DEPARTAMENTO DE MEDICINA PREVENTIVA

DETERMINACIÓN DE HERBICIDAS DERIVADAS DE LA UREA EN AGUAS Y VEGETALES

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UNIVERSITAT DE VALÈNCIA

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Determinación de herbicidas derivados de la urea en aguas y vegetales

TESIS DOCTORAL

Presentada por:

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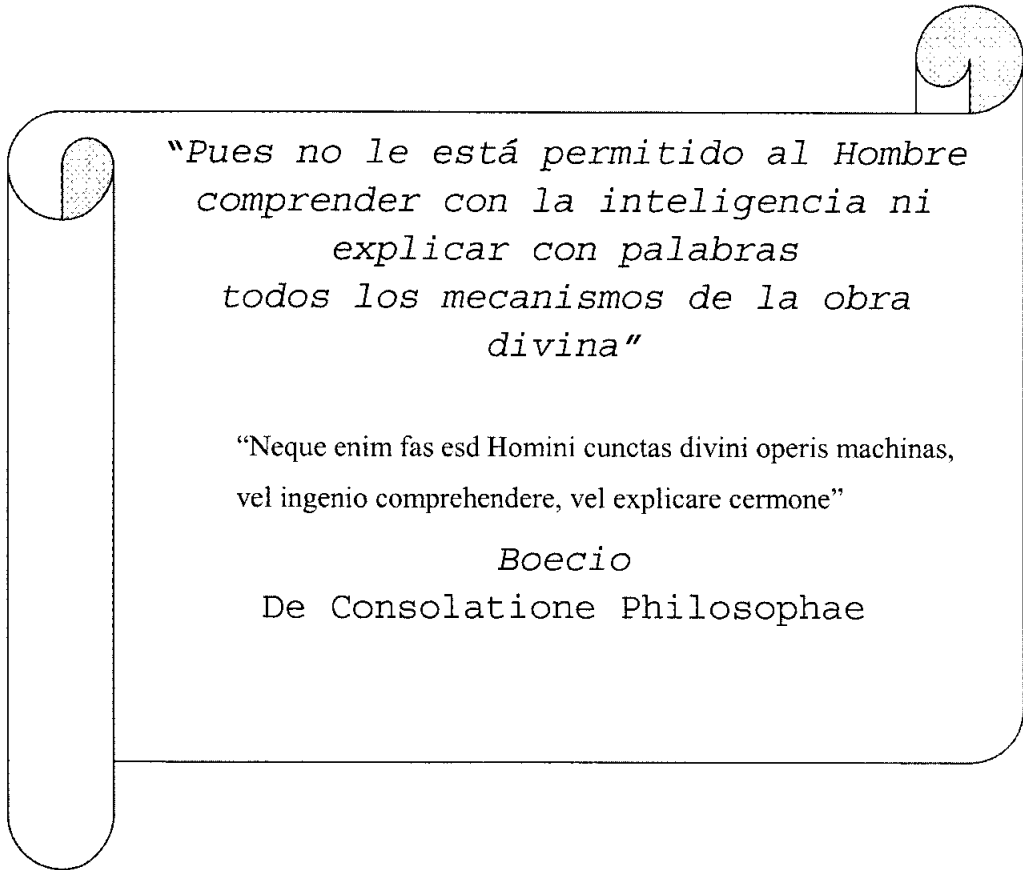
La doctoranda figura en todas las publicaciones como primer autor.

Por lo que autorizan su presentación para optar al grado de Doctor.

Burjassot, 22 de octubre de 2001

Guillermina Font Pérez   Juan Carlos Moltó Cortés

"*Pues no le está permitido al Hombre
comprender con la inteligencia ni
explicar con palabras
todos los mecanismos de la obra
divina*"

"Neque enim fas esd Homini cunctas divini operis machinas,

vel ingenio comprehendere, vel explicare cermone"

Boecio
De Consolatione Philosophae

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ABREVIATURAS

ADI: Admissible Dairy Intake

APCI: Atmospheric Pressure Chemical Ionization

API: Atmospheric Pressure Ionization

BUI: Benzoylurea Insecticide

CCPR: Comité del Codex sobre Residuos de Plaguicidas

CG: Cromatografía de Gases

CL: Cromatografía Líquida

CL_{50}: Concentración Letal 50

CZE: Capillary Zone Electrophoresis

DCE: Detector de Captura de Electrones

DEM: Detector de Espectrometría de Masas

DL_{50}: Dosis Letal 50

DMFS: Dispersión de Matriz en Fase Sólida

DNF: Detector de Nitrógeno-Fósforo

EC: Electroforesis Capilar

ECD: Electron Capture Detector

EFS: Extracción en Fase Sólida

EKC: Electrokinetic Chromatography

ELL: Extracción Líquido-Líquido

EM: Espectrometría de Masas

EPA: Environmental Protection Agency

ES: Electrospray

ESI: Electrospray Ionization

FAO: Food and Agriculture Organization

IARC: Internacional Agency for Research on Cancer

IDA: Ingestion Diaria Admisible

IDL: Instrument Detection Limit

LMR: Límite Máximo de Residuos

LOQ: Limit of Quantification

MAC: Maximum Admisible Concentration

MCPA: Methyl Chloro Phenoxyacetic Acid

MDL: Method Detection Limit

MEFS: Microextracción en Fase Sólida

MEKC: Micellar Electrokinetic Chromatography

MRL: Maximum Residue Level

MSD: Mass Spectrometry Detector

NPD: Nitrogen-phosphorus Detector

NSEO: Nivel Sin Efecto Observable

OM: Organic Matter

OMS: Organización Mundial de la Salud

PA: Poliacrilato

PDMS: Polidimetilsiloxano

PUH: Phenylurea Herbicide

RSD: Relative Standard Deviation

SDS: Sodium Dodecyl Sulfate

SFC: Supercritical Fluid Chromatography

SIM: Selected Ion Monitoring

SIR: Selected Ion Registering

SPE: Solid Phase Extraction

SPME: Solid-Phase Microextraction

SUH: Sulfonylurea Herbicide

INTRODUCCIÓN

I.1. CONSIDERACIONES HISTORICAS

A lo largo de la historia, el hombre ha estado preocupado constantemente por los daños que pueden sufrir los cultivos y ha utilizado diferentes agentes con objeto de protegerlos de las plagas y enfermedades. Hasta 1940 se emplearon sustancias inorgánicas u orgánicas naturales de baja eficacia y poco impacto ambiental. La madera se cubría con cloruro de mercurio, las caries del trigo se trataban con cloruro de sodio y se aprovechaban las propiedades fungicidas del azufre y del cobre, insecticidas del tabaco y del arsénico y la actividad herbicida del ácido sulfúrico y del sulfato de hierro.

A partir de 1940 se usaron sustancias artificiales para la protección de las plantas iniciándose la era de "los plaguicidas orgánicos de síntesis" que continua hasta nuestros días y que engloba a un conjunto de sustancias muy heterogéneo que se aplica en grandes cantidades y extensiones de cultivos.

En la actualidad existen unas 100.000 formulaciones distintas de plaguicidas constituidas fundamentalmente por unos 1500 principios activos diferentes, entre los que se encuentran compuestos de elevada actividad cuyas propiedades resultarían impensables en los años 40, como los herbicidas altamente selectivos, insecticidas sistémicos, etc.

I.2. DEFINICIÓN DE PLAGUICIDA

En la "Reglamentación Técnica Sanitaria para la fabricación, comercialización y utilización de plaguicidas" se define plaguicida como [1]:

"Las sustancias o ingredientes activos, así como las formulaciones o preparados que contengan uno o varios de ellos, destinados a cualquiera de los fines siguientes:

- Combatir los agentes nocivos para los vegetales o prevenir sus acciones.

- Favorecer o regular la producción vegetal, con excepción de los nutrientes y los destinados a la enmienda de los suelos.

- Conservar los productos vegetales, incluida la protección de las maderas.

- Destruir los agentes indeseables.

- Destruir parte de los vegetales o prevenir un crecimiento indeseable de los mismos.

- Hacer inofensivos, destruir o prevenir la acción de otros organismos nocivos o indeseables distintos de los que atacan los vegetales."

Asimismo se define residuos de plaguicidas como "los restos de ellos y los eventuales productos tóxicos de su metabolización o degradación que se presentan en o sobre los alimentos destinados al hombre o al ganado".

La Organización Mundial de la Salud (OMS), define plaguicida como "cualquier sustancia, o mezcla de sustancias destinadas a prevenir o controlar toda especie indeseable de plantas y animales que se utilice como defoliante, desecante o reguladora del crecimiento vegetal" [2]. La OMS aclara que el término plaguicida abarca, además cualquier sustancia que se emplee para combatir plagas durante la producción, almacenamiento, transporte, comercialización o elaboración de alimentos para el hombre y animales, o que se administre a estos últimos para combatir insectos o arácnidos que se encuentren dentro o sobre sus cuerpos. El término no se aplica a los antibióticos u otros productos químicos administrados a los animales con otros fines, como el de estimular su crecimiento o modificar el comportamiento reproductivo, ni a los fertilizantes.

La OMS entiende igualmente por residuo de plaguicida cualquier sustancia especificada presente en alimentos, productos agrícolas o alimentos para animales como consecuencia del uso de un plaguicida. El término incluye cualquier derivado de un plaguicida, como productos de degradación, metabolitos y productos de reacción y las impurezas consideradas de importancia toxicológica.

Los términos plaguicidas, pesticidas, productos fitosanitarios, parasiticidas, productos agroquímicos y productos fitofarmacéuticos se suelen considerar sinónimos.

El término pesticida deriva de la palabra inglesa "pest" que se asigna a todo animal o planta (virus, bacterias, hongos, hierbas, gusanos, moluscos, insectos, roedores, pájaros o mamíferos) susceptible de modificar al medio ambiente del hombre. Etimológicamente, pesticida es cualquier producto químico destinado a luchar contra

parásitos, animales o vegetales, que amenacen los cultivos agrícolas, la ganadería o la salud humana, se excluyen los medicamentos y productos farmacéuticos.

La determinación "plaguicida" es la más correcta ya que es el término más general y estrictamente engloba al de producto fitosanitario que es un tipo particular de plaguicida, ya que también existen plaguicidas de uso ganadero, ambiental, para la industria alimentaría e higiene personal.

I.3. DATOS SOBRE EL CONSUMO DE PRODUCTOS FITOSANITARIOS

I.3.1. Cifras mundiales

En la década de los noventa, el mercado mundial [3] de fitosanitarios (excluyendo las ventas de productos de biotecnología agrícola) aumentó de 18.306 millones de Euros en 1990 a un valor de 26.405 millones en 1999.

I.3.2. Cifras europeas

Como se observa en la figura 1, en los países de Europa occidental, el mercado global

Figura 1. Evolución de las ventas anuales de plaguicidas en Europa
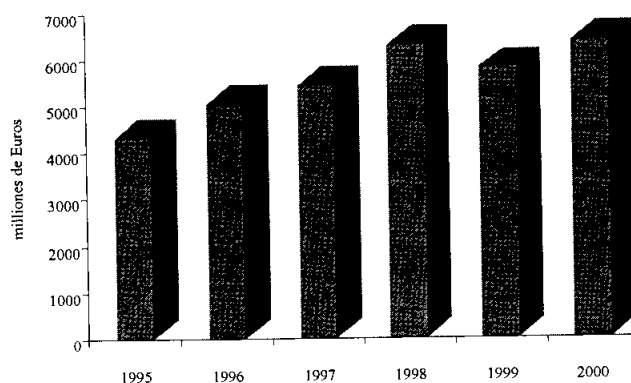
de productos fitosanitarios registró un descenso marginal del 0'2% en 1999, alcanzando 6.175 millones de Euros [4]. Después de la eliminación de los factores referentes al cambio y la inflación, el descenso resultante equivale a una reducción del consumo del 2'1% en el mercado de Europa Occidental. Este ligero descenso se debe a que los estados europeos han incluido la adopción de estrategias de reducción de costes de los agricultores, en respuesta a las decrecientes rentabilidades de las explotaciones y a situaciones climatológicas particulares.

II.3.3. El mercado español

La evolución de las ventas de plaguicidas en España se refleja en la figura 2. En años anteriores (quinquenio 1989-1993) se produce una disminución del 35% en la cantidad fabricada de plaguicidas, este descenso se debe a la creciente presencia en el mercado de productos más concentrados, con una dosis de aplicación en constante reducción, logrando con poca cantidad de producto conciliar la eficacia en la protección vegetal con la reducción de daños al medio ambiente [5].

Figura 2. Evolución de las ventas anuales de plaguicidas en
España
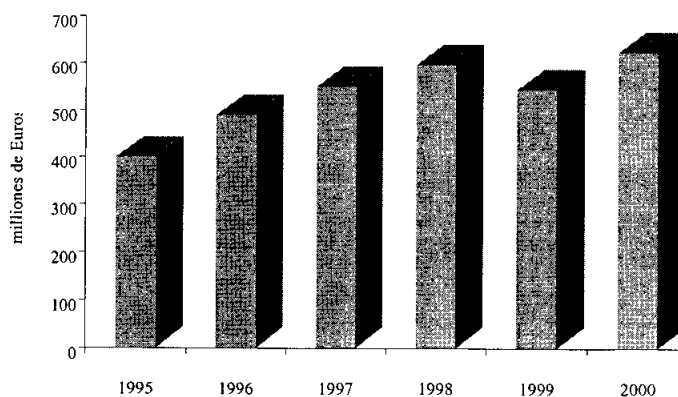
Las ventas de fitosanitarios en España en los últimos años han experimentado un crecimiento estable a excepción de 1999, año en el que todas las familias de productos sufrieron un descenso global del 3'46 % debido a unas condiciones agroclimáticas atípicas, con una primavera particularmente seca, por lo que las ventas de fungicidas y herbicidas sufrieron las más importantes caídas, -8'12% y -4% respectivamente.

España es uno de los principales países consumidores de plaguicidas, con una ventas totales el año 2000 de 626 millones de Euros, mientras que el mercado europeo facturó 6393 millones de euros, mostrando claramente la importancia de su agricultura. En el año 2000 se vuelve a una tendencia estable con un crecimiento del 1'11% en las ventas de 2000 con respecto a 1999. Este cambio se debe sin duda a la mejora de las condiciones climatológicas del año 2000, año con una cierta mejora pluviométrica en toda la geografía española.

Dentro del Estado Español (figura 3), Andalucía es la Comunidad Autónoma que mayor cantidad de plaguicidas consume, con un 32'25% del mercado nacional seguida de la Comunidad Valenciana con un 15'49% y la Comunidad de Murcia sigue en el tercer lugar con un 9'55% del mercado al igual que el año pasado seguida en cuarto lugar por Cataluña con el 9'36% del mercado [3,6].

Figura 3. Consumo de plaguicidas por Comunidades
Autonomas en el año 2000
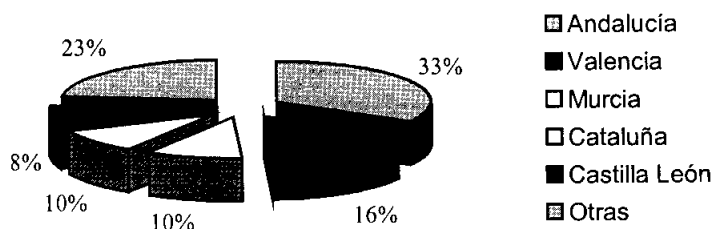
I.4. NECESIDAD DEL EMPLEO DE PLAGUICIDAS

El empleo de plaguicidas es hoy por hoy una constante en la producción industrial agroalimentaria que genera importantes beneficios económicos. Sin embargo existe una creciente oposición a su uso indiscriminado debido al impacto medioambiental y

sobre la salud del consumidor. La utilización de plaguicidas presenta tanto ventajas como inconvenientes, entre los beneficios que aportan se pueden citar [7-10]:

- Aumento del rendimiento de las producciones agrícolas, al disminuir las pérdidas por plagas y proteger los cultivos durante su transporte y almacenamiento. Actualmente son imprescindibles para mantener la producción mundial, un aumento de la superficie de cultivo no repercute proporcionalmente en un aumento de producción, ya que a más área de cultivo, más perdidas por plagas.

- Razones comerciales y económicas ya que mejoran el aspecto del producto, ahorran mano de obra y mantienen los precios más estables.

- Razones sanitarias para el hombre, los animales domésticos y la ganadería, eliminan tanto a los parásitos como a las enfermedades transmisibles por vectores así que no se debe olvidar la utilidad de los pesticidas en la mejora de la situación sanitaria de las poblaciones, como la lucha contra la malaria, el paludismo y el tifus.

Pero como no puede ser de otra manera, su aplicación también presenta los inconvenientes siguientes:

- Se trata de sustancias artificiales con una toxicidad inherente a veces elevada, resultando especialmente peligrosos para los formuladores, manipuladores y aplicadores.

- Sus residuos permanecen en los productos alimentarios consumidos por toda la población.

- Algunas especies indeseables han desarrollado resistencias.

- Los plaguicidas poco selectivos causan la muerte de especies inocuas e incluso de beneficiosas (abejas, lombrices).

- Causan desequilibrios biológicos y alteran la sucesión ecológica.

- Los compuestos persistentes presentan el fenómeno de bioacumulación, bioconcentración y/o biomagnificación.

Con este difícil balance, El País Semanal del 22-04-01 publicó un articulo titulado "¿Qué estamos comiendo?" donde se afirmaba que las frutas y verduras no tratadas con productos de síntesis "son más sanas". Esto sirve para evocar el tema de la valoración de los alimentos llamados "naturales", "biológicos" o "ecológicos" sobre los tratados.

Actualmente no se puede deslindar la producción de alimentos de la industria fitosanitaria, excepto en pequeñas empresas artesanales, incapaces de dar abasto para todas las necesidades de la población. Por otro lado aparecen en el mercado, otros productos modificados genéticamente que pueden no requerir plaguicidas, pero que provocan una gran desconfianza por parte de los consumidores.

No hay que olvidar que el 98% de las intoxicaciones alimentarias se deben a bacterias, virus, parásitos y toxinas eliminadas por los tratamientos y que confundir lo artesano con lo saludable puede conllevar un riesgo muy grande para la salud de la población. "Un alimento de calidad" es aquel que presenta las condiciones de conservación e higiene necesarias para una nutrición sana y segura a un precio asequible [4].

La seguridad alimentaría, en lo que se refiere al control de los residuos de productos fitosanitarios en los productos agrarios, está y debe estar perfectamente garantizada y los organismos internacionales velan por su cumplimiento.

I.5. CLASIFICACIÓN DE PLAGUICIDAS

Los plaguicidas constituyen un grupo de compuestos muy amplio y heterogéneo, por lo que existen diversas posibilidades de clasificación: según el tipo de organismo a controlar, un criterio físico-químico, su toxicidad o según sus aplicaciones, propósito del tratamiento, forma de acción, etc... [7-8]

Debido a la gran variedad de plaguicidas que existen en la actualidad, ninguna clasificación resulta completa. Una de las clasificaciones más practicas es la basada en el organismo que se intenta controlar, siguiendo esta clasificación, las categorías más frecuentes en España son:

- Acaricidas: combaten las distintas variedades de ácaros.

- Atrayentes: actúan como reclamo sobre cebos envenenados y se emplean contra insectos y roedores.

- Desinfectantes del suelo: se destinan a la desinfección de los terrenos de cultivo. Su acción general se dirige contra gusanos, insectos y hongos, que se encuentran en superficie y en profundidad.

- Esterilizantes: inhiben la fertilidad de los huevos de insectos.

- Fungicidas: con acción contra los hongos, también se conocen como anticriptogámicos.

- Herbicidas: destruyen las malas hierbas, impidiendo el crecimiento y respetando el cultivo.

- Insecticidas: destinados a combatir los insectos.

- Molusquicidas: exterminan los caracoles, limacos y babosas.

- Nematicidas: se emplean para eliminar toda clase de gusanos del suelo.

- Repelentes: ahuyentan a los insectos nocivos.

- Rodenticidas: se usan para eliminar los roedores.

En la Unión Europea, los herbicidas son el grupo de plaguicidas más consumidos [5] (figura 4).

En España, a diferencia del mercado europeo y por claros motivos climáticos, la familia de productos de mayor consumo es la de insecticidas que incluye también acaricidas y nematicidas cuyas ventas han superado los 254 millones de Euros.; seguida por herbicidas, fungicidas con ventas de 240 y 172 millones de Euros. respectivamente, otros productos que incluyen fitorreguladores, molusquicidas y otros han facturado 69 millones de Euros.

Figura 4. Mercado europeo por familias de plaguicidas
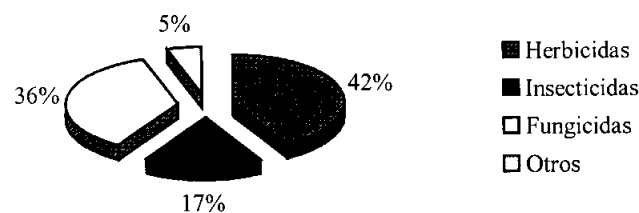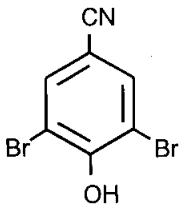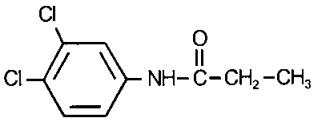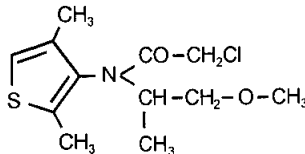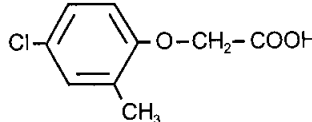
España por su situación climática-geográfica, es un país donde las plagas tienen una gran importancia con aparición de resistencias [5].

Figura 5. Mercado español por familias de plaguicidas
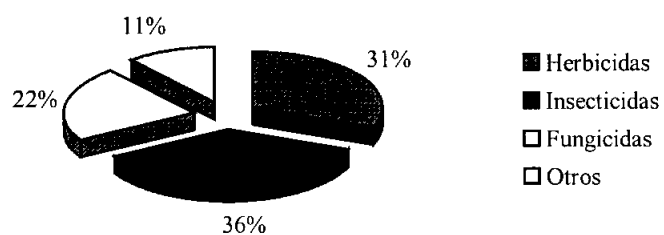
En la tabla 1, se recogen algunos ejemplos de los herbicidas más usados por su acción herbicida.

Tabla 1. Ejemplos de herbicidas y sus mecanismos de acción.

Grupo	Ejemplo de estructura	Acción herbicida
Acidos alifáticos clorados	CH_3-CCl_2-COOH **Dalapón**	Interfiere la respiración celular a nivel de la producción del ácido pantoténico y la actividad meristematica
Ácidos aromáticos halogenados	**Bromoxinil**	Impide la segunda reacción lumínica de la fotosíntesis y también desacopla la fosforilación oxidativa de la respiración
Anilidas sustituidas	**Propanil**	Se descompone a 3,4 dicloroacetanilida que impide la fotosíntesis
Cloroacetamidas sustituidas	**Dimetenamida**	Metila los grupos sulfhídrilos de ciertas enzimas reduciendo la división celular
Fenoxiacidos y derivados	**MCPA**	Interfiere la división y diferenciación celular así como la síntesis de proteínas

Tiadiazinas
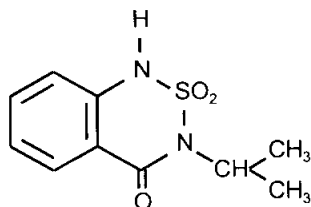
Bentazone

Impide la fotosíntesis a nivel de la reacción de Hill

Tiocarbamatos
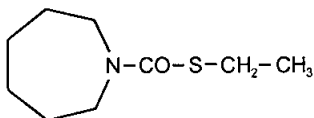
Molinato

Actúa sobre la síntesis de los lípidos de las plantas así como sobre la fotosíntesis y la respiración

Triazinas
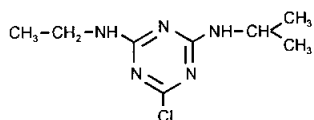
Atrazina

Impide la fotosíntesis en la fase de absorción de CO_2 en la reacción de Hill

Ureas sustituidas
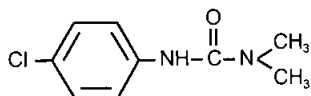
Monuron

Impide la función clorofílica

Otros compuestos
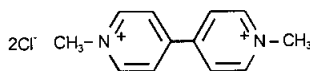
Paracuat

Actúa sobre el sistema fotosintético de la membrana denominado fotosistema 1, desintegrando las membranas celulares y tejidos

I.6. HERBICIDAS DERIVADOS DE LA UREA

I.6.1. Generalidades

Las ureas sustituidas son un importante grupo de fitosanitarios que responde a la estructura general [9-11].

$$R_1 \underset{R_2}{N} \overset{O}{\underset{}{\parallel}} \underset{R_4}{N} R_3$$
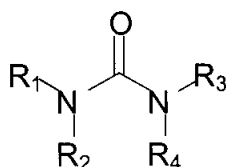
Según esta estructura, se puede distinguir:

Fenilureas que tienen en común un grupo fenil en R_2 y en general un hidrógeno en el sustituyente R_1. Entre las fenilureas se incluyen los N-fenil-N',N'-dialquilureas como diuron, clortoluron y fluometuron donde R_3 y R_4 son dos metilos. Otro grupo es el formado por N-fenil-N'-alquil-N'-metoxilurea como linuron, monolinuron y metobromuron con un metilo en el R_3 y un metoxilo en el R_4.

Algunas ureas como benzotiazuron y metabenzotiazuron ofrecen otro perfil estructural con un grupo heterocíclico en el sustituyente R_2 y un metilo en el R_1.
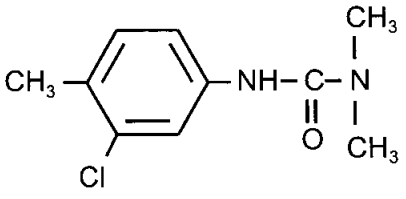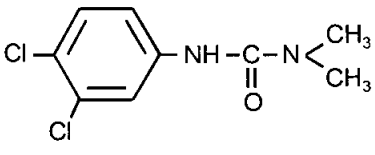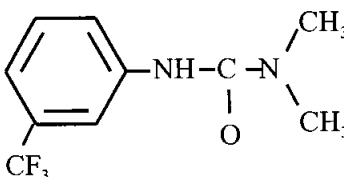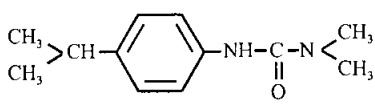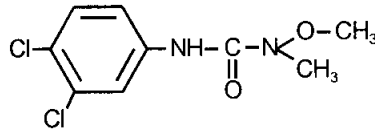
Sulfonilureas incorporan un grupo sulfonilico en R_1, que puede ser sulfonilfenil como en el caso del clorsulfuron. En la tabla 2 se recogen las estructuras de las distintas ureas seleccionadas en el presente trabajo.

En la búsqueda de nuevas estructuras derivadas de la urea con actividad plaguicida, se descubrió que algunas benzoilureas presentaban una importante actividad insecticida. Ejemplos ya comercializados de esta estructura son el diflubenzuron y el clorfluazuron.

I.6.2. Actividad herbicida

Las **fenilureas** dan lugar a una pérdida del vigor de la planta por inhibición de la fotosíntesis [12].

Tabla 2. Estructuras de las ureas herbicidas seleccionadas

Tipo	Ejemplo
Fenilurea	Clortoluron Diuron Fluometuron Isoproturon Linuron

$$Br-\text{C}_6\text{H}_4-NH-CO-\underset{\underset{CH_3}{|}}{N}-O-CH_3$$

Metobromuron

$$Cl-\text{C}_6\text{H}_4-NH-CO-\underset{\underset{CH_3}{|}}{N}-O-CH_3$$

Monolinuron

$$Cl-\text{C}_6\text{H}_4-NH-\underset{\underset{O}{\|}}{C}-N{\underset{CH_3}{\overset{CH_3}{<}}}$$
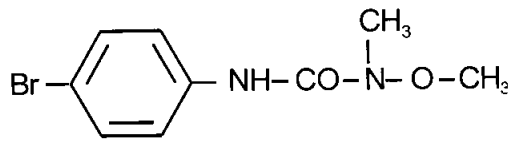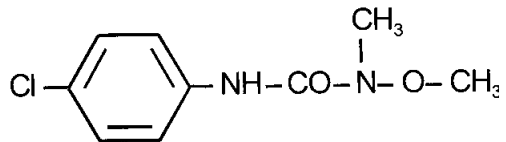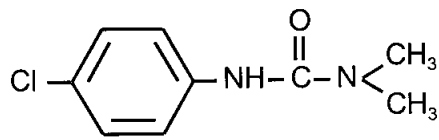
Monuron

Urea heterocíclica
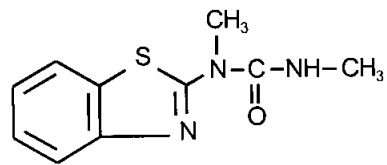
Metabenzotiazuron

Sulfonilurea
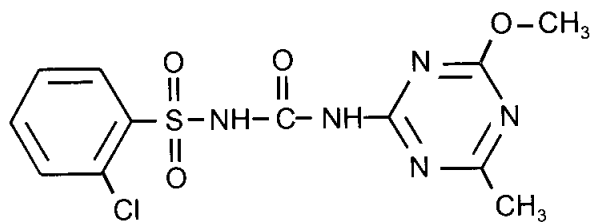
Clorsulfuron

El primer síntoma que aparece es la clorosis de las puntas de las hojas, comenzando por las más viejas, la cual se va extendiendo hasta la pérdida total del color verde y la muerte de las plantas.

Los herbicidas de este grupo apenas tienen efecto sobre las semillas en germinación, pero sí sobre las plantas que se desarrollan en terrenos tratados. Se absorben fácilmente por las raíces, y son transportados lentamente a las hojas, a través del xilema, acumulándose en los brotes.

La acción tóxica se debe a que inhiben la reacción de Hill, impidiendo la fotosíntesis. Además, algunas fenilureas sustituidas reducen la absorción de nitratos por la planta.

La actividad herbicida de las fenilureas aumenta notablemente con la presencia de átomos de halógeno en la molécula. La fitotoxicidad aumenta en la serie fenuron < monuron < diuron. En la serie de compuestos citada, la máxima fitotoxicidad se da cuando hay dos átomos de cloro unidos a un núcleo aromático y dos grupos metilo unidos al nitrógeno. Al remplazar un grupo metilo por un hidrógeno o un butilo se reduce la fitotoxicidad .

Las **ureas heterocíclicas** actúan tanto a través, de las hojas como de las raíces de las plantas. Se traslocan por toda la planta a través del xilema, impidiendo la función clorofílica al frenar la segunda reacción de la luz interfiriendo la reacción de Hill. Se supone que la urea heterocíclica reduce la liberación de los electrones en esta segunda reacción de la luz.

Más tarde, se comercializó otro importante grupo de ureas sustituidas, las **sulfonilureas**, estas inhiben la enzima que sintetiza la acetolactasa, enzima clave en la biosíntesis de aminoácidos ramificados. Se trata de potentes herbicidas sistémicos y selectivos, que se absorben por vía radicular y foliar.

Las sulfonilureas actúan inhibiendo la enzima acetolactasa sintasa que cataliza los primeros pasos de la biosíntesis de los aminoácidos esenciales valina, leucina e isoleucina provocando la detención de la mitosis. Por tanto impide la división celular y el crecimiento de las puntas de las raíces y brotes de las plantas sensibles. Se caracterizan por su alta actividad a bajas dosis y larga persistencia. A las dosis a las que impide el crecimiento, los principales procesos fisiológicos como fotosíntesis, respiración y síntesis de ADN, ARN y proteínas, y la distensión celular no se ven

afectados directamente al principio, pero pueden disminuir de modo gradual en los días siguientes. Las células animales no son sensibles a su acción. La inhibición es rápida, apareciendo a la 1-2 horas del tratamiento. Los síntomas visibles se desarrollan durante 1-3 semanas al menos en forma de clorosis y antocianosis en los tejidos más jóvenes seguidos de necrosis de los brotes apicales y otros tejidos. Es más eficaz sobre especies de hoja ancha que sobre gramíneas.

Aunque, son estables en el suelo, las ureas se descomponen por la actividad microbiana favorecida por la presencia de materia orgánica. Por ejemplo algunas pseudomonas son capaces de utilizar al monuron como fuente de carbono para su desarrollo.

I.6.3. Aplicaciones

La selectividad de los herbicidas derivados de la urea depende, fundamentalmente, de la tolerancia de ciertos cultivos y de la profundidad a que germinan las semillas o se desarrollan las raíces.

En la tabla 3 se exponen los principales cultivos en los que se utilizan los herbicidas seleccionados junto con las dosis de aplicación y los tipos de tratamiento [12].

Los cultivos cuyas semillas o raíces se desarrollan a mayor profundidad que las hierbas no se afectan por las aplicaciones superficiales. Por ello, el mayor empleo de **las fenilureas** se da en los cultivos de raíces profundas, perennes, en los que resultan muy fitotóxicas para otras plantas jóvenes que se deben desarrollar a partir de semillas que germinan en la zona donde se realiza el tratamiento con el herbicida.

Sin embargo, también se usan en cultivos anuales que sean tolerantes, como, por ejemplo, en plantaciones de cebolla.

En tratamiento de preemergencia se utilizan, sobre todo, en los cultivos de azúcar y cebollas y, por su gran actividad y su persistencia, se utilizan como desherbantes de suelos, técnica llamada en barbecho.

Las **sulfonilureas** son fitotóxicas para la mayoría de los cultivos de hoja ancha, especialmente crucíferas y remolacha azucarera

Tabla 3. Aplicaciones de los herbicidas seleccionados

Herbicida	Cultivos preferentes de aplicación	Dosis	Tipo de tratamiento
Clorsulfuron	Cebada, trigo	15-20 g/ha	Pre y post emergencia
Clortoluron	Trigo blando, semiduro y cebada	1'5-2'8 kg/ha	Pre y post emergencia temprana
Diuron	Alcachofa, algodón, cítricos, espárrago, frutales de pepita, olivo, parral de vid y vid	0'4-25 kg/ha	Pre y post emergencia
Fluometuron	Algodón, caña de azucar y trigo de invierno	1'25-2 kg/ha	Pre y post emergencia
Isoproturon	Cebada, trigo de invierno y de primavera	1'5-2 kg/ha	Pre y post emergencia
Linuron	Alcachofa, espárrago, girasol, haba, maíz, patata, zanahoria	0'5-3 kg/ha	Preemergencia o post emergencia temprana
Metabenzotiazuron	Ajos de diente, cebolla, guisante, haba y patata	2-3 kg/ha	Preemergencia y post emergencia temprana
Metobromuron	Judía, maíz , patata, tabaco	1-2'5 kg/ha	Preemergencia
monolinuron	Judía, maíz, patatá, cebolla, cítricos y frutales de pepita	0'5-2 kg/ha	Preemergencia y post emergencia temprana
Monuron	Control total de hierbas en zonas sin cultivo	4-40 kg/ha	Premergencia o post emergencia

El metabenzotiazuron se utiliza en el control de diversas monocotiledóneas y dicotiledóneas, sus mejores efectos, se obtienen cuando se aplica durante los primeros estadios de desarrollo de la planta y la temperatura ambiental es elevada.

I.6.4. Selectividad

La selectividad consiste en que en la planta resistente, el herbicida se descompone antes de que llegue al lugar en que va actuar, o bien que, por acumulación de otra sustancia en la planta se anule el efecto del herbicida o, incluso, que existan diferencias en el transporte. Por ejemplo el metabenzotiazuron, es selectivo en el trigo, porque en el se descompone rápidamente [12].

I.6.5. Toxicidad

I.6.5.1. Intoxicaciones agudas

Se producen cuando la sustancia se administra en una sola dosis. Los síntomas desencadenantes se observan en condiciones de laboratorio en animales de experimentación, en exposiciones incontroladas o en suicidios. En la tabla 4 se recogen los datos de toxicidad de los plaguicidas seleccionados [13-15].

La toxicidad oral aguda: Se expresa por la dosis letal 50 (DL50) y se define como la cantidad de plaguicida que es necesario ingerir en una sola dosis para producir la muerte al 50% de los animales del ensayo.

La toxicidad dérmica: Se expresa como la DL50 dérmica aguda, y se define por la cantidad de tóxico en mg/kg que en contacto durante 24 horas con la piel del animal es capaz de producir la muerte del 50% de los individuos del ensayo en un plazo de 14 días.

La toxicidad por inhalación: Se expresa por la concentración letal (CL50) en mg de sustancia por metro cúbico de aire.

Tabla 4. Toxicidad aguda de los herbicidas seleccionados en rata

Herbicida	DL $_{50}$		CL $_{50}$
	Oral	Dérmica	Inhalación
	(mg/kg)	(mg/kg)	(mg/L)
Clorsulfuron	3053-5545	>2000	>5'9
Clortoluron	>10000	>2000	>5'3
Diuron	3400	>5000	>5
Fluometuron	6416-8900	>2000	>2'07
Isoproturon	2417	>3170	>0'67
Linuron	1500-4000	>2000	>0'849
Metabenzotiazuron	>2500	>500	0'5 sin efectos
Metobromuron	3000	>3000	>1'1
Monolinuron	1800	>2000	>3'39
Monuron	1480	-	-

La toxicidad de los herbicidas derivados de la urea para mamíferos es baja, oscilando su DL50 oral en rata, entre 1.480 mg/kg para el monuron y 11.000 para el clortoluron.

I.6.5.2. Intoxicaciones crónicas

Se producen como consecuencia de una exposición continuada al tóxico en pequeñas cantidades. Algunos plaguicidas que son poco tóxicos cuando se ingieren en una sola dosis, pueden resultar peligrosos si se absorben de forma repetida y continuada. En Toxicología Alimentaría el riesgo de los residuos de plaguicidas es debido fundamentalmente a su toxicidad crónica. En la tabla 5 se exponen datos de toxicidad crónica y los síntomas de intoxicaciones de los herbicidas objeto de estudio [13-15].

Entre los efectos crónicos más importantes y más graves de los plaguicidas se encuentran sus posibles acciones mutagénas, carcinógenas, teratogénas y efectos sobre la reproducción. La relación entre los plaguicidas en general y el riesgo de cáncer, como consecuencia de la ingestión repetida de pequeñas cantidades de plaguicidas

presentes en los alimentos, se evalúa, entre otros organismos, por la IARC (Internacional Agency for Research on Cancer) y la EPA (Environmental Protection Agency). Los únicos datos utilizables para abordar estos casos son los resultados de los ensayos experimentales en laboratorio, pero su posible extrapolación al hombre, la fijación de la dosis umbral, la relación entre mutagénesis y carcinogénesis y la intervención de factores individuales (sobre todo genéticos) y del medio ambiente, hace que la interpretación de laboratorio sea difícil.

Los herbicidas seleccionados son en general persistentes en suelos y muy fitotoxicos por lo que al manejarlos se deben cumplir estrictamente las precauciones generales de seguridad.

Como no son volátiles, no ofrecen peligro para los cultivos colindantes, si se toma la precaución de evitar que sean arrastrados por el viento durante su aplicación, pero su máximo riesgo estriba en la aplicación de dosis demasiado altas, ya que su gran persistencia en el suelo puede perjudicar a cultivos posteriores.

I.7. LEGISLACIÓN

Los plaguicidas son sustancias peligrosas y distintas regulaciones tratan de conseguir que su empleo resulte relativamente seguro. Antes de comercializar un nuevo producto, se exigen para su registro estudios biológicos que incluyen ensayos de laboratorio para conocer su selectividad, en invernadero, en campo y bajo diferentes condiciones climáticas. Se observa su eficacia para cada cultivo y tipo de plaga. Se realizan estudios toxicológicos para conocer la toxicidad aguda, subcrónica y crónica que presenta tanto el plaguicida como sus metabolitos para el aplicador y para el consumidor. Y por ultimo se exigen estudios ecotoxicológicos que evalúan los riesgos para la fauna y los ecosistemas, observando la toxicidad aguda sobre aves, peces, abejas y otros insectos, microcrustáceos (Daphnia spp), etc.., y los efectos a largo plazo sobre la reproducción y bioacumulación en animales.

Tomando como base los estudios toxicológicos sobre animales y observaciones en humanos y basándose en el conocimiento del NSEO (nivel sin efecto observable) o

cantidad que puede ingerirse sin que aparezcan efectos tóxicos apreciables y teniendo en cuenta un factor de seguridad amplio admisible, las legislaciones de distintos países

Tabla 5. Toxicidad crónica de los herbicidas seleccionados.

Herbicida	Especie: tiempo - dosis	Síntomas de intoxicaciones	Carcinogénesis, teratogénesis, mutagénesis, reproducción
Clorsulfuron	Rata: 2 años - 100 mg/kg dieta Ratón: 2 años - 500 mg/kg dieta	Irritación de ojos , nariz, garganta y piel	Sin efectos cancerígenos, ni en la reproducción, en ratas a dosis altas
Clortoluron	Rata: 90 días - 53 mg/kg/dieta Perro: 90 días - 23 mg/kg/dieta	Puede irritar piel y ojos	-
Diuron	Rata: 2 años - 250 mg/kg dieta Perro: 2 años - 125 mg/kg dieta	Puede irritar ojos, nariz, garganta y piel	Efectos embriotóxicos y en la madre a 500 mg/kg, pero no teratógenos
Fluometuron	Rata: 90 días - 100 mg/kg dieta Perro: 90 días - 400 mg/kg dieta	-	No teratogéno hasta 500 mg/kg
Isoproturon	Rata: 90 días - 400 mg/kg dieta Perro: 90 días - 50 mg/kg dieta	Metahemoglobinemia, cianosis.	El registro español lo considera carcinogénico categoría 3
Linuron	Rata: 2 años - 50 mg/kg dieta Perro: 2 años - 125 mg/kg dieta	Metahemoglobinemia, cianosis. Irrita piel, ojos y sistema respiratorio	El registro español lo considera carcinogénico categoría 3
Metabenztiazuron	Rata: 3 meses - 150 mg/kg dieta Perro: 3 meses - 500 mg/kg dieta	-	-
Metobromuron	Rata: 2 años - 250 mg/kg dieta Perro: 2 años - 100 mg/kg dieta	-	-
Monolinuron	Rata: 2 años - 250 mg/kg dieta	Por ingestión, cianosis, anemia	-

		hemolítica tóxica, dolor de cabeza, shock	
Monuron	Rata: 1 año - 500 mg/kg dieta	-	-

y organizaciones como la FAO/OMS establecen la IDA (Ingesta Diaria Admisible) ó cantidad de una sustancia que se puede ingerir diariamente durante un periodo prolongado de la vida sin manifestar efectos tóxicos. La IDA se expresa en mg de sustancia química por kg de peso corporal.

Debido a la potencial toxicidad de los residuos para la salud, la mayoría de países establecen límites a su presencia en alimentos abarcando desde prohibiciones de empleo a la fijación de unos Límites Máximos de Residuos (LMR) [16-21].

El LMR se define como la máxima concentración de residuos de un plaguicida, permitida por la legislación, en o sobre un alimento, que puede aceptarse para el consumo humano o animal a largo plazo. Este LMR se expresa como miligramo de plaguicidas por kilo de alimento fresco. Para establecer los LMR, se toman como base las IDA y se tienen en cuenta la proporción con que los alimentos con residuos contribuyen a la dieta de la población asi como las prácticas agrícolas.

En la tabla 6 se indican los LMR de los herbicidas seleccionados para los principales alimentos en que se aplican.

A nivel internacional, la Organización de la Naciones Unidas para la Agricultura y la Alimentación (FAO) y la OMS en 1996 crearon el *Comité del Codex sobre Residuos de Plaguicidas* (CCPR) cuyo primer objetivo es establecer mas recomendaciones sobre los LMRs, a la vez que regular los métodos de muestreo, análisis y ensayo [2]. No existe la obligación de incorporar dichos LMR a las distintas legislaciones, sin embargo, deben ser respetadas en la exportación de alimentos a países poco desarrollados, donde no existe normativa legal al respecto [22].

Con el fin de alcanzar niveles de residuos por debajo de los LMR establecidos en el momento de la comercialización del cultivo, se establecen "plazos de seguridad" o tiempo que hay que esperar desde el tratamiento hasta la cosecha. Estos plazos se fijan en base a estudios de cinéticas de degradación de plaguicidas a las dosis recomendadas de aplicación.

En el marco de la Unión Europea, existe una legislación armonizada sobre los LMR, comprometiéndose los diferentes estados a establecer un sistema de vigilancia para el control de los residuos de plaguicidas en productos que se comercializan en el mercado único, bien sean de producción propia, o procedente de terceros países

Tabla 6. Límites Máximos de Residuos (LMR)

Herbicida	Cereales	cítricos	Cebolla	Fruta de hueso	Patata	Zanahoria
Clorsulfuron	0'05	0'05	0'05	0'05	0'05	0'05
Clortoluron	0'05	0'05	0'05	0'05	0'05	0'05
Diuron*	0'05	0'2	0'1	0'1	0'05	0'1
Fluometuron	0'05	0'05	0'05	0'05	0'05	0'05
Isoproturon	0,05	0'05	0'05	0'05	0'05	0'05
Linuron*	0,05	0'2	0'05	0'1	0'05	0'1
Metabenzotiazuron	0'05	0'05	0'02	0'05	0'05	0'05
Metobromuron	0'02	0'02	0'02	0'02	0'1	0'02
Monolinuron**	0'02	0'02	0'2	0'02	0'05	0'2
Monuron**	0'02	0'02	0'2	0'02	0'05	0'2

* Suma de diuron, linuron y neburon expresada como 3,4-dicloroanilina.

** Suma de monolinuron, buturon y monuron expresada como 4-cloroanilina.

I.8. ANÁLISIS DE HERBICIDAS DERIVADOS DE LA UREA

Debido a las propiedades físico-químicas particulares de los herbicidas derivados de la urea, los LMR legislados suelen ser muy bajos, ya que no existe un método oficial, los análisis de estos residuos resultan particularmente difíciles. Las técnicas cromatográficas, son de elección para el control de estos compuestos, pero es necesario una preparación muy laboriosa de las muestras.

I.8.1. Extracción:

Entre los métodos de preparación de muestra, destacan la extracción líquido-líquido, (ELL) y la extracción en fase sólida, (EFS).

La extracción líquido-líquido, es una técnica clásica muy utilizada, consistente en la extracción de los plaguicidas gracias al reparto entre la matríz acuosa y un disolvente. Los plaguicidas se extraen dependiendo de su solubilidad en cada una de las fases. Se ha usado disolventes de diferentes polaridades como acetonitrilo, acetona, diclorometano, acetato de etilo y metanol para extraer las ureas desde, agua [23-24] vegetales [25] y suelo [26]. La selección del disolvente se realiza dependiendo de la polaridad de la urea y de la naturaleza de la muestra. La adición de sales y los cambios de pH contribuyen a la obtención de extractos más limpios.

La ELL, por su elevado consumo de disolventes (cuyo uso se está restringiendo cada vez más), por la laboriosa preparación y manipulación de la muestra y por su dificultad de automatización está siendo reemplazada cada vez más por la EFS.

La extracción en fase sólida se realiza forzando el paso de las ureas disueltas en una matriz acuosa a través de un soporte sólido extractante. Esta técnica se basa en un reparto en el que la fase extractante puede presentarse sobre discos o cartuchos rellenos por un material absorbente. Las fases más utilizadas son los rellenos de octil (C8), octadecilsilice (C18), etilsílice, ciano y aminopropilsílice seguidas de carbono grafito. La elección dependerá de la polaridad de los plaguicidas a extraer y el tipo de matriz analizada [27-29]. Las principales ventajas de la extracción en fase sólida

frente a la extracción líquido-líquido son la reducción en el consumo de disolventes y la menor cantidad de muestra, disminuyendo el tiempo de preparación y reduciendo la contaminación del extracto, lo cual proporciona una reproducibilidad y recuperación del analito adecuadas. Además, mediante un accesorio sencillo, es posible realizar la extracción simultánea de varias muestras. Sin embargo, el proceso de preparación sigue siendo laborioso y su automatización y acoplamiento en linea requiere de instrumentación compleja y costosa. No estan resueltas las aplicaciones en las que se requiere un acoplamiento para cromatografía de gases. En el análisis de muestras acuosas con particulas en suspensión, el principal inconveniente radica en la obturación de los poros de la fase extractiva.

En la actualidad, hay un interés creciente, por la simplificación, y miniaturización de las técnicas de análisis, con la consiguiente reducción de la cantidad de muestra utilizada en la extracción y del consumo de disolventes orgánicos, por ello las técnicas como la dispersión de matriz en fase sólida (DMFS) y la microextracción en fase sólida (MEFS), se están aplicando cada vez más en el análisis de plaguicidas.

La dispersión de matriz en fase sólida (DMFS) permite la extracción de estos herbicidas de matrices sólidas y semisólidas. En esta técnica se disgrega la muestra y se mezcla con la fase sólida extractante hasta conseguir una mezcla homogénea que se vierte en una pequeña columna de vidrio para su posterior elución con la ayuda de un disolvente orgánico. Existen varios tipos de fases sólidas comercializadas como C_{18}, C_8, fenil, ciano, amino y florisil Los resultados obtenidos son comparables con los obtenidos con otras técnicas [30]. La DMFS ha reducido considerablemente el tiempo de análisis, aunque el pequeño tamaño de la muestra (0'5 g) puede ser un inconveniente si no se ha homogenizado la muestra cuidadosamente.

La utilización de sistemas de extracción líquido-sólido, absorbentes con cierta especificidad para extraer determinados compuestos, ha proporcionado muy buenos resultados, y se utiliza habitualmente para el análisis de compuestos orgánicos en aguas y otras matrices líquidas. En esta línea, J. Pankow y L.M. Isabelle desarrollaron las primeras aproximaciones a la microextracción en fase sólida. Poco después J. Pawliszyn empleó una fibra óptica para ensayar la desorción mediante láser de diferentes compuestos (trietilen glicol, polietilen glicol y carbowax) [33-35]. La

microextracción se basa en un proceso de reparto y/o absorción entre una microfibra formada por una fase polimérica y una disolución acuosa. Los analitos de la muestra, se extraen mediante la microfibra que se sumerge en la muestra a analizar, MEFS directa o sobre el espacio en cabeza de la muestra (headspace SPME ó HS-SPME). Los analitos difunden hacia la fibra hasta que transcurrido un tiempo, se alcanza el equilibrio para cada compuesto entre el agua y la fibra. Pasado este tiempo de equilibrio, la fibra se introduce con ayuda de una jeringa modificada, en el inyector del cromatógrafo de gases donde se desorben instantáneamente a temperaturas entre 150-300°C.

Las microfibras comerciales consisten en un pequeño cilindro de 1 cm de longitud y un espesor entre 7 y 100 μm de un polímero determinado. El tipo de polímero empleado es el que confiere la especificidad y eficacia de extracción. En la actualidad existen 5 tipos de polímeros comerciales: Polidimetilsiloxano (PDMS) con 3 tipos de espesores de 7, 30 y 100 μm, polímero bien conocido por su empleo como fase estacionaria de las columnas capilares, Poliacrilato (PA) con espesor de 85 μm, y los polímeros mixtos más recientes en el mercado como polidimetilsiloxano-divinilbenceno (PDMS-DVB), carbowax- divinilbenceno (CW-PDMS y carboxen polidimetilsiloxano (CAR-PDMS) todas con 65μm de espesor. Aunque la MEFS presenta extraordinarias posibilidades para la extracción de microcontaminantes en muestras acuosas, sólo existen referencias bibliográficas del uso de las tres primeras fibras para extraer las ureas herbicidas [36-37].

Asimismo, hacia 1997 se ha empezado a estudiar la desorción de la fibra mediante disolventes para su acoplamiento con cromatografía líquida. Para ello se ha desarrollado una interfase consistente en un bucle en el que se lleva a cabo la desorción con la propia fase móvil de la CL [38].

La microextracción en fase sólida (MEFS) ofrece muchas posibilidades, es una técnica sencilla, rápida, no precisa de disolventes orgánicos y requiere volumenes de muestra de tan sólo 2 mL [33-35]. Dada su simplicidad, la manipulación de la muestra es prácticamente nula, y la automatización de todo el proceso es sencilla. Existen actualmente comercializados muestreadores automáticos, que incorporan estas jeringas modificadas, y mediante un programa informático se controlan los diferentes

parámetros de extracción, desorción, etc. En este sentido la MEFS se presenta como una alternativa a los métodos clásicos de preparación de muestras para el análisis de microcontaminantes orgánicos en aguas.

Los fluidos supercríticos se caracterizan por tener la capacidad de difusión y transporte propia de los gases, pero además hacen la función de un líquido al disolver el analito. Estas características se han aprovechado para la extracción con fluidos supercríticos de algunas sulfonilureas [31-32].

El fluido más utilizado es el CO_2 por ser químicamente inerte, barato, no tóxico ni inflamable, y poseer un punto crítico de temperatura y presión fácilmente accesible. Después de despresurizar, el fluido vuelve a su estado gaseoso, y el analito se recoge sobre una superficie sólida. Sin embargo, el CO_2 no es eficaz en la extracción de los plaguicidas más polares, por lo que se utiliza metanol o acetona como cosolventes.

A pesar de estas ventajas, la extracción con fluidos supercríticos presenta problemas de reproducibilidad, e interferencias de matriz y pueden aparecer obturaciones por formación de hielo en muestras con alto contenido en agua.

I.8.2. Purificación

En ocasiones, dependiendo del tipo de compuesto, la naturaleza de la muestra y de la selectividad del equipo analítico usado para la determinación se requiere una purificación de los extractos como paso previo a la determinación de los herbicidas. El uso de detectores selectivos como el de nitrógeno fósforo (DNF), y el de espectrometría de masas (DEM) reduce la necesidad de la purificación en algunos casos.

Los principales procedimientos de purificación se basan en establecer un reparto líquido-líquido con dos disolventes inmiscibles, cromatografía en columna o cromatografía de permeación sobre gel o de exclusión molecular. La elección del método de purificación depende de varios factores como la solubilidad en agua, las propiedades iónicas y polares, la estabilidad térmica y el peso molecular de los compuestos.

La partición líquido-líquido es la técnica de purificación más sencilla que se lleva a cabo en un embudo de decantación. Suele utilizarse como una purificación parcial previa a la cromatografía de adsorción. El extracto inicial se extrae con otro disolvente y el analito se queda en una de las dos fases, mientras que la mayoría de los componentes interferentes quedan en la otra.

Cuando se emplea la cromatografía en columna, las fases más utilizadas son gel de sílice, Florisil, alúmina y aminopropilo para retener las sustancias interferentes más polares, mientras que el carbón activo se utiliza por su gran afinidad por los pigmentos de las plantas [9-11].

La cromatografía de permeación sobre gel o de exclusión molecular separa los plaguicidas de los componentes interferentes de la matriz dependiendo del tamaño molecular e independientemente de su polaridad. La fase estacionaria es un gel con una estructura porosa e hinchada, siendo las mas utilizadas las fases Bio-Beads SX-3 y SX-8. Este tipo de purificación presenta la ventaja de que se puede automatizar, y conociendo exactamente el tiempo de elución de cada plaguicida, permite obtener resultados muy reproducibles [39-40]. Sin embargo, al basarse la separación en el tamaño molecular, puede haber una purificación incompleta de los compuestos de bajo peso molecular, una inadecuada separación de los plaguicidas de elevado peso molecular y además un importante consumo de disolvente.

I.8.3. Derivatización

Muchas de las ureas seleccionadas son termolábiles y se descomponen a las temperaturas usuales para el análisis por cromatografía gaseosa (CG). Para aumentar la estabilidad térmica de estos compuestos y poderlos determinar por CG, se han desarrollado diversos métodos de derivatización que sustituyen los hidrógenos libres del puente nitrogenado de la urea.

Los métodos de derivatización más empleados se basan en la alquilación. Otros métodos ocasionan la hidrólisis del grupo urea para obtener anilinas derivadas que ya pueden analizarse por CG.

La reacción de alquilación sustituye los hidrógenos libres del grupo urea con grupos alquilo. Los reactivos más usados son hidroxido de trimetil anilina, yoduro de alquilo y diazometano [40].

La reacción con trimetil anilina pueden llevarse a cabo dentro del inyector con una inyección directa de una mezcla de la urea, el reactivo y el metanol.

I.8.4. Técnicas de separación y determinación

I.8.4.1. Electroforesis capilar

La electroforesis capilar (EC) es una técnica de separación que se ha utilizado con éxito en biología y en biomedicina. Se basa en la diferente movilidad de las sustancias en disolución bajo la acción de un campo eléctrico. La separación de las sustancias se lleva a cabo en el interior de un tubo capilar, normalmente de sílice fundido, lleno de una disolución tampón. Así para las ureas herbicidas, se utiliza la electroforesis capilar de zona cuando el capilar contiene sólo un medio tamponado lo que hace que la movilidad de las ureas dentro del capilar dependa fundamentalmente de la relación carga/masa. La electromatografía micelar es una variedad en la que se añade al medio tamponado un tensioactivo, formandose micelas que se pueden considerar como una pseudofase estacionaria que ayuda a la separación. Debido a que no precisa de disolventes orgánicos y su mantenimiento es más económico que las técnicas cromatográficas clásicas, se encuentra entre las técnicas que se pueden utilizar para la separación de plaguicidas. La principal limitación de la EC es la baja sensibilidad, por lo que la clave parece estar en el empleo de procesos de extracción, purificación y concentración más eficaces [41].

I.8.4.2. Cromatografía líquida

La cromatografía líquida permite la separación y cuantificación directa de compuestos no volátiles y termolábiles sin necesidad de derivatización. La flexibilidad en el manejo de las fases móviles y la gran variedad de columnas disponibles hace que sea

la técnica más empleada para determinar las ureas. El detector ultravioleta (UV) ha sido muy utilizado, sin embargo, presenta numerosas interferencias procedentea de las matrices, y de los disolventes ya que son muchas las sustancias que absorben a las longitudes de onda seleccionadas. Este hecho contribuye a que este detector no sea muy selectivo, y los límites de detección sean peores que los obtenidos con otros detectores empleados en otras técnicas. El detector de filas de diodos es capaz de medir simultáneamente varias longitudes de onda, lo que posibilita la obtención de un espectro completo cada 0'1 sg, con ello, se ha superado en parte el inconveniente de su baja selectividad [42]. El detector de fluorescencia ofrece una gran sensibilidad y selectividad para las sustancias fluorescentes o para las que previa derivatización pasan a ser fluorescentes.

La CL se puede acoplar a un sistema de EFS automático cuando la matriz es acuosa.

I.8.4.3. Cromatografía gaseosa

La cromatografía gaseosa (CG) presenta la ventaja de utilizar columnas capilares de gran poder de resolución y detectores selectivos y muy sensibles como el detector de captura de electrones (DCE), y el detector de nitrógeno fósforo (DNF) por lo que resulta una técnica de primera elección. Aunque la mayoría de ureas herbicidas son termolábiles y se descomponen durante el análisis por CG, la determinación directa de estos compuestos sin degradación es posible bajo unas condiciones cromatográficas muy controladas [43-45]. El DCE es uno de los detectores más utilizado a nivel mundial, pero su amplio espectro de respuestas hace a veces difícil la interpretación de los cromatogramas y por ello, resulta recomendable una confirmación adicional con el DNF en los análisis de estos herbicidas en productos agroalimentarios.

Un aspecto importante es la inyección. En la cromatografía gaseosa capilar se pueden utilizar tres tipos de inyección: con división de flujo, sin división de flujo y en columna. En los dos primeros la muestra se introduce en disolución en el bloque caliente del inyector y allí se vaporiza. Para las ureas, ésta es una etapa crítica, en la cual el grado de descomposición puede ser muy importante. En algunos casos, se usan estos mismos productos de degradación para cuantificar la urea de origen. En otros, se

intenta resolver este problema con una inyección en frío y en cabeza de la columna capilar, es decir, en columna. Existen inyectores automáticos, desarrollados por algunas casas comerciales que permiten la inyección "en columna" así como su acoplamiento con el dispositivo de microextracción en fase sólida lo que mejora tanto la rapidez como la reproducibilidad de los análisis.

La presencia de átomos de halógenos y de nitrógeno en la estructura química de los herbicidas permite su determinación con los detectores selectivos de la CG a concentraciones muy bajas, a pesar que existan otras sustancias presentes en los extractos de las diferentes matrices.

I.8.5. Presente y futuro en el análisis de residuos de herbicidas derivados de la urea

La combinación de la espectrometría de masas con la cromatografía de gases (CG-EM) y más tarde con la cromatografía líquida (CL-EM) permite la determinación de estos plaguicidas en diversas matricesasi como la identificación y la de sus metabolitos a muy baja concentración. La selectividad y la sensibilidad del análisis se puede mejorar seleccionando los iones más característicos y abundantes de la molécula mediante el sistema de monitorización de iones seleccionados [46].

La automatización aplicada a los análisis de los residuos de los pesticidas ha mejorado en los últimos años, el uso de inyectores automáticos en CG y CL es corriente. Diversas etapas del procedimiento analítico como la adición de reactivos y dilución de la muestra se pueden realizar automáticamente. La extracción y la preparación de la muestra también se pueden automatizar con procedimientos "en línea" o independientes. La microextracción en fase sólida con la variedad de fibras comerciales existentes en el mercado, presenta como ventajas el ahorro de tiempo, disolventes y mano de obra sí bien queda limitada por el momento a matrices acuosas.

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OBJETIVOS

Y

PLAN DE TRABAJO

II. OBJETIVOS Y PLAN DE TRABAJO

Los plaguicidas son productos químicos que se utilizan en grandes cantidades en todo el mundo por los enormes beneficios económicos que proporcionan a la industria agroalimentaria, sin embargo su aplicación produce un impacto negativo sobre el medio ambiente y sus residuos en alimentos y aguas constituyen un riesgo para la salud de los consumidores. Los plaguicidas de mayor consumo en el conjunto de Europa son los herbicidas, mientras que en el Estado Español se sitúan en el segundo lugar tras los insecticidas. Dentro del grupo de los herbicidas, ocupan un lugar destacado los que presentan estructuras derivadas de la urea.

Con el fin de proteger la salud de sus ciudadanos y favorecer el comercio, los países de la Unión Europea establecen en su legislación límites muy rigurosos para la presencia de residuos de plaguicidas en aguas y en alimentos. Únicamente se pueden comercializar y consumir los productos con residuos menores a los exigidos en la legislación. La vigilancia del cumplimiento de dicha legislación obliga a los laboratorios a analizar un elevado número de muestras y a disponer de técnicas no sólo muy sensibles y específicas, sino también rápidas y fiables.

Muchos de los herbicidas derivados de la urea son termolábiles, por lo que la mayoría de métodos citados en la bibliografía utilizan la cromatografía líquida para su determinación, desaprovechando la alta sensibilidad y especificidad de los detectores de la cromatografía gaseosa y el gran poder de resolución de las columnas capilares. El conocimiento de la degradación térmica producida durante la cromatografía de gases podría permitir la determinación indirecta de la urea herbicida en base a los productos derivados de su degradación.

Por otro lado, las técnicas clásicas de extracción de los residuos en aguas y alimentos utilizan volúmenes considerables de disolventes orgánicos contaminantes, requieren en ocasiones de procesos adicionales de purificación y resultan difícilmente automatizables, mientras que la extracción en fase sólida es rápida y consume poco volumen de disolventes, y la microextracción en fase sólida ni siquiera utiliza

disolventes y además permite realizar la extracción y la determinación de forma automática.

Con estos antecedentes, el presente trabajo se propone como objetivos:

- Estudiar el comportamiento de herbicidas derivados de la urea cuando se inyectan en un cromatógrafo de gases con columnas capilares.

- Evaluar la posibilidad de cuantificar dichos herbicidas en base a sus productos de degradación térmica.

- La puesta a punto de un nuevo método de análisis de herbicidas derivados de la urea en aguas y vegetales basado en la microextracción en fase sólida y la determinación por cromatografía de gases.

- Aplicar el método al análisis de herbicidas en muestras reales de aguas y vegetales.

Para la consecución de dichos objetivos, se propone el siguiente plan de trabajo:

1.- Estudio bibliográfico de las propiedades físico-químicas y de las técnicas de extracción y de determinación de plaguicidas derivados de la urea.

2.- Estudio de los factores que influyen en el comportamiento de herbicidas derivados de la urea cuando se determinan por cromatografía de gases.

3.- Estudio de los factores que influyen en la microextracción en fase sólida de los herbicidas seleccionados desde matrices acuosas.

4.- Cálculo experimental de las constantes de distribución aparentes fibra/agua que rigen la extracción de los herbicidas seleccionados.

5.- Aplicación de la microextracción en fase sólida acoplada a la cromatografía de gases al análisis de residuos de herbicidas en aguas naturales y en muestras de vegetales.

ESTUDIO
BIBLIOGRÁFICO

III. Determination of Urea Pesticide Residues in Vegetable, Soil and Water Samples

Houda Berrada, Guillermina Font and Juan Carlos Moltó

Enviado a

Critical Reviews in Analytical Chemistry

ABSTRACT

Main physico-chemical, toxicological and environmental properties of urea pesticides are summarized. General characteristics of analytical methods for residues of phenylurea herbicides (PUHs), sulfonylurea herbicides (SUHs) and Benzoylurea insecticides (BUIs) in crops, soil and water samples, employed in the last five years are reviewed. It is provided information about liquid-liquid and solid-phase extraction of the samples and clean up steps. Applications of gas chromatography (GC), liquid chromatography (LC) and capillary electrophoresis (CE) techniques in the analysis of these compounds are exposed in tabular form and commented. Sensitivity and instrument conditions of liquid and gas chromatographic techniques coupled to mass spectrometric detectors are outlined. Advantages and drawbacks of the analytical methods recently developed are discussed.
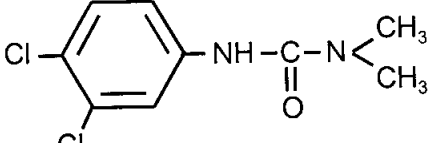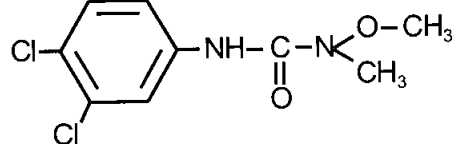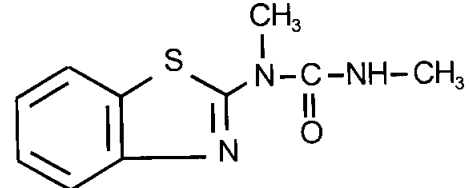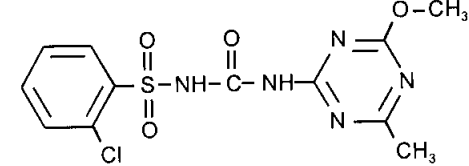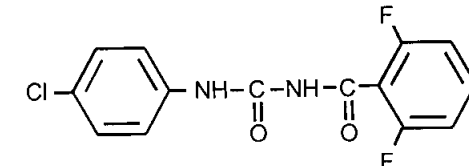
KEYWORDS phenylurea herbicides, sulfonylurea herbicides, benzoylurea insecticides, liquid chromatography, gas chromatography, capillary electrophoresis, foods, water.

III.1. INTRODUCTION

Substituted ureas are an important group of pesticides that are used as herbicides (phenylureas and sulfonylureas) and insecticides (benzoylureas). Phenylurea herbicides (PUHs) are largely used in field applications for pre- and post-emergence weed control in a variety of crops. The main groups of phenylurea herbicides are the N-phenyl-N',N'-dialkylureas, N-phenyl-N'-methoxyureas and compounds containing a heterocyclic group. More recently a group of sulfonylurea herbicides (SUHs) has been developed. SUHs are very specific to the target organisms and thus the applied amounts in the field are much smaller than with conventional pesticides. The mechanism of action of all ureas is common consisting in act inhibiting the photosynthesis producing herbicidal activity, impeding the chlorophyll's function due to the inhibition light reaction at level of Hill reaction. Sulfonylureas act inhibiting acetolactate synthasa. This enzyme intervenes in the biosynthesis of branched chain amino acids conducting to the photosynthesis inhibition. Benzoylurea compounds are promising insecticides (BUIs) used because their ability to act as insect growth regulators which inhibit the synthesis of cuticle chitin in target pests. Figure 6 shows some examples of structures of the main groups of urea pesticides and tables 7 and 8 outline the physicochemical and toxicological properties of these compounds.

For the determination of urea pesticides several techniques can be used [1,2]. Gas chromatography (GC) is used because of its high sensitivity and selectivity for the detection of these compounds, but the thermal instability of some urea pesticides makes it necessary, to first prepare stable derivatives, indirectly determine them in the form of their derivatives or to use other techniques such as liquid chromatographic (LC) or capillary electrophoresis (CE). Although in recent years the preference for LC methods has increased in the applications for urea compounds, CG methods, when applicable, still have the advantages of great separation efficiency, high speed of analysis and the availability of a wide range of highly sensitive detectors [3].

Figure 6: Examples of the chemical structures of the main groups of substituted urea pesticides

Urea pesticide group	Chemical structure of typical examples
Phenylurea (PUH) N-phenyl -N', N'- dialkylurea	 Diuron
N-phenyl-N'-alkyl-N'- Methoxyurea	 Linuron
Substituted urea with an heterocyclic group	 Metabenzthiazuron
Sulfonylurea (SUH)	 Chlorsulfuron
Benzoylurea (BUI)	 Diflubenzuron

Capillary electrophoresis (CE) is a promising analytical tool that provides improved resolution over LC with similar sensitivity at trace levels of urea pesticides in a variety of matrices with several different sets of CE conditions [4].

Multiresidue and single residue methods generally consist of the same basic steps, but multiresidue methods allow the determination of a large number of pesticides in a single analysis, reducing thus time and cost of analysis. The Maximum Admissible Concentration (MAC) of pesticides in drinking water, defined by European Community Directive as 0.1 μg L^{-1} for individual pesticides and 0.5 μg L^{-1} for the sum of pesticides posed certain demands to the analytical methods for pesticide residue determination, this subject has been reviewed by several authors [5-7]. Analytical methods have also been reviewed to screen, quantify, and confirm pesticide residues in agricultural products under Maximum Residue Limits (MRLs) established by regulations [8-10].

The aim of this review is to summarize and discuss the extraction methods developed to isolate and preconcentrate urea pesticides, and the methods used to determine these compounds the last five years in water and food samples.

III.2. EXTRACTION AND CLEAN-UP

Several of the substituted urea pesticides are highly persistent in the environment and can contaminate surface waters damaging crops if water contaminated is used for irrigation or increasing residues in drinking water. Extraction and clean-up procedures play an important role in the determination of urea pesticides in food and water samples. An important advantage of reversed-phase column LC in conjunction with aqueous samples is that the low eluotropic strength of water samples allows the injection of large sample volume [11]. Phenylureas can be determined using a GC method by large volume injection [12]. Aqueous samples can be analyzed directly by CE [13].

Table 7. Characteristic physico-chemical properties of urea pesticides

Common name	IUPAC name, Molecular Formula	Molecular weight	Melting Point (°C)	Vapour pressure μPa (Temp°C)	Log P_{ow} (Temp°C)	Solubility g/L (Temp °C)			
						Water	Methanol	Dichloro methane	Acetone
Diuron	3-(3,4-Dichlorophenyl)-1,1-dimethylurea $C_9H_{10}Cl_2N_2O$	233.1	180-189*	0.230	2.8-2.9 (25)	42 10^{-3} (25)	-	1-2 (25)	53 (27)
Fluometuron	1,1-Dimethyl-3-(α, α, α -trifluoro-m-tolyl)urea $C_{10}H_{11}F_3N_2O$	232.2	163	67 (20)	2.23	105 10^{-3} (20)	110-140 (20)	23 (20)	105-150 (20)
Linuron	3-(3,4-Dichlorophenyl)-1-methoxy-1-methylurea $C_9H_{10}Cl_2N_2O_2$	249.1	180-190*	1470 (24)	3 (22)	81 10^{-3} (25)	-	-	500 (25)
Metobromuron	3-(4-Bromophenyl)-1-methoxy-1-methylurea $C_9H_{11}BrN_2O_2$	259.11	95.5-96	400 (20)	2.41	0.33 (20)	240 (20)	550 (20)	500 (20)
Methabenzthiazuron	1-(Benzothiazol-2-yl)-1,3-dimethylurea $C_{10}H_{11}N_3OS$	221.29	119-121	5.9 (20)	2.64	59 10^{-3} (20)	65.9 (20)	>200 (20)	115.9 (20)

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Common name	IUPAC name, Molecular Formula	Molecular weight	Melting Point (°C)	Vapour pressure μPa (Temp°C)	Log P_{ow} (Temp°C)	Solubility g/L (Temp °C) Water	Methanol	Dichloro methane	Acetone
Chlorsulfuron	1-(2-Chlorophenylsulfonyl)-3-(4-methoxy-6-methyl-1,3,5-triazin-2-yl) urea $C_{12}H_{12}ClN_5O_4S$	357.8	192*	613 (25)	-	27.9 (25)	14 (22)	102 (22)	57 (25)
Triasulfuron	3-(6-Methoxy-4-methyl-1,3,5-triazin-2-yl)-1-(2-(2-chloroethoxy) phenylsulfonyl) urea $C_{14}H_{16}ClN_5O_5S$	401.83	186	$0.1\ 10^{-3}$ (20)	-0.96	1.5 (20)	3.4 (25)	15 (20)	16 (25)
Chlorfluazuron	1-(3,5-Dichloro-4-(3-chloro-5-trifluoro-methyl-2-pyridiloxy) phenyl)3-(2,6-difluorobenzoyl) urea $C_{20}H_9\ Cl_3F_5N_3O_3$	540.66	228*	$<13.3\ 10^{-3}$ (20)	5.8	$16\ 10^{-6}$ (25)	2.2	22 (25)	52.1 (25)
Diflubenzuron	1-(4-Chlorophenyl)-3-(2,6-difluorobenzoyl) urea $C_{14}H_9ClF_2N_2O_2$	310.69	230-232*	<1000 (20)	1.63 (22) pH 3	$0.14\ 10^{-3}$ (20)	-	<10 (20)	6.5 (20)

* decomposition beginnings

Table 8. Characteristic toxicological and environmental properties of urea pesticides

Common name	Oral toxicity (rats, mg/ kg)	ADI (mg/ kg)	MRL (mg/kg)		Hydrolysis per day (Temp, pH)	Photolysis per day (Temp, pH)	Field Dissipation half life in day (pH- % OM)	Half life in soil in days	Application (kg /ha)
			Cereals	Fruit and Vegetable					
Diuron	3400	0.002	0.2	0.5	<0.0014 (25, 5-9)	0.016 (25,7)	90	372	8 - 25
Fluometuron	6416-8900	0.005	0.05	0.05	Stable (25, 5-9)	Stable (25, 5-9)	95	189	1.25 - 2
Linuron	1500-4000	0.00625	0.2	0.5	0.00086 (25, 5)	0.014 (25, 5)	60 (6.4 - 2.7)	81	0.5 - 3
					0.00051 (25,9)		30 (5.5 - 1.3)		
Metobromuron	2000	0.03	0.02	0.02	-	-	90	-	1 - 2.5
Methabenzthiazuron	>2500	0.075	0.05	0.05	-	-	60 - 180	-	2 - 3

Common name	Oral toxicity (rats, mg/ kg)	ADI (mg/ kg)	MRL (mg/kg)		Hydrolysis per day (Temp, pH)	Photolysis per day (Temp, pH)	Field Dissipation half life in day (pH- % OM)	Half life in soil in days	Application (kg /ha)
			Cereals	Fruit and Vegetable					
Chlorsulfuron	3053-5545	0.05	0.05	0.05	0.0289 (25, 5)	0.0034 (25, 5)	39 (6.9 - 1.0)	20 - 46	15 - 20 10^{-3}
					0.0005 (25, 7)	Stable (25, 7.9)	185 (5.6 - 3.8)		
					0.0010 (25, 9)				
Triasulfuron	>5000	0.005	0.05	0.05	0.023 (20, 5)	0.0080 (25, 9)	10 - 87	114 -161	10 - 20 10^{-3}
					Stable (20, 7-9)				
Chlorfluazuron	>8500	0.025	0.05	0.05	-	-	-	-	10 - 100 10^{-3}
Diflubenzuron	>4640	0.011	0.05	0.05	0.0046 (22, 5.7)	0.1	8 (2 - 35)	4	20 - 125 10^{-3}

ADI: Admissible Dairy Intake
MRL: Maximum Residue Level
OM: Organic Matter

Table 9. Liquid-liquid extraction and clean up procedures of urea pesticides

Type of compound	Matrix (Volume)	Extracting solvent	Clean up sequence	Method of determination	Ref.
Phenylurea	Water (500mL)	3x 50mL dichloromethane	_	GC	[18]
Phenylurea	Water (500mL)	3x50mL dichloromethane	_	GC	[12]
Phenylurea	Crops (100g)	200mL acetone	Solvent exchange 2 x 100mL dichloromethane Gel permeation chromatography Ethyl acetate-cyclohexane (1:1)	GC	[24]
Benzoylurea	Apples (25g)	125mL dichloromethane	_	GC	[26]
Phenylurea and benzoylurea	Water	3x100mL dichloromethane	_	LC	[14]
Phenylurea	Water (300mL)	30mL dichloromethane	_	LC	[15]
Phenylurea and sulphonylurea	Water (1L)	3x60mL dichloromethane	_	LC	[16]
Benzoylurea	Grapes (50mL)	100mL ethyl acetate	Silica cartridges Dichloromethane-isopropanol (9:1)	LC	[19]

Type of compound	Matrix (Volume)	Extracting solvent	Clean up sequence	Method of determination	Ref.
Benzoylurea	Plant material (50g)	100mL ethyl acetate	Gel permeation chromatography Ethyl acetate-cyclohexane (1:1)	LC	[21]
Benzoylurea	Apples and pears (50g)	100mL ethyl acetate	Silica cartridge Dichloromethane-2-propanol (9:1)	LC	[22]
Phenylurea	Carrot and potato (20g)	40mL acetone 20mL Ethyl acetate-cyclohexane (1:1)	Gel permeation chromatography Ethyl acetate-cyclohexane (1:1) Florisil cartridge Ethyl acetate-n-hexane Acetone-n-hexane	LC	[23]
Sulfonylurea	Rice and crayfish (5g)	100mL dichloromethane	Hexane Silica cartridges Isopropyl alcohol-hexane (1:9)	LC	[25]
Benzoylurea	Apples (50g)	100mL acetone	Solvent exchange 50mL dichloromethane Gel permeation chromatography Cyclohexane-chloroform (3:2)	LC	[27]

Type of compound	Matrix (Volume)	Extracting solvent	Clean up sequence	Method of determination	Ref.
Sulfonylurea	Soil (50g)	Methanol-0.1M NaOH (1:1)	Dichloromethane washing (pH 11) Solvent exchange dichloromethane (pH 3-4)	LC	[35]
Sulfonylurea	Cottonseed and cotton gin trash (5g)	50mL acetonitrile-0.1M ammonium carbonate (8:2)	–	LC	[45]
	Water (1L)	pH 6.0 3x 60mL dichloromethane	–	CE	[17]
	Soil (20g)	2 x 100mL Methanol-0.1M Potassium phosphate (1:1)	Solvent exchange 3 x 30mL dichloromethane		
Sulfonylurea	Grains (10g)	2 x 50mL acetonitrile	2 x 20mL Hexane washing Isolute SCX cartridge 0.1M ammonium acetate Solvent exchange 2 x 20 mL acetonitrile-dichlorometane (5:95)	CE	[30]

Table 10. Solid phase extraction and cleanup procedures of urea pesticides

Type of compound	Matrix (Volume)	Extracting phase (Eluting solvent)	Clean up sequence	Method of determination	Ref.
Phenylurea	Water (1 L)	C_{18} cartridges (30 mL Ethyl acetate)	-	GC	[38]
Phenylurea	Water (1 L)	C_{18} column (Hexane-2-propanol (3:1))	-	GC	[39]
Phenylurea	Water (1 L)	C_{18} column (2 x 10 mL ethyl acetate)	-	GC	[31]
Phenylurea Sulfonylurea	Water (2 mL)	85 μm Polyacrylate (SPME) 14,3% NaCl pH 4, 60 min	-	GC	[48]
Benzoylurea	Wines (3 mL)	100 μm Polydimethylsiloxane (SPME) 12.5% Ethanol T^a 45°C, 30 min	-	GC	[49]
Phenylurea	Water (1 L)	47 mm Empore disks 2 x 5 mL Ethyl acetate	-	LC	[32]
Phenylurea	Water (100 mL)	25 mm C_{18} Empore disk 1.5 mL Acetonitrile-methanol (1:1)	-	LC	[33]
Phenylurea	Water (1 L)	C_{18} Bond Elut column 3 x 7 mL Ethyl acetate	-	LC	[34]

Type of compound	Matrix (Volume)	Extracting phase (Eluting solvent)	Clean up sequence	Method of determination	Ref.
Sulfonylurea	Water (100 mL)	C_{18} Bakerbond column 5 mL Methanol	-	LC	[35]
	Soil (50 g)	2 x 100 mL 0.1M Sodium hydrogen carbonate C_{18} Bakerbond column (pH 2.5) 5 mL Methanol		LC	
Sulfonylurea	Water (200 mL)	2 x C_{18} column 10 mL 0.1% Acetic acid in ethyl acetate	Silica cartridges 0.1% acetic acid in ethyl acetate	LC	[41]
	Soil (50 g)	100 mL 0.1M Ammonium carbonate-acetone (8:2) C_{18} column 10 mL 0.1% Acetic acid in ethyl acetate			
Sulfonylurea	Water (1 L)	SAX-RP 102 tandem cartridges (pH3) 3 x 4 mL 1% Acetic acid in acetone	-	LC	[42]
Phenylurea	Water (1 L)	Carbograph 4 SPE cartridges 1.5 mL Methanol 6 mL Dichloromethane	-	LC	[37]
Phenylurea	Water (1 L)	SPE column Hexane-2propanol (3:1)	-	LC	[40]
Benzoylurea	Wine (10 mL)	Isolute C_{18} 2 mL Methanol	-	LC	[19]

Type of compound	Matrix (Volume)	Extracting phase (Eluting solvent)	Clean up sequence	Method of determination	Ref.
Benzoylurea	Citrus fruits (0.5 g)	0.5 g C$_8$ (MSPD) 10mL Dichloromethane	-	LC	[46,47]
Phenylurea	Water (25-50 mL)	On line SPE 30 x 4.6 Silica-based immunosorbent Gradient acetonitrile with phosphate buffer (pH7)		LC	[51]
Sulfonylurea	Water (100 mL)	On line SPE 10 x 2 mm C$_{18}$ (pH3) Gradient methanol-100 mM acetic acid	-	LC	[52]
Phenylurea	Water (100 mL)	On line SPE 10 x 2 mm C$_{18}$ 10 x 2 mm PLRP Gradient acetonitrile-water	-	LC	[53]
Sulfonylurea	Water (100 mL)	On line SPE C$_{18}$ column	-	SFC	[54]
Sulfonylurea	Water (500 mL)	RP-102 Column 10 mL Methanol	SAX-Alumina Bakerbond cartridges 17mL 0.5% acetic acid-dichloromethane	CE and LC	[36,44]

SPME: Solid phase micro-extraction
MSPD: Matrix solid-phase dispersion

Although the traditional liquid–liquid extraction (LLE) requires the handling of a large volume of solvents, some methods are proposed for the analysis of waters with dichloromethane [14-17], ethyl acetate [18,19] or acetonitrile [20], for vegetables with ethyl acetate [21,22], acetone [23,24], dichloromethane [12,16,25-27] or acetonitrile [28-30]. The extracts from vegetal samples are cleaned up by gel permeation chromatography [21,23,24,27] or solid-phase extraction (SPE) to clean up with silica [19,22,25], aminopropyl cartridges [28] or cation exchange SPE [13].

SPE is a very useful preconcentration technique, which allows both extraction and concentration of pesticide residues. Water samples can be extracted using SPE bulk C_{18} sorbent [31], disks [32,33] columns [34-36] or cartridges [37-45]. After SPE extraction extracts can be cleaned up with alumina cartridges [36]. Considerable reduction of solvent consumption can be achieved by miniaturizing the scale of sample extraction. Adoption techniques such as matrix solid-phase dispersion (MSPD) can help to reduce considerably the size of sample and the solvent consumption. C_8 bonded silica is used to extract BUIs from citrus fruits [46,47]. Solid-phase microextraction (SPME), a solvent free, easy sample preparation method, is used to extract urea pesticides from water [48,49].

Automatic devices, which couple on-line the sample pre-treatment by SPE-LC in one analytical run, are nowadays commercially available. This method is well suited for multiresidue analysis of water samples [50-53]. Both parent and breakdown products of sulfonylureas were on-line extracted from large water samples by supercritical fluid chromatography SFC [54]. The combination of an on-line concentration and extraction and microbore LC is capable of providing good recoveries in the determination of herbicides in environmental water samples [55].

Tables 9 and 10 summarize the main liquid-liquid and solid-phase extraction procedures found in the literature and the cleaning-up steps used before determination.

In general, analytical procedures based in GC detectors do not need extracts of great purity and cleaning-up can usually be avoided. Dichloromethane is the most frequently used solvent for extraction and clean-up purposes in methods based on liquid-liquid partition whereas octadecylsilica is largely preferred over other supports in SPE methods.

III.3. DETERMINATION SYSTEMS

A variety of analytical methods have been used for the analysis of pesticides. Most analytical schemes are currently based on GC, LC and CE techniques.

III.3.A. Gas Chromatography

The behavior of the analytes in the GC or LC columns is the main criterion for selection of the separation method. Most of the phenylurea herbicides are non-GC amenable compounds, due to thermal instability but in some cases in spite of their degradation under GC conditions the precision of analysis is not significantly affected. Some compounds can be determined unchanged by GC with electron capture detector (ECD) [24,26,31,49,54] and nitrogen phosphorus detector (NPD) [31,54]. In other cases, a degradation product is quantified [48,56] or the pesticide is derivatized before GC determination [31]. GC coupled with a quadrupole MSD [24,26,39,48,56] or an ion trap (IT) MSD operating in electronic impact mode [12,38] performs sometimes confirmation of identity of pesticide residues. Table 11 summarizes the use of GC methods in the determination of urea pesticides.

Table 11. Gas chromatographic methods for determining urea pesticides

Urea pesticide	Matrix	Derivative conditions (Solvent of injection)	Injector Temp (°C)	Column	Detector	LOD	Determined compound	Ref
Chlorsulfuron Triasulfuron	Soil (50g)	Diazomethane/Ethyl acetate (Ethyl acetate)	250	25m x 0.2mm 0.33μm HP-5	NPD	0.1 ppb 0.2 ppb	Methyl derivative	[77]
Metabenzthiazuron Diuron	Water (0.5L)	(Cyclohexane-acetone)	250	20m x 0.32mm 0.25μm CP-sil 19CB 20m x 0.32mm 0.25μm CP-sil 5CB	NPD ECD	-	-	[31]
Chlorsulfuron Fluometuron Isoproturon Linuron Metobromuron Monuron	Water (2mL)	(Solvent free)	300	30m x 0.25mm 0.25μm BP10.	NPD ECD	0.05 ppb	Amine triazine Anilines	[48]
Diflubenzuron	Apple (25g)	Toluene/HFBA/Pyridine Room Temp/1.5-2h (Toluene)	225 240	25m x 0.32mm 0.52μm NB-1701. 25m x 0.32mm 0.52μm HP-5.	ECD	0.03 ppb	Heptafluorobutyryl derivative	[26]
Linuron Metobromuron	Crop samples (100g)	(Acetone-hexane)	250	30m x 0.25mm 0.25μm SPB-608	ECD	0.01 ppb	-	[24]

Urea pesticide	Matrix	Derivative conditions (Solvent of injection)	Injector Temp (°C)	Column	Detector	LOD	Determined compound	Ref
Flufenoxuron	Wine (3mL)	(Solvent free)	250	25m x 0.32mm 0.25μm HP-PAS 1701	ECD	13 ppb	-	[49]
Diuron	Sugar cane Orange (1kg)	(Acetone)	250	30m x 0.25mm 0.35μm 5% phenylmethylpolys iloxane	ECD		-	[78]
Chlortoluron Diuron Fluometuron Isoproturon Linuron Metobromuron Monuron	Standard and Water (100mL)	(Methanol)	300	30m x 0.25mm 0.25μm BP-10	NPD ECD MS (SIM)	0.1 pg	Carbamic acid methyl esters	[64,76]
Metabenzthiazuron							Benzothiazolami ne	
Diuron Linuron Monuron	Water (1L)	(n-Hexane)	220	30 m x 0.32mm DB-1	FTD	0.08 ppb 0.002 ppb 0.02 ppb	-	[63]
Linuron Monuron	Water (1 L)		240	25m x 0.25mm HP-5	MS (SIM)	0.01 ppb 0.005 ppb		
Fenuron Monuron Isoproturon Linuron	Standards	(Dichloromethane)	280	30m x 0.25mm 0.25μm DB5	MS	20.0 ppb	Phenyl isocyanates	[56]

Urea pesticide	Matrix	Derivative conditions (Solvent of injection)	Injector Temp (°C)	Column	Detector	LOD	Determined compound	Ref
Linuron Tebuthiuron	Water (1L)	(Hexane-2-propanol)			MS	0.02 ppb	-	[39]
Fluometuron Linuron Metobromuron Monolinuron	Water (1L)	(Ethyl acetate)	230	30m x 0.25mm 0.25µm DB-5ms	MS-ITD	0.005 ppb 0.05 ppb 0.005 ppb 0.005 ppb	Phenyl isocyanates	[38]
Diuron Isoproturon Chlortoluron Linuron	Water (500mL)	Dichloromethane/HFBA 37°C/ 1h (Dichloromethane)	Programmed 35°C (0.7min), 12°C/s -300°C	30m x 0.25mm 0.25µm DB-5ms	MS-ITD	0.09 ppb 0.09 ppb 0.08 ppb 0.15 ppb	Heptafluorobutyryl derivative	[12]
Linuron Diuron Neburon Isoproturon	Water (500mL)	HFBA/Pyridine 37°C/ 1h (Acetonitrile)		30m x 0.25mm 0.25µm DB-5	MS-ITD	0.1ppb	Heptafluorobutyryl derivative	[18]
Tebuthiuron	Milk (1L)	MBTFA/Toluene 90°C/2h (Toluene)	250	30m x 0.25mm 0.25µm DB-5	MS (SIM)	0.01 ppb	Trifluoroacetyl derivative	[79]
Tebuthiuron	Water (100mL)	Acetic anhydride/Toluene 270°C/2h (Acetone)	240	30m x 0.25mm 0.25µm DB5	MS (SIM)	0.02 ppb	Acetyl derivative	[80]

HFBA: Heptafluorobutyric anhydride
MBTFA: N-Methylbis-(trifluoroacetamide)

III.3.B. Liquid Chromatography

LC is the most commonly adapted technique for analyzing urea pesticides. A summary of methods based on LC is given in table 12. Many of these applications employ UV or diode array detection (DAD). PUHs [11,32,33,51] and SUHs [41,44,54] are determined currently in water with UV detection. PUHs [23], SUHs [25] and BUIs [21,22,46] can also been determined in fruits and vegetables with UV detection thanks to the previous processes of concentration and purification. LC with DAD performed satisfactorily for determining PUHs, SUHs and BUIs in water samples [14,15,18,34,35,40], fruits [27] and wine [19].

LC-MS technique is now widely applied to determine urea pesticides in food and water analysis [57]. Due to completely different ionization techniques like electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI), in contrast to electron impact ionization (EI), as well as different fragmentation mechanisms, new mass spectral libraries have been constructed [58]. In the last few years, interfaces based on atmospheric pressure ionization (API) increased in the number of applications [59].

The most abundant ions in the MS spectra of PUHs and sulfonylureas are produced in positive-ion mode $[M+H]^+$. ESI is an effective interface for assessing trace levels of PUHs and their major related degradation products [37,55] and sulfonylureas [42,43,52] in water and in cottonseed and cotton gin trash [29]. SUHs can also been determined in water using ESI in negative ion mode [16]. PUHs are best ionized as positive ions with APCI [16,60] and determined in water samples. APCI spectra of BUIs typically provided intense molecular ions in negative-ion mode allowing acquisition of primarily molecular weight information rather than promoting fragmentation. An LC/APCI-MS method was applied to determine these compounds in mushrooms [61], plums, strawberries and blackcurrant-based fruit drinks [62] and in citrus fruits [47].

Table 12. Liquid chromatographic methods for determining urea pesticides

Type of compound	Matrix	Stationary phase	Mobile phase	Detection	LOD	Ref.
Phenylureas	Water	Whatman C_{18} Partisphere	Methanol/water/buffer pH 7	UV (225, 254nm)	0.4 ppb	[32]
Phenylureas	Water	4.6 x 150 mm Supelcosil LC-18-DB	Gradient acetonitrile/water	UV (252nm)	1.6 ppb	[33]
Phenylureas	Water	4.6 x 30 mm, 5 μm Supelcosil LC-18DB	Gradient acetonitrile/phosphate buffer pH 7	UV (244nm)	0.05-0.5 ppb	[51]
Phenylurea	Water	4.6 x 30 mm, 5 μm Spherisorb ODS-2 4.6 x 100 mm, 3 μm Microsphere C_{18}	Gradient methanol/water	UV (250, 277 nm)	0.1 ppb	[11]
Sulfonylureas	Soil and water	4.6 x 250 mm, 5 μm Zorbax SB-Phenyl	Water/acetonitrile	UV (245nm)	0.1 ppb	[41]
Sulfonylureas	Water	4.6 x 250 mm, 5 μm Zorbax SB-Phenyl	Phosphoric acid-potassium phosphate pH 2.7	UV (245nm)	0.1 ppb	[44]
Benzoylureas	Plants	4.6 x 250 mm, 5 μm LiChrosorb RP-18	Methanol/water	UV (245nm)	1 ppm	[21]
Benzoylureas	Citrus	4.6 x 250 mm, 5 μm Kromasil C_{18}	Gradient acetonitrile/water	UV (200 nm)	0.2 ppm	[46]
Phenylureas	Vegetables	4.6 x 250 mm, 5μm LiChrospher R18	Gradient methanol-acetonitrile/water	UV (242 nm)	0.01 ppm	[23]
Sulfonylureas	Rice and crayfish	4.6 x 150 mm, 5 μm Zorbax SB-Phenyl 4.6 x 250 mm, 5 μm Zorbax RX-C$_8$	Potassium phosphate pH 7.6 Potassium phosphate pH 3.2 Acetonitrile	UV (254 nm)	8 ppb	[25]
Benzoylureas	Apples and pears	2.1 x 250 mm, 5 μm ODS Hypersil C_{18}	Gradient methanol/water	UV (260 nm)	0.02 ppm	[22]

Type of compound	Matrix	Stationary phase	Mobile phase	Detection	LOD	Ref.
Phenylureas Benzoylureas	Water	4.6 x 250 mm, 5 μm Zorbax SB-C$_{18}$	Gradient acetonitrile/water	DAD (240nm)	0.5 ppm	[14]
Phenylureas	Water	250 mm TSK ODS 80TM C$_{18}$	Gradient acetonitrile/water	DAD (220nm)	0.1 ppm	[18]
Phenylureas	Water	4.6 x 250 mm, 5 μm LiChrosorb RP-18	Gradient methanol/water	DAD (250nm)	20 ppb	[34]
Sulfonylureas	Soil and water	4.6 x 120 mm, 5 μm Viospher C$_6$ 4.6 x 250 mm, 5 μm Beckman C$_{18}$	Gradient 0.01% HClO$_4$ in water-methanol	DAD (224-234nm)	1ppb	[35]
Phenylureas	Soil and water	4.6 x 250 mm, 5 μm Toso Haas 80 TM C$_{18}$	Gradient acetonitrile/water	DAD (249nm)	0.3 ppb	[15]
Phenylureas	Water	46 x 250 mm, 5 μm Baker C$_{18}$	Methanol/water	DAD (249nm)	0.1 ppb	[40]
Benzoylureas	Apples	3 x 150 mm, 5 μm Separon SGX C$_8$	Methanol-water	DAD (260nm)	0.1 ppm	[27]
Benzoylureas	Grapes and wines	2.1 x 250 mm, 5 μm ODS Hypersil C$_{18}$	Gradient methanol-water	DAD (260nm)	0.01ppm	[19]
Phenylureas	Water	4.6 x 250 mm, 5 μm Alltima C$_{18}$	Gradient acetonitrile/water	ESI-MS	0.08 ppb	[37]
Phenylureas	Water	2.1 x 150 mm, Zorbax RX-C$_{18}$	Gradient acetonitrile/water	ESI-MS	0.04 ppb	[55]
Sulfonylureas	Water	3 x 125 mm, 5 μm LiChrospher 60 RP select B	Gradient methanol/water	ESI-MS	0.05 ppb	[52]
Sulfonylureas	Water	2 x 150 mm, 3 μm Metasil basic	Gradient acetonitrile/ammonium formate-formic acid buffer pH 3.7	ESI-MS	0.01ppb	[42,43]

Type of compound	Matrix	Stationary phase	Mobile phase	Detection	LOD	Ref.
Phenylureas	Water	4.6 x 250 mm, 5 μm Hypersil ODS	Gradient acetonitrile/water	APCI-MS	0.02 ppb	[16]
Phenylureas	Water	4.6 x 250 mm, 5 μm Alltima C_{18}	Methanol/water	APCI-MS	0.5 ppb	[60]
Benzoylurea	Mushrooms	4.6 x 250 mm, S5ODS2	Gradient methanol/water	APCI-MS	17 ppb	[61]
Benzoylurea	Citrus	4.6 x 30 mm, 5 μm Kromasil C_{18}	Gradient methanol/water	APCI-MS	2 ppb	[47]

III.3.C. Mass Spectrometry

GC-MS is routinely used for monitoring pesticides in aqueous samples. Several methods have been reported for the determination of either the intact ureas [38,63] or their breakdown products [56,64]. The mass spectra of the urea derivatives obtained with electronic impact mode (-70 eV) show few fragments and an intense molecular ion $[M]^+$, for this, an adequate selection of few m/z ions (two or three) to perform a SIM provides both good sensitivity and correct identification of such derivatives.

Polar pesticides are the most likely to leach to ground water that is way analytical methods based on LC are often preferred and, in particular methods using LC-MS. Different interfaces such as particle beam (PB) [65], thermospray, and atmospheric pressure ionization (API) have been used for urea pesticides. However during the last few years, API techniques, high flow pneumatically assisted electrospray ionization (ES) [36,66-69], or ionspray (ISP) [70] and atmospheric pressure chemical ionization (APCI) [65,71], both of these soft ionization modes, have become the most popular interfacing techniques. Table 13 details the instrument sensitivity of several GC-MS and LC-MS methods using different interfaces employed to analyze urea pesticides in several matrices as well as the searched m/z ions. The sensitivity of GC-MS is very good, instrumental conditions are completely reproducible and the high separation power of capillary columns can be used. On the other hand LC-MS techniques become more and more sensitive and allow the determination of intact urea pesticides.

III.3.D. Capillary Electrophoresis

Capillary electrophoresis (CE) is rapidly becoming an important tool for the separation of a wide variety of compounds (Table 14). To apply CE for monitoring studies of pesticides, particular attention has to be devoted to the optimization of the separation in order to obtain the best selectivity in a complex matrix as vegetables, where many potential compounds may interfere. There are several separation modes. In micellar electrokinetic chromatography (MEKC), the potential applied across a fused-silica capillary is the driving for migration, as in capillary zone electrophoresis

(CZE) but MEKC involves the addition of an anionic micellar additive as sodium dodecyl sulfate (SDS) to the working electrolyte. This allows the separation of non-ionic compounds [72]. Experimental retention factors of the phenylurea herbicides, measured using MEKC with working electrolytes containing various micellar concentrations of SDS shows a good agreement with the retention data predicted using the polarity and the lipophilicity indices. MEKC can be used for separations using working electrolytes containing SDS at concentration upper than the critical micellar concentration (CMC) [73]. Micellar, mixed micellar and microemulsion electrokinetic chromatography (EKC) was applied to the separation of phenylureas and chlorsulfuron showing that the separation efficiencies in mixed micellar and microemulsion EKC were higher than in micellar EKC [74,75]. Sulfonylurea herbicides and their degradation products may be simultaneously detected and separated by MEKC permitting the quantitation of these compounds at the ng/g in water [13,17], mushrooms [61] and grains [30]. CZE offers also a fast and reliable method to determine sulfonylurea herbicides in water [20,36,44].

Table 13. Instrument sensitivity of liquid and gas chromatographic techniques coupled to mass spectrometric detectors

Technique	Matrix	Injected herbicide	Selected *m/z* ions	IDL (ng injected) *	Ref.
GC-IT-MS	Water	Fluometuron	72	≈ 0.084	[38]
		Linuron	61	1	
		Metobromuron	61	≈ 0.200	
		Monolinuron	61	0.010	
GC-MS	Water	Linuron	248, 250	0.0075	[63]
		Monuron	198, 200	0.015	
GC-MS	Water	Isoproturon	Scan (29-350)	0.25	[56]
		Fenuron		0.3	
		Monuron		0.35	
		Linuron		0.15	
GC-MS	Water	Chlorsulfuron	191, 128, 111	0.0001	[64]
		Chlortoluron	199, 167, 154	0.0006	
		Diuron	219, 187	0.001	
		Fluometuron	219, 187, 174	0.0009	
		Isoproturon	193, 178, 146	0.0004	
		Linuron	219, 187, 174	0.001	
		Metabenzthiazuron	164, 136	0.0004	
		Metobromuron	229, 197, 184	0.0004	
		Monuron	185, 153, 140	0.0005	
LC-APCI-MS	Water	Isoproturon	207	0.4	[65]
LC-PB(EI)-MS			72	10	
LC-PB(PChI)-MS			205	4	

Technique	Matrix	Injected herbicide	Selected m/z ions	IDL (ng injected) *	Ref.
LC-ES-MS	Water	Bensulfuron-methyl	411	1.875	[36]
		Chlorsulfuron	358	9.375	
		Chlorimuron-ethyl	415	5.625	
		Halosulfuron-methyl	435	7.5	
		Metsulfuron-methyl	382	5.625	
		Nicosulfuron	411	22.5	
		Primisulfuron-methyl	469	9.375	
		Prosulfuron	420	3.75	
		Sulfometuron-methyl	365	3.75	
		Thifensulfuron-methyl	388	7.5	
		Triasulfuron	402	7.5	
		Triflusulfuron-methyl	493	3.75	
LC/ES/MS	Water	Chlortoluron	213	0.288	[66]
		Isoproturon	207	0.065	
		Diuron	233	0.317	
		Linuron	249	0.561	
		Methabenzthiazuron	165	0.211	
		Neburon	276	0.252	
LC-ES-MS	Water	Bensulfuron-methyl	182, 213, 411	1.08	[67]
		Chlorsulfuron	141, 167, 358	1.89	
		Metsulfuron-methyl	167, 199, 382	2.16	
		Rimsulfuron	182, 325, 432	4.05	
		Thifensulfuron-methyl	141, 167, 388	1.89	
		Triasulfuron	141, 167, 402	1.62	
		Tribenuron-nethyl	155, 181, 396	2.97	

Technique	Matrix	Injected herbicide	Selected *m/z* ions	IDL (ng injected) *	Ref.
LC-ESNI-MS	Soil and water	Chlorsulfuron Metsulfuron-methyl Thifensulfuron-methyl Tribenuron-methyl	356 380 386 394	≈ 0.040 ≈ 0.040 ≈ 0.040 ≈ 0.040	[68]
LC-ES-MS-MS	Water	Chlorsulfuron Metsulfuron-methyl Thifensulfuron-methyl Triasulfuron	356 (primary), 139 (secondary) 380 (primary), 139 (secondary) 386 (primary), 139 (secondary) 400 (primary), 139 (secondary)	0.350 0.300 0.300 0.15	[69]
LC-ISP-MS	Water	Linuron Monolinuron	249, 161 215, 127	0.0215 0.0134	[70]
LC-APCI-MS	Citrus	Diflubenzuron Flufenoxuron Hexaflumuron	309, 289 487, 467 459, 439	0.01 0.01 0.01	[47]
LC-APCI-MS-MS	Water	Chlorbromuron Chlortoluron Diuron Linuron Metoxuron Monuron Neburon	292.9 (primary) 182.2 (secondary) 213.0 (primary) 72.0 (secondary) 233.0 (primary) 72.0 (secondary) 249.0 (primary) 282.2 (secondary) 229.1 (primary) 72.0 (secondary) 199.1 (primary) 72.1 (secondary) 275.3 (primary) 88.1 (secondary)	0.030-0.200 (QqQ) 1-2 (Ion Trap)	[71]
LC-ES-MS	Soil	Chlorsulfuron Metsulfuron methyl Thifensulfuron Triasulfuron	141, 167, 358 141,167, 264, 388 141, 167, 264, 382 141, 167, 402	0.3125	[81]

* Calculated from data reported by authors

** Determined as phenylisocyanate

IDL Instrument detection limit

Table 14. Capillary Electrophoresis methods for determining urea pesticides

CE method	Matrix	Buffer (pH)	Detection (λ nm)	Injected herbicide	LOD	Ref.
CE-MEKC	Standards	0.1M SDS (8.5)	UV (234)	Fenuron Metoxuron Linuron	-	[73]
CE-MEKC	Standards	12.4 mM Potassium dihydrogen-phosphate 3.8 mM Sodium Borate 250 mM SDS (7.0)	UV (254)	Chlorsulfuron Chlortoluron Diuron Linuron Fenuron Fluometuron Monuron Linuron	-	[74]
CE-MEKC	Water Soil	25 mM Sodium phosphate 50 mM Lithium dodecyl sulfate (6.5)	UV (214)	Primisulfuron Triasulfuron	50 ppb	[17]
CE-MEKC	Soil	30 mM Sodium borate 80 mM SDS (7.0)	UV (239)	Metsulfuron Chlorimuron Chlorsulfuron	10 ppt	[13]
CE-MEKC	Grains	50 mM SDS 25 mM Sodium phosphate (6.15)	UV (234)	Chlorsulfuron Metsulfuron-methyl Rimsulfuron Thifensulfuron Tribenuron-methyl	0.02 ppt 0.02 ppt 0.035 ppt 0.02 ppt 0.035 ppt	[30]

CE method	Matrix	Buffer (pH)	Detection (λ nm)	Injected herbicide	LOD	Ref.
CE-CZE	Water	50 mM Ammonium acetate (4.75)	UV (240)	Bensulfuron-methyl Chlorimuron-ethyl Chlorsulfuron Halosulfuron-methyl Metsulfuron-methyl Prosulfuron Primisulfuron-methyl Sulfometuron-methyl Thifensulfuron-methyl Triasulfuron Triflusulfuron-methyl	0.2 ppt	[36]
CE- CZE	Water	50 mM Ammonium acetate (4.76)	UV (240)	Bensulfuron-methyl Chlorimuron-ethyl Chlorsulfuron Ethametsulfuron Halosulfuron-methyl Metsulfuron-methyl Primisulfuron-methyl Sulfometuron-methyl Thifensulfuron-methyl Triasulfuron Triflusulfuron-methyl	0.1 ppt	[44]

CE method	Matrix	Buffer (pH)	Detection (λ nm)	Injected herbicide	LOD	Ref.
CE-CZE	Water Sediments	25 mM Acetic acid 25 mM sodium acetate (4.7)	UV (239)	Amidosulfuron Bensulfuron-methyl Chlorimuron-ethyl Chlorsulfuron Ethametsulfuron-methyl Metsulfuron-methyl Nicosulfuron Sulfometuron-methyl Thifensulfuron-methyl Triasulfuron	0.1 ppb 0.02 ppb	[20]
CE-CZE	Soil	50 mM Ammonium acetate (4.76)	UV (239)	Amidosulfuron Bensulfuron-methyl Chlorimuron-ethyl Chlorsulfuron Ethametsulfuron-methyl Metsulfuron-methyl Nicosulfuron Primisulfuron-methyl Sulfometuron-methyl Thifensulfuron-methyl Triasulfuron Tribenuron methyl	2 ppb	[82]

III.4. REAL SAMPLES

Considerable research activities have been done to increase the sensitivity of the methods and their reability at trace levels to accomplish the exigencies of the laws. At very low concentrations, matrix interference from complexes samples as foods could cause errors in the final results and consequently a purification step is required. Water is usually a more simple matrix and clean-up can be avoided if selective extraction and detection techniques are used (see tables 9 and 10).

III.4.A. Water and Soil

The levels of urea herbicides found in real samples are very low (μg L^{-1} order), that is because its own physico-chemical properties (see the octanol/water partition coefficient and the water solubility of PUHs and BUIs in table 1), its environmental behaviour (see the field dissipation half life and half life in soil in table 8), and its low application rate (see table 8).

Real water samples have been analyzed by GC using direct and indirect determination of urea herbicides in surface and ground water [18,39,48,76]. In the indirect analysis of phenylurea pesticides the solvent used for injection has influence on the determined compound. In this sense, phenylcarbamic acid alkyl ester derivatives are determined when methanol or ethanol is used, phenylisocyanates are determined when acetonitrile or dichloromethane is used [64] and phenylamines are formed when water is present as occurs in SPME [48]. For samples analyzed by GC techniques, typical reported levels in waters are as low as 3.5-11.4 μg L^{-1} for chlortoluron in irrigation channels for citrus fields [76], and 0.28 μg L^{-1} for isoproturon and 1.55 μg L^{-1} for diuron in surface waters [18].

LC was applied to the determination of PUHs [11,15,32-34,40,41,51], BUIs [14] and SUHs [35,41-44,52] in surface and ground water samples. Nicosulfuron is one of the most frequently detected herbicide in surface waters from the Midwestern of USA [43], whereas diuron is a commonly detected compound in surface waters from central and southeastern regions of France [16]. A residue of 23.1 μg L^{-1} of diuron was found

in Lake Creek stream water [33]. It is one of the highest concentrations reported for urea herbicides in waters when analysis is performed following LC techniques.

III.4.B. Foods

Urea pesticide residues in food have been researched by GC, particularly diflubenzuron in apples [26], linuron, metobromuron and monolinuron in routine crop monitoring [24], diuron in orange and sugar cane [78] and flufenoxuron in wines [49]. After a field treatment applied 55 days before sampling, 0.10 mg kg^{-1} of diflubenzuron residue was found in apples [26]. As a result of a controlled application of diuron (6400g ha^{-1}) following a good agricultural practice in orange and sugar cane fields from Sao Paulo (Brazil) the diuron residues quantified were of 8.9-11.3 μg kg^{-1} for orange samples and 5.3-6.6 μg kg^{-1} for sugar cane samples [78]. 7 Portuguese red wine and 5 white wine samples were analyzed for flufenoxuron and other pesticides and no residue was found [49].

LC permits the determination of bensulfuron methyl in rice and crayfish [25] and BUIs in citrus fruits [47] grapes and wine [19]. Diflubenzuron, hexaflumuron and flufenoxuron were found in several citrus fruit samples from Spain but none sample exceeded the maximum residue levels established in such State [47]. Teflubenzuron and flufenoxuron were determined in grapes from treated Greek fields and produced wines. Their residues were higher in grapes (0.52 mg kg^{-1} for teflubenzuron and 0.20 mg kg^{-1} for flufenoxuron) than in wines (0.012 mg L^{-1} for teflubenzuron and 0.010 μg kg^{-1} for flufenoxuron) [19].

III.5. CONCLUSIONS

The extraction with organic solvents is still used from the isolation of urea pesticides from food and water samples. The solid-phases are now preferred to analyze water samples as well as food samples by using solid-phase extraction and matrix solid-phase dispersion procedures.

GC is preferably used for analysis of volatile and thermostable pesticides but the well-established methods with selective detectors allow the determination of some urea pesticides. However, multiresidue analysis including thermally labile compounds over a wide range of polarity is generally performed by LC and CE techniques. The larger number of applications has been performed by LC. Approximately half uses UV and DAD detectors and the other half MS detector. In the last few years LC-MS methods operating with different interfaces have been proposed showing the suitability of these techniques. The interfaces ESI and APCI are considered to have a promising future.

CE is an emerging technique complementary to GC and LC with applications in the area of urea pesticides. The advantages of CE for monitoring routine analyses are the simplicity of the instrumentation, the low solvent consumption and the easy equipment maintenance with respect to chromatographic techniques. The main limitation of CE is the low loadability of the system that allows the achievement of impressive detection limits without preconcentration or sample stacking techniques to enhance sensitivity. In recent years the employ of MSD configured with ESI or APCI interfaces will permit to extend the applications of this technique. Nevertheless, the potential of CE has non been fully exploited in many fields of research, including environmental analyses for urea pesticides.

Monitoring of vegetables and water sources will become extremely important and the methods applied will be intended to achieve efficient and rapid separations whatever the technique may be employed.

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ESTUDIO

EXPERIMENTAL

IV.1. Influence of the solvent on the Gas Chromatographic

Behaviour of Urea Herbicides

H. Berrada, G. Font and J.C. Moltó.

ESTUDIO EXPERIMENTAL

ABSTRACT

Degradation products of chlorsulfuron, chlortoluron, diuron, fluometuron, isoproturon, linuron, metabenzthiazuron, metobromuron, and monuron formed in the gas chromatographic injector were used for identification of their respective herbicide. The spectra of the derived compounds were obtained with a quadrupole mass spectrometric detector working in scan mode (20-450 amu). The solvent used for phenylurea herbicide injection (ethanol, methanol, dichloromethane, and acetonitrile) had influence on the generated compounds. When methanol and ethanol were used as solvents, the major products formed from phenylureas were carbamic acid esters. When acetonitrile or dichloromethane were used, the main derivatives were phenylisocyanates. However, chlorsulfuron and metabenzthiazuron generated a triazine plus a phenylsulfonamide and a benzothiazolamine respectively regardless of the assayed solvent. Linuron and diuron showed the same behaviour and gave degradation products with the same mass spectra. The thermal reactions occurred instantaneously in the injector block favoured by the high selected temperature (300°C).

The derived compounds from urea herbicides can be determined using a BP10-30m column and a Selected Ion Registering (SIR) program based on two to three m/z ions as a way to sensitively detect the presence of urea herbicides in environmental extracts. With standards in methanol the instrumental detection limits ranged from 0.1 pg for chlorsulfuron (detected as 2-chlorobenzensulfonamide) to 1 pg for monuron and metobromuron (both detected as their analogous carbamic acid methyl esters). The RSD were below 9 % at the 5 ng·L^{-1} level. The response was linear ($r > 0.9986$) within the 5 ng·L^{-1} to 25 μg·L^{-1} range. The unequivocal identification of some phenylurea herbicides was not always possible because some herbicides having analogous structures such as diuron and linuron gave the same derivative.

KEYWORDS Gas chromatography-mass spectrometry, Urea herbicides, Thermal degradation, Phenylisocyanates.

IV.1.1. INTRODUCTION

Urea pesticides are very useful in weed control. They are intensely applied on cereal, vegetable, citrus, cotton, sunflower and other crops. Chemically they are formed by a urea bridge substituted by a triazine, benzothiazol, sulfonyl, phenyl, alkyl or other moieties. Besides herbicidal activity, some analogous structures present other biological activities. For example, some derivatives of sulfonylurea are anti-diabetic drugs. In addition, the possible activity as carcinogens makes it necessary to control their residues in crops and the environment. The environmental fate of some urea herbicides has been investigated either using laboratory experiences or field studies [1, 2].

The most sensitive methods for identifying and determining intact urea herbicides at trace levels are based on liquid chromatography with mass spectrometric techniques (LC-MS). Different LC-MS interfaces like thermospray (TS) [3, 4], particle beam (PB) [5, 6], and atmospheric pressure ionization (API) either with electrospray (ES) [7-11], or atmospheric pressure chemical ionization (APCI) [6, 12] have been proposed to determine phenyl- and sulfonylurea herbicides.

Analysis of urea herbicide residues is difficult. Liquid chromatographic methods allow determination of intact urea pesticides, but the sensitivity and separation efficiency are poorer than with gas chromatography (GC). Moreover, LC-MS mass spectra are dependent on systems and conditions, and matrix effects sometimes modify the signal intensity. LC coupled to a triple quadrupole is a powerful tool in pesticide residue analysis [13], but its use is reduced at present to a few laboratories. On the other hand, thermal instability of urea herbicides make it difficult to determine them by GC. In spite of that, several gas chromatographic methods to determine either intact urea or their breakdown products in water [14-18], soil [19, 20], milk [21], cereals [22] and fruits and vegetables [23-28] have been reported. Some of these breakdown products have been identified as triazines [21, 29], sulfonamides [29], isocyanates [16, 20, 22, 27, 28] and anilines [16, 20, 22, 28].

The products generated by thermal decomposition can be useful as a fast sensitive way to detect their respective urea herbicide precursors. The objective of this study was to ascertain the identity of the compounds formed during capillary gas chromatography and their ability to be used for environmental residue analysis. Urea pesticides including substituted phenyl- (chlortoluron, diuron, fluometuron, isoproturon, linuron, metobromuron, monuron), benzothiazol- (metabenzthiazuron), and triazine-/sulfonyl-(chlorsulfuron) structures were selected for this purpose.

IV.1.2. EXPERIMENTAL

IV.1.2.1. Chemicals

Herbicide standards, chlorsulfuron (1-(2-chlorophenylsulfonyl)-3-(4-methoxy-6-methyl-1,3,5-triazin-2-yl)urea) (Chlors), chlortoluron (3-(3-chloro-p-tolyl)-1,1-dimethylurea) (Chlort), diuron (3-(3,4-dichlorophenyl)-1,1-dimethylurea) (Diu), fluometuron (1,1-dimethyl-3-(α,α,α-trifluoro-m-tolylurea) (Fluo), isoproturon (1,1-dimethyl-3-(4-isopropylphenyl) urea) (Isop), linuron (3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea) (Lin), metabenzthiazuron (1-(benzothiazol-2-yl)-1,3-dimethylurea) (Mbz), metobromuron (3-(4-bromophenyl)-1-methoxy-1-methyl-urea) (Mbro), and monuron (3-(4-chlorophenyl)-1,1-dimethylurea) (Mon) were produced by Riedel de Haën (Seelze, Germany) with purities up to 95%.

Phenylisocyanate standards, 4-bromophenylisocyanate (Mbro1), 3,4-dichlorophenylisocyanate (Diu1), 4-isopropylphenylisocyanate (Isop1), 4-chlorophenylisocyanate (Mon1), and 3-trifluoromethylphenylisocyanate (Fluo1) with purities up to 97% were from Aldrich (Milwaukee, WI, USA).

Methanol, ethanol, acetonitrile and dichloromethane for residue analysis were purchased from Merck (Darmstadt, Germany).

Stock (500 $\mu g \cdot mL^{-1}$) and working solutions (25 $\mu g \cdot mL^{-1}$) of herbicides and isocyanates were freshly prepared in methanol, ethanol, acetonitrile and dichloromethane.

IV.1.2.2. Apparatus

Analyses were performed in a Fisons 8000 series gas chromatograph equipped with a conventional split/splitless injector and a quadrupole mass spectrometer Trio 1000 (Fisons Instruments, Milano, Italy). Spectra were operated on a LAB-BASE data station with NBS, NIST, and Wiley 6 spectral libraries.

The fused silica capillary columns were a 30 m BPX35 (35% phenylpolysilphenylene-siloxane) and a 30 m BP10 (14% cyanopropylphenyl 86% dimethyl polysiloxane), both with 0.25 μm thickness film and 0.25 mm I.D. from SGE (Austin, TX, USA).

IV.1.2.3. Gas Chromatography Mass-Spectrometric Conditions

The injector, transfer line and source temperatures were 300°C, 280°C and 200°C respectively. The oven temperature was programmed as follows: the initial temperature of 100°C was maintained for 2 min and then increased by 10°C·min^{-1} to 280°C and held for 15 min. The splitless time was 1 min. Helium was used as carrier gas at a flow rate of 2.7 mL·min^{-1}. The volume injected was 1 μL.

The mass spectrometer was used in electron impact mode (-70 eV). 20 to 450 amu were scanned to obtain full spectra of interesting compounds.

IV.1.3. RESULTS AND DISCUSSION

IV.1.3.1. Identification of the Peaks and Dependence on the Solvent
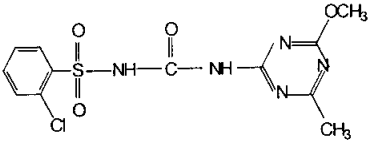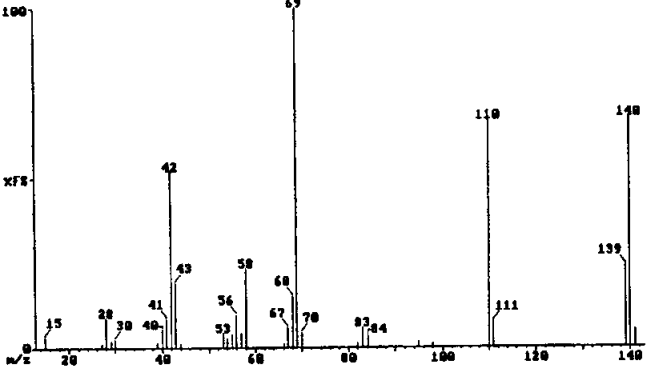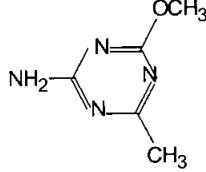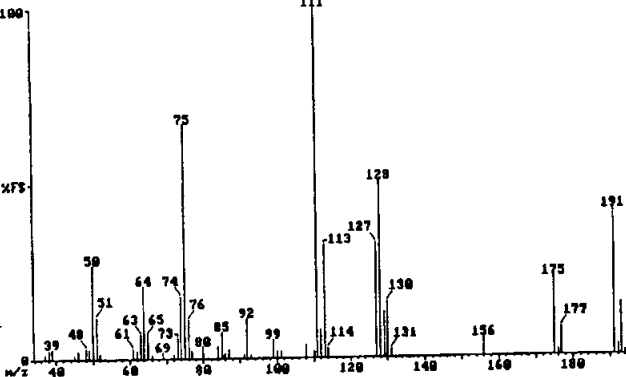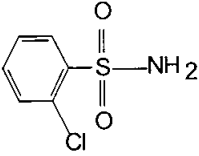
Gas chromatographic-mass spectrometric analysis performed in any column of standards of a phenylurea (chlortoluron, diuron, isoproturon, fluometuron, linuron, metobromuron, and monuron) or a benzothiazolurea (metabenzthiazuron) produced only one major peak on the chromatogram, whereas injection of sulfonylurea chlorsulfuron generated two peaks. Herbicides injected as single standard or as a mix of them showed the same reproducible behaviour. Nevertheless, changes in the retention times and different fragmentation patterns were observed when standards

were prepared in different solvents. Table 15 shows the structure of the herbicides injected on the BP10 column, the mass spectra of the major peak observed using different solvents for injection and the structures assigned to these spectra. Other non-interfering minor peaks (5% lower than the major peak) were not included in this table.

In the present study, the products formed from sulfonyl and benzothiazol ureas were not dependent on the solvent used for injection. On the contrary, the compounds generated from phenylureas were strongly dependent on the solvent. The major products from phenylureas were isocyanates when using dichloromethane or acetonitrile, methyl esters of carbamic acid when using methanol, and ethyl esters of carbamic acid when using ethanol. These compounds can be used for the detection of their respective urea precursors.

Since the urea bridge is quite labile, thermal cleavage on both sides of the carbonyl group to give derivated compounds was a priori expected. It is essentially what happened, but in practice only structures having a relatively high weight were useful for gas chromatographic analysis. For example, dimethyl- and methylamine fragments were ignored for this purpose. All the identified structures were substituted benzenes, triazines or a benzothiazol. Hence, herbicide structure cleavage conserved the stable aromatic ring with its respective moieties. Using the optimized analytical conditions, the high injector temperature (300°C) propitiated the degradation of herbicides, and intact ureas were practically not observed. Only traces of intact urea were identified when 100 ng of linuron and metobromuron were injected instead of 25 ng. It seems that the N-methoxyl group in the urea bridge of linuron and metobromuron make the molecule slightly more thermostable than the N-dimethyl substitution of the chlortoluron, diuron, isoproturon, metabenzthiazuron, and monuron structures. Other observed minor products were the homologous anilines.

Table 15. Herbicide structures. Mass spectra of the major compound generated in capillary g- chromatographic analysis and their assigned structural formulae

HERBICIDE STRUCTURE	DERIVATIVE COMPOUNDS	
	SPECTRUM	ASSIGNED STRUCTURE
Chlorsulfuron (Chlors)		2-amino-4-methoxy-6-methyl-1,3,5-triazine (Chlors1) MW=140 Formed in any solvent
MW = 357		
		2-chlorobenzensulfonamide (Chlors2) MW=191 Formed in any solvent

Table 15. Continued
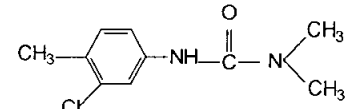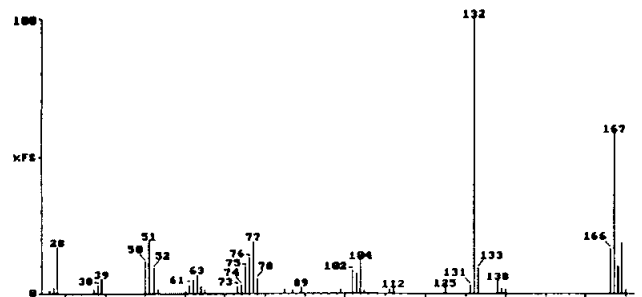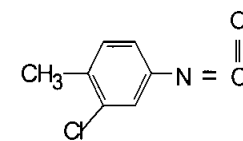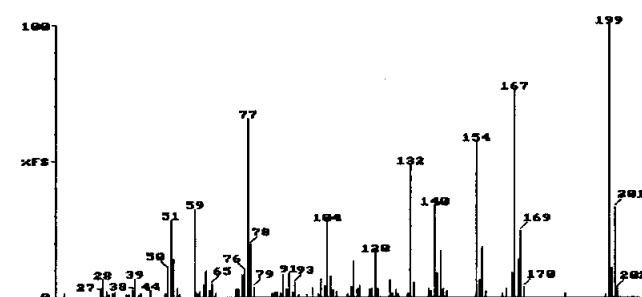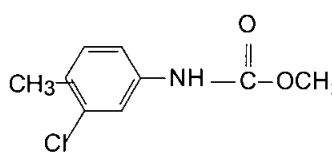
DERIVATIVE COMPOUNDS

HERBICIDE STRUCTURE	SPECTRUM	ASSIGNED STRUCTURE
Chlortoluron (Chlort) MW = 212		3-chloro-4-methylphenylisocyanate (Chlort1) MW=167 Formed in DCM and in ACN

3-chloro-4-methylphenylcarbamic acid, methyl ester
(Chlort2) MW=199
Formed in methanol

Table I. Continued.

HERBICIDE STRUCTURE	DERIVATIVE COMPOUNDS	
	SPECTRUM	ASSIGNED STRUCTURE

3-chloro-4-methylphenylcarbamic acid,
ethyl ester
(Chlort3) MW=213
Formed in ethanol

Diuron (Diu)
MW=232

3,4-dichlorophenylisocyanate
(Diu1) MW=187
Formed in DCM and in ACN

Table 15. Continued

DERIVATIVE COMPOUNDS

HERBICIDE STRUCTURE	SPECTRUM	ASSIGNED STRUCTURE
		3,4-dichlorophenylcarbamic acid, methyl ester (Diu2) MW=219 Formed in methanol
		3,4-dichlorophenylcarbamic acid, ethyl ester (Diu3) MW=233 Formed in ethanol

Table 15. Continued
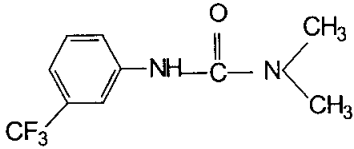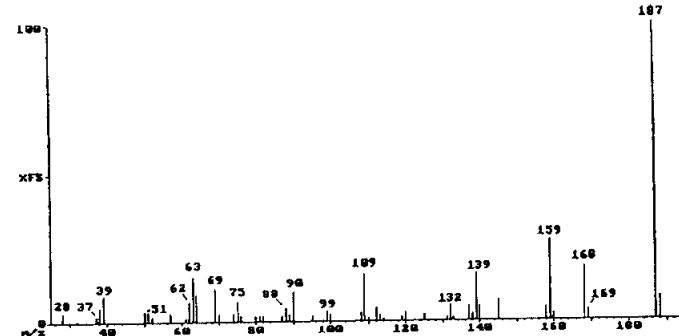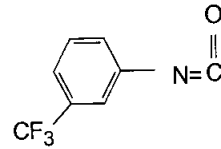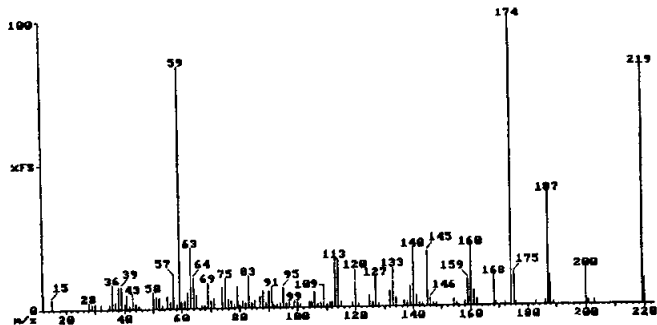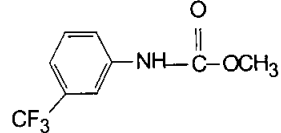
DERIVATIVE COMPOUNDS

HERBICIDE STRUCTURE	SPECTRUM	ASSIGNED STRUCTURE
Fluometuron (Fluo) MW=232		3-trifluoromethylisocyanate (Fluo1) MW=187 Formed in DCM and in ACN
		3-trifluomethylphenylcarbamic acid, methyl ester (Fluo2) MW = 219 Formed in methanol

Table 15. Continued

DERIVATIVE COMPOUNDS

HERBICIDE STRUCTURE	SPECTRUM	ASSIGNED STRUCTURE

3-trifluomethylphenylcarbamic acid,
ethyl ester
(Fluo3) MW = 233
Formed in ethanol

Isoproturon (Isop)
MW = 206

4-isopropylphenylisocyanate
(Isop1) MW = 161
Formed in DCM and in ACN

Table 15. Continued

DERIVATIVE COMPOUNDS

HERBICIDE STRUCTURE	SPECTRUM	ASSIGNED STRUCTURE
		4-isopropylphenylcarbamic acid, methyl ester (Isop2) MW = 193 Formed in methanol
		4-isopropylphenylcarbamic acid, ethyl ester (Isop3) MW = 207 Formed in ethanol

69

Table 15. Continued.

DERIVATIVE COMPOUNDS

HERBICIDE STRUCTURE	SPECTRUM	ASSIGNED STRUCTURE
Linuron (Lin) MW = 248		3,4-dichlorophenylisocyanate (Lin1) MW = 187 (identical to Diu1) Formed in DCM and in ACN
		3,4-dichlorophenylcarbamic acid, methyl ester (Lin2) MW = 219 (identical to Diu2) Formed in methanol

Table 15. Continued

HERBICIDE STRUCTURE	DERIVATIVE COMPOUNDS	
	SPECTRUM	ASSIGNED STRUCTURE

3,4-dichlorophenylcarbamic acid,
ethyl ester
(Lin3) MW = 233 (identical to Diu3)
Formed in ethanol

Metabenzthiazuron (Mbz)
MW = 221

N-methylbenzothiazolamine
(Mbz1) MW = 164
Formed in any solvent

71

Table 15. Continued
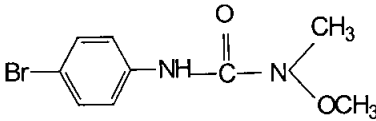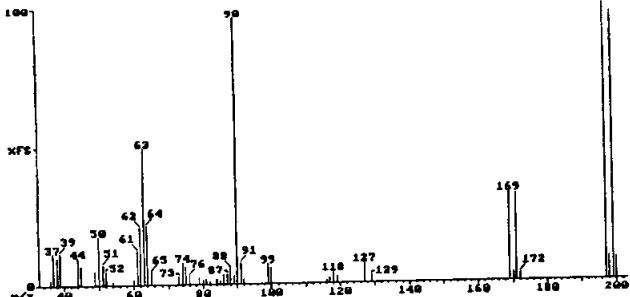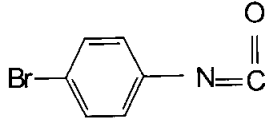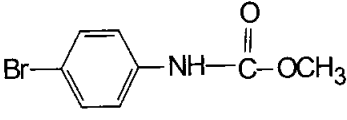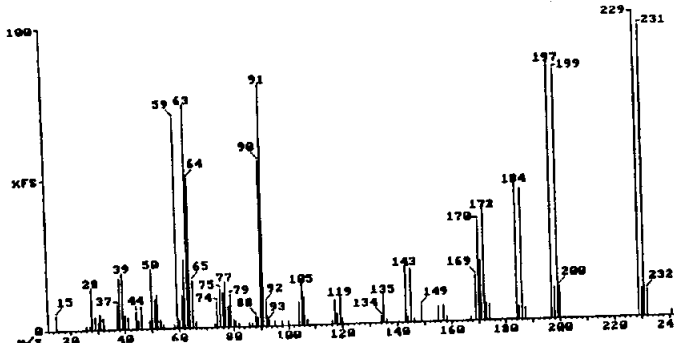
DERIVATIVE COMPOUNDS

HERBICIDE STRUCTURE	SPECTRUM	ASSIGNED STRUCTURE

Metobromuron (Mbro)
MW = 258

4-bromophenylisocyanate
(Mbro1) MW = 197
Formed in DCM and in ACN

4-bromophenylcarbamic acid,
methyl ester
(Mbro2) MW = 229
Formed in methanol

Table 15. continued.

HERBICIDE STRUCTURE	DERIVATIVE COMPOUNDS	
	SPECTRUM	ASSIGNED STRUCTURE

4-bromophenylcarbamic acid,
ethyl ester
(Mbro3) MW = 243
Formed in ethanol

Monuron (Mon)
MW = 198

4-chlorophenylisocyanate
(Mon1) MW = 153
Formed in DCM and in ACN

Table 15. Continued

HERBICIDE STRUCTURE	SPECTRUM	ASSIGNED STRUCTURE

ASSIGNED STRUCTURE

4-chlorophenylcarbamic acid,
methyl ester
(Mon2) MW = 185
Formed in methanol
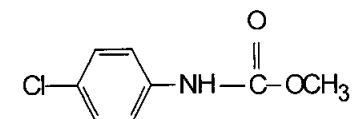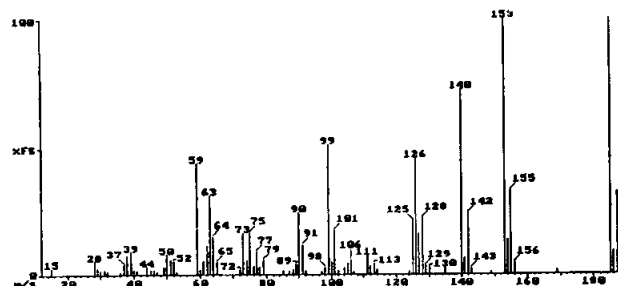
4-chlorophenylcarbamic acid,
ethyl ester
(Mon3) MW = 199
Formed in ethanol
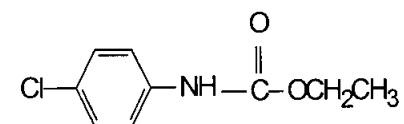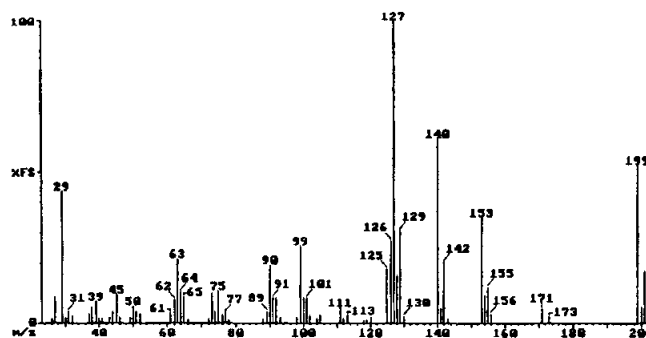
The presence of water in the injecting solvent could facilitate aniline formation by hydrolysis and decarboxylation of the isocyanates. This fact must be present when water-miscible solvents such as alcohols, acetone, acetonitrile or ethyl acetate are utilized. Since water, alcohols and other solvents tend to form distinct products, a mixture of solvents should be carefully avoided in gas chromatographic analysis of phenylurea pesticides.

Among the studied solvents, methanol was selected because it is water free, economical, and selected ureas have good solubility in it.

Injections of the selected urea herbicides in the BPX35 column generated the same products described before. All the identified compounds were completely separated either in the PBX35 or in the PB10 column. The BP10 was finally selected because it produced better chromatographic peaks for the methyl esters of the carbamic acid.

Some of the products formed from urea herbicides in gas chromatographic analysis have already been identified in the literature. An aminotriazine [21, 29] and a sulfonamide [29] were formed from chlorsulfuron. Hydrolysis of chlorsulfuron also produced chlorobenzenesulfonamide and aminotriazine as the major metabolites both in laboratory [1] and in field [2] studies. Phenylisocyanates [16, 20, 22, 27, 28] and anilines [16, 20, 22, 28] have also been reported as derivatives of phenylurea herbicides. Anilines were only detected as minor products. In these reports the influence of the solvent was not mentioned. Formation of substituted phenylisocyanates from their respective phenylurea precursors coincided with the use of acetone [27], ethyl acetate [16, 22], hexane [20] or dichloromethane [28]. Gas chromatographic determination of metabenzthiazuron has not been reported.

Phenylisocyanates have been reported to be the compounds determined in gas chromatographic analysis of phenylurea herbicides. Nevertheless, injections of 3,4-dichlorophenylisocyanate (Diu1), 3-trifluoromethylphenylisocyanate (Fluo1), 4-isopropylphenylisocyanate (Isop1), 4-bromophenylisocyanate (Mbro1), and 4-chlorophenylisocyanate (Mon1) in methanol generated the respective methyl esters of the carbamic acid, Diu2, Fluo2, Isop2, Mbro2, and Mon2.

Intact phenylisocyanates were not detected. Thus, the thermal reaction forms the more thermostable products. In the analytical conditions, they were the methyl esters of carbamic acid. Such reaction occurred at the injector block almost instantaneously at the selected temperature (300°C).

IV.1.3.2. Interpretation of Mass Spectra

As can be seen in Table 15, the mass spectra of the all identified derived compounds contained the molecular ion $[M]^{+\cdot}$ of the major derivated product with a relative abundance of more than 31% (Isop2). The molecular ion was the base peak for nine identified compounds (Chlort2, Diu1, Fluo1, Lin1, Mbz1, Mbro1, Mbro2, Mon1 and Mon2). According to the stability of the aromatic rings, fragmentation generally showed characteristic ions with m/z values higher than 77, which are recommended as diagnostic ions.

Chlors1 mass spectra showed a base peak at m/z 69 $[C_3H_5N_2]^+$ and two other abundant ions, m/z 140 $[M]^{+\cdot}$ and 110 $[M-30, CH_2O]^+$. The mass spectra of Chlors2 displayed ions at m/z 111 (base peak), 128, 175 and 191 corresponding to $[M-80, SO_2$ and $NH_2]^+$, $[M-64, SO_2]^+$, $[M-16, NH_2]^+$, and $[M]^{+\cdot}$ respectively.

The mass spectra of all the observed phenylisocyanates (Chlort1, Diu1, Fluo1, Isop1, Lin1, Mbro1 and Mon1) had similar fragmentation patterns. The molecular ion was always intense. Phenylisocyanates bearing halogen atoms gave intense ions corresponding to losses of halogens and/or a CO fragments generating ions at m/z 132 $[M-35, Cl]^+$ for Chlort1, at m/z 124 $[M-63, CO$ and $Cl]^+$ for Diu1 and Lin1, at m/z 159 $[M-19, F]^+$ for Fluo1, at m/z 90 $[M-107, CO$ and $Br]^+$ for Mbro1, and at m/z 125 $[M-28, CO]^+$ for Mon1. The phenylisocyanate Isop1 do not contain halogen atoms; its main ions corresponded to m/z 128 $[M-33, CH_3$ and $H_2O]^+$, 146 $[M-15, CH_3]^+$, and 161 $[M]^{+\cdot}$.

The mass spectra of the all methyl esters of carbamic acid containing halogen atoms showed losses of characteristic fragments that produced the most interesting ions. The

molecular ion was intense, it was the base peak $[M]^{+\cdot}$ of Chlort2 (m/z 199), Mbro2 (m/z 229) and Mon2 (m/z 185). One important ion was produced by the loss of a methanol fragment $[M-32, CH_3OH]^+$. This loss explained the ions at m/z 167, 187 (base peak), 174 (base peak), 187 (base peak), 197 and 153 of the mass spectra of Chlort2, Diu2, Fluo2, Lin2, Mbro2 and Mon2 respectively. The main ions from the Isop2, which is halogenless, appeared at m/z 146 $[M-47, CH_3OH$ and $CH_3]^+$, 178 $[M-15, CH_3]^+$, and 193 $[M]^{+\cdot}$.

Analogously, the behaviour of the ethyl esters of carbamic acid was close. The molecular ion was intense. The loss of ethanol $[M-46, CH_3CH_2OH]^+$ produced signals at m/z 167, 187, 187, 187, 197 and 153 in the mass spectra of Chlort3, Diu3, Fluo3, Lin3, Mbro3 and Mon3 respectively. The loss of the $[M-72, C_3H_4O_2]^+$ fragment generated the base peaks at m/z 161, 161, 161, 171 and 127 for Diu3, Fluo3, Lin3, Mbro3 and Mon3 respectively. The most intense ions in the Isop3 mass spectra were 207 $[M-61, CH_3CH_2OH$ and $CH_3]^+$, 192 $[M-15, CH_3]^+$ and 146 $[M]^{+\cdot}$.

Metabenzthiazuron had a specific behaviour according to its structure. The base peak was the molecular ion $[M]^{+\cdot}$, and the other intense signals at m/z 136 and 108 corresponded to $[M-28, HCNH^+]$ and $[M-56, C_2H_4N_2]^+$.

IV.1.3.3. Gas Chromatographic Determination of Urea Herbicides as their Derived Compounds

The derived compounds formed from urea herbicides when injected in methanol were selected to determine the respective urea precursor. Figure 7 shows the SIM chromatograms of these derived compounds.

As can be seen in Table 15, the three main m/z signals observed in the phenylisocyanate spectra —104, 132, and 167 for Chlort1, 124, 159 and 187 for both Diu1 and Lin1, 159, 168, and 187 for Fluo1, 128, 146, and 161 for Isop1, 90, 169, and 197 for Mbro1, and 90, 125, and 153 for Mon1— were also present with different intensities in the spectra of the homologous esters of the carbamic acid.
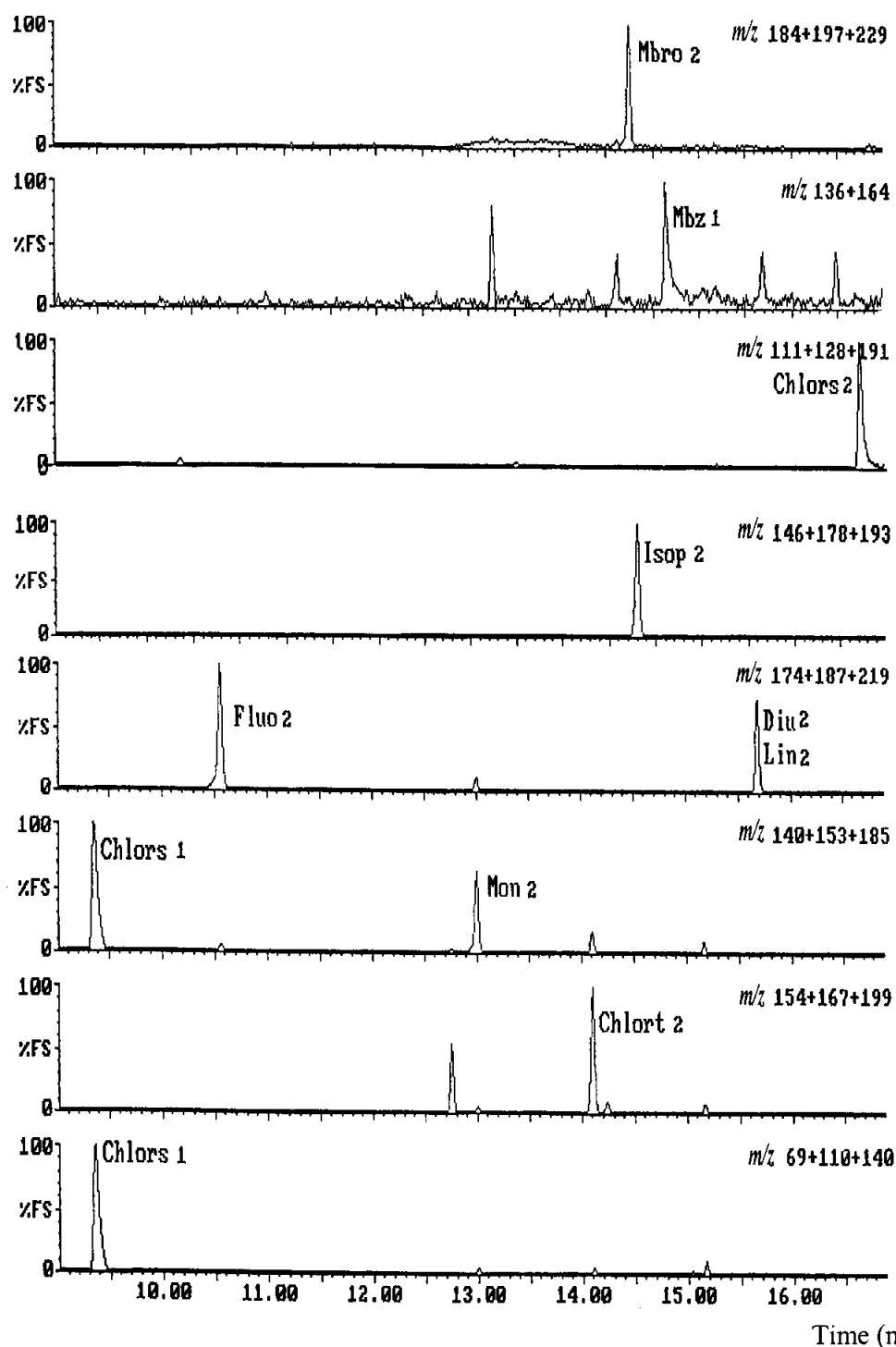
Figure 7. SIM chromatograms of the selected urea herbicides injected in methanol. Abbreviations as in table 15.

Table 16 shows the instrument detection limits (IDL), the relative standard deviation (RSD), the coefficients of correlation (r), and the conditions for the selected ion monitoring (SIM) program which determines the urea herbicides by means of their derivative compounds. The ions selected have high m/z masses and intensities and, at the same time, make the methyl esters of the carbamic acid distinguishable from the phenylisocyanates.

As seen in Table 16, the method was very sensitive, reaching IDLs of the pg and sub-pg order. Response was linear ($r>0.9986$) at the studied range (from 5 ng·L^{-1} to 25 µg·L^{-1}), and variability was also good (<9%). The IDLs of the proposed method (Table II) compare favourably with those reported by other authors that used GC-MS techniques (Table 17). In such table the IDLs were calculated from the data indicated by the authors and only the IDLs corresponding to the selected herbicides were included in it. Several factors explain the very good sensitivity for the proposed method. It is based on a capillary gas chromatographic technique, the ions selected to perform the SIM program were abundant, of relative high mass, and fragmentation was scarce, the high injector temperature facilitated the complete conversion of the original urea to the derived compounds, and the moderately polar BP10 column produced symmetrical narrow chromatographic peaks for the derived chosen for quantification.

Other authors who reported the use of gas-chromatography-mass spectrometric techniques utilized lower injector temperatures, such as 220°C [14] or 230°C [16], and less polar columns, such as HP-5 [14] or DB-5ms [16], to determine intact ureas. For studies in which phenylureas were determined as phenylisocyanates, the injector temperature was 250°C [22] and the column was of very low polarity such as the BP-1 [20, 22].

Table 16. Gas chromatographic-mass spectrometric determination of urea herbicide standards in methanol. SIM conditions, instrument detection limit (IDL) at a signal-to-noise ratio of 3, relative standard deviation (RSD, n =4), and correlation coefficient (r) for five calibration levels tested in quadruplicate form 5 ng/mL^{-1} to 25 µg mL^{-1}.

Injected herbicide	Determined derivative	SIM conditions Channel mass (m/z)	Retention window (min)	IDL (pg injected)	RSD% (at 5 ng·mL^{-1} level)	r
Chlors	Chlors1	69*, 110, 140$^+$	9.0-9.9	0.1	7	0.9990
	Chlors2	111*, 128, 191$^+$	17-18.5	0.1	9	0.9986
Chlort	Chlort2	154, 167, 199*$^+$	13.5-14.8	0.6	4	0.9995
Diu	Diu2	187*, 219$^+$	15.4-17	1	5	0.9991
Fluo	Fluo2	174*, 187, 219$^+$	9.9-11.5	0.9	7	0.9993
Isop	Isop2	146, 178*, 193$^+$	15.2-15.4	0.4	8	0.9987
Lin	Lin2	187*, 219$^+$	15.4-17	1	4	0.9992
Mbz	Mbz1	136, 164*$^+$	15.4-17	0.8	4	0.9990
Mbro	Mbro2	184, 197, 229*$^+$	14.8-15.2	0.4	5	0.9993
Mon	Mon2	140, 153, 185*$^+$	11.5-13.5	0.5	6	0.9989

* base peak; $^+$ molecular ion; Abbreviations as in Table 15.

Table III. Instrument sensitivity of gas chromatographic-mass spectrometric techniques from the literature
* Calaculated from data reported by other authors

Technique	Matrix	Injected herbicide	Selected m/z ions	IDL (pg injected) *	Ref.
GC-IT-MS	Soil	Isoproturon**	146	≈ 100	[20]
		Chlortoluron**	132		
GC-IT-MS	Cereals	Chlortoluron**	167, 132	< 100	[22]
GC-MS	Water	Linuron	248, 250	7.5	[14]
		Monuron	198, 200	15	
GC-IT-MS	Water	Fluometuron	72	≈ 84	[16]
		Linuron	61	1000	
		Metobromuron	61	≈ 200	

** determined as phenylisocianate
IDL = instrument detection limit
IT = ion trap

The main drawback of the proposed method was that different herbicides such as diuron and linuron gave the same derived product. If the reaction pattern of the studied compounds is extrapolated to the structures of all the commercial urea herbicides, chlorsulfuron, metsulfuron, prosulfuron, triasulfuron and tifensulfuron would generate the same aminotriazine (Chlors1), but the o-chlorophenylsulfonamide (Chlors2) would be specific of chlorsulfuron. Thus, only Chlors2 should be chosen for determination of chlorsulfuron. Diuron, linuron and neburon would produce the same derived compound (Diu2) and monuron, monolinuron and diflubenzuron would produce Mon2 and thus be indistinguishable from one another. In contrast, the compounds derived from chlortoluron, fluometuron, isoproturon, metabenzthiazuron, and metobromuron would be specific of their respective urea herbicide.

IV.1.4. CONCLUSIONS

Urea herbicides can be determined by GC-MS in the SIM mode at pg and sub-pg levels in form of their respective derived products that are generated by thermal reaction in the injector block. Chlorsulfuron produces two derivatives, 2-chlorobenzensulfonamide and aminotriazine, the first one is the most useful fragment to determine chlorsulfuron. Metabenzthiazuron produces a characteristic benzothiazolamine. The derived products formed from phenylurea herbicides depended on the solvent used for injection. Anhydrous methanol is recommended as a solvent for the extracts. This solvent produces the homologous methyl esters of carbamic acid from their respective phenylurea precursor. The phenylurea herbicides diuron and linuron, which have such similar structures close that they are indistinguishable from each other even when such a powerful tool as mass spectrometry is used. Nevertheless, the sensitivity of GC-MS, the fully reproducible spectra and the disposable data base are additional advantages over LC-MS.

Acknowledgements

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IV.2. Gas Chromatographic Behaviour of Urea Herbicides

H. Berrada, J.C. Moltó and G. Font.

ABSTRACT

Gas chromatographic conditions for determining eight phenylurea —chlortoluron, diuron, fluometuron, isoproturon, linuron, metabenzthiazuron, metobromuron and monuron— and one sulfonylurea —chlorsulfuron— herbicides were assessed. Degradation products of herbicides formed in the injector were used for identification. Most phenylureas formed their respective carbamic acid methyl esters, metabenzthiazuron formed an aminobenzothiazol and chlorsulfuron formed an aminotriazine plus a phenylsulfonamide.

On-column injections of standards using a BP10 capillary column were evaluated to identify the chromatographic behaviour. Instrument detection limits (IDLs) ranged from 0.05 ng for chlorsulfuron to 3 ng for monuron with the NPD and, from 0.01 ng for chlorsulfuron to 5 ng for metabenzthiazuron with the ECD. The RSDs (n=4) were lower than 4% at the 12-25 ng level.

The method was applied to analyse surface waters extracted with C18 Empore disks with recoveries higher than 85%. Down to 0.1 $\mu g \cdot L^{-1}$ of each herbicide could be determined in water. Chlortoluron was found (11.4 $\mu g \cdot L^{-1}$) in a water sample and its presence was confirmed by gas chromatography-mass spectrometry.

KEYWORDS Gas chromatography, Solid-phase extraction, Urea pesticides, Surface water

IV.2.1. Introduction

Most urea pesticides have a high herbicidal activity, they are an heterogeneous class of chemicals including triazine, benzothiazol, sulfonyl, phenyl, alkyl and other moieties. Only the presence of a substituted urea is common to the group. In general, urea pesticides are thermolabile, have a low water solubility and of low volatility [1].

The widespread agricultural use of urea pesticides and their toxicity stimulate the development of methods for the detection of their residues in water, soil and food. However owing to their physicochemical characteristics their determination at trace levels is difficult.

In the literature most urea herbicide analyses are performed by liquid chromatographic (LC) techniques with UV [2-5] and mass spectrometric (MS) detectors. Several LC-MS interfaces have been compared for determination of phenylurea and other pesticides [6]. In general, atmospheric pressure chemical ionisation (APCI) [7], ionspray [8] and electrospray [9-12] are more sensitive than thermospray [13] and particle beam [14] interfaces. Capillary electrophoresis (CE) is a emerging technique that has been applied to separate sulfonyl- [15] and phenylureas [16].

To make profit of selectivity and sensitivity of gas chromatographic detectors some authors selected the gas chromatography (GC) for determining urea pesticides even if these compounds are thermally unstable. Forming more stable acetyl- [17], alkyl- [17,18], perfluoroacyl- [19], or silyl- [20] derivatives solves the problem of the thermal degradation. Other way to analyse urea herbicides by GC is to minimise thermal degradation by using packed columns [21] or short capillary columns [22] at low temperatures. Thermal degradation products and intact urea herbicides have been detected by ECD [22], NPD [21, 23-28], and Ion Trap [21, 27, 29] or Quadrupole Mass Spectrometric Detectors [28, 30] at ng or sub-ng levels. Several products of thermal decomposition such as isocyanates and anilines [21, 27, 29] from phenylurea pesticides, and a triazine [25, 26] and a sulfonamide [26] from chlorsulfuron have been identified and used for quantification purposes.

Extraction of urea herbicides from water is usually done by Solid Phase Extraction (SPE) either with silica based phases [2, 3, 5, 10, 15, 18, 31], polymeric [4, 12, 31-33], carbon [9] or other solid supports [11, 34]. Solid phase microextraction coupled to either LC [35] or GC [36] has recently been reported for some urea herbicides.

The purpose of this study was to investigate the ability of capillary GC for identifying and quantifying nine urea herbicides including substituted phenyl- (chlortoluron, diuron, fluometuron, isoproturon, linuron, metobromuron, monuron), benzothiazol- (metabenzthiazuron), and triazin/sulfonyl- (chlorsulfuron) structures. The products of the target pesticides formed in the GC injector were detected by NPD and ECD in parallel. A multiresidue method based on SPE in combination with GC was developed for rapid trace quantification of selected herbicides in water samples. Analysis of surface water samples and GC-MS identification of the determined compounds was carried out to check the applicability of the method to real samples.

IV.2.2. EXPERIMENTAL

IV.2.2.1. Chemicals

The selected pesticides chlorsulfuron (1-(2-chlorophenylsulfonyl)-3-(4-methoxy-6-methyl-1,3,5-triazin-2-yl)urea), chlortoluron (3-(3-chloro-p-tolyl)-1,1-dimethylurea), diuron (3-(3,4-dichlorophenyl)-1,1-dimethylurea), fluometuron (1,1-dimethyl-3-(α,α,α-trifluoro-m-tolylurea), isoproturon (3-(4-isopropylphenyl)-1,1-dimethylurea), linuron (3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea), metabenzthiazuron (1-(benzothiazol-2-yl)-1,3-dimethylurea), metobromuron (3-(4-bromophenyl)-1-methoxy-1-methyl-urea), and monuron (3-(4-chlorophenyl)-1,1-dimethylurea) with purities between 95 and 99% were purchased from Riedel de Haën (Seelze, Germany). 4-bromophenylisocyanate, 3,4-dichlorophenylisocyanate, 4-isopropylphenylisocyanate, 4-monochlorophenylisocyanate, and 3-trifluoromethylphenylisocyanate with purities higher than 97% were from Aldrich (Milwaukee, WI, USA). Ethyl acetate and methanol for residue analysis was from

Merck (Darmstadt, Germany). Ultrapure water was obtained from a MilliQ system (Millipore Corporation, Bedford, MA, USA). Stock (500 $\mu g \cdot mL^{-1}$) and working solutions of herbicides and isocyanates were monthly prepared in methanol and preserved at 4°C.

5% DMDCS in toluene (Sylon CT) from Supelco (Bellefonte, PA, USA) was used to deactivate glass inserts. 32% hydrochloric acid and anhydrous sodium sulphate both of analytical grade were from Merck.

47 mm C18 Empore disks from Varian (Harbor City, CA, USA) were used with a standard Millipore 47-mm filtration apparatus. 0.45 μm HVLP filters from Millipore were also utilised for water sample filtration.

IV.2.2. Apparatus

Routine analyses were carried out on a Varian Star model 3400CX gas chromatograph (Varian Inc., Walnut Creek, CA, USA) equipped with an 8200CX autosampler, an on-column injector (SPI) model 1093, a conventional split/splitless injector model 1078, a thermoionic specific detector (NPD), an electron capture detector (ECD), and a Varian Star Chromatography Workstation version 4.51.

A Hewlett-Packard 5890 series II (Palo Alto, CA, USA), with an automatic sampler model HP-7673 and a Konik Instruments 2000-C (Sant Cugat del Vallés, Spain) gas chromatographs both equipped with ECD and NPD detectors were utilised to study the reproduction of the chromatographic behaviour of the selected herbicides.

Identification of peaks was performed in a Fisons 8000 series gas chromatograph (Fisons Instruments, Rodano, Italy) coupled with a Trio 1000 quadrupole mass spectrometer. A LAB-BASE data station and NBS spectra library was used to handle data.

A fused silica capillary column 30 m x 0.25 mm I.D., 0.25 μm BP10 (14% cyanopropylphenyl + 86% dimethylpolysiloxane) obtained from Scientific Glass Engineering (SGE) (Austin, TX, USA) was used.

IV.2.2.3. Gas Chromatographic Conditions

At the Varian gas chromatograph, injector and detector temperatures were set at 300°C. The oven temperature was programmed as follows, the initial temperature (100°C) was increased by $10°C·min^{-1}$ to 240°C and held for 15 min. Helium was used as carrier gas at a flow rate of 2.7 $mL·min^{-1}$. Parallel detection was performed by splitting the effluent from the column to both detectors by a steel splitter from SGE.

The Hewlett-Packard and the Konik gas chromatograph conditions reproduced those for the Varian gas chromatograph.

Mass spectrometer worked in electron impact (-70 eV) and SCAN (20-410) mode. Source and transfer line temperatures were 200 and 250°C respectively. Injector and oven temperatures were the same as the Varian gas chromatograph.

IV.2.3. RESULTS AND DISCUSSION

The high temperature used at the injector (300°C) facilitated the herbicide transformation into its respective thermostable analogous. The identity of such compounds was studied by GC-MS analysis in the same conditions. The analogous carbamic acid methyl esters were found instead of the phenylurea herbicides. 2-aminobenzothiazol was found instead of metabenzthiazuron and 2-amino-4-methoxy-6-methyl-1,3,5-triazine instead of chlorsulfuron [37].

It has been described in the literature that phenylurea herbicides are transformed during the gas chromatographic analysis producing the corresponding phenylisocyanates and anilines in different proportions according to the compound and the analytical conditions [21, 27, 29, 30]. Since phenylisocyanates have been quoted as the main derived products from phenylureas and some of them are commercially available, we proceeded to inject phenylisocyanate standards in methanol in order to know the efficiency of conversion of each phenylurea to its respective phenylisocyanate. Results obtained are shown in Table 18 Differences in the urea herbicide and the phenylisocyanate molecular weights were taken into consideration for calculation. For example if 20 ng of diuron (MW=233) were

completely transformed to 3,4-dichlorophenylisocyanate (MW=188), 16.1 ng of the later should be found (100%).

As seen in Table 18 when a phenylurea is injected in the proposed conditions, 73-87 % of its mass is detected as the same compound that the one detected after the analogous phenylisocyanate injection with good reproducibility (RDS lower than 5%). Actually, both phenylureas and phenylisocyanates are converted to the analogous phenylcarbamic acid methyl esters as observed by GC-MS. Such conversion occurred in presence of methanol because the phenylcarbamic acid methyl esters are more thermostable than the analogous phenylisocyanates. As a consequence, diuron, linuron and 3,4-dichlorophenylisocyanate gave peaks at the same t_R in agreement to their analogous structures. Other authors [21, 27, 29, 30] reported the phenylurea determination in form of their analogous phenylisocyanates but they used different non-alcoholic solvents for injection. The importance of the solvent in the GC analysis of urea herbicides has been recently studied [37].

Figure 8 shows the chromatograms obtained from the three chromatographs using different injection modes. On-column mode was performed at the Varian chromatograph (Figure 8 (x)) with the analytical conditions described in gas chromatographic conditions section. Splitless mode was performed with the Konik apparatus (Figure 8 (y)), in such occasion the oven program was started at 50°C (1min), then increased by 30°C·min^{-1} to 100°C and at this point the temperature rate was changed to 10°C·min^{-1} to reach 240°C which was held for 15 min. The splitless time was 0.9 min. Split mode was performed with the Hewlett-Packard chromatograph (Figure 8 (z)). The temperature program was the same used with the Varian but the amount injected was increased by ten-fold and the split relation was 10:1.

Table18 . Efficiency (E %) and relative standard desviation (RSD %) of selected phenylurea herbicides to produce the same compound that the injection of their analogous phenylisocianates in the BP10 – 30m column.[a]

Injected phenylisocianate analogous	Injected herbicide	E (%)	RSD(%)
3,4-dichlorophenylisocyanate	*Diuron*	80	4
3-trifluoromethylphenylisocyanate	Fluometuron	75	4
4-isopropylphenylisocyanate	Isoproturon	73	4
3,4-dichlorophenylisocyanate	Linuron	78	4
4-bromophenylisocyanate	Metobromuron	87	5
4-chlorophenylisocyanate	Monuron	80	3

[a] E and RSD (n =4) were calculated with NPD. The amount injected was 12 – 25 ng for phenylurea herbicide and 9 – 20 ng (corresponding to 100% efficiency) for phenylisocianate standards in methanol.
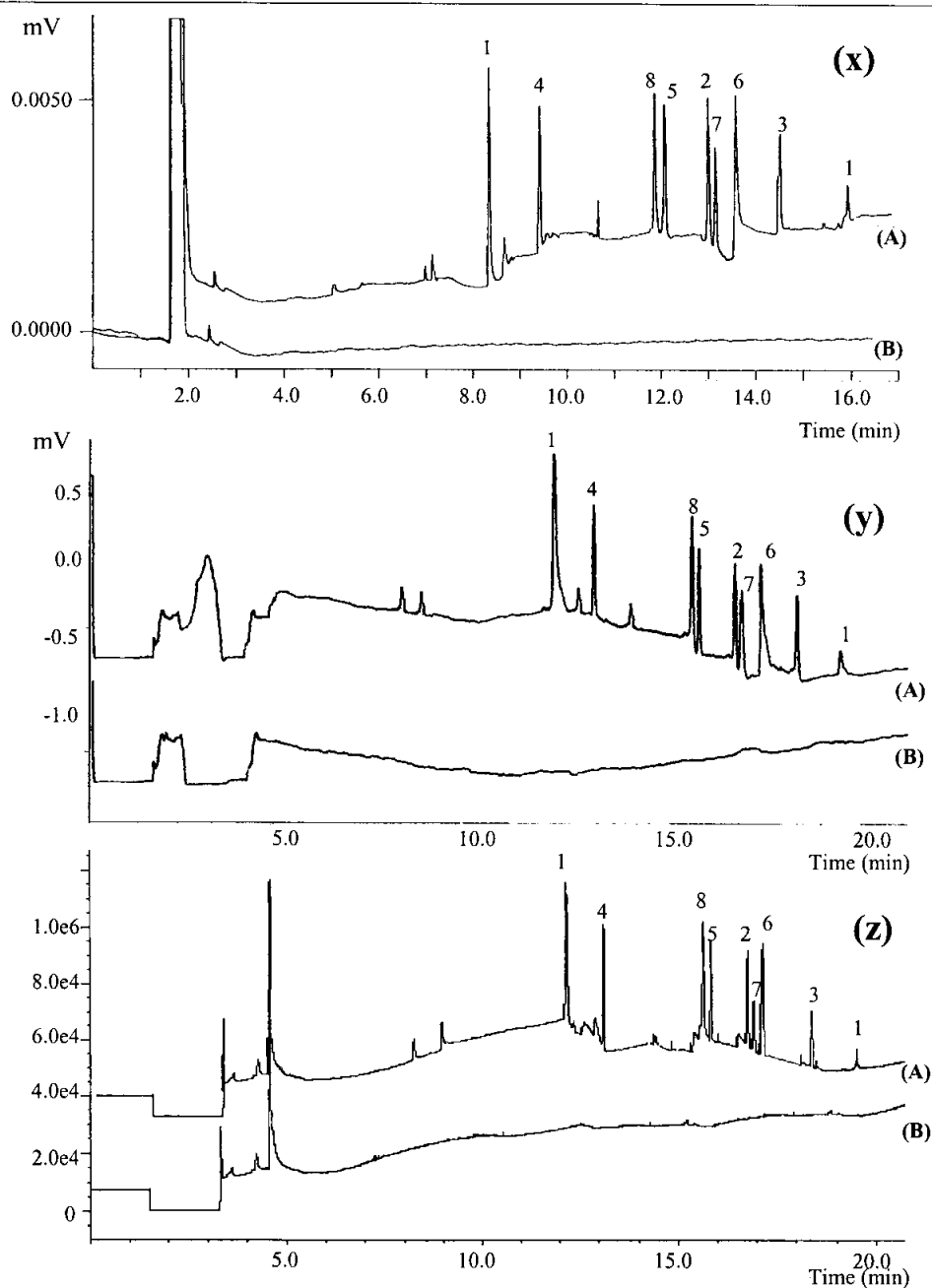
Figure 8. NPD chromatograms of (A) standards mixture and (B) methanol obtained with the BP10 column: (x) on-column injection with the Varian Star 3400CX, (y) splitless injection with the Konik 2000C and (z) split (10:1) injection with the Hewlett-Packard 5890 series II. Full conditions are detailed in the text. Identification of peaks: 1 = chlorsulfuron, 2 = chlortoluron, 3 = diuron / linuron, 4 = fluometuron, 5 = isoproturon, 6 = metabenzthiazuron, 7 = metobromuron, 8 = monuron.

It was observed the same chromatographic behaviour for the three injection modes. The relative abundance between the peaks of the herbicide derivatives differed less than 20% (n=4). This variability is low taking into consideration that detector designs were also different. No differences were observed between silanized and non-silanized liners for a determined gas chromatograph.

The good response of the derived carbamic acid methyl esters in NPD and their satisfactory chromatographic behaviour allowed the determination of the herbicide predecessors. Instrument detection limits (IDLs) obtained with NPD ranged from 0.05 ng for chlorsulfuron to 3.0 ng for monuron and were better than those obtained in the ECD except for chlorsulfuron (IDL 0.01 ng) and for monuron (IDL 0.5 ng). Nevertheless the ECD working in parallel is an interesting tool to confirm the presence of chlorsulfuron, chlortoluron, diuron/linuron, metabenzthiazuron, metobromuron and monuron and to determine sensitively chlorsulfuron and monuron.

IV.2.4. WATER ANALYSIS

Water samples (1000 mL) were filtered through a Whatman No. 1 and a 0.45 μm HVLP filters and then acidified to pH 2 with diluted hydrochloric acid (1:50 dilution of the hydrochloric acid analytical grade). Just after addition of the acid the sample was extracted by passing through an Empore C18 disk at 15-30 mL·min^{-1} with aid of vacuum. The disk was previously prewashed with 10 mL of methanol followed by 10 mL of deionized water, leaving some water on the disk. After the sample was extracted, the disk was dried by air suction for 5 min. Elution was performed slowly (1-2 mL·min^{-1}) with 10 mL of ethyl acetate. The eluate was carefully dried over anhydrous sodium sulphate and concentrated just to dryness using a gentle stream of nitrogen. The residue was dissolved with 50 μL of methanol and 1 μL of it was analyzed by gas chromatography.

Five water samples (7.5 L) were collected from different channels for citrus field irrigation which are placed in the protected area of the Albufera Lake (Valencia, Spain), transported and stored at 4°C until analysis. All the samples had low organic matter content (3.2-6.3 mg $O_2 \cdot L^{-1}$) and pH near neutrality (7.2-7.8). Selected urea herbicides present octanol/water partition constants (K_{ow}) ranging from 1.98 for monuron to 3 for linuron [38]. In addition, they have not neat acid or basic properties. Thus, high breakthrough volumes allowing the extraction of large volumes of sample and no influence of the pH on the extraction is to be expected. The sulfonylurea chlorsulfuron is different from the other selected urea herbicides considering its acidic character ($pK_a=3.6$) [1]. An acidic pH is required for extracting the molecular form of chlorsulfuron but, at the same time it facilitates sulfonylurea hydrolysis. For this, the pH control is done just before extraction. At acidic pH other basic compounds such as anilines that are phenylurea herbicide metabolites are not extracted since at such pH their ionic form are largely predominant.

Recovery assays were done using both MilliQ water and the previously analyzed sample No. 2 at two different fortified levels. In such sample no interfering peaks were found. Average recoveries ($R\%$) and relative standard deviations (RSD%) are summarized in Table 19. The NPD was used to quantify results except for monuron in which ECD was utilized. Recoveries were higher than 85% with RSD lower than 11%, no appreciable difference in method performance with the sample No. 2 and Ultrapure water matrices was found. Results were in agreement with those reported by other authors using C18 columns [3, 5, 10, 31] and C18 disks [18].

The method detection limits (MDLs) (blank background noise + 3 SD) were studied using the free of interfering peaks sample No. 5 as a blank. MDLs were in the range of 0.004 $\mu g \cdot L^{-1}$ for chlorsulfuron to 0.095 for metobromuron using the NPD. The less sensitive monuron which is detected at 0.19 $\mu g \cdot L^{-1}$ in NPD can be detected at 0.04$\mu g \cdot L^{-1}$ level with ECD.

Table 19. Mean recoveries ($R\%$) and relative standard deviation (RSD%) obtained after solid-phase extraction with C18 disks of 1000 mL of Ultrapure water and water sample No. 2 spiked at 0.2 and 10 $\mu g \cdot L^{-1}$ levels. The BP 10-30m column and NPD were utilized for determination.

Herbicide	Spiked at 0.2 $\mu g \cdot L^{-1}$				Spiked at 10 $\mu g \cdot L^{-1}$			
	Reagent water[a]		Water sample No.2[b]		Reagent water[a]		Water sample No. 2[b]	
	$R\%$	RSD%	$R\%$	RSD%	$R\%$	RSD%	$R\%$	RSD%
Chlorsulfuron	91	6	88	9	96	6	93	5
Chlortoluron	89	5	90	7	92	6	94	7
Diuron	92	4	93	6	91	5	93	6
Fluometuron	90	5	89	8	98	4	94	6
Isoproturon	87	7	85	9	91	5	93	9
Linuron[c]	96	4	90[d]	8[d]	95	5	90[d]	7[d]
Metabenzthiazuron	86	8	85	11	90	8	89	9
Metobromuron	89	9	93	10	90	7	87	9
Monuron	90[e]	8[e]	88[e]	9[e]	99	5	95	8

[a] mean of three replicates
[b] mean of two replicates
[c] compound extracted individually
[d] water sample No.3 was used
[e] determined by ECD
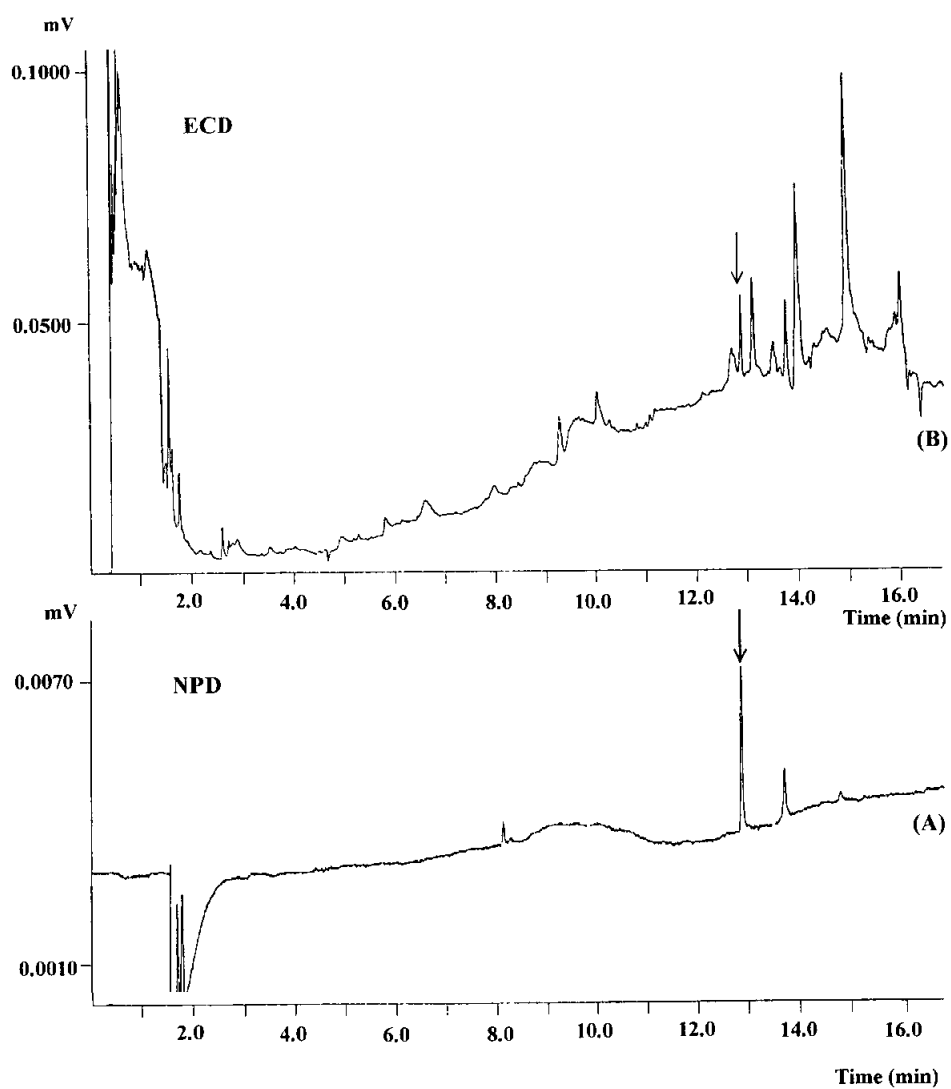
Figure 9. NPD (A) and ECD (B) chromatograms obtained with the BP 10 column corresponding to the water sample No. 1 containing 11.4 µg . L^{-1} of chlortoluron.

Samples 2, 3 and 5 did not contain peaks from selected herbicides. Chlortoluron was found in the samples No.1 (11.4 $\mu g \cdot L^{-1}$) and No. 4 (3.5 $\mu g \cdot L^{-1}$). Such herbicide was utilized in weed control near the sampling points. The corresponding ECD and NPD profiles from analysis of the sample No.1 are shown in Figure 9. GC-MS analysis identified the 3-chloro-4- methylphenylcarbamic acid methyl ester in both samples 1 and 4. Such compound generated the same spectrum as the one obtained from the chlortoluron standard.

IV.2.5. CONCLUSIONS

Injection of urea herbicides under controlled gas chromatographic conditions produces characteristic urea derivative peaks allowing the urea herbicide determination. In this way, 0.05 ng to 3 ng of the selected urea herbicides can be detected by GC/NPD.

The use of C18 solid-phase extraction disks for urea herbicide preconcentration from waters followed by gas chromatographic determination is a well suited method for the fast and easy analysis of this kind of pesticides. The results obtained indicate that it is adequate for monitoring urea herbicides in the low $\mu g \cdot L^{-1}$ range.

Following the proposed procedure chlortoluron has been determined in surface water samples by quantifying its carbamic acid methyl ester derivative as confirmed by GC-MS.

Acknowledgements

IV.2.6. REFERENCES

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IV.3. Indirect analysis of urea herbicides from environmental water using

solid-phase microextraction

H. Berrada, G. Font and J.C. Moltó.

ABSTRACT

We described here a solid-phase microextraction (SPME) procedure used to extract six urea pesticides —chlorsulfuron, fluometuron, isoproturon, linuron, metobromuron and monuron— from environmental samples. Two polydimethylsiloxanes (PDMS) and a polyacrylate fiber (PA) are compared. The extraction time, pH control, addition of NaCl to the water and the influence of organic matter such as humic acid on extraction efficiency were examined to achieve a sensitive method. Determination was carried out by gas chromatography with a nitrogen-phosphorus detector (NPD). The proposed method requires the extraction of 2 ml of sample (pH 4, 14.3 % w/v NaCl) for 60 min with the PA fiber. The limits of detection range from 0.04 for linuron to 0.1 μg/l for fluometuron and monuron and the relative standard deviations at the 1 μg/l level are between 15% and 9%. The apparent fiber/water distribution constants (K_{fw}) calculated in the proposed conditions were in the order of 10^3. Phenylurea herbicides were indirectly determined in the form of their derived anilines and chlorsulfuron in the form of an aminotriazine as confirmed by gas chromatography mass spectrometry. Natural waters were utilized to validate the final procedure. However, a unequivocal identification in unknown environmental samples should be done by LC-MS. The presence of dissolved organic matter such as humic acid produces losses during the extraction step. Adding sodium chloride to the sample compensates for this effect.

KEYWORDS SPME, Urea pesticides, Water Analysis, Gas Chromatography, Humic Acid.

IV.3.1. INTRODUCTION

Although extraction procedures that use little organic solvent have been developed, techniques that use no solvent have appeared only recently. Solid-phase microextraction (SPME), a solvent-free, easy sample preparation method, has been successfully applied to many contaminants in environmental studies ever since Pawliszyn and Liu [1] perfected it in their work on optical fibers. SPME is based on analyte distribution between the extracting fiber and the water sample volumes. As this process is dominated by the fiber/water (K_{fw}) partition coefficients, identifying the K_{fw} is of great importance in choosing the best extracting fiber. The K_{fw} of some fibers have been studied for several pesticides [2-7] and for other contaminants such as PAHs [8,9], PCBs [3,9,10] and phenols [11]. The utility of SPME combined with GC using selective detectors has been proven for the three major classes of pesticides, i.e. organochlorine, [3, 4, 12-15], organophosphorus [2-5, 13, 15-20] and organonitrogen pesticides [2-4, 6, 13, 17-19, 21-25].

Polydimethylsiloxane (PDMS), polyacrylate (PA), polydimethylsiloxane-divinylbenzene (PDMS-DVB), Carboxen-polydimethylsiloxane (CAR-PDMS), Carbowax-polydimethylsiloxane (CW-PDMS) and Carbowax-divinylbenzene CW-DVB are commercially available fibers covering a whole range of polarities, thicknesses and affinities to pesticides. PDMS and PA appeared on the market earlier, and consequently are the two most frequently cited. Several papers comparing fibers of different polarities have been reported. A high affinity for N-containing pesticides was found in fibers containing DVB [2]. Therefore, CW-DVB was chosen for an inter-laboratory study to determine triazines [24]. PDMS-DVB was found to have the highest affinity for triazine herbicides [3], whereas PA and PDMS-DVB were the best choice for extraction of several organophosphorus pesticides [3, 20]. Regardless of which fiber coating is being worked with, adding sodium chloride to water samples is an extended practice meant to improve the K_{fw} of non-ionized pesticides. This practice has been reported for quite different pesticides such as organophosphorus [5, 17, 18],

triazine [6, 17, 18, 24], other N-containing pesticides [6, 17, 18], and even for HS-SPME of low-polarity pesticides such as organochlorine insecticides [12].

Phenyl- and sulfonylureas are extensively used in agriculture as selective herbicides. The residues of these compounds in matrices can be investigated by both high-performance liquid chromatography (HPLC) and gas chromatography (GC) methods [26, 27]. Since sulfonylureas are weak acids having pK_a values ranging from 3.3 to 5.2 [28], extraction from waters is affected by the pH. For this reason, solid phase extraction (SPE) from water is usually done at an acid pH [29]. As phenylureas do not present acid or basic properties, SPE is executed at a neutral pH. Consequently, a change in pH from 7 to 3 in the aqueous mobile phase did not modify their retention times in HPLC determination [30].

SPME of phenylureas has not been extensively studied, and there are few reports in which SPME is coupled with HPLC to test for these compounds [31, 32]. It was precisely the lack of studies on SPME of urea herbicides that prompted us to assess the feasibility of SPME coupled with GC using commercial equipment to determine urea herbicides from aqueous matrices.

IV.3.2. EXPERIMENTAL

IV.3.2.1. Materials

Thwo PDMS fibers with thicknesses of 7 and 100 μm and a (PA) with a thickness of 85 μm (Supelco, Bellefonte, CA, USA) were used with an SPME holder for an 8200 CX autosampler (Varian Inc., Palo Alto, CA, USA).

All the coated fibers were previously conditioned in the injector port according to the manufacturer's instructions until a stable baseline was obtained. The PA fiber was conditioned before initial application by heating it at 300°C for 2 h and the PDMS fibers at 250°C for 1 h.

Salt saturated (35.7 % w/v NaCl), humic acid sodium salt (10 mg/l), and pH 4 (0.5% w/v sodium hydrogentartrate) and pH 9.2 (0.1 M sodium tetraborate decahydrate)

buffered solutions were used in several experiences. Sodium chloride, sodium hydrogentartrate, sodium tetraborate with purities up to 99.5% and technical grade humic acid were purchased from Aldrich (Milwaukee, WI, USA).

Methanol (Suprasolv quality) was from Merck (Darmstadt, Germany). Ultra pure water was obtained from a Milli-Q purification system (Millipore, Milford, MA, USA).

IV.3.2.2. Standards

The selected pesticides chlorsulfuron, fluometuron, isoproturon, linuron, metobromuron and monuron were purchased from Riedel de Häen (Seelze, Germany) with purities between 95 and 99%.

1000 µg/l stock solutions of each of the six phenylurea herbicides were prepared separately in methanol. These stock solutions were stored at 4°C and diluted daily with ultra pure water to prepare 100 µg/l intermediate solutions. Working water solutions were freshly prepared by spiking appropriate amounts of the intermediate solutions. The methanol contained in the spiked working water samples was always below 0.1%.

IV.3.2.3. Apparatus

Analysis was carried out in a Varian 3400 CX gas chromatograph equipped with a nitrogen-phosphorus detector (NPD) working at 3.2 A intensity with hydrogen at 4 ml/min, air at 175 ml/min and nitrogen at 30 ml/min, a septum programmable injector (SPI), a Varian 8200 CX autosampler with an SPME agitation accessory and Varian Star 4.51 software to control the parameters. The SPME agitation accessory provides continuous contact of the fiber with fresh sample by vibrating the fiber protective sheath in the water solution without increasing temperature. A BP10 (30m x 0.25 mm, 0.25 µm 14% cyanopropylphenyl 86% dimethylpolysiloxane) produced by Scientific Glass Engineering (SGE) (Austin, TX, USA) was used with helium as the carrier gas at 2.4 ml/min. SPI and NPD were kept at 300°C. The oven temperature was

programmed as follows, the initial temperature (100°C) was increased by 10°C/min to 240°C and held for 15 min. The total run time was 29 min.

To identify of the determined compounds a Fisons 8000 series gas chromatograph coupled with a Trio 1000 quadrupole mass spectrometer (Fisons Instruments, Milano, Italy) was used. The transfer line and source temperatures were 250 and 200°C respectively. The mass spectrometer worked in electron impact mode (-70 eV) by scanning from 50 to 450 amu to obtain full spectra of the detected compounds. The spectra were handled with a LAB-BASE data station with NBS, Wiley 6 and NIST spectral libraries. The rest of the chromatographic conditions reproduced those of the Varian 3400 CX.

IV.3.2.4. Analytical procedure

Two ml autosampler vials were filled with 2 ml of the water samples at pH 4 containing 14.3% w/v sodium chloride and were sealed with hole caps with Teflon faced silicone septa (Supelco). The fiber was immersed in the sample for 60 min under needle agitation at room temperature (22±2°C). After extraction the fiber was directly exposed to the hot injector port for subsequent analysis, and at this time the oven program was started. Thermal desorption of pesticides was held for 5 min. Quadruplicate analyses were performed for all experiments except for the precision study, where six extractions were made. Blank analyses were carried out to ensure the absence of memory effects.

IV.3.3. RESULTS AND DISCUSSION

IV.3.3.1. Fiber coating selection

In contrast with other extraction methods, SPME consists of an equilibrium process in which analytes are not quantitatively extracted from water. The amount of extracted analyte depends strongly on partitioning from the water matrix to the fiber coating, and this process is controlled by the distribution constant (K_{fw}). Since the amount of

extracted analyte determines the sensitivity of the method, the choice of an appropriate polymeric coating fiber is of great importance.

We compared three fibers (85 μm PA, 100 μm PDMS and 7 μm PDMS) for extracting the urea herbicides from water. Both PDMS fibers have the same polymer coating and their K_{fw} values are identical. The PDMS fibers differ in film thickness, which results in different coating volumes. The 100 μm fiber presents a larger volume than the 7 μm, and as analytes must diffuse through it, the time needed to reach equilibrium should be longer for the 100 μm fiber. On the other hand, a large fiber volume increases the capacity to retain an analyte, and therefore an improvement in the sensitivity is to be expected using the 100 μm fiber.

For each fiber coating, the maximum sensitivity is reached when the extraction time is almost equal to the equilibrium time. Time absorption profiles for each analyte were investigated by exposing the selected fibers to working water samples (50 μg/l) for times between 5 and 240 min in order to determine the optimum extraction time. Equilibration time was established by plotting the response of the gas chromatograph in area counts for each herbicide against the exposure time. As can be seen in the curves in figure 10, the selected herbicides showed similar tendencies with the three assayed fibers. Nevertheless, the PA coating gave the longest equilibrium times (120 min), the 100 μm PDMS reached equilibrium within 50-60 min and the 7 μm PDMS within 30-40 min. The equilibrium times are different for the different fibers, but for a determined fiber they are close for all selected herbicides. The equilibrium times remained unchanged regardless of whether a single pesticide or a mixture of them was extracted.
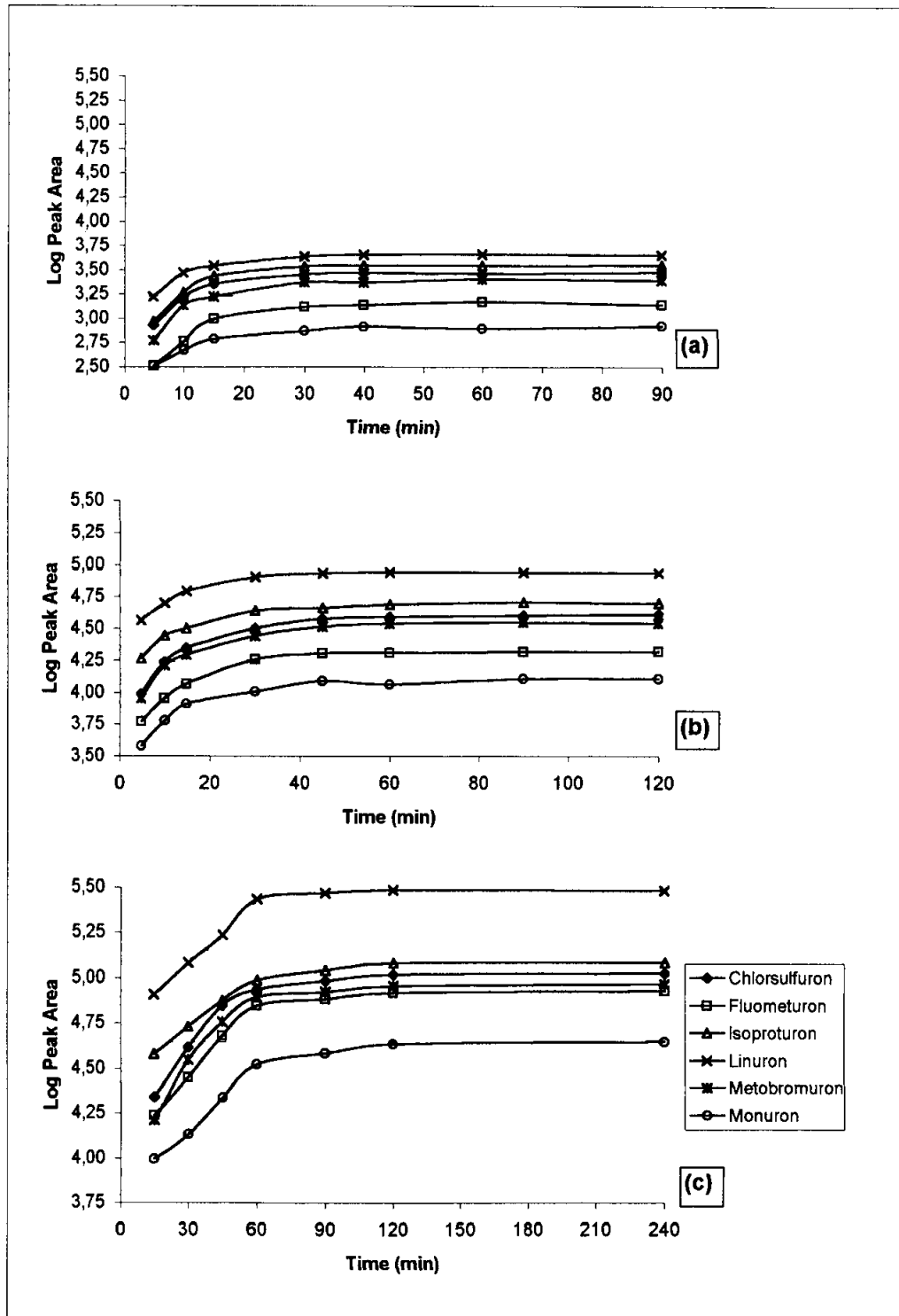
Figure 10. **Absorption time profiles for selected herbicides using 7μm PDMS (a), 100 μm PDMS (b), and 85 μm PA (c).**

Because of their water solubility, the selected urea herbicides were considered polar compounds. Therefore, a higher affinity for a polar coating such as PA than for a non-polar one such as PDMS was to be expected. This hypothesis is confirmed in figure 10, in which PA extracts the selected herbicides more efficiently than the PDMS fibers. This is true even at 60 minutes (equilibrium for PA not reached); at this time the amount of extracted herbicide represented 75-90% of the maximum extracted at the equilibrium time (100%).

The volume of the coating was of minor importance. As expected according to the fiber volumes, PDMS 100 μm (0.65 μl) extracts 15 to 19-fold more herbicide than the PDMS 7 μm (0.028 μl).

In order to reconcile good sensitivity with a reasonable extraction time, all further method development and validation were done with the PA fiber at 60 min.

IV.3.3.2. Optimization of the extraction

The influence of ionic strength (salting out effect) and pH on extraction efficiency were investigated. The ionic strength was modified by diluting NaCl saturated water (35.7 % w/v) with ultra pure water to obtain more diluted solutions (7.1, 10.8 and 14.3% w/v). To study the salting out effect, working water was prepared with salted water instead of pure water. The pH effect was studied at pH 4 and pH 9.2, with working water obtained by spiking buffered solutions as opposed to pure water. Extractions at various salt concentrations and pH were compared with control samples without salt or buffer addition.

Figure 11 shows the salting out effect on SPME of selected herbicides with the PA fiber. The amount of herbicide extracted depended on the salt addition. At 14.3% w/v salt addition, the areas increased within 13 and 107%. On the whole, the areas from herbicides with low K_{ow} such as chlorsulfuron or monuron (see table 20), which are difficult to extract, were enhanced more. The best extractions were observed at salt saturation, where area growth was between 55 and 230%. Only the most hydrophobic compound (linuron) did not show this improvement.
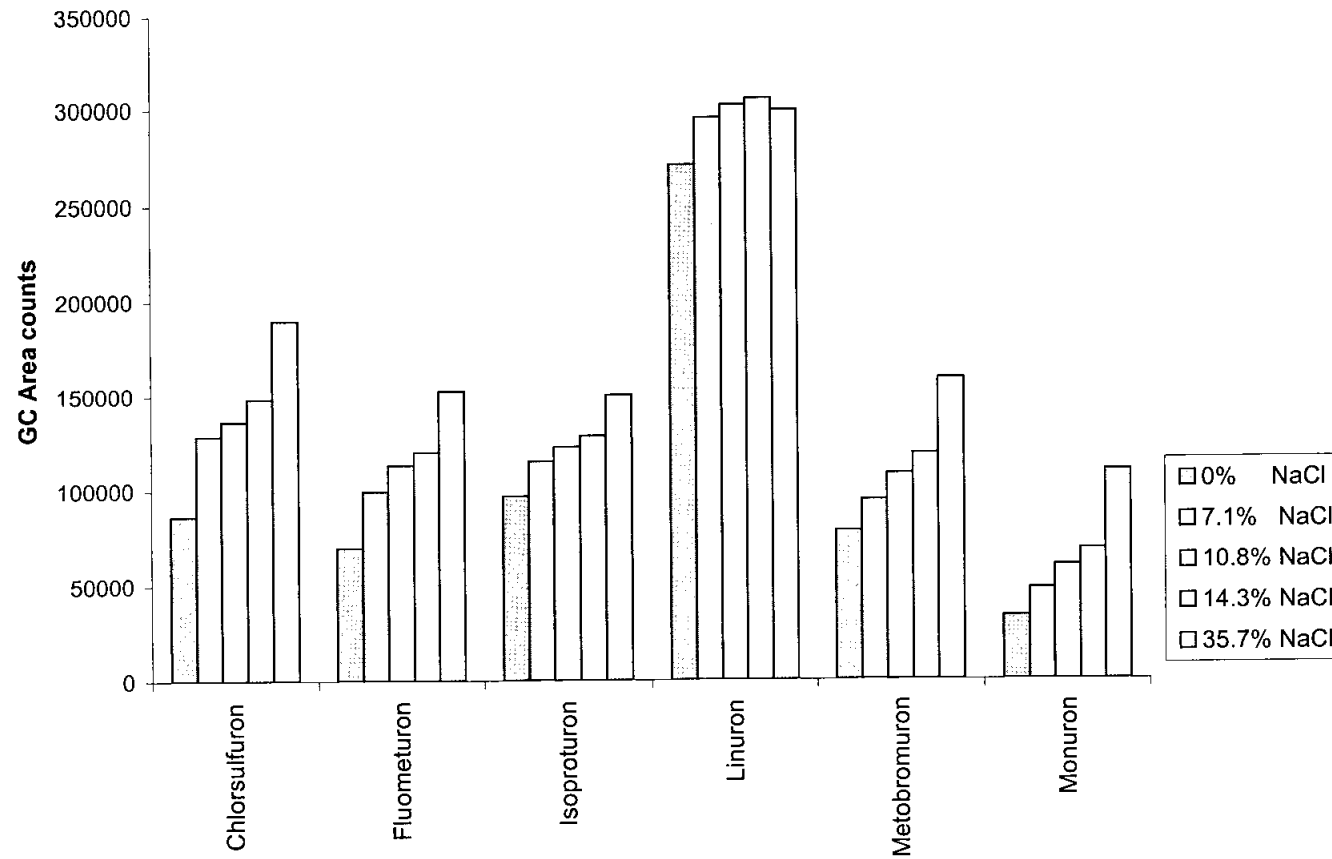
Figure 11. Efect of NaCl addition on herbicide peak areas with the PA fiber

However, working at a high salt concentration facilitates crystal formation thus blocking the fiber protection mechanism and producing a mechanical failure. Other authors [24, 31] have already reported this effect. To reduce salt precipitation, further assays were carried out at 14.3% w/v sodium chloride and the fiber was submerged in clean water after each run.

On the other hand, buffering at pH 4 increased the chlorsulfuron area by 105% as compared with experiments without pH control, whereas the rest of the herbicides were not affected. Since extraction at pH 9.2 drastically reduced the herbicide areas, all the subsequent SPME extractions were performed at pH 4.0. At acid pH, neutral molecules that have more affinity for the fiber increased in the water sample and extraction improved. In contrast, phenylureas are not acid compounds, and therefore decreasing the pH does not modify the molecular form and extraction remains unaltered.

These observations are consistent with the weak acid properties of sulfonylurea herbicides that have pK_a ranging from 3.3 to 5.2 [28]. In a recent study, Young [29] buffered at pH 3.5 to extract chlorsulfuron and other sulfonylureas from water samples by SPE using a cartridge containing a polymeric sorbent. But Sanchis-Mallols et al. [30] performed SPE of phenylurea herbicides from drinking waters without buffer addition. In that study the authors found no significant changes in the retention factors when the pH of the mobile phase was in the 3-7 range, which means that ionization of phenylureas does not occur at these pH.

IV.3.3.3. Linearity, sensitivity, precision and efficiency of the fiber

Linearity, sensitivity and precision were studied using natural water (Acequia Dreta), which was Whatman No. 1 filtered and then spiked. This water sample was previously analyzed and no herbicide was detected. The results are given in table 21. The linearity of the method was tested with the NPD by extracting spiked samples in quadruplicate at 1, 5, 10, 25, 50, 100 and 250 µg/l levels; correlation coefficients (r) were better than 0.994.

Table 20. Physical properties of the selected herbicides. Formula, molecular weight, water solubility, and octanol/water (K_{ow}) and calculated fiber/water (K_{fw}) distribution constants.

Herbicide	*Formula*	*Molecular Weight*	Solubility (mg/l)[a]	K_{ow}	$K_{fw}{}^c$
Chlorsulfuron	$C_{12}H_{12}ClN_5O_4S$	357.78	100-125[d] (25°C)	12.3[e, b]	1028
Fluometuron	$C_{10}H_{11}F_3N_2O$	232.2	105 (20°C)	263[b], 169[a]	624
Isoproturon	$C_{12}H_{18}N_2O$	206.29	72 (20°C)	316[a]	1857
Linuron	$C_9H_{10}Cl_2N_2O_2$	249.1	75-81 (25°C)	575[b], 1000[a]	1613
Metobromuron	$C_9H_{11}BrN_2O_2$	259.11	330 (20°C)	240[b], 257[a]	1343
Monuron	$C_9H_{11}ClN_2O$	198.66	230 (25°C)	96[b]	757

[a] from reference [38]
[b] from reference [39]
[c] PA fiber, pH 4, 14.3% w/v NaCl, 180 min extracting time
[d] at pH 4.1
[e] at pH 4.5

Successive extractions at levels below 1 µg/l provided the information necessary to estimate the limits of detection (LOD) based on the lowest peak with S/N =3. The LODs obtained allow the detection of selected herbicides at 0.1 µg/l or less. This is the maximum level of an individual pesticide permitted in the European Union for drinking water. If required, sensitivity can be improved by working at NaCl saturation, by prolonging the extracting time to 120 min, and by using a more sensitive detector system such as mass spectrometry with selected ion monitoring mode (MS-SIM).

As seen in table 21, the relative standard deviations (RSD %) at three levels calculated from four replicates ranged from 5.9 to 15.4%. No carryover effect was observed at blanks within the extractions.

Fiber efficiency was studied with the working water samples in ultra pure water. Calculation of extraction coefficients can not be based on external standard injection because the selected herbicides are degraded at the injector port to anilines and a triazine (as discussed below), whereas injection of ureas prepared in organic solvents generates other compounds such as isocyanates or esters of carbamic acid [33]. For calculation of extraction coefficients (E), the spiked water is successively extracted four times. Assays are performed in quadruplicate.

The extraction coefficient E_1 (per unit) of the first extraction can be calculated from,

$$E_1 = A_1/A_0$$

where A_0 is the area corresponding to the total amount of analyte in the water sample before the extraction (unknown) and A_1 the area corresponding to the amount extracted at the first extraction (known). Similarly, for the second extraction and successive "i" extractions E can be calculated from,

$$E_2 = A_2/(A_0-A_1) \text{ and}$$

$$E_i = A_i/[A_0-(A_1 +A_2 +...+A_{i-1})]$$

Assuming that extraction coefficients remain constant at the assay levels:

$$E_1 = E_2 = ... = E_i$$

This allows "E" to be calculated by re-extracting the same sample twice. Nevertheless, each sample was re-extracted four times to assure that extraction coefficients remained unaltered at the assayed levels. The extraction coefficients (E) calculated in this form

are given in table 21. With the proposed SPME conditions, extraction coefficients ranged from 15 to 38%. These extraction coefficients correspond typically to compounds having a medium affinity for the fiber. This affinity was artificially increased by pH control and adding salt. Affinity and therefore recoveries are lower when the matrix is not modified (ultra pure water). A similar method allowing the calculation of the amount of analyte extracted by a fiber by running successive SPMEs has recently been published by L. Urruty and M. Montury [7].

IV.3.3.4. Relation between the octanol/water (K_{ow}) and the fiber/water (K_{fw}) distribution constants

Several authors found the octanol/water (K_{ow}) and fiber/water (K_{fw}) partition coefficients to be directly related in the case of compounds such as organochlorines [9, 34], PAH [8, 9] and triazines [18]. For other contaminants such as PCB [10] or organochlorine pesticides [3], the K_{ow}/K_{fw} relationship was not evident. These last authors [3, 10] therefore suggest that if adsorption processes predominate or coexist with absorption ones, the K_{ow}/K_{fw} interrelation disappears. Other authors disagree and indicate that these adsorption phenomena were due to adsorption onto materials such as stir bars and glass vials but not onto the PDMS fiber [34].

Since K_{fw} is directly related to the amount of extracted analyte, and thus to the signal generated in the detector, we examined the question of whether a similar K_{ow}/K_{fw} association exists in the case of the selected urea herbicides.

Extractions at 50 µg/l level were performed for 180 min (over equilibrium time) and vials were completely filled to avoid headspace. The resulting NPD signal in area counts affected by the number of nitrogen atoms included in the molecule was plotted as a function of the K_{ow} for each herbicide obtained from the literature. Linear relationships with correlation coefficients (r) ranging from 0.98 to 0.96 were found depending on the K_{ow} bibliographic source. This seems to indicate that herbicide extraction using PA fiber is mainly governed by absorption processes, and we therefore expected at this point that the K_{ow} and the apparent K_{fw} would correlate for

the selected herbicides. In consequence, we proceeded to the K_{fw} calculation to confirm this hypothesis.

The following equation,

$$K_{fw} = nV_s/V_f(C_0V_s-n)$$

where "n" is the amount of the extracted analyte, V_s is the sample volume, V_f is the fiber volume and C_0 is the initial concentration, makes it possible to calculate the K_{fw}. Here, K_{fw} is apparent because the extractions were carried out with modified water (pH 4, 14.3 % sodium chloride). According to the extraction coefficients, low K_{fw} were to be expected. The calculated K_{fw} are shown in table 21 together with the K_{ow} obtained from the literature. The expected linear relationship between K_{fw} and K_{ow} for the selected herbicides presented correlation coefficients ranging from 0.59 to 0.66. This decrease in the linear correlation was attributed to both the different K_{ow} values obtained from the literature and the uncertainty of the indirect K_{fw} calculation.

The selected herbicides showed K_{fw} ranging from 624 for fluometuron to 1857 for isoproturon, thus indicating an intermediate affinity for the PA fiber. Salting out and pH control had an equalizing effect because affinity for the fiber in the case of the herbicides with low K_{ow} increased more, whereas herbicides with higher K_{ow} were influenced less.

IV.3.3.5. Identification of the determined products

Urea herbicides are decomposed at the injector to products with good gas chromatographic properties. To identify these compounds, the fiber was manually inserted immediately after extraction at the injector port of the Fisons 8000/Trio 1000 gas chromatograph/mass spectrometer. Extractions were performed either with individual herbicides (200 μg/l) or a mixture of them (200 μg/l each).

The homologous anilines from phenylureas and a triazine from chlorsulfuron were identified. The characteristic ions and the abundance of these derived compounds are shown in table 21. Mass spectra of anilines had intense molecular ions that coincided with the base peak $[M]^+$, except for the aniline formed from isoproturon, in which the

base peak corresponded to the loss of a methyl group [120; M-CH$_3$]$^+$. The typical abundance of some isotopes revealed the presence of halogen atoms on the base peak of the anilines formed from linuron [163 (64%), 2Cl], metobromuron [173 (98%), 1Br] and monuron [129 (32%), 1Cl]. Other characteristic but less intense m/z ions corresponded to the loss of halogen atoms. This was observed in the anilines derived from fluometuron [142; M-F]$^+$, linuron [126; M-Cl]$^+$, metobromuron [92; M-Br]$^+$, and monuron [92; M-Cl]$^+$.

The spectra show low fragmentation with characteristic high masses. These conditions make it possible to reach very good sensitivity using the SIM detection mode. Fluometuron and linuron had the same base peak (m/z 161), but their retention times were clearly different.

IV.3.3.6. Environmental water analysis

Two and a half litres of water samples were taken at five sites (Gola de Puçol, Racó de l'Olla, Acequia Dreta, Acequia Rodena, and Acequia del Canal) from the Albufera Lake (Valencia, Spain). The Albufera lake is a protected area of great environmental interest, where an abundant biomass including unique species coexists with intense agricultural activity, mainly rice growing. Sampling was done just before the rice was planted. At that time the organic content of the samples ranged from 3 to 7 mg O$_2$/l, and the pH from 6.8 to 7.8. The samples were passed through a Whatman No. 1 filter prior to analysis. An appropriate amount of sodium hydrogentartrate and sodium chloride was added to all the samples to reach pH 4 and 14.3% sodium chloride content. None of the selected herbicides were detected in the Albufera water samples. The profiles of the chromatograms were practically as clean as those of pure Milli-Q water, which indicates that other contaminants giving NPD response were not co-extracted. Therefore, the sample from the Acequia Dreta was utilized to study linearity, sensitivity and precision. The figure 12 (a) shows the chromatogram corresponding to the non spiked Acequia Dreta sample. To prevent sodium chloride precipitation several assays with the spiked sample were performed with lower sodium chloride content (10.8% w/v) than that proposed in the final method (14.3% w/v).
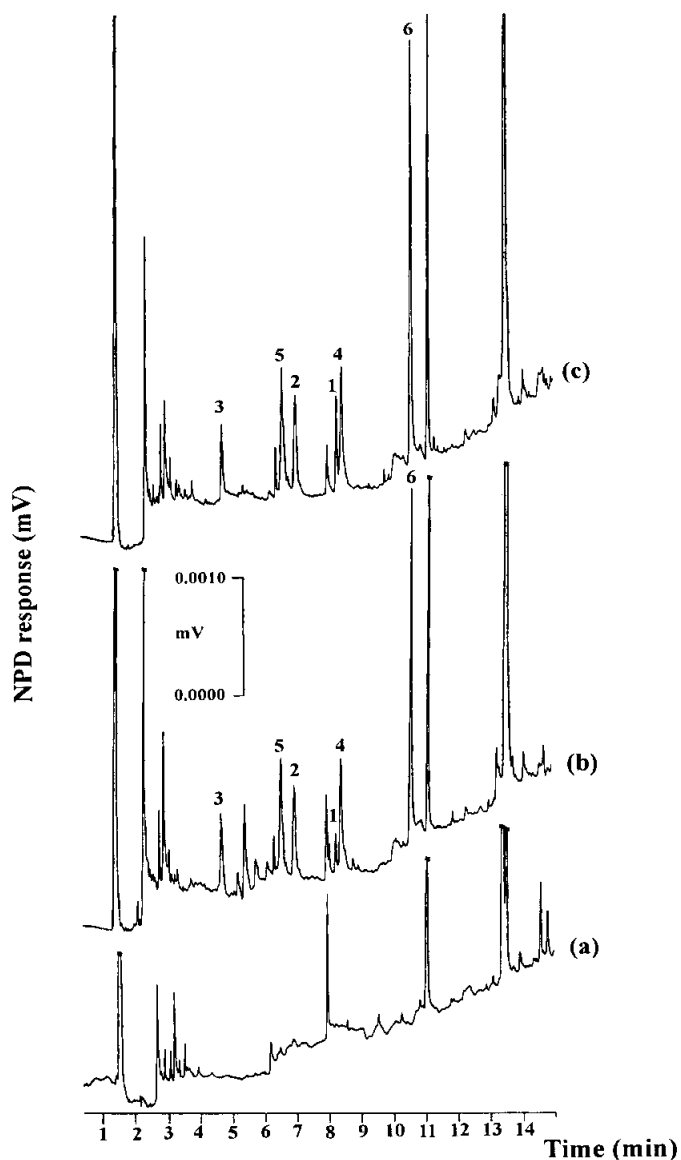
Figure 12. Chromatograms from the SPME of Acequia Dreta water sample buffered at pH 4; non spiked and containing 14.3% w/v NaCl **(a)**, spiked and containing only 10.8% w/v of NaCl **(b)**, spiked and containing 14.3% w/v NaCl **(c)**.

Peak assignment and level of spike; **1** chlorsulfuron (0.32 µg/l), **2** monuron (0.5 µg/l), **3** fluometuron (0.5 µg/l), **4** metobromuron (0.32 µg/l), **5** isoproturon (0.30 µg/l), **6** linuron (0.5 µg/l).

The corresponding chromatograms are shown in figure 12 (b) and (c) respectively. In these assays the losses of chlorsulfuron (peak No. 1) and linuron (peak No. 6) were greater than those due to the differences in salt content. This negative effect was initially attributed to the dissolved organic matter. To corroborate the hypothesis, an assay with spiked ultra pure water containing 10.8% sodium chloride and humic acid added to 10 mg/l (equivalent to 7.3 mg O_2/l) was run. In this assay extraction of chlorsulfuron and linuron decreased by 53% and 20% respectively, while the use of 10.8% instead of 14.3% sodium chloride without humic acid only produced 9% losses of chlorsulfuron and 2% of linuron. When the assays were performed with 14.3% w/v salt saturation, the recoveries from ultra pure water, water containing 10 mg/l humic acid and Acequia Dreta water were indistinguishable. Humic acid was not extracted by the fiber or if it was, it was only to a small extent. Humic acid modifies the fiber/water partition by increasing the water solubility of the herbicides which are bonded to the humic acid macromolecule. The presence of a large amount of ions liberated the herbicides from humic acid union. For this reason, when natural water is to be analyzed, the organic matter should be evaluated and sodium chloride may need to be added to correct losses generated by the presence of humic acid. The effect of humic acid was first observed when carbamates were extracted from natural waters by SPE [35], and sodium chloride addition was proposed to improve recoveries. The influence of the presence of organic matter on SPME has also been studied by Pörschmann et al. [36, 37] for some organic pollutants. In these studies the bonded fraction of PAHs and phenols to humic organic matter was calculated using PDMS fibers.

In summary, SPME using a PA coating fiber is a sensitive and reproducible technique for the detection of the selected urea pesticides in water. The affinity of the herbicides for the PA fiber is increased by NaCl addition and pH control, and allows detection of almost 0.1 µg/l of the selected urea herbicides. In these conditions the apparent K_{fw} range from 624 to 1857. The presence of humic acid in environmental waters reduces the extraction efficiency, but reinforcing the ionic strength prevents this effect.

Since the proposed method is based on an indirect determination, unequivocal identification of herbicides should be done by LC-MS. The proposed method is a simple, rapid and economical way to rule out the presence of selected herbicides in water at trace levels, leaving the more sophisticated equipment for confirming positive samples.

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IV.4. Application of Solid-Phase Microextraction for Determining

Phenylurea Herbicides and their Homologous Anilines from Vegetables

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Enviado a

Journal of Chromatography A

ABSTRACT

Metobromuron, monolinuron and linuron and their aniline homologous were analyzed in carrots, onions and potatoes by SPME performed with a polyacrylate fiber. Food samples were liquefied diluted, and an aliquot was extracted after sodium chloride (14%) addition and pH control. At pH 4 only phenylureas were extracted. A new extraction at pH 11 allowed extraction of phenylureas plus homologous aniline metabolites. Determination was carried out by gas chromatography with NPD and identity of the determined compounds was studied by gas chromatography-mass spectrometry. Limits of quantification obtained with NPD and MS (SIM) were in the µg/kg order allowing determination of maximum residue levels (MRL) established in the European Union regulations. MRLs ranged from 0.02 mg/kg to 0.2 mg/kg depending on the kind of food and herbicide. Under the proposed conditions matrix effects were low enough to permit calibration with samples proceeding from ecological (non-pesticide treated) crops. Twelve commercial samples of each carrots, onions and potatoes were analyzed and only three samples of potatoes contained residues of linuron at levels below MRL.

KEYWORDS Food analysis, Solid-phase microextraction, Urea pesticides, Anilines, Carrots, Potatoes, Onions.

IV.4.1. INTRODUCTION

Solid-phase microextraction (SPME) is very useful for determining pesticide residues from water samples [1]. Direct SPME is limited to liquid aqueous matrixes, only beginning studies to use it in non-polar matrixes have been done [2]. Although the fiber can not be submerged in a solid, some applications of SPME to analyze pesticide residues in solid matrices have been reported. The problem of the solid state of the sample has been resolved by using Head Space (HS) SPME [3] or by obtaining an aqueous extract from the sample [4-9]. Direct SPME has been applied to determine pesticide residues in liquid foods as wine [10] and juice fruit [5] and, HS-SPME has been used in wines [11,12]. It has been reported that complex matrices such as soil [13], vegetables [9], fruits [4,5] and honey [6,8] can cause interference in SPME. The use of deuterated surrogates or internal standard calibration may correct such matrix effects.

Phenylureas are an important group of pesticides utilized in weed control. Their residues appear in foods where they are applied [14]. Direct SPME with a polyacrylate fiber (PA) has been used to extract phenyl- and sulfonylurea herbicides from water [15]. Phenylureas are thermolabile and SPME is usually performed with automated instruments that are coupled with a gas chromatograph working at high temperatures. Under controlled conditions phenylureas can be determined from waters by SPME-GC in form of their respective derived anilines [15]. The gas chromatographic behaviour of the urea herbicides has been recently studied [16,17], the use of high temperatures at the injector port facilitates the degradation of the herbicide in a derived product which depends on the solvent used for injection [18].
In the environment, the main metabolites of phenylurea herbicides are anilines. Anilines and other compounds have been analyzed from soil using a PA fiber [13] by HS-SPME and from water by direct SPME [19].

The European Union legislation establishes Maximum Residue Levels (MRL) in foods considering like residue the sum of all commercial phenylurea whose chemical

structures are close giving the same aniline metabolites. Results must be expressed as the homologous aniline. For example, residues of linuron are the sum of diuron, linuron, and neburon expressed as 3,4-dichloroaniline.

Metobromuron, monolinuron and linuron are utilized in carrot (*Daucus carota*), onion (*Allium cepa*) and potato (*Solanum tuberosum*) crops and their MRL fixed in the European Union for these foods ranged from 0.02 to 0.2 mg/kg. Monolinuron and linuron have been included in the black list of the Council Directive 76/464/EU as dangerous substances for aquatic environment [20]. Metobromuron and linuron have been considered herbicides of potential concern in the Mediterranean region. No research in analyzing phenylurea herbicides from vegetables by SPME has been reported,so the utility of SPME with a PA fiber for determining such phenylurea herbicides as well as their aniline homologous in such foods with high water content is studied.

IV.4.2. EXPERIMENTAL

IV.4.2.1. Materials

A Polyacrylate fiber (PA) with a thickness film of 85 μm (Supelco, Bellefonte, CA, USA) was used with a SPME holder for an 8200 CX autosampler (Varian, Palo Alto, CA, USA). The fiber was previously conditioned in the injector port by heating it at 300°C for 2h, after that a stable baseline was obtained.

Sodium chloride 98%, sodium hydrogentartrate 98%, and sodium carbonate 99.5% were purchased from Aldrich (Milwaukee, WI, USA).

Methanol (Suprasolv quality) was provided by Merck (Darmstadt, Germany). Ultra-pure water was obtained from a Milli-Q purification system (Millipore, Milford, MA, USA).

IV.4.2.2. Standards

The pesticides buturon, diuron, linuron, metobromuron, monolinuron, monuron and neburon were purchased from Riedel-de Häen (Seelze, Germany) with purity up to 99%. 4-bromoaniline, 4-cloroaniline and 3,4-dichloroaniline were purchased from Aldrich with purity up to 97 %.

Stock solutions (1000µg/l) of each phenylurea herbicide were prepared separately in methanol. These stock solutions were stored at 4°C and diluted daily with ultra-pure water to prepare 100µg/l intermediate solutions. Appropriate aliquots of intermediate solutions were used for spiking food samples. Methanol contained in such spiked samples was always below 0.1%.

IV.4.2.3. Apparatus and conditions

Two gas chromatographs were used, the first was a Varian 3400 CX equipped with a nitrogen-phosphorous detector (NPD) working at 3.2 A intensity with hydrogen at 4 ml/min, air at 175 ml/min and nitrogen at 30 ml/min, a septum programmable injector (SPI), a Varian 8200 CX autosampler with an SPME agitation accessory and Varian Star 4.51 software to control the parameters. The SPME agitation accessory provides continuous contact of the fiber with fresh sample by vibrating the fiber protective sheath in the water solution without increasing temperature. A BP10 (30 m x 0.25mm, 0.25µm 14% cyanopropylphenyl+86% dimethylpolysiloxane) column produced by Scientific Glass Engineering) (SGE) (Austin, TX, USA) was used with helium as the carrier gas at 2.4 ml/min. SPI and NPD were kept at 300°C. The oven temperature was programmed as follows, the initial temperature (100°C) was increased by 10°C/min to 240°C and held for 15 min. The total run time was 29 min.

The second gas chromatograph was a Fisons 8000 series coupled with a Trio 1000 Quadrupole mass spectrometer (Fisons, Milan, Italy). The transfer line and source temperatures were 250 and 200°C respectively. The mass spectrometer worked in

electron impact mode (-70eV) by scanning from 50 to 450 amu for obtaining full spectra of the detected compounds or by selected ion monitoring (SIM) for quantifying purposes. The spectra were handled with a LAB-BASE data station with NBS, Wiley 6 and NIST spectral libraries. The rest of the chromatographic conditions reproduced those of the Varian 3400 CX.

IV.4.2.4. Sample preparation

50.0 g of fresh vegetable (carrots, potatoes, onions) were cut into small pieces. The sample was liquefied with a home liquefier and made up to 50 ml with Ultra-pure water. An aliquot of 5 ml was added with NaCl, buffered and diluted to 25 ml with water. The final aqueous extract contained 14% w/v NaCl and pH 4 (for phenylurea determination) or 14% w/v NaCl and pH 11 (for phenylurea plus aniline metabolite determination).

To obtain fortified samples, ecological samples of vegetables (non-pesticide treated) without peticides were spiked with known amounts of standards of urea herbicides and/or anilines.

IV.4.2.5. Analytical procedure

2 ml autosampler vials were filled with 2 ml of the final aqueous extracts containing the appropriate buffer and 14% w/v sodium chloride and sealed with hole caps with Teflon faced silicone septa (Supelco). The fiber was immersed in the sample for 60 min under needle agitation at room temperature (22±2°C). After extraction the fiber was directly exposed to the hot injector port for subsequent analysis, and at this time the oven program was started. Thermal desorption of pesticides was held for 5 min. Experiences were performed in quadruplicate. Some runs with pure Ultra-pure water were carried out after spiked food sample analyses to ensure the absence of memory effects.

IV.4.3. RESULTS AND DISCUSSION

IV.4.3.1. SPME

Some factors affecting SPME of phenylureas from water and soil have been already studied. The use of a polyacrylate (PA) fiber produces best extraction efficiency for phenylureas [15] and anilines [13] than polydimethylsiloxane. Equilibrium times for PA are typically of near 90-120 min, but 60 min is usually enough for most analytical purposes. A large desorption time (5 min) and a high injector temperature (300°C) reduces memory effects. Salting out effect is positive for improving extraction except those for linuron that remains practically unaltered [15].

The pH of the sample extract affects the extraction of anilines that are basic compounds but not those of the phenylureas that are neutral substances. For example the main metabolites of linuron, metobromuron and monolinuron are 3,4-dichloroaniline (pK 2.97), 4-bromoaniline (pK 4.72) and 4-chloroaniline (pK 3.98). At pH 4 the ionic molecules of the anilines predominates largely over the non-ionic ones, that is because at pH 4 only extraction of phenylureas was produced. At pH 11, the proportion between non-ionic and ionic molecules of anilines was sufficient to allow aniline extraction thus, at such pH both anilines and phenylureas were extracted.

Anilines can be found in foods proceeding from phenylurea herbicides but also from industrial activities. A double analysis of the sample performed at pH 4 and at pH 11 allows know separately the amount of phenylurea herbicide and the aniline residues in food.

SPME must be performed from aqueous solutions. Water contained in carrots, onions and potatoes is 88.2%, 88% and 77.8% respectively, this fact allows to liquefy easily the samples in order to obtain an aqueous solution available to perform SPME. However, the presence of endogenous substances and solid particles affects the equilibrium fiber/water and the integrity of the fiber. It was observed that if the liquefied sample is directly extracted without further dilution, recoveries were 40-70% lower than with proposed method and the fiber deteriorates quickly. Dilution of the
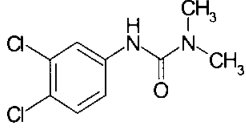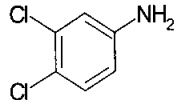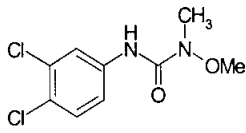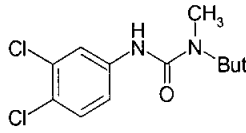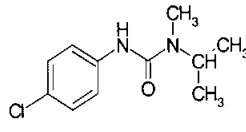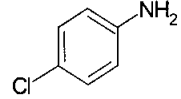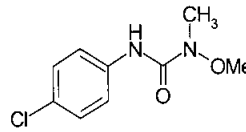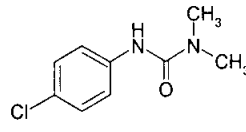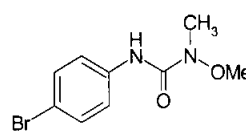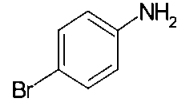
primary liquefied extract improved extraction and protected the PA fiber from deterioration but at the same time reduced sensitivity. A dilution of 1/5 was sufficient to reduce matrix interference and to reach enough sensitivity. Dilution was also chosen by other authors who extracted aqueous matrices with suspended matter such as fruits [4,5], vegetables [9] and honey [6,8].

IV.4.3.2. Identify of the determined compounds

Phenylureas that were analyzed by SPME-GC were actually detected as the corresponding analogous anilines. Consequently, phenylurea herbicides with close structures giving the same derivative aniline can not be distinguished. This analytical trouble is an advantage when analyzing samples for MRLs because the European Union legislation indicates that linuron residue is the sum of diuron, linuron and neburon expressed as 3,4-dichloroaniline. The three commercial herbicides have analogous structures and were determined in form of 3,4-dichloroaniline in SPME-GC analysis. The high temperature facilitated the degradation that occurred in the injector. Similarly the residue of monolinuron is the sum of buturon, monolinuron and monuron expressed as 4-chloroaniline, those herbicides generated 4-chloroaniline in SPME-GC, and metobromuron is determined as 4-bromoaniline in SPME-GC. The identity of the determined compounds was known by inserting the PA fiber immediately after extraction in the injector of the Fisons gas chromatograph with mass spectrometric detector to obtain the full mass spectra. Our interest is focussed on phenylurea herbicides currently applied on carrots, onions and potatoes, that are linuron, monolinuron and metobromuron. The rest of pesticides (diuron, neburon, buturon and monuron) are not applied on selected crops and were excluded of validation assays.

On the other hand, 4-bromoaniline, 4-chloroaniline and 3,4-dichloroaniline were extracted at pH 11 and determined as intact compounds as observed by mass spectrometry. A summary of the structures, recommended crops to be applied and identity of the determined compounds is done in table 22.

Table 22. Recommended crops for application of the herbicides and identity of the determined compounds.

Crops recommended,	Herbicide	Determined by SPME-GC as,
Cotton, asparagus, olive trees, vine, fruit trees (with stone), citric trees and others	Diuron (MW 233.1)	
Potatoes, carrots, onions, sunflower, maize, artichoke, asparagus and others	Linuron (MW 249.1)	3,4-dichloroaniline (MW 162.02)
Garlic, cereals, strawberries and others	Neburon (MW 275.18)	
Not used in Spain	Buturon (MW 227.72)	
Potatoes, onions, carrots, maize, asparagus, vine, fruit trees (with stone), citric trees and others	Monolinuron (MW 214.65)	4-chloroaniline (MW 127.57)
Weed control in uncultivated lands	Monuron (MW 198.65)	
Potatoes, onions, carrots, artichoke, maize, tobacco, tomato and others	Metobromuron (MW 259.1)	4-bromoaniline (MW 172.03)

IV.4.3.3. Validation of the analytical method

Samples of carrots, onions and potatoes proceeding from crops in which synthetic products were not applied were taken for studying analytical parameters.

SPME is not an exhaustive process, it is based in equilibrium between fiber and water governed by the K_{fw} constant. Under similar conditions (pH 4, 14% w/v NaCl) phenylureas showed apparent K_{fw} corresponding to moderate to low affinities to the PA fiber [15]. For this reason, the same extractive conditions from ultra-pure water was taken as reference (100% of recovery) to evaluate extraction from diluted liquefied extracts. Hence, samples of selected matrixes were fortified with the selected herbicides at three levels and analyzed. Parallel analyses at the same levels and conditions were accomplished with ultra-pure water. Results of these assays performed with selected herbicides are shown in table 23, and those performed with selected anilines are shown in table 24. Recoveries were greater than 76% for selected phenylurea and greater than 79% for the homologous anilines. Recoveries of anilines at pH 4 were below 4%. No differences between analysed matrices were observed. The RSDs observed at any of the three levels of spike studied (n=4) were lower than 10% for the selected herbicides at lower than 9% for anilines. Chromatograms corresponding to the SPME of an ecological carrot sample non-spiked (a) and spiked (b) with the selected herbicides are shown in figure 13. Some non-identified peaks were present but they did not interfere in herbicide determination.
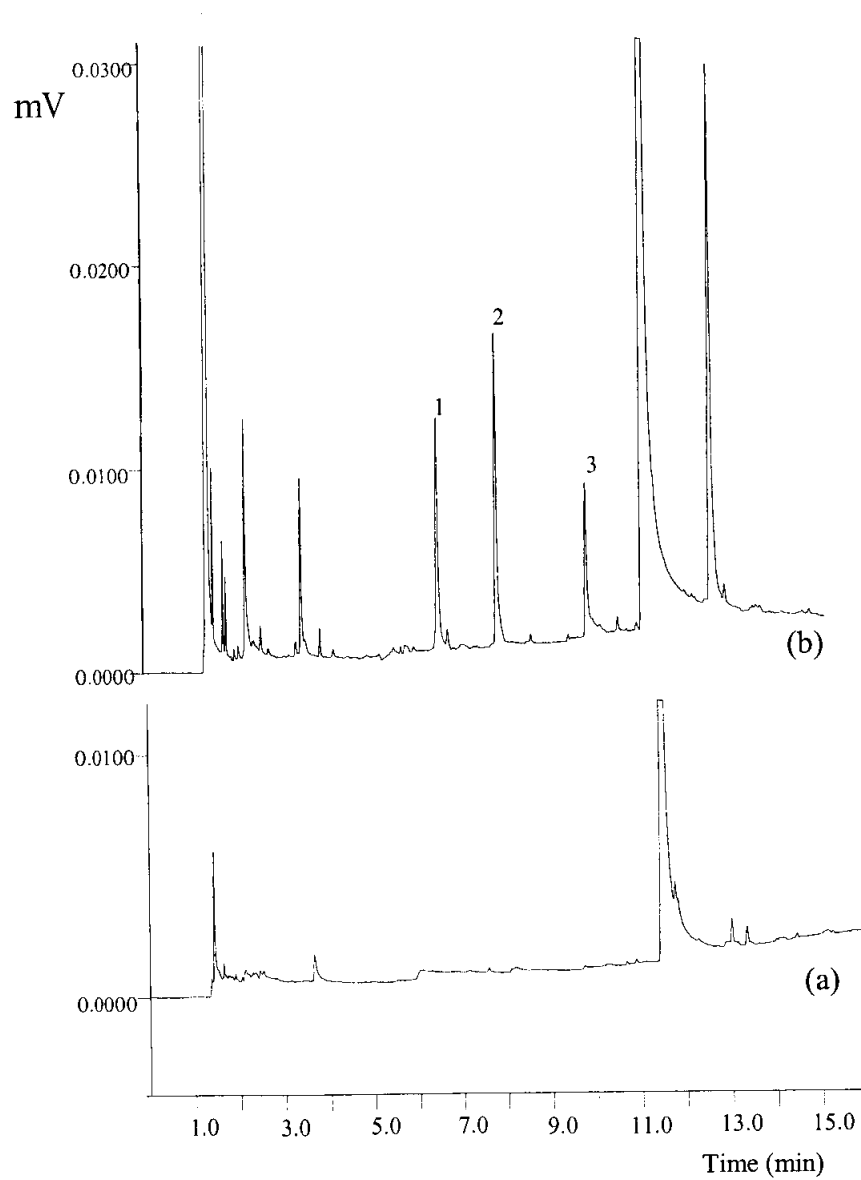
Figure 13. NPD profiles corresponding to the SPME of a pesticide-free carrot sample non-spiked (a), and spiked (b). Peak identification; 1 monolinuron (30 µg/kg), 2 metobromuron (50 µg/kg) and 3 linuron (10 µg/kg)

Table 23. Percent recoveries and relative standard deviations (in parenthesis) of the SPME (NPD) at pH 4 of selected herbicides from fortified samples.

| Herbicide | Carrots | | | Onions | | | Potatoes | | |
| | Level (mg/kg) | | | Level (mg/kg) | | | Level (mg/kg) | | |
	0.020	0.250	1.250	0.020	0.250	1.250	0.020	0.250	1.250
Metobromuron	88 (4)	85 (7)	90 (6)	89 (5)	87 (5)	80 (6)	83 (8)	77 (7)	78 (10)
Monolinuron	92 (5)	93 (4)	91 (4)	90 (4)	95 (5)	84 (5)	76 (5)	85 (6)	81 (9)
Linuron	90 (4)	89 (3)	87 (5)	87 (4)	85 (7)	91 (4)	79 (6)	76 (6)	76 (5)

n = 4

Table 24. Percent recoveries and relative standard deviations (in parenthesis) of the SPME (NPD) at pH 11 of selected anilines from fortified samples.

Aniline	Carrots			Onions			Potatoes		
	Level (mg/kg)			Level (mg/kg)			Level (mg/kg)		
	0.020	0.250	1.250	0.020	0.250	1.250	0.020	0.250	1.250
4-Bromoaniline	87 (6)	89 (8)	93 (5)	89 (5)	91 (7)	89 (6)	82 (7)	84 (6)	81 (9)
4-Chloroaniline	94 (5)	93 (5)	89 (4)	95 (3)	93 (4)	90 (5)	81 (6)	82 (7)	79 (7)
3,4-Dichloroaniline	95 (5)	94 (6)	96 (5)	89 (6)	88 (6)	94 (4)	85 (8)	83 (6)	79 (6)

n = 4
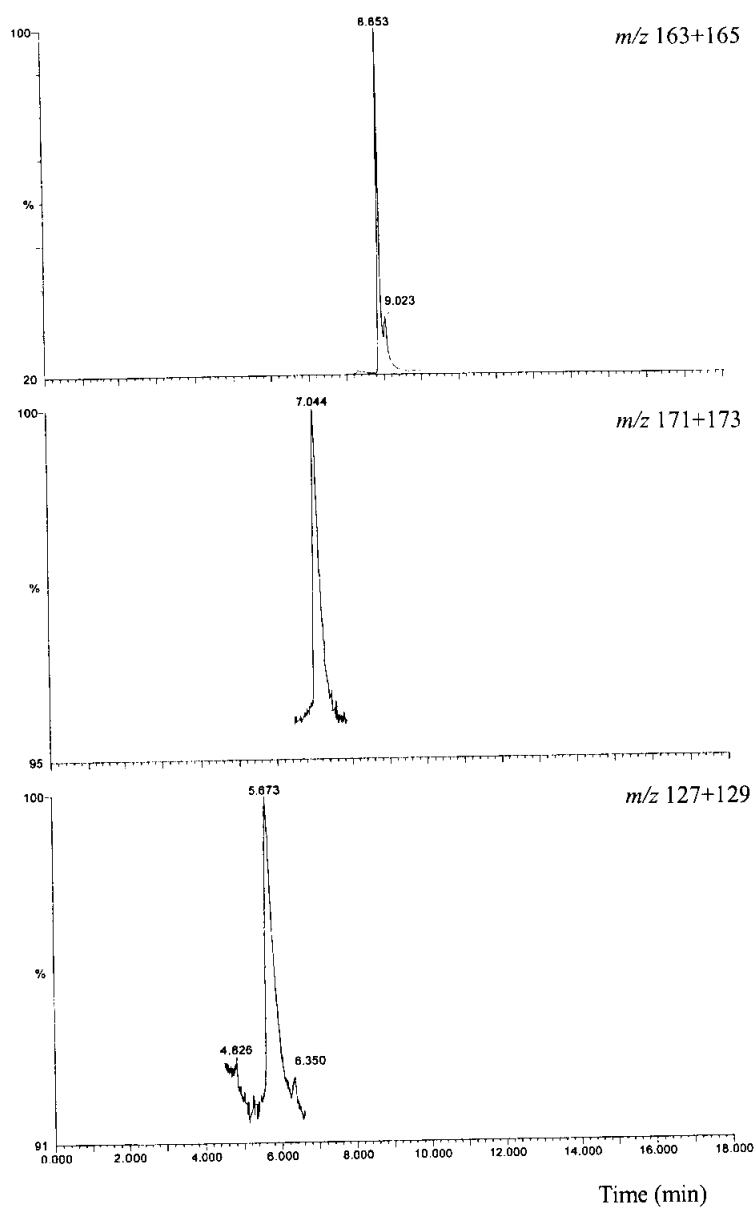
Figure 14. **Mass chromatograms corresponding to the SPME of a carrot sample spiked with 0.3 µg/kg of monolinuron (m/z 127+129), 0.3 µg/kg of metobromuron (m/z 171+173) and 0.1 µg/kg of linuron (m/z 163+165).**

Linearity was studied with NPD by analyzing fortified samples of carrots, onions and potatoes in quadruplicate at five levels (2.5, 25, 100, 250 and 2500 μg/kg). As seen in table 25, the coefficients of correlation were better than 0.994 for the herbicides and better than 0.995 for the anilines.

Limits of quantification (LOQs) (S/N=10) were evaluated for each herbicide and aniline with NPD and MS (SIM), results are shown in table 25. The *m/z* ions monitorized were 161 and 163 to determine linuron residues as 3,4-dichloroaniline, 171 and 173 for metobromuron as 4-bromoaniline and 127 and 129 for monolinuron as 4-chloroaniline. The SIM program included the molecular mass of anilines that were also the base peaks. Figure 14 shows the mass chromatograms corresponding to the analysis of a carrot sample spiked with the herbicides at very low levels. LOQs reached with NPD were good enough to determine residues at the MRL (see table 25). The injector of the Fisons gas chromatograph is not specially designed to be used with SPME fibers but LOQs obtained with MS (SIM) were better than those obtained with NPD.

IV.4.3.4. Analysis of commercial samples

Twelve commercial samples (2 kg) of each carrots, onions and potatoes were acquired from different markets of the Comunitat Valenciana (Spain). No herbicide residue was detected by SPME (NPD) at pH 4 in samples of carrots and onions. Residues of linuron expressed as 3,4-dichloroaniline were found by SPME (NPD) at pH 4 in three samples of potato at 5 μg/kg, 9 μg/kg and 4 μg/kg levels (below MRLs). Such quantified residues did not proceed from aniline metabolites because new extractions of these samples at pH 11 quantified the same levels of linuron. Since diuron and neburon that could generate same aniline are not applied on potato crops the quantified residues should be assigned to linuron. The linear equation obtained in the validation studies by spiking pesticide-free potatoes with 3,4-dichloroaniline was used for quantifying linuron residues in commercial samples of potatoes. Results were confirmed in such samples by mass spectrometry.
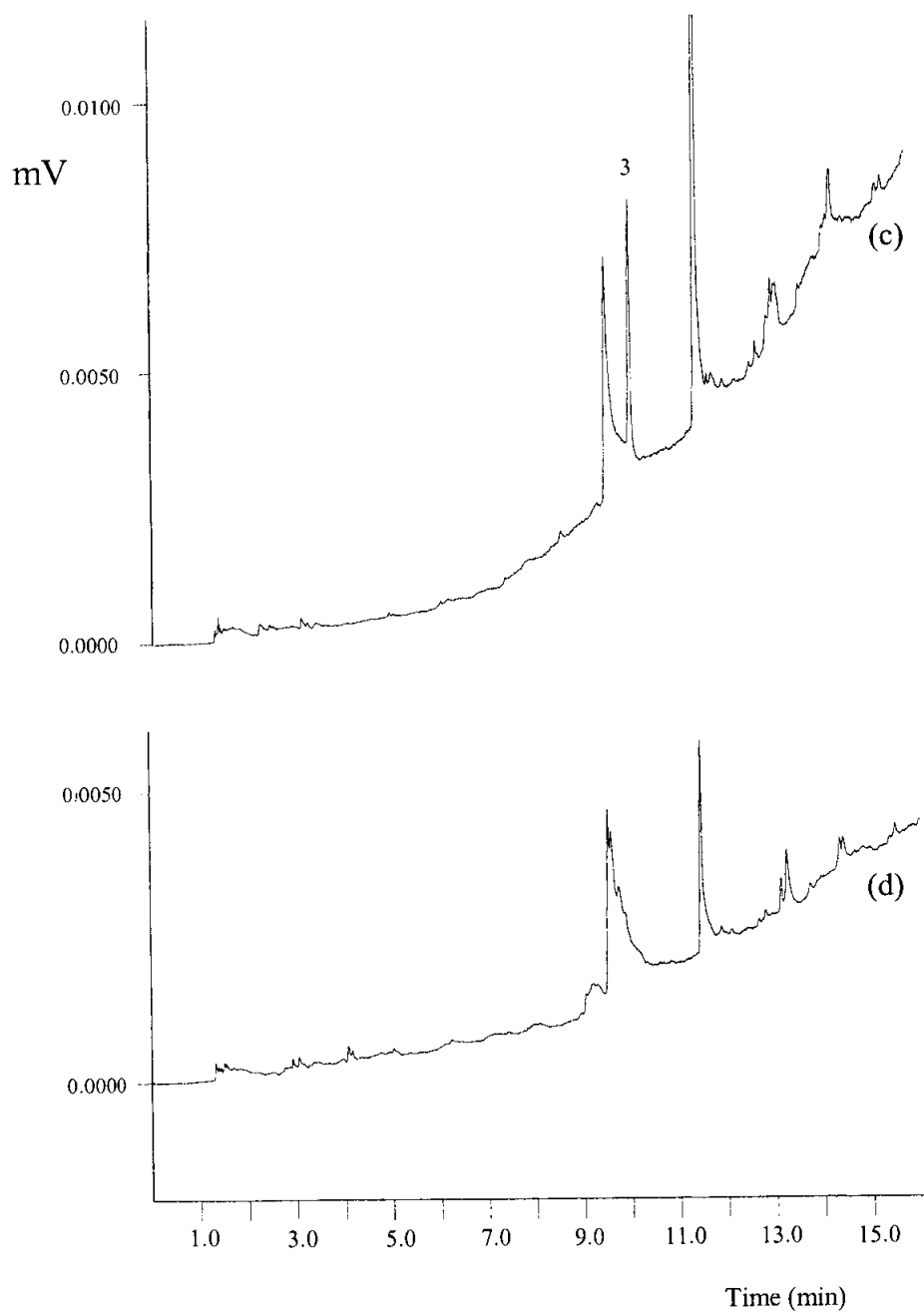
Figure 15. **SPME (NPD) analysis of potato sample No. 4 containing 5 µg/kg of linuron (c) and a pesticide-free potato sample (d).**

Table 25. Limits of quantification (LOQ, µg/kg), coefficients of regression (r^2) and maximum residue levels from European regulation (MRL, µg/kg)

Compound	Carrots LOQ NPD	Carrots LOQ MS (SIM)	Carrots MRL	Carrots r^2 *	Onions LOQ NPD	Onions LOQ MS (SIM)	Onions MRL	Onions r^2 *	Potatoes LOQ NPD	Potatoes LOQ MS (SIM)	Potatoes MRL	Potatoes r^2 *
Metobromuron	1.55	0.45	20	0.994	1.53	0.50	20	0.995	1.65	0.48	100	0.994
Monolinuron	1.85	0.61	200	0.999	1.87	0.63	200	0.998	2.21	0.67	50	0.997
Linuron	0.81	0.08	100	0.998	0.82	0.07	100	0.998	0.88	0.08	50	0.998
4-Bromoaniline	1.28	0.40	-	0.996	1.27	0.41	-	0.997	1.34	0.42	-	0.995
4-Chloroaniline	1.38	0.59	-	0.998	1.40	0.59	-	0.999	1.61	0.58	-	0.997
3,4-Dichloroaniline	0.79	0.07	-	0.999	0.78	0.07	-	0.999	0.86	0.07	-	0.999

MRL of monolinuron is the sum of buturon, monolinuron and monuron expressed as 4-chloroaniline
MRL of linuron is the sum of diuron, linuron and neburon expressed as 3,4-dichloroaniline
* (2.5, 25, 100, 250 and 2500 µg/kg, n=4), obtained with NPD.

NPD profiles corresponding to the analysis of samples of potatoes No. 4 (c) and potatoes pesticide-free (d) are shown in figure 15.

IV.4.4. CONCLUSIONS

The SPME coupled to a gas chromatograph with NPD or MS detectors allows determination of urea herbicide residues from carrots, onions and potatoes according to European regulation on MRLs exigencies. Samples must be liquefied, diluted, added with sodium chloride and buffered. Such sample preparation is easy, economic, and solvent free. Once the sample is prepared the extraction and determination can be performed automatically. Residues from phenylurea herbicide and from their aniline homologous can be known by performing two SPME, at pH 4 only phenylureas are determined and at pH 11 phenylureas plus anilines are quantified.

Very low levels of linuron were found in three of the twelve commercial samples of potatoes. Liquefying and diluting foods with high water content makes it possible to perform SPME of such kind of samples, nevertheless the influence of different matrices can affect the extraction process and should be particularly studied.

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RESUMEN DE RESULTADOS

Y

DISCUSIÓN

V. DISCUSIÓN

V.1. Factores que influyen en el comportamiento cromatográfico

Los herbicidas con estructura derivada de la urea son termolábiles, a las temperaturas de trabajo habituales en la cromatografía de gases sufren una degradación térmica, lo que dificulta su determinación por esta técnica. Si la degradación térmica es incompleta, la reproducibilidad de los picos detectados es mala y en estas condiciones la técnica no es útil para cuantificarlas.

En este trabajo se estudian los factores que facilitan la formación de una estructura derivada que permita la cuantificación de la urea herbicida original, aprovechando la alta selectividad y sensibilidad de los detectores DNF, DCE y DEM. Se buscan las condiciones de análisis que permitan la generación de un único producto de degradación que será el empleado para la determinación de la urea precursora.

Muchos factores pueden influir en esta reacción térmica, entre ellas la temperatura, técnica de inyección, longitud y fase de la columna, el disolvente usado, la presencia de otras ureas, etc...

V.1.1. Influencia de la temperatura del inyector

El inyector es el primer lugar físico de contacto de estos compuestos con las temperaturas altas. Si la degradación aquí se produce cuantitativamente y se genera un compuesto termostable, los demás factores serán menos relevantes. Para estudiar este factor, se inyecta en columna la misma cantidad (25ng) y el mismo volumen (1 μL) de las nueve ureas estudiadas a diferentes temperaturas de inyector (–30°C hasta 400°C).

La figura 16 muestra la influencia de la temperatura del inyector en esta degradación. El eje "Y" representa el area del pico mayoritario de degradación de cada herbicida, y el eje "X" representa la temperatura del inyector de –30 hasta 400°C.
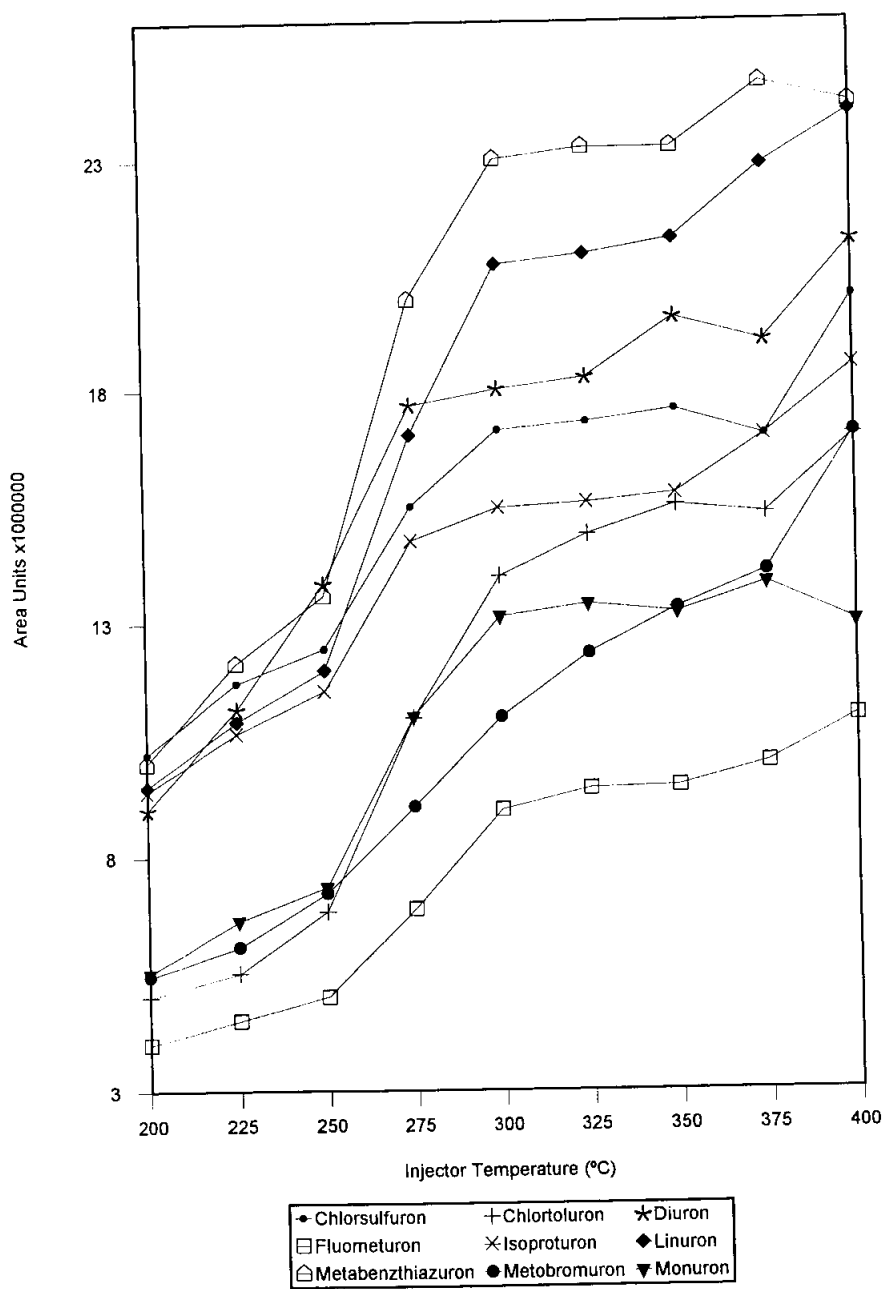
Se observa en esta figura la evolución del area del producto mayoritario de degradación de cada urea. De 200°C a 250°C, el area aumenta lentamente. De 250 a 300°C, el area aumenta de manera exponencial lo que indica que la mayor degradación térmica ocurre en este intervalo de temperaturas, siendo para este rango, el coeficiente de variación (n = 4) inferior al 4%. A temperaturas superiores a 325°C las líneas de base son irregulares y la precisión es inadecuada.

La inyección en frío a –30°C (nieve carbónica) seguida de un aumento balístico de la temperatura del inyector hasta alcanzar 300°C, no muestra ninguna ventaja, produciendo degradaciones parciales de mala reproducibilidad. La degradación ocurre a todas las temperaturas ensayadas pero a bajas temperaturas (< 250°C) sólo es parcial. La inyección a 300°C facilita la degradación completa y reproducible de los herbicidas estudiados antes de su interacción con la fase estacionaria de la columna, por lo que fue esta la temperatura elegida para llevar a cabo el resto de los análisis.

V.1.2. Técnicas de inyección

Se inyectan las ureas a 300°C utilizando la misma columna BP10, en tres cromatógrafos, dos de ellos equipados con inyectores capilares convencionales y uno con un inyector de temperatura programable que inyecta en columna.

Los resultados obtenidos tenían el mismo perfil que el que se muestra en la figura 16. Así los mejores datos analíticos se obtienen entre 250°C y 300°C con independencia de la técnica de inyección y cromatográfo utilizado. Como se observa en la figura 8, los cromatogramas obtenidos en tres equipos diferentes resultan semejantes.

V.1.3. Columnas

Se ensayan las siguientes columnas capilares de 30 m de longitud x 0.25 μm de diámetro interno: BPX70 (70% cianopropilo polisilfenil-siloxano), BPX35 (35% fenilpolisilfenileno-siloxano) y BPX5 (5% fenilpolisilfenileno-siloxano), y otra BPX5 de 12 m x 0.25μm de d.i. La polaridad de las columnas ensayadas disminuye en el orden siguiente; BPX70, BPX35, BP10 y la BPX 5. No se encontró ninguna relación

entre la polaridad de la fase estacionaria y el perfil cromatográfico. Se elige la BP10 por conseguir una resolución completa de todos los herbicidas con un menor tiempo de análisis.

V.1.4. Disolvente empleado en la inyección

Los disolventes ensayados son: metanol, etanol, diclorometano acetonitrilo y acetato de etilo.

La identidad del compuesto producido en la degradación térmica de las ureas herbicidas depende del disolvente empleado en la inyección de estas.

Las fenilureas generan con los alcoholes los ésteres carbámicos de estructura análoga, es decir ésteres metílicos con metanol y etílicos con etanol [1]. Mientras que en acetonitrilo, acetato de etilo y diclorometano, se generan los isocianatos análogos.

La presencia de agua favorece la formación de anilinas, tal como se comprueba al realizar SPME desde aguas donde no se utiliza disolvente para la inyección. En este sentido, se conoce desde hace tiempo la inmediata descomposición de isocianatos a anilinas, en presencia de agua a temperatura elevada [2].

Clorsulfuron y metabenzotiazuron se comportan de igual manera con todos los disolventes empleados. El chlorsulfuron se rompe en dos fragmentos, la clorosulfonamida y la triazina, mientras que el metabenzotiazuron se detecta como benzotiazol.

Tras estos estudios, se seleccionan como condiciones de trabajo, la columna BP10, metanol como disolvente de inyección y la inyección en columna a 300ºC.

V.1.5. Detectores

Se utilizan para este estudio diversos detectores de gases, DNF, DCE y DEM. La reproducibilidad y linealidad obtenidas con las condiciones seleccionadas en cantidades inyectadas de 1 a 50 ng son adecuados para el análisis con CV siempre inferiores al 4% y coeficientes de correlación r^2 siempre superiores a 0.995. Se establecen comparaciones entre los detectores a base a su sensibilidad y selectividad

con vistas a identificar y cuantificar residuos de estos compuestos. La tabla 26 muestra los límites de detección para DCE, DNF y DEM.

Como se puede observar, el DCE es generalmente más sensible que DNF, pero también menos selectivo. Esta menor selectividad por parte de DCE ha sido mencionada por varios autores que recomiendan generalmente un trabajo en paralelo con DNF y DCE. [3]

Todos los plaguicidas seleccionados, se analizan también por CG-EM con ionización por impacto electrónico positivo realizando ó bien un barrido completo o bien una monitorización de iones seleccionados. Los iones elegidos para la monitorización (m/z) y sus abundancias relativas, se muestran en la tabla 16.

Se elige el trabajo con los dos detectores DNF y DCE en paralelo para los análisis de rutina y el DEM para la confirmación adicional de los resultados.

V.2. Métodos de extracción

Los dos métodos de extracción que se estudian son la EFS y la MEFS. Ambos generan extractos lo suficientemente puros como para eliminar procesos de purificación largos y tediosos, que constituyen una fuente de pérdidas para los plaguicidas estudiados.

V.2.1. EFS

La extracción en fase sólida es una técnica que se ha utilizado ampliamente para el análisis de residuos de plaguicidas en una gran variedad de matrices [4]. Se aplica el procedimiento descrito por Klafenbach y col. usando discos de C18 modificado con el empleo del acetato de etilo como disolvente de elución [5]. . La etapa de la concentración es crítica, ya que hay que evitar las pérdidas relacionadas con la inestabilidad térmica de las ureas.

Las fenilureas estudiadas tienen un valor de Kow que varía entre 1'98 para el monuron y 3 para linuron, y no tienen propiedades ácidas ni básicas relevantes, lo que permite una buena extracción desde el agua con pHs desde 2 hasta 11.

Tabla 26. Limites de detección de los herbicidas estudiados con los tres detectores empleados.

Herbicide	NPD (ng)	ECD (ng)	MS-SIM (pg)
Clorsulfuron	0'01	0.01	0.1
Clortoluron	1	0.1	0.6
Diuron	0'1	0.01	1
Fluometuron	0.5	0.05	0.9
Isoproturon	1	0.7	0.4
Linuron	0.1	0.01	1
Metabenzotiazuron	0.5	0.1	0.8
Metobromuron	1	0.5	0.4
Monuron	3	0.5	0.5

El clorsulfuron es una sulfonilurea que presenta un pKa = 3'6, por lo tanto precisa pH ácido para que predomine en disolución su forma molecular y se pueda realizar la extracción con la fase sólida. Por lo que se utiliza pH = 2 para la extracción desde agua.

Para calcular la exactitud y reproducibilidad del método propuesto, se realizan ensayos de recuperación a niveles de adición muy bajos (entre 0.2 y 10 µg/L). Las recuperaciones para las fenilureas varian entre 87% para isoproturon y 96 % para linuron, mientras que para el metabenzotiazuron son del orden de 90% y clorsulfuron el del 96% (Tabla 19).

V.2.1.1. Aplicación a muestras reales

La utilidad del método se evalua aplicándolo a la determinación de los herbicidas en aguas superficiales. Las muestras de agua presentan un bajo nivel de materia orgánica (3'6-6'3 mg O_2/L) y pH entre 7'2 y 7'8 por lo que no se necesita recurrir a la adición de sales para eliminar la interferencia que una concentración superior de materia organica podría producir [6].. Las recuperaciones obtenidas para estas aguas con este método aparecen en la tabla 19.

En los cromatogramas obtenidos de muestras reales, el clortoluron aparece claramente en la muestra 1 y 4 (figura 9). La buena resolución de los picos y la ausencia de compuestos que interfieren en la determinación por DNF y DCE permite la detección de estos herbicidas a niveles inferiores a 0.1 µg/L como exige la legislación vigente para aguas potables de consumo publico.

V.2.2. MEFS

La MEFS está basada en un equilibrio entre la fase acuosa y el polímero que constituye la fibra. Con el fin de desarrollar un método de análisis de ureas seleccionadas mediante MEFS acoplada a cromatografía de gases, se estudian los posibles factores que puedan influir.

V.2.2.1. Modo de extracción

Existen dos modos posibles de MEFS; la MEFS directa y la MEFS de espacio en cabeza.

La MEFS directa se basa en un equilibrio entre dos fases, fibra y agua y para ello se sumerge la fibra directamente en la muestra acuosa a analizar.

La ecuacion que refleja la eficiencia de la extracción es la siguiente:

$n = K_{fw}V_fV_sC_0 / (K_{fw}V_f + V_s)$

Donde K_{fw} es la contante de distribución fibra/agua del analito, V_f y V_s son los volúmenes de la fibra y de la muestra respectivamente, C_0 la concentración inicial del analito en la muestra.

La MEFS de espacio en cabeza es un equilibrio entre tres fases; agua, espacio en cabeza y fibra. Se consigue situando la fibra en el espacio en cabeza del vial que contiene la muestra. La eficiencia de esta técnica responde a la ecuación siguiente:

$n = K_{fw}V_fVsC_0 / (K_{fw}V_f + K_{gw}V_h + V_s)$

Donde K_{gw} es, un término adicional que expresa la constante de distribución del analito entre las fases gaseosa y acuosa [7].. En este caso, el factor limitante en la extracción es la difusión de las ureas en la fase vapor, lo que hace que su concentración sea muy baja en el espacio en cabeza y los tiempos de equilibrio muy elevados.

Se selecciona la MEFS directa para estudiar las ureas por su mayor capacidad de extracción y el menor tiempo necesario para alcanzar el equilibrio.

V.2.2.2. Factores que afectan la difusión: Agitación y temperatura

En el inicio de la extracción, la concentración de un analito en la fibra será cero. A continuación, se produce una transferencia del analito desde la fase acuosa hacia el polímero, hasta producirse un reparto entre ambas fases agua/fibra, en un tiempo de equilibrio característico para cada analito y cada tipo de fibra. Los factores que favorecen la difusión del analito en la fase acuosa, acortan el tiempo de equilibrio. Los dos parametros manejables en la práctica para este objetivo son la agitación y la

tamperatura. Existen fundamentalmente dos métodos prácticos para llevar a cabo la agitación. El primero es la agitación magnética, consistente en colocar una barra magnética recubierta de teflón en el interior del vial. Otro método habitual que ha sido incorporado en los muestreadores automáticos para MEFS se basa en la vibración de la fibra. Mediante este sistema la fibra al vibrar transmite una agitación a la muestra acuosa más eficaz que la agitación magnética, cuando se trabaja con viales de 2 mL [8].

El aumento de la temperatura de la muestra durante la extracción presenta dos efectos contrarios. Por un lado, un efecto beneficioso al aumentar considerablemente la difusión de los analitos, disminuyendo los tiempos de equilibrio. Por otro lado, a temperaturas por encima de 60°C, existe un efecto negativo ya que la transferencia del analito de la fase acuosa al polímero es un fenómeno exotérmico con lo que por encima de 60°C este proceso se enlentece. Aunque la combinación de agitación y aumento de temperatura en torno a 60°C sería una situación óptima, por cuestiones prácticas se trabaja solamente con sistemas de agitación de muestra, consiguiendose tiempos de extracción suficientemente bajos y aplicables al análisis rutinario.

V.2.2.3. Establecimiento del tiempo de extracción

La cantidad de analito extraído aumenta con el tiempo de extracción hasta alcanzar un tiempo denominado tiempo de equilibrio. Para determinarlo, se realizan los análisis con tiempos crecientes de extracción y se representa la cantidad de analito extraído frente al tiempo de extracción para obtener lo que se denomina perfil de tiempos de absorción (figura 10).

El polidimetilsiloxano (PDMS), polimero que se comporta como un líquido, presenta tiempos de equilibrio inferiores al poliacrilato (PA) que por tratarse de un polímero mucho más viscoso tiene propiedades similares a un sólido y por ello presenta tiempos de equilibrio más largos. Este hecho se confirmó en la práctica, como se puede observar en la figura 10 , donde el polidimetilsiloxano alcanza el equilibrio a los 60 minutos mientras que el poliacrilato no lo alcanza hasta los 120 minutos.

V.2.2.4. Desorción

Una vez transcurrido el tiempo de absorción, los analitos se desorben térmicamente en el inyector del cromátografo de gases. Para que la desorción se lleve a cabo de manera rapida y a la vez se favorezca la degradación de fenilureas a las anilinas correspondientes, se controla el factor tiempo/temperatura del inyector para que no se manifiesten problemas de ensanchamiento de los picos cromatográficos. Así, y también para evitar efectos de memoria, se seleccionan tiempos de desorción entre 2-5 minutos a las máximas temperaturas recomendadas por el fabricante de cada polímero que coinciden con la temperatura de inyección 300°C.

V.2.2.5. Selección de la fibra

La fibra consiste en un pequeño cilindro de 1 cm de longitud y un espesor entre 7 a 100 μm de un polímero determinado. El tipo de polímero empleado es el que confiere la especificidad y eficacia de extracción en cuanto a cantidad de analito extraido, por lo que la selección del polímero es decir, el tipo de fibra, es de crucial importancia para la puesta a punto de un método.

La eficacia de extracción de una fibra para un determinado compuesto se basa en la polaridad del polímero. El PDMS es el polímero mas apolar y el PA más polar. Por lo tanto, de manera orientativa, el polímero polar sería más adecuado para la extracción de compuestos polares con propiedades relativamente polares como las ureas herbicidas [9]. Pero, esta aproximación necesita demostración ya que en ciertas fibras, no sólo interviene la absorción sino tambien la adsorción.

En cuanto al espesor de fibra en el caso de PDMS, un mayor espesor proporciona un mayor volumen de fibra V_f y por lo tanto la cantidad de analito extraído "n" es también mayor. En el caso de las ureas estudiadas, se observa cómo con la fibra de 100 μm se obtiene una extracción promedio 15 a 19 veces superior a la obtenida con la fibra de 7 μm. Pero por otro lado mayor espesor de fibra hace que la transferencia

del analito sea más lenta en el propio polimero y por tanto los tiempos de equilibrio relativamente mayores (figura 10).

La ventaja de llevar a cabo la extracción con tiempos de absorción por encima del tiempo de equilibrio es que se consiga la máxima eficacia de extracción (máxima cantidad de analito extraído). Sin embargo, con objeto de obtener un método más rapido, el tiempo de absorción se establece llegando a un compromiso entre cantidad de analito extraído y sensibilidad requerida para el método sin alcanzar los tiempos de equilibrio.

Así, la fibra PA es la que mejor concilia buena sensibilidad con razonable tiempo de extracción en cada momento de la extracción como se ve en la figura 10, y es por lo tanto la fibra elegida para el resto de los ensayos.

Tras 60 min de absorción, la cantidad de analito extraido en la mayoria de los casos es superior al 75% de la cantidad que se extraía en equilibrio. Se selecciona 60 minutos por tambien permitir que una muestra pueda estar extrayendose mientras se realiza el análisis cromatográfico de la muestra previa. Con este tiempo de extracción, se consiguen límites de detección inferiores a 0.1μg/L (Tabla 21). Cuando se trabaja con tiempos de extracción por debajo del tiempo de equilibrio deben controlarse todos los factores de forma precisa, para que no se afecte la repetibilidad del análisis, con el empleo del muestreador automático Varian 8200 para MEFS, esta cuestión queda resuelta.

Se han estimado las constantes de distribución fibra/agua aparentes y comparado con las constantes de distribución octanol/agua (Tabla 20). Se observa una buena correlación entre ambos. Por otro lado, se observa una buena correlación lineal entre la cantidad de analito extraído y las K_{ow} (ver figura 17). Esta buena correlación se interpreta como que los fenómenos que predominan en el proceso de extracción son los de absorción.
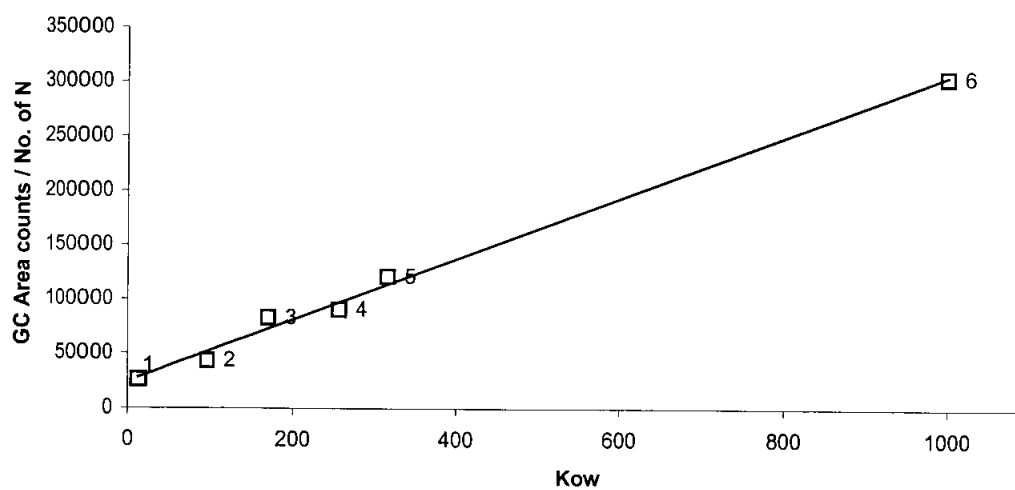
Figura 17. Correlación entre la cantidad de analito extraído y K_{ow}

V.2.2.6. Adición de sal

La adición de sal a la muestra generalmente aumenta la constante de distribución fibra/agua de las moléculas orgánicas no disociadas, lo que explica su uso frecuente para aumentar la extracción de plaguicidas polares. En este caso se demuestra como la adición de cloruro sódico al 14'3% incrementa la extracción en un factor de hasta 10 veces (Figura 12).

Para las ureas más apolares con log Kow de 2 como diuron y linuron , el efecto puede llegar a ser negativo [10].

V.2.2.7. Ajuste de pH

El ajuste del pH de la matriz acuosa resulta muy eficaz para incrementar la extracción de analitos ácidos o basicos. El pH afecta al equilibrio de disociación en el medio acuoso. Una disminución del pH permite el incremento en la extracción de los analitos ácidos. Para que exista un desplazamiento del equilibrio hacia las formas no disociadas, el pH debe ser al menos 2 unidades inferiores al pKa del analito [11]. Así, se aplicó esta estrategia para optimizar la extracción de del clorsulfuron (pKa=3'6). Este ajuste de pH sirve también para hacer una extracción selectiva de las ureas, respecto a sus correpondientes anilinas que son sus principales metabolitos. A pH=4 las anilinas se encuentran mayoritariamente en forma iónica y no se extraen, sin embargo a pH=11 se extraen tanto las fenilureas como las anilinas.

V.2.2.8. Presencia de materia orgánica

Para estudiar el efecto de la materia orgánica, se añadió 10 mg/L de ácido húmico sobre una muestra de agua y se vió como se reducia la cantidad de herbicida extraido entre un 20 y 50%. La misma operación en una muestra con 10'8 % de cloruro sódico sólo reducia la extracción un 10 % y en una muestra con 14'3 % de sal el efecto pasa a ser insignificante. El ácido húmico modifica el reparto fibra/agua aumentando la solubilidad de los herbicidas en el agua que quedan adheridas a las macromoleculas

del ácido húmico. El aumento de la fuerza ionica que produce la adición de cloruro sódico, libera la unión de los herbicidas con el ácido húmico [12].

Para comprobar la versatilidad de este método de extracción, se estudia la reproducibilidad, linealidad y exactitud sobre muestras de agua de acequia añadida. Los resultados se muestran en la tabla 21 donde se observa como los límites de detección oscilan entre 0'1 y 0'04 μg/L, y se obtiene una buena respuesta lineal y reproducibilidad de este método. Estos resultados prueban la utilidad de la MEFS para analizar herbicidas en muestras de agua reales.

V.2.2.9. Presencia de partículas de origen vegetal

Las partículas en suspensión de origen vegetal afectan a la extracción de dos maneras: Disminuyen la eficacia de la fibra alterando el reparto fibra/agua y Dañan físicamente su estructura formando depositos sobre su superficia. Simplició y col. han estudiado este efecto concluyendo que la dilución es el mejor remedio [13].

Con una dilución de 1:5 se consiguen unas recuperaciones superiores al 85% a los niveles de adició estudiadas (1-250 μg/L).

V.2.2.10. Aplicación a muestras reales

Se aplica el método a la determinación de herbicidas en aguas superficiales y muestras comerciales de hortalizas. Se analizan cinco muestras de agua de la Albufera de Valencia. No se detectan residuos de herbicidas en ninguna muestra. La figura 12 (a) representa el cromatograma de la muestra sin residuos detectables de agua de la Acequia Dreta. Esta muestra se tomó como base para realizar todos los ensayos de validación del método. Los datos obtenidos de recuperación se observan en la tabla 21.

Se analizan doce muestras comerciales de zanahoria, de cebolla y de patata de la Comunidad Valenciana. Solo se detecta linuron en tres muestras de patatas a los

niveles siguientes; 4, 5 y 9 μg/kg expresado en 3,4-dicloroanilina. Ninguna muestra supera el LMR (50 μg/kg).

La figura 15 reproduce cromatogramas obtenidos con muestras reales. La figura 15 B recoge el cromatograma que corresponde a una muestra de patata con 5 μg/kg de linuron.

Los límites de cuantificación obtenidos se observan en la tabla 25 donde se aprecia la sensibilidad del método utilizando el DNF. Esta sensibilidad es incluso mejor cuando se utiliza el DEM en modo SIM, como se puede apreciar en la figura 14, con niveles de adición extremamente bajos (0'3 μg/kg)

El tipo de matriz no influye en la recuperación de los herbicidas (Tabla 23). Las hortalizas estudiadas tienen una composición similar en cuanto a macronutrientes con un 0'3 % de lípidos , 9-12 % de proteinas, 1-4 % de carbohidratos y 77'8-88'2 % de agua. Otros componentes endogenos no interfieren en el análisis.

A la vista de los resultados, este método muestra muy buenas espectativas para su aplicación en otros alimentos de alto contenido en agua.

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CONCLUSIONES

CONCLUSIONES

Primera - Los herbicidas derivados de la urea se pueden determinar por cromatografía de gases con detectores de nitrógeno-fósforo, captura electrónica y espectrometría de masas a partir de los productos de degradación térmica formados en el inyector con buena sensibilidad y reproducibilidad.

Segunda - La temperatura del inyector es el factor más importante de todos los que influyen en la degradación térmica. La inyección á 300 °C facilita la degradación completa y reproducible de los herbicidas estudiados antes de su interacción con la fase estacionaria de la columna, por lo que es la temperatura elegida para llevar a cabo el resto de los análisis.

Tercera - El disolvente empleado en la inyección de fenilureas es muy importante, así se forman los ésteres carbámicos análogos con metanol y etanol y los isocianatos análogos con acetonitrilo, diclorometano y acetato de etilo mientras que metabenzotiazuron forma un benzotiazol, clorsulfuron una triazina y una fenilsulfonamida con independencia del disolvente empleado..

Cuarta - La extracción en fase sólida con discos de octilsílice y posterior análisis de los extractos por cromatografía de gases permite la detección de los herbicidas seleccionados en aguas a concentraciones inferiores a 0'1 µg/L con recuperaciones superiores al 87 %.

Quinta - La fibra de poliacrilato muestra mejor eficacia que las de polidimetilsiloxano para la extracción de los herbicidas permitiendo con un tiempo de extracción razonable (60 min) alcanzar límites de detección inferiores a 0'1 µg/L.

Sexta - El aumento de la fuerza ionica y la dilución de la muestra favorecen la MEFS de los herbicidas, disminuyendo las interferencias que ocasionan la presencia de acidos humicos en aguas y la de las particulas sólidas de origen vegetal en hortalizas.

Séptima - La MEFS acoplada con la cromatografía de gases permite el análisis de ureas herbicidas en muestras de cebollas, patatas y zanahorias a concentraciones muy por debajo de las exigidas en los Límites Máximos de Residuos legislados. Una doble extracción realizada a pH 4 y a pH 11, es suficiente para conocer por separado los residuos que proceden de las fenilureas herbicidas y los residuos de anilinas que son sus metabolitos.

Octava - La MEFS acoplada con la cromatografía de gases no emplea disolventes y permite realizar la extracción desde muestras acuosas y la determinación de manera automática. Su aplicación en alimentos con alto contenido en agua, abre muchas posibilidades de futuro en el campo del análisis de residuos de plaguicidas en alimentos.