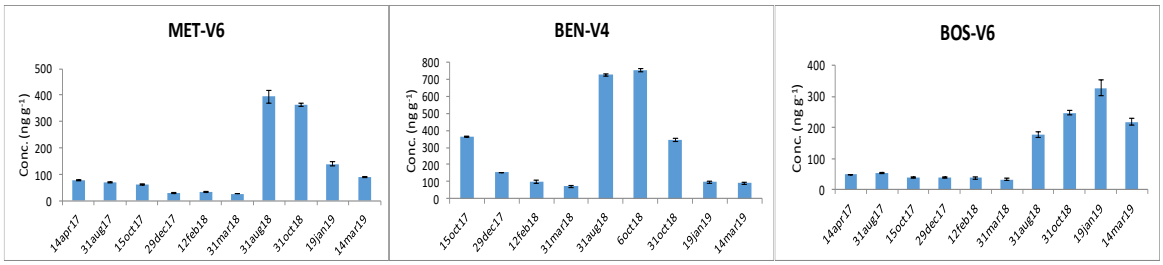

Figure 35. Temporary control of MET, BEN and BOSC from 2017 to 2019 (P. VI).

<u>CHAPTER 4</u>: Evaluation of SFC as a green alternative to detect the presence of PPPs in environmental samples

The objective of this chapter was to assess the suitability of the SFC-ESI-MS for the determination of a selection of pesticides in wine and soil samples. This technique has been proved to increase selectivity and sample throughput, as it reduces solvent consumption. Some preliminary studies showed that under SFC conditions, low polar fungicides and insecticides were poorly retained by tested polar columns and C18 type; so, as a preliminary conclusion, SFC is not expected to become an alternative to RPLC to this kind of studies. Further applications were focussed on selected groups of compounds with some particular features. First, SFC was focussed on the determination of neonicotinoid insecticides, which are relatively polar compounds, comparing its performance as separation technique, and also in combination with MS detection, with that achieved using RPLC. Furthermore, chromatographic separation was tested for five chiral fungicides widely employed to treat different fungal diseases and usually commercialized either as racemates (azolic fungicides), or in both forms: racemates and formulations containing only the active enantiomer. Such distinction is relevant, since racemate formulations contain the double of dose than those using just the most active enantiomer.

The first work developed with the use of SFC-ESI-MS/MS was focused on the determination of seven neonicotinoids and one TP (imidacloprid-olefin, IMI-OLE) in wine (**Publication VII**, **P. VII**). Those compounds have been widely employed, but their association with de disappearance of bees was proved. Thus, nowadays (2022), only ACE is allowed to be used in agricultural practices.

The first step was the optimization of the SFC conditions for compounds. Firstly, three different polar columns were tested, and the silica column was selected as it provided the best separation (45ºC). Then, different mobile phase modifiers (ACN and MeOH) and additives (FA and ammonium acetate, NH_4Ac) were tested, with the final selection of MeOH and NH_4Ac, as a result of shorter retention times and a better signal response (Figure 36A). Those conditions were compared to RPLC (Figure 36B), employing the same analytical method optimized in **P. I**. No correlation on compounds retention was found, and a better separation was achieved with SFC.

Parameters such as the back-flush pressure (BPR) and injection volume were also optimized. BPR was established at 140 bar due to the slight reduction of retentions times, while obtained responses raised. In the case of the injection volume, 3µL were adopted as a compromise of signal intensity and baseline peak width. The last parameter affecting the ionization process is the make-up composition and flowrate. Thus, different mixtures of MeOH with NH_4Ac and water were tested, achieving better results with a make-up composition of MeOH:H_2O (25:75) and a flowrate of 0.2 mL/min. Those results reinforce the reference of SFC as a green analytical approach, as it reduces the use of organic solvents.

The extraction protocol for neonicotinoids was optimized from a previous one developed by Rodríguez-Cabo et al. [34]. Under final conditions, 2 mL of wine were passed thought an Oasis HLB cartridge, the rinse solvent was ultrapure water and 2 mL of ACN were employed for elution. SPE recoveries were tested, achieving results in the range from 87 % to 113 %.

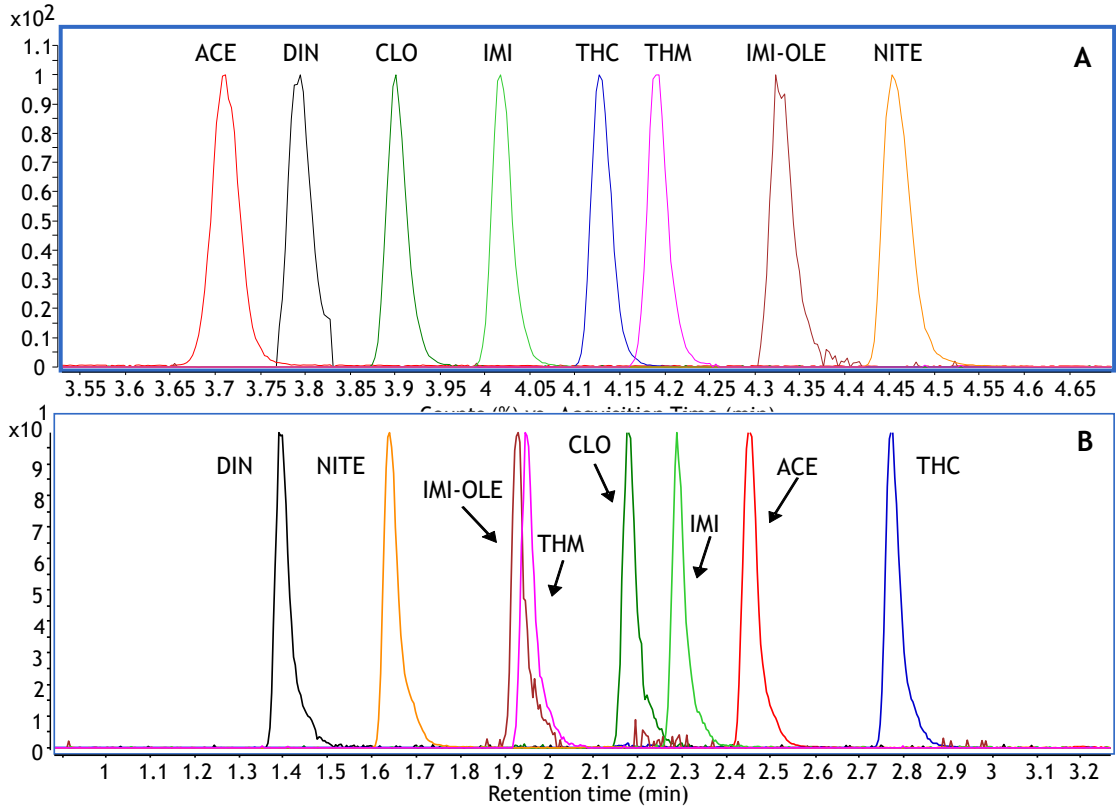

Figure 36. Chromatograms for the $[M+H]^+$ ion of analytes using the Viridis SFC column (A) and the RPLC column (B) (P. VII).

Once the SFC-ESI-MS procedure was optimized, the method was evaluated in terms of linearity and LOQs, compared to UPLC. SFC provided good linearity in the range from 1ng/mL to 500 ng/mL ($R^2 > 0.996$) and instrumental LOQs were situated in 1 ng/mL (CLOT, THC, IMI, nitenpyram (NITE) and THM), 5 ng/mL for ACE and 10 ng/mL (dinotefuran (DINO) and IMI-OLE). Employing the sample injection volume, SFC and UPLC responses (peak area and height) were compared, with a clear advantage of the SFC approach (SFC/UPLC response ratios from 1-5). Thus, SFC provided best peak separation and a more efficient ionization.

Validation of the overall methodology was performed in terms of EEs, MEs and LOQs. MEs were calculated as the ratio for the calibration curve of spiked sample extracts (white and red wine) and solvent-based standards. SFC MEs varied from 73% to 105 %, which represents no signal attenuation. Conversely, UPLC MEs were situated between 35% and 92%, with a higher attenuation for those compounds at lower retention times (Figure 37). Global recoveries were performed for two wine samples at three addition levels in triplicate against solvent-based standards (after IS correction). Results were between 74% and 118%, except NITE at the lowest addition level (67% and 63%). Thus, this methodology is suitable for quantification of neonicotinoids in wine. The procedural LOQs achieved were situated between 1.1 ng/mL-1.2 ng/mL, except ACE (6 ng/mL) and 11 ng/mL for DINO and IMI-OLE.

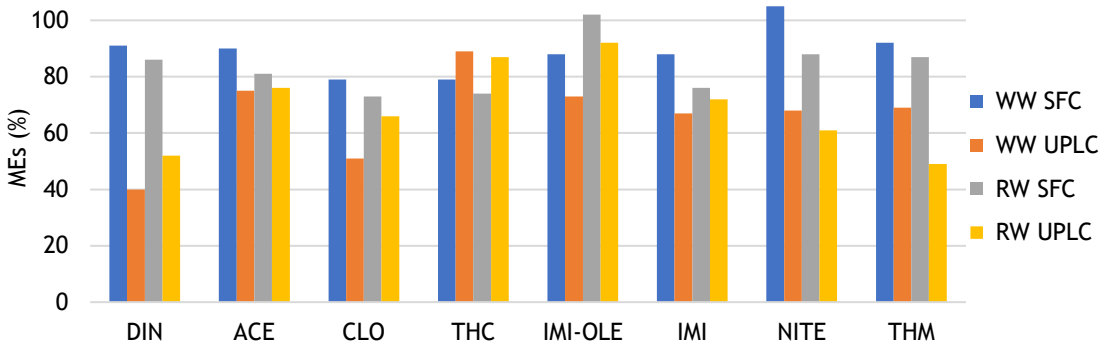

Figure 37. Average MEs obtained for neonicotinoids using SFC-ESI-MS and UPLC-ESI-MS approaches (WW: white wine, RW: red wine) (P.VII).

The optimized and validated methodology was applied to a set of 25 commercial wines (8 red and 17 white wines) from the same geographical area produced in 2018. IMI was detected in 48% of samples above its LOQ (Table 17). The maximum concentration of IMI (33 ng/mL, code 12) was a hundred times below the MRL set for this insecticide in vinification grapes (1 mg/Kg, until May 2022). Furthermore, those values are in concordance with those obtained in **P. I** from previous campaigns (0.7 ng/mL -17 ng/mL). Conversely, IMI-OLE remained below its LOQ in all samples, yet its presence has been associated with IMI residues in wine. Finally, ACE was detected in samples 8 and 9 at concentrations below its LOQ.

Table 17. Concentrations of IMI detected in wine samples (P. VII).

Code	Concentration (ng/mL) ± SD	Code	Concentration (ng/mL) ± SD
1	17.0 ± 0.5	**7**	14.6 ± 0.4
2	2.7 ± 0.1	**8**	8.3 ± 0.3
3	14.6 ± 0.3	**9**	2.8 ± 0.1
4	7.5 ± 0.2	**10**	29.1 ± 0.4
5	4.8 ± 0.1	**11**	28.0 ± 1.1
6	1.3 ± 0.1	**12**	33.8 ± 2.0

A large percentage of PPPs employed in agriculture have, at least, a chiral centre. Furthermore, one isomer is usually much more effective than the other, although the majority are still commercialized as racemates. This practice involves the application of a higher amount of pesticide, and it is expected to lead to higher residue levels in crops and soil. For instance, azolic compounds, used in the treatment of *oidium* infections (powdery mildew), were proved to be persistent in soils (**P. VI**), although their PFs from grapes to wine are smaller than those for the acylalanine fungicides (MET and BEN) applied against downy mildew. Usually, determination of chiral pesticides was restricted to a single compound or a multianalyte approach involving long analysis times. Thus, research conducted in **Publication VIII** (**P. VIII**) was focused on the use of SFC-ESI-MS for the chiral determination of two acylalanine and three azolic fungicides with a chiral structure.

Firstly, SFC parameters were optimized for the five analytes. To this aim, three chiral columns (amylose-1, amylose-3, cellulose-5) and two modifiers (MeOH and ACN) were tested. MeOH showed better separation of compounds and a higher desolvation efficiency. Resolution factors (Rs) were column and modifier dependent (Table 18). Moreover, different additives (NH_3 0.1%, FA 0.1% and NH_4Ac 5mM) were also tested for each modifier and they

showed an important influence in the ionization of compounds. Finally, NH_4Ac (5 mM) was selected as additive and 140 bar of BPR was established.

Table 18. Resolution factors (Rs) for compounds using two different chiral columns and two modifiers (MeOH, ACN) (P. VIII).

Compound	Amylose-1		i-Cellulose-5	
	MeOH	ACN	MeOH	ACN
TEBU	1.62	0.91	-	-
MET	1.06	0.94	-	-
BEN	2.48	0.99	1.56	2.56
PEN	-	1	1.2	1.56
MYC	-	0.76	-	1.27

On the basis of those results, two chromatographic methods were set. On one hand, the amylose-1 column was selected for the determination of MET, BEN and TEBU using CO_2:MeOH (5 mM in NH_4Ac) as mobile phases and the S-form as the first eluted isomer for the three species. The make-up flowrate (MeOH: FA, 99.5: 0.5) was set at 0.3 mL/min. On the other hand, cellule-5 column was used for MYC and PEN determination, with ACN (5 mM in NH_4Ac) as modifier. In this column no isomer identification was performed, except in the case of BEN, also detected with this column in the opposite elution order (BEN-R first). The make-up flowrate (MeOH: FA, 99.5: 0.5) was established at 0.1 mL/min.

Then, linearity was assessed in the range from 1 ng/mL to 200 ng/mL (sum of enantiomers), achieving good results (R^2 >0.99). Instrumental LOQs varied from 0.5 ng/mL (MET, TEB, MYC) to 2.5 ng/mL (PEN), values just a little higher than those reported in **P.I** by UPLC-ESI-QqQ-MS.

Sample preparation methods for wine and soil samples were developed in **P.I** and **P. VI**, respectively. Thus, method validation was performed just in terms of MEs and accuracy, since their extraction yield was previously demonstrated. Signal suppression was noticed for some compounds, yet both enantiomers were affected in the same manner. For instance, the enantiomers of TEB and BEN showed a moderate signal attenuation for soil extracts and, in a lesser extent, during analysis of red wine. Recoveries varied in the range from 80% to 117% in wine and 84% - 112% in soil. In the case of wines, the procedural LOQs are similar to the instrumental values (0.5 ng/mL-2.5 ng/mL). For soils, LOQs varied in the range from 1.3 ng/g to 6.3 ng/g.

The optimized methodology was applied to 17 wines produced in Galicia (Spain) and 7 vineyard soils from the same region. In the case of wine samples, MET was found in all samples above its LOQ, followed by TEBU (53 % of positive samples) and MYC (24% of positive samples) (Figure 38). Conversely, BEN and PEN were detected below LOQs in the samples analyzed. MRLs of compounds in vinification grapes were established in 1 mg/kg for MET and TEB and 1.5 mg/kg for MYC. Thus, maximum residues found for MET (412 ng/g), TEB (76 ng/g) and MYC (106 ng/g) represent the 41%, 8% and 7% of their MRLs, respectively. Regarding EFs, values around 0.5 for TEB and MYC confirms their commercialization as racemates and the absence of enantioselective dissipation processes. Conversely, EFs of MET varied as function of the sample, from 0.05 to 0.57. For instance, the sample with EF = 0.05 (R2) shows that only MET-M (R-enantiomer) was applied. The opposite case can be seen for R3 (EF = 0.57), where MET-S (inactive isomer) is supposed to be enriched during the fermentation process after racemate application to vines. In the case of the rest of wine samples with EF < 0.5, more information about the vineyard treatments is

required in order to draw some conclusions about the potential accumulation of MET-M (R form).

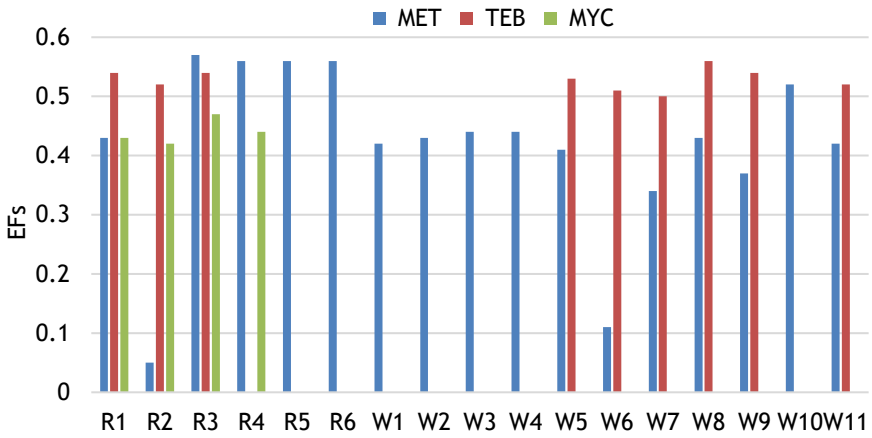

Figure 38. Average EFs for chiral pesticides in positive wine samples in P. VIII.

Soil samples contained at least one of the compounds involved in this study, and they were re-analyzed using the chiral SFC-ESI-MS procedure. Four out of the seven samples were analyzed in autumn and at the end of winter to assess their tentative dissipation (soils 1 to 4, Figure 39). BEN, TEBU and MYC showed EFs around 0.5, which means absence of enantioselective degradation processes. PEN was detected in two samples with slight variations between autumn and spring, one of them with values around 0.4 (sample 1) and the other situated in 0.5. MET was again the compound with the highest variability between samples. For instance, soil 3 showed prevalence of MET-R (EF =0.01-0.03), while soils 4 and 5 displayed MET-S as the dominant form (faster degradation of MET-R, as MET-S is not commercially available). In soils 1 and 7, a faster degradation of MET-S is noticed during wintertime, EFs 0.37→ 0.28 and 0.96→0.65, respectively.

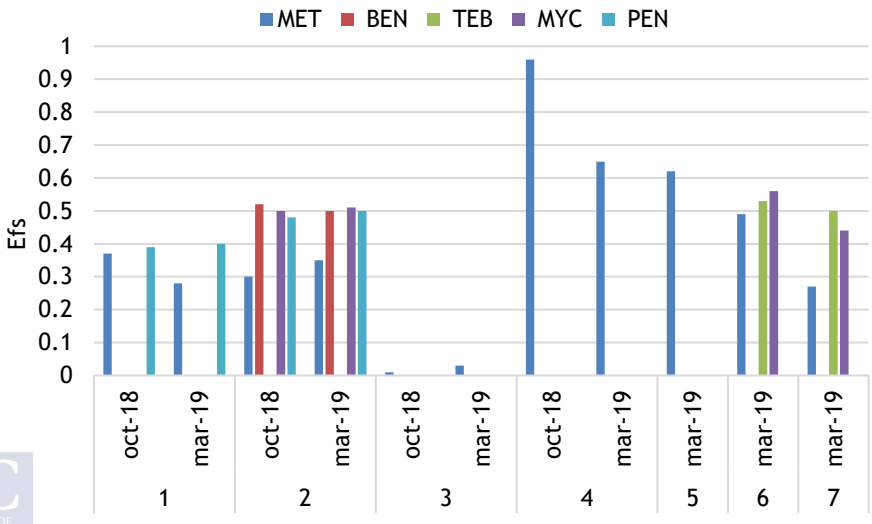

Figure 39. Average EFs of chiral compounds detected in soil samples in P. VIII.

VI. CONCLUSION

The objectives initially proposed were achieved through exhaustive research regarding the development of analytical methodologies in order to detect a wide variety of pesticides applied to vineyards.

Along this Thesis, different extraction approaches were performed, with the added toughness of working with very complex samples, such as wine and soil. The use of low- and high-resolution mass spectrometry (QqQ and QTOF) instruments allowed obtaining sensitive, selective and robust methods for the determination of pesticide residues.

The general conclusions extracted from each chapter are summarized below.

Chapter 1. Development of multianalyte methodologies for the determination of plant protection products in wine samples.

- Nearly fifteen compounds were determined by SPE and UPLC-ESI-QqQ-MS, improving previous methods in terms of analysis time, accuracy and LOQs. Analysis of real samples pointed out the presence of PPPs, although at relative low concentrations. Pesticide residues were detected in various samples identified with the ecological stamp. Thus, this methodology is expected to be a useful tool regarding pesticide control in commercial wines under conventional and ecological production.

- FPSE was developed and applied for the first time to wine samples combined with UPLC-ESI-MS/MS for the determination of fungicides (19) and insecticides (3) with the minimum use of organic solvents. Parameters related to the extraction yield were optimized, achieving LOQs below 1 ng/mL, even considering extraction times lower than those required to achieve equilibrium conditions. However, the technique showed a very low extraction efficiency for most polar compounds integrated in SPE-based methods. Commercial samples analyzed showed the presence of pesticide residues in the range from 0.2 ng/mL to 130 ng/mL.

- The simultaneous determination of PYRI, CYP and their 4-hydroxyanilino derivatives was carried out in wine samples for the first time. A large number of commercial wines were analyzed and the ratios between the derivative forms and the parent fungicides varied for white and red wines, being higher in the latter case. Furthermore, the coexistence of derivatives with the hydroxyl group in different positions of the pyrimidine ring was proved in wines and tested in grapes previously sprayed with CYP formulations. Glycosylated metabolites were also detected in wine samples. Further research is required regarding those derivatives and their potential toxicity compared to the parent fungicides.

- The automated monitoring of 48 pesticides has been optimized employing on-line SPE and LC-MS/MS. A really sensitive and selective methodology was established,

with the advantages of a reduction in sample manipulation, analysis cost and generation of solid wastes. LOQs were situated below 1 ng/mL using just 3 µL of sample volume and values almost ten times lower can be reached for ecological samples if 25 µL are used. Commercial wines analyzed showed the presence of 25% of compounds above 10 ng/mL in, at least, one sample. Furthermore, only one ecological sample was totally free of pesticide residues and values between 0.1 ng/mL and 6 ng/mL were quantified on the rest of samples with the ecological label.

Considering results obtained with the different methodologies applied to the determination of pesticide residues in wine samples, SPE seems to be the most robust technique to extract and/or concentrate pesticide residues from this matrix. Major advantages are the repeatability of the process, combined with shorter extraction times than those required for the tested microextraction technique. In case of receiving a large number of samples, the online combination between SPE and LC-MS/MS is probably the most appealing approach in terms of application cost, low residues generation and minimum sample pre-treatment, just filtration. Its major limitation is the longer LC separation time compared to methods developed under UPLC conditions.

As a general statement, wine samples do not contain pesticide residues above the recommended threshold (10% of MRLs set for vinification grapes). However, residues of some compounds reached quite often values in the range of 100 ng/mL. In “my opinion” these active ingredients are candidates to be replaced by other species, offering similar plant protection efficiencies, but lower PFs during the vinification process. Results obtained in case of CYP and PYRI suggest that other pesticides could be partially metabolized by vine plants and/or transformed during the vinification process. So, further research is required to identify these transformation products and to fully assess the residues of PPPs remaining in wine.

Chapter 2. Development of methodologies for the determination of polar pesticides in wine samples.

- Two different approaches were applied to the determination of anionic pesticides in wine samples.

- A combination of a molecularly imprinted polymer (MIP) followed by FMOC derivatization permitted the effective extraction and sensitive determination of GLY and AMPA in wine samples by UPLC-MS/MS. LOQs were situated in 0.5 ng/mL for GLY and 1 ng/mL for AMPA.

- The direct determination of GLY, AMPA and Fosetyl in wine was attained using a strong anionic exchange column in combination with a QqQ instrument. LOQs achieved were 0.2 ng/mL (Fosetyl), 1 ng/mL (GLY) and 8.3 ng/mL (AMPA). Even though AMPA showed a higher LOQ with this approach, this value is suitable to control its presence in wines in a safe level. So, this direct methodology can be used for the rapid control of GLY residues in wine. In further studies, its suitability for the simultaneous determination of Fosetyl and its major degradation product, phosphonic acid, needs to be assessed.

- Commercial wine samples analyzed showed the presence of GLY and Fosetyl in around 70% of samples, yet AMPA residues were absent (except one sample below its LOQ). Fosetyl was quantified in the range from 0.5 ng/mL to 63.8 ng/mL, whilst GLY concentrations stayed between 1.4 ng/mL and 31.4 ng/mL. From a scientific point of view, it arises relevant identifying GLY levels existing in some wines correspond to direct uptake of this species from vineyard soils, or it is the result of drifts from aerosols during application of the herbicide in the surrounding of vineyards.

Chapter 3. Development of extraction methods for the determination of pesticides in soil matrices.

- A multiresidue methodology was developed for the determination of pesticides in vineyard soils. PLE extraction followed by UPLC-ESI-MS/MS achieved the accurate determination of 44 compounds.

- The validated method was applied to soil samples collected in Galicia (Spain) at the beginning of spring. Results noticed the presence of twelve fungicides and one insecticide (IMI) in concentration values above 10 ng/g. The highest concentrations were found for DIM, CAR, MYC, PYRA and FLUO.

- Dissipation rates from the end of autumn to spring were situated below 50% for half of the analytes, which means that those compounds have a potential to be accumulated if sprayed in consecutive wine campaigns. In case of DIM, it could be verified that soils were enriched in one of its E/Z forms.

- Time-course evolution of pesticide residues have highlighted the pollution levels of vineyard soils in the region of interest, with compounds remaining in soil above 100 ng/g in consecutive years. These levels might suppose a hazard for the biodiversity of these soils, compromising their medium-term productivity. Also, compounds found in the surface of vineyards present a risk to contaminate surface water reservoirs due to soil erosion by wind and/or during intense raining events. Migration to groundwater and accumulation in micro-invertebrates are also potential pathways of pesticides found in surface vineyard soils depending on their groundwater ubiquity score (GUS) and bioaccumulation potential, respectively.

Chapter 4. Assessment of SFC as an alternative to determine insecticides and chiral fungicides in viticulture-related samples.

- A combination of SPE and SFC-ESI-MS/MS was developed and optimized for the determination of seven neonicotinoid insecticides in wine. LOQs were situated in the range from 1 ng/mL and 11 ng/mL. SFC data pointed out to a better separation and peak shape, a lower signal suppression and, thus, a more convenient technique for neonicotinoids in wine samples compared to RPLC. Although the importance of this group of insecticides has been reduced significantly after limiting their open uses, they are expected to be present in the environment, particularly the aquatic environment, for years, considering their stability in agriculture soils (demonstrated for IMI in vineyard soils) and their further leaching/run-off to water streams. Likely, alternative fenozide-

type insecticides are also amenable to SFC separation given their moderate polarity, and high GUS index (case of methoxyfenozide).

- Twenty-five white commercial wines produced in Galicia were analyzed using the optimized methodology for neonicotinoids. ACE was detected in two samples below its LOQ. Conversely, IMI was quantified in around half of the samples processed in the range from 1.3 ng/mL to 33 ng/mL.

- Five chiral fungicides (acylalanine and azolic families) were successfully detected in wine and soil samples using two chromatographic methods. LOQs were situated in the range from 0.5 ng/mL to 2.5 ng/mL for wine, and 1.3 ng/g to 6.5 ng/g for soil.

- Seventeen commercial wines were analyzed and showed the presence of TEBU and MYC, applied as racemates and no enantioselective degradation. MET was detected in all samples with variations in the enantiomeric fraction (EF from 0.05 to 0.57), which implies the use of a racemate mixture or the application of the active form in some cases. Further information about application treatments is required to draw more conclusions about the degradation rate of the enantiomers of MET in wine. Taking into account that MET is one of the fungicides most often employed for the treatment of mildew infections in vineyards, and that the compound has a high PF, replacement of PPP commercial formulations based on the racemate by those containing only MET-M would serve to reduce the residues of this compound in commercial wines. Preliminary conclusions derived from its EF in real samples pointed out to the fact that MET-M (the active enantiomer) degrades faster in soil and wine samples.

- Seven soil samples were processed, and analytes were detected at least in one sample. No variation was found for MYC, TEBU and BEN in terms of enantiomeric fraction. Conversely, MET showed differences in dissipation rates for its isomers as function of the soil and sampling point, leading to an enantioselective degradation.

INDEX OF FIGURES

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LIST OF PUBLICATIONS AND JOURNAL PERMISSIONS

❖ PUBLICATION I:

Gabriela Castro, Leticia Pérez-Mayán, Tamara Rodríguez-Cabo, María Ramil, Isaac Rodríguez, Rafael Cela.

Multianalyte, high-throughput liquid chromatography tandem mass spectrometry method for the sensitive determination of fungicides and insecticides in wine.

Analytical and Bioanalytical Chemistry 410**,** 1139–1150 (2018).
DOI: 10.1007/s00216-017-0724-9

Affiliation: Departamento de Química Analítica, Nutrición y Bromatología, Instituto de Investigación y Análisis Alimentario (IIAA), Universidad de Santiago de Compostela, Avenida Ciencias sn, 15782 Santiago de Compostela, Spain

Electronic ISSN: 1618-2650 (Springer)

Impact factor: 3.286 (2018)
Q1 - Analytical chemistry (JCI)

Contribution: investigation, methodology, review & editing.

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Multianalyte, high-throughput liquid chromatography tandem mass spectrometry method for the sensitive determination of fungicides and insecticides in wine

Author: Gabriela Castro et al
Publication: Analytical and Bioanalytical Chemistry
Publisher: Springer Nature
Date: Nov 16, 2017

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❖ PUBLICATION II:

Leticia Pérez-Mayán[1], Isaac Rodríguez[1], Maria Ramil[1], Abuzar Kabir[2], Kenneth G. Furton[2], Rafael Cela[1]

Fabric phase sorptive extraction followed by ultra-performance liquid chromatography-tandem mass spectrometry for the determination of fungicides and insecticides in wine. Journal of Chromatography A 1584, 13-23 (2019).

DOI: 10.1016/j.chroma.2018.11.025

Affiliation: [1]Department of Analytical Chemistry, Nutrition and Food Sciences, IIAA-Institute for Food Analysis and Research, Universidade de Santiago de Compostela,R/Constantino Candeira SN, 15782, Santiago de Compostela, Spain. [2]International Forensic Research Institute, Department of Chemistry and Biochemistry, Florida International University, 11200 SW 8thStreet, Miami, FL,33199, USA.

ISSN: 0021-9673 (Elsevier)

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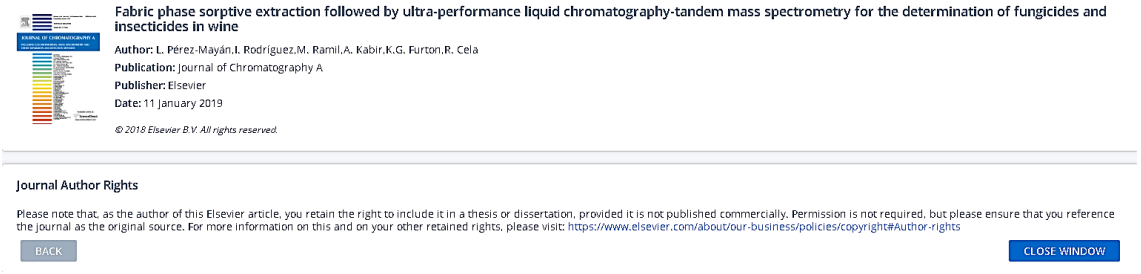

❖ PUBLICATION III:

Gabriela Castro, Leticia Pérez-Mayán, Inma Carpinteiro, María Ramil, Rafael Cela, Isaac Rodríguez.

Residues of anilinopyrimidine fungicides and suspected metabolites in wine samples. Journal of Chromatography A, 1622, 461104 (2020).

DOI: 10.1016/j.chroma.2020.461104

Affiliation: Department of Analytical Chemistry, Nutrition and Food Sciences. Institute of Research on Chemical and Biological Analysis (IAQBUS). Universidade de Santiago de Compostela, Santiago de Compostela 15782, Spain.

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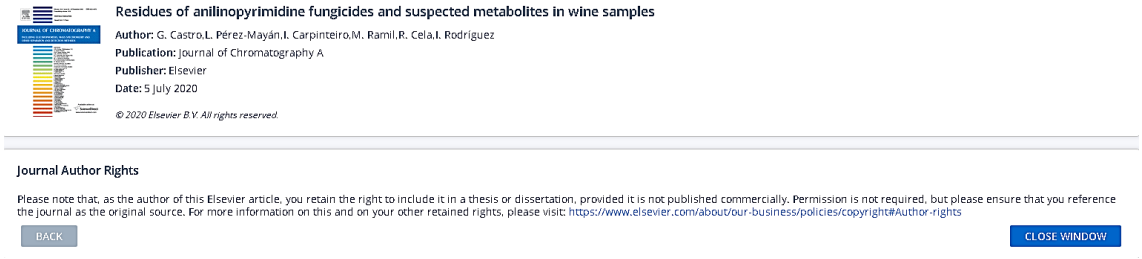

❖ PUBLICATION IV:

Leticia Pérez-Mayán, María Ramil, Rafael Cela, Isaac Rodríguez.
Determination of pesticide residues in wine by solid-phase extraction on-line combined with liquid chromatography tandem mass spectrometry.
Journal of Food Composition and Analysis, 104, 104184 (2021).
DOI: 10.1016/j.jfca.2021.104184.
Affiliation: Department of Analytical Chemistry, Nutrition and Food Sciences. Institute of Research on Chemical and Biological Analysis (IAQBUS). Universidade de Santiago de Compostela, Santiago de Compostela 15782, Spain.
ISSN: 0889-1575 (Elsevier)

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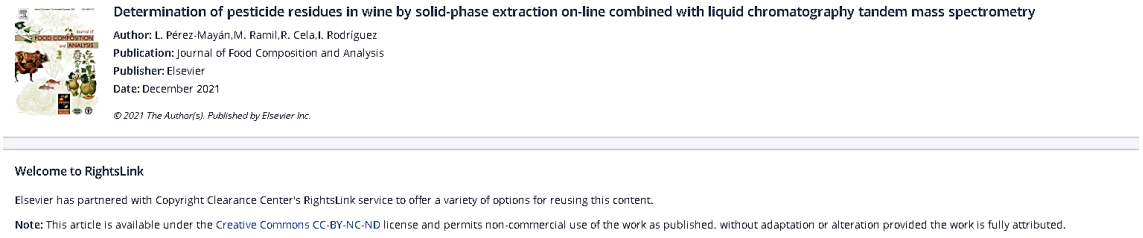

❖ PUBLICATION V:

Leticia Pérez-Mayán, Gabriela Castro, María Ramil, Rafael Cela, Isaac Rodríguez.
Approaches to liquid chromatography tandem mass spectrometry assessment of glyphosate residues in wine.
Analytical and bioanalytical chemistry, 414, 1445-1455 (2022)
DOI: 10.1007/s00216-021-03775-w
Affiliation: Department of Analytical Chemistry, Nutrition and Food Sciences. Institute of Research on Chemical and Biological Analysis (IAQBUS). Universidade de Santiago de Compostela, Santiago de Compostela 15782, Spain.
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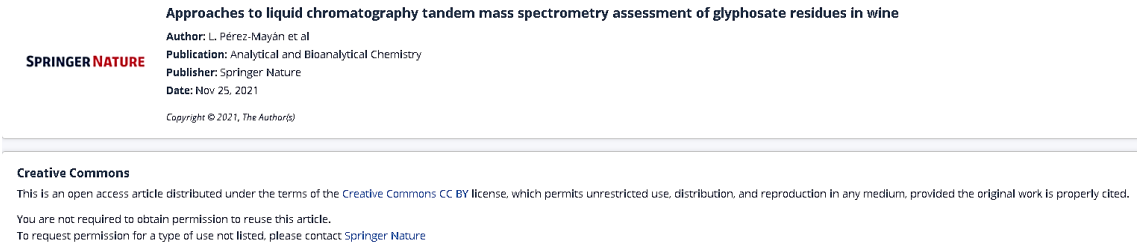

❖ PUBLICATION VI:

Leticia Pérez-Mayán, María Ramil, Rafael Cela, Isaac Rodríguez.
Multiresidue procedure to assess the occurrence and dissipation of fungicides and insecticides in vineyard soils from Northwest Spain.
Chemosphere, 261, 127696 (2020).
DOI: 10.1016/j.chemosphere.2020.127696
Affiliation: Department of Analytical Chemistry, Nutrition and Food Sciences. Institute of Research on Chemical and Biological Analysis (IAQBUS). Universidade de Santiago de Compostela, Santiago de Compostela 15782, Spain.
ISNN: 0045-6535 (Elsevier)

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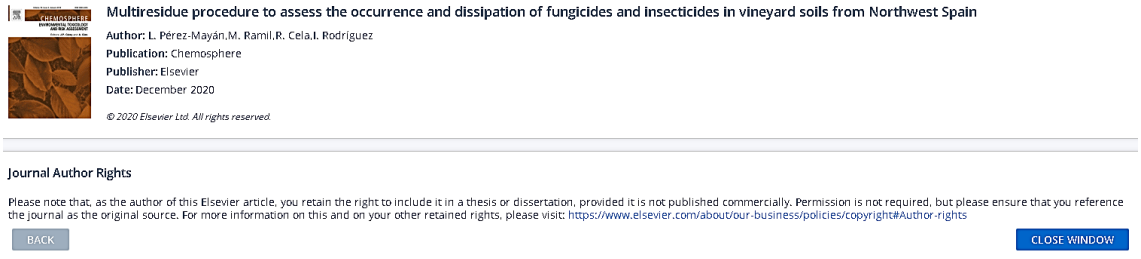

❖ PUBLICATION VII:

Leticia Pérez-Mayán, Miguel Cobo-Golpe, María Ramil, Rafael Cela, Isaac Rodríguez.
Evaluation of supercritical fluid chromatography accurate mass spectrometry for neonicotinoid compounds determination in wine samples.
Journal of Chromatography A, 1620, 460963 (2020).
DOI: 10.1016/j.chroma.2020.460963
Affiliation: Department of Analytical Chemistry, Nutrition and Food Sciences. Institute of Research on Chemical and Biological Analysis (IAQBUS). Universidade de Santiago de Compostela, Santiago de Compostela 15782, Spain
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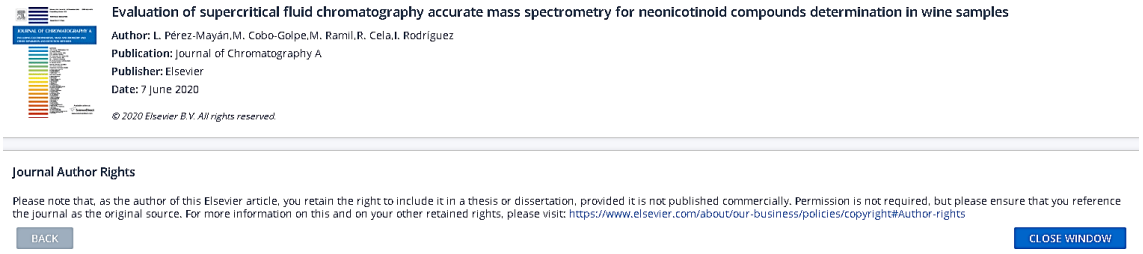

Evaluation of supercritical fluid chromatography accurate mass spectrometry for neonicotinoid compounds determination in wine samples
Author: L. Pérez-Mayán,M. Cobo-Golpe,M. Ramil,R. Cela,I. Rodríguez
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❖ PUBLICATION VIII:

Leticia Pérez-Mayán, María Ramil, Rafael Cela, Isaac Rodríguez.
Supercritical fluid chromatography-mass spectrometric determination of chiral fungicides in viticulture-related samples.
Journal of Chromatography A, 1644, 462124 (2021).
DOI: 10.1016/j.chroma.2021.462124
Affiliation: Department of Analytical Chemistry, Nutrition and Food Sciences. Institute of Research on Chemical and Biological Analysis (IAQBUS). Universidade de Santiago de Compostela, Santiago de Compostela 15782, Spain.
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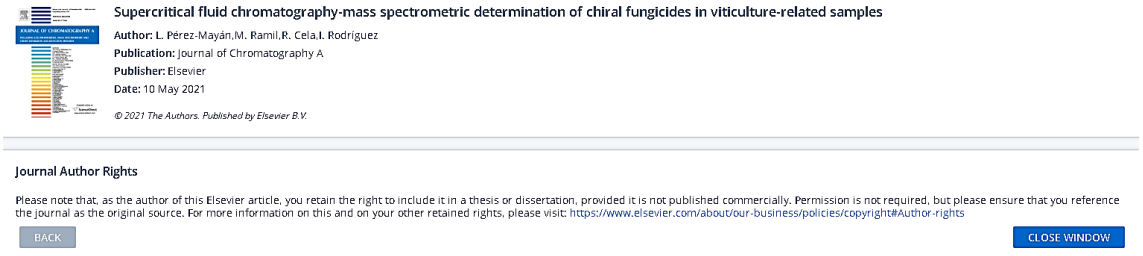

Supercritical fluid chromatography-mass spectrometric determination of chiral fungicides in viticulture-related samples
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Plant protection products used to ensure the product quality and agricultural production can remain on crops as incurred residues, be transferred to environmental compartments, or even reach elaborated foodstuffs. This Thesis is focused on the optimization and validation of analytical methodologies for the determination of a relevant number of pesticides in vineyard soil and wines, as so as transformation products. Application of developed methods has contributed to advance in the understanding of their processing factors, degradation rates, potential pollution of the soil, and the identification of new transformation products. In this vein, eight publications were divided into four chapters regarding the matrix analyzed, the family of the compounds studied, or the analytical technique employed.