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*Alla strada percorsa,
che mi ha insegnato a cadere e a rialzarmi,
ricordandomi che ogni passo
è parte del viaggio.*

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General abbreviations

2D	Two-dimensional
3D	Three-dimensional
ACN	Acetonitrile
APCI	Atmospheric pressure chemical ionization
API	Atmospheric pressure ionization
BP-3	Benzophenone-3
Cap-EI	Capillary-EI
CF	Commercial formulation
CH ₄	Methane
CI	Chemical ionization
CP-MIMS	Condensed phase membrane introduction MS
CUPs	Current-use pesticides
DEI	Direct-EI
DI-SPME	Direct solid phase microextraction
DLI	Direct liquid introduction
DLLME	Dispersive liquid-liquid microextraction
d-MRM	Dynamic multiple ion monitoring
d-SPE	Dispersive solid-phase extraction
EI	Electron ionization
E-LEI-MS	Extractive Liquid-Sampling Electron Ionization MS

ESI	Electrospray ionization
FA	Formic acid
FAO	Food and Agriculture Organization
FIA	Flow injection analysis
GC	Gas chromatography
H ₂ O	Water
He	Helium
Hex	Hexane
HILIC	Hydrophilic interaction liquid chromatography
HR	High resolution
Htyr	Hydroxytyrosol
I.D.	Inner diameter
IS	Internal standard
LC	Liquid chromatography
LEI	Liquid electron ionization
LLE	Liquid-liquid extraction
LOD	Limit of detection
LOQ	Limit of quantification
LP-GC	Low-pressure gas chromatography
LR	Low resolution
LVI	Large volume injection
MAE	Microwave-assisted extraction
MB	Moving belt

MD-LC	Multidimensional liquid chromatography
ME	Matrix effects
MeOH	Methanol
MOI	Microfluidic open interface
MRLs	Maximum Residue Limits
MRM	Multiple ion monitoring
MS	Mass spectrometry
M-WATF	Water-assisted trap focusing
N	Nitrogen
Nb	Narrow-bore
NCI	Negative chemical ionization
NPLC	Normal-phase liquid chromatography
NTA	Non-targeted analyses
O.D.	Outside diameter
OCPs	Organochlorine
OPPs	Organophosphate
PA	Phosphoric acid
PAHs	Polycyclic aromatic hydrocarbons
PAS	Passive air samplers
PB	Particle beam
PCI	Positive chemical ionization
PDMS	Poly-dimethyl siloxane
PFS	Passive flow splitter

PLE	Pressurised liquid extraction
PUF	Polyurethane foam
QqQ	Triple quadrupole
Q-TOF	Quadrupole Time-of-flight
QuEChERS	Quick Easy Cheap Effective Rugged, and Safe
QuPPE	Quik polar pesticide
RPLC	Reversed-phase liquid chromatography
S/N	Signal-to-noise ratio
SBM	Supersonic molecular beam
SBSE	Stir bar sorptive extraction
SHLLE	Salting-out homogenous liquid-liquid extraction
SLE	Solid-Liquid extraction
SPE	Solid phase extraction
SPME	Solid phase micro-extraction
SRM	Selected ion monitoring
T _R	Retention time
Tyr	Tyrosol
UAE	Ultrasound extraction
UAME	Ultrasound assisted microextraction
UHPLC	High-performance liquid chromatography
VMC	Vaporization microchannel
WHO	World Health Organization

Abstract

To safeguard public safety and preserve biodiversity, it is crucial to closely observe pesticide concentrations. Mass Spectrometry (MS), combined with liquid chromatography (LC) has emerged as the preferred detection for pesticide detection and quantification. However, atmospheric pressure ionization (API) methods have limitations, including reduced spectral information, reproducibility issues, and challenges with poorly polar molecules and matrix effects, which complicate quantitative analyses and increase costs. To address these issues, alternative approaches have been explored, leading to the development of the Liquid Electron Ionization (LEI) interface, known for its robustness and consistency. LEI interface allows coupling the LC with the electron ionization (EI), extending it to low volatile and thermolabile molecules.

In this dissertation, the LEI interface was involved in different qualitative, quantitative, and untargeted applications. The first part of the project, regarded the development of an innovative, rapid, and green pesticide quantification method based on LEI-MS. Two pesticides with different physical/chemical properties were analyzed simultaneously in a complex matrix. The system was centred on the direct coupling of a solid phase microextraction (SPME) fiber with LEI-MS, due to the use of a microfluidic open interface (MOI); the LC separation was not involved, allowing to perform compound extraction and analyses in about 5 minutes. The method proved to be reliable and reproducible.

The second part of the project was focused on improving performance and demonstrating the versatility and advantages of the LEI interface. Coupling normal phase liquid chromatography (NPLC) with MS is a challenge due to the non-compatibility of the non-polar solvents with API

techniques. For the first time, the system LC-LEI-MS was coupled with the NPLC. Different model compounds were analyzed, and the results showed that non-polar solvents did not affect the EI spectra, which were easily recognized using the NIST library. In addition, a real problem was solved using NPLC coupled with the LEI interface; a non-polar impurity was separated from the main compounds of the mixture in about 5 minutes, instead of the 50 minutes required in RPLC.

In the last part of the project, the LEI interface was coupled with a high-resolution mass spectrometer (HRMS), a quadrupole-time-of-flight, hybrid instrument (Q-TOF), which was used for targeted and untargeted analysis in a forensic case involving Carbofuran (CBF), a carbamate pesticide banned by the EU. A novel workflow for untargeted analysis was developed by using HRMS, allowing for the identification of different compounds in different CBF formulations, required to understand the provenience of the formulation used. In addition, a quantitative method was developed to quantify CBF in animal samples. The use of CBF was confirmed, and the concentration of pesticide found in animal samples was compatible with the lethal dose (LD50). The same workflow was applied to identify an impurity in a formulation that had to be commercialized.

So far, the LEI interface demonstrated its robustness and versatility in several applications, solving some problems that affect conventional ionization techniques. The next steps will focus on improving the sensitivity and making the interface more user-friendly; achieving these goals will expand the use of the LEI interface, establishing it as a crucial alternative for solving analytical challenges.

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List of publications

- Nicole Marittimo, **Genny Grasselli**, Adriana Arigò, Giorgio Famiglini, Pierangela Palma, Mansoor Saeed, Simon Perry, Pablo Navarro, Phil Clarke, Mark Brittin, Achille Cappiello. Sustainable and Rapid Determination of Two Halogenated Pesticides in a Commercial Formulation by Solid Phase Microextraction and Liquid Phase Chemical Ionization Mass Spectrometry. *Separation*. 2023,10,325.
- Nicole Marittimo, **Genny Grasselli**, Adriana Arigò, Giorgio Famiglini, Marco Agostini, Caterina Renzoni, Pierangela Palma, Achille Cappiello. Liquid electron ionization-mass spectrometry as a novel strategy for integrating normal-phase liquid chromatography with low and high-resolution mass spectrometry. *Analyst*. 2024,149, 2664.
- **Genny Grasselli**, Adriana Arigò, Pierangela Palma, Giorgio Famiglini, and Achille Cappiello. Latest Developments in Direct and Non-Direct LC-MS Methods Based on Liquid Electron Ionization (LEI). *Critical Reviews In Analytical Chemistry*. 1-18.
- Adriana Arigò, **Genny Grasselli**. Chapter: Alkaloids' wide world (extraction, quantification, biological activities). Book: *Phytochemicals for Health*. Elsevier. **[In press]**.
- **Genny Grasselli**, Adriana Arigò, Giorgio Famiglini, Zdena Skrob, Artur Sniegón, Tomáš Cajtham, Achille Cappiello. Targeted and Non-Targeted Analysis Workflow in Animal Poisoning Cases: Challenges in Source Tracing. *Environmental Science & Technology*. **[Under review]**.

- Tommaso Grazioso, **Genny Grasselli**, Adriana Arigò, Giorgio Famiglini, Achille Cappiello. Optimizing Liquid Electron Ionization Interface to Boost LC-MS Instrumental Efficiency. Journal of the American Society for Mass Spectrometry. **[Under review]**.

List of Conference Presentations and Posters

- **G. Grasselli**, N. Marittimo, G. Famiglini, P. Palma, A. Cappiello. High-Resolution MS Detection Of Pesticides Using The Direct Coupling Of A SPME Fiber With A Liquid-EI Interface. XXIX Congresso della Divisione di Chimica Analitica. Milazzo, Italy, 11-15 September 2022. **Oral Presentation.**
- **Grasselli G.**, Arigò A., Marittimo N., Famiglini G., Palma P., Saeed M., Perry S., Navarro P., Clarke P., Brittin M., Cappiello A. Coupling Normal Phase Chromatographic Separation With Electron Ionization In Low And High-Resolution Mass Spectrometry Via Liquid Electron Ionization Interface. 71st conference Mass Spectrometry and Allied Topics. American Society For Mass Spectrometry. Houston, Texas, 4-8 June 2023. **Poster Presentation.**
- **G. Grasselli**, A. Arigò, N. Marittimo, G. Famiglini, P. Palma, A. Cappiello. Innovative Approaches For Impurity Identification: Liquid Electron Ionization Interface Coupled With High-Resolution Mass Spectrometry And Normal/Reversed Phase UHPLC. XXX Congresso della Divisione di Chimica Analitica. Vasto, Italy, 17-19 September 2023. **Oral Presentation.**
- **G. Grasselli**, A. Arigò, N. Marittimo, G. Famiglini, P. Palma, A. Cappiello. “Normal and Reversed Phase Liquid Chromatography Coupled To High-Resolution Electron Ionization Mass Spectrometry In Non-Targeted Analysis”. Incontri di Scienza delle Separazioni 2023, Società Chimica Italiana (SCI). Termoli, Italy. 12-13 October 2023. **Oral Presentation.**
- **G. Grasselli**, A. Arigò, G. Famiglini, P. Palma, Z. Skob, T. Cajthaml, A. Cappiello. “Novel strategy based on liquid electron ionization (LEI) interface for targeted and

untargeted analysis in forensic application. 28th International Symposium on Separation Sciences. Messina, Italy. 22-29 September 2024. **Oral Presentation.**

Chapter 1

1.1. Pesticides: role, benefit, classification, and EU regulations

The world's population is continuously growing, expected to reach 10.3 billion people in the mid-2080s (1). This means that an increase in high-quality food demand, such as meat and milk products, leads continuously to an increment of grain requests for livestock feed (2). Therefore, a rise in crop production will be required (3). It has been proved that, in infestation of weeds in the early stage of crops can reduce the yield by up to 40% (4). In addition, in a year the world losses attributed to pests and pathogens, and indirectly to climate change that enhances the incidence of diseases, insects, and viruses, is estimated between 20 and 40% (5). Different strategies are applied to increase productivity and protect crops; advanced fertilizer production techniques, genetically modified cultivars, and pesticide use are the main approaches fielded by land managers (3).

Pesticide is a group of substances including insecticides, fungicides, herbicides, rodenticides, molluscicides, and nematocides (6). They play a role in reducing losses from diseases and insect pests, which lead to a loss in productivity; without their use, it has been estimated a loss of 78% of fruit production, 58% of vegetable production, and 32% of cereal production (7). In addition, pesticide usage also leads to several secondary benefits; one of them regards the increase of productivity of high-quality food, such as fresh fruit and vegetables. Their growth facilitates the increase of the consumption of these foods proved able to reduce the incidence of cancer, hypertension, and cardiac problems, therefore improving the health and life expectancy of

the population (8). Pesticides also play a part against insect disease vectors; their use helps to fight deadly diseases such as malaria (9).

Despite the advantages of their use, pesticides can threaten public health and the environment; their effect on targeted organisms can sometimes be toxic for non-targeted organisms as well. The risks of pesticide usage are explained in detail in the following paragraph.

Pesticides classification. Pesticides feature compounds with different chemical and physical properties. The first differentiation is between natural and synthetic (**Figure 1.1**). Natural pesticides include compounds found in nature or compounds derived from living organisms' structures, such as plants, fungi, bacteria, and so on. Synthetics are instead man-made compounds that are not present in nature (10).

Natural pesticides are classified as biochemical, microbial, and plant-incorporated protectants. The advantages of using naturals rely on their low toxicity, easy decomposition, and low susceptibility to genetic modification in plant populations (10). Regarding synthetic pesticides, there are three different types of classifications: (I) chemical structure; (II) entry mode; (III) mode of action. Being the most widespread and the most significant for deciding an appropriate analytical method, herein the classification is based on the chemical structure (11). Organic pesticides can be divided into four main classes: Organochlorine (OCPs), Organophosphate (OPPs), Carbamate, and Pyrethroid (11).

OCPs typically share aliphatic or aromatic structures heavily substituted with chlorines. They are scarcely soluble in water and semi-volatile, and due to their high stability, some are among persistent organic pollutants (POPs) (12). OCPs were found in different matrices, such as soils and waters, and can travel long distances from the application point through wind. Due to their

lipophilic ability, OCPs accumulate in human, animal, and fish bodies, becoming a risk to public health (12,13).

OPPs are esters derived from phosphoric acid (14). OPPs are highly soluble in water and, therefore, widely found in water bodies, such as surface water, groundwater, wastewater, and drinking water (15). OPPs are found in food crops as well, which are the first source of contamination for humans and animals (16). Other contaminations can occur by direct and indirect inhalation, and by dermal contact. In addition, the short half-life, and quick metabolization of their metabolites, make their detection in biological tissues and fluids a challenge (17).

Carbamate pesticide structures are derived from carbamic acid, and, as OPPs, they affect the transmission of nerve signals inducing the death of pests (10). Due to their low bioaccumulation and low persistence characteristics, in addition to the broad spectrum of action, they are largely used in modern agriculture (18). However, their large application, in addition to their solubility in water, raised questions regarding public health (19). Carbamates show high toxicity in non-targeted organisms as well (10); several cases of intentional and accidental poisoning occurred over the years, leading to the ban of some pesticides belonging to this class, such as carbofuran and carbosulfan (20-24).

The last class of the pesticide classification discussed herein is Pyrethroids. They derive from pyrethrins and they are divided into two major categories: agricultural pyrethroids and urban pyrethroids. Both categories are used against pests (25). Despite they are considered less environmentally polluting than the other classes, they show high toxicity to aquatic organisms and, due to their high lipophilicity, they are persistent in organisms (25-27). Different studies reported the presence of pyrethroids in different environmental media (28), residential

environments (29), and foods (30). Consequently, some of them were detected in the human body as well (25).

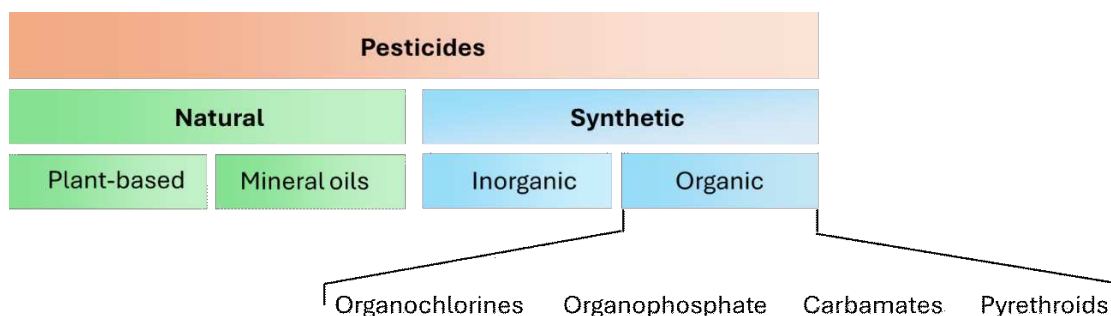


Figure 1.1. Pesticides classification.

EU Regulation. As explained previously, pesticides are fundamental to increasing crop yields; however, their negative effect on public health and the environment are well known. Therefore, the presence of pesticides, and their impact on the ecosystem, have been largely studied in different matrices, such as soil, water (groundwater and surface water), air, food, sediments, plants, and organisms, like fishes, and insects.

Concerning public health, food is the most regulated matrix. The international trade of food helps to utilize the advantages of different growing conditions and is a key element of economies (31). Therefore, with its expansion, it became fundamental that the use of pesticides is strictly regulated and controlled. The United Nations for Food and Agriculture Organization (FAO) is a specialized agency, that includes different countries, responsible for the regulation of food safety. The FAO, together with the World Health Organization (WHO), established in 1963 a Codex Alimentarius (Codex), which includes international guidelines and codes with

the scope of protecting the health of customers (32). The use of pesticides is fundamental in most agriculture products, therefore Codex established the Maximum Residue Limits (MRLs), expressed in mg/kg, essential to ensure the safety of food containing pesticide residues (33). MRLs refer to the maximum concentrations of pesticide residues permitted in food (34).

The limits of pesticides in groundwater and surface water, including drinking water, are dictated by the EU Water Directive Framework (Directive 2000/60/EC of the European Parliament and of the Council) (35); in this case, the concentration of each pesticide and metabolites has to be below 0.1 µg/L, while the sum of pesticides and their metabolites below 0.5 µg/L (35).

Despite the expanded contamination of soil and air by pesticides can lead to several implications, there are no EU legislations concerning the maximum levels of pesticides on them (36). In particular, in soil, it has been estimated that less than 0.1% of pesticides applied reach pests; the rest can contaminate water, air, and soil, causing adverse effects on non-targeted organisms (37). Therefore, extensive monitoring of pesticide levels, particularly concerning persistent and water-soluble pesticides, could be required to reduce the risks to public health and biodiversity.

1.2.The role of Mass Spectrometry for Pesticides Analysis

To ensure public safety and the integrity of biodiversity, pesticide concentrations must be strictly monitored. Several analytical methods have been developed over the years; however, Mass Spectrometry (MS) has been established as a preferable detection technique due to its high identification power and low limit of quantification (LOQ) (38).

After the development of the electron ionization (EI) technique, gas chromatography (GC) coupled with MS was at the beginning the most used gas-phase technique. The ionization of the molecules under high vacuum and high temperatures, in addition to a fixed electron energy (70 eV), allows for reproducible and highly specific molecule fragmentations and constructing a library for easy compound identification (39). Alternative to EI, chemical ionization (CI) is another high-vacuum ionization technique employed in GC-MS. In this case, the ionization is given by the ionized reagent gas, introduced in large quantities in the ion source, which reduces the fragmentation. CI can be used in positive (PCI) and negative (NCI) modes. The NCI allows better sensitivity to EI for compounds containing electronegative atoms, increasing the signal-to-noise ratio (S/N). Recently, a novel strategy using EI to minimize fragmentation and increase the intensity of the molecular ion was developed by Amirav et. al. (40). The Cold-EI keeps the EI fragmentation, but the presence of the molecular ion facilitates the identification of unknown compounds using GC-MS (40). However, GC allows the analysis of volatile and thermostable molecules; it has been estimated that only 20% of organic molecules are GC amenable (41). Most recently, liquid chromatography (LC) coupled with MS has taken over, extending the MS to molecules non-GC amenable. The soft ionization techniques used with the LC, mainly based on electrospray ionization (ESI), show high sensitivity and versatility, allowing the ionization of thermolabile, high-molecular weight, and non-volatile compounds. However, ESI provides less informative spectra, producing mainly molecular ions, hence making more difficult the determination of unknown structures (39).

So far, LC-MS did not replace completely GC-MS in pesticide analysis; although is the preferred technique, GC-MS remains the most suitable technique for the analysis of pyrethrins and organochlorines (42,43).

Over the years, different strategies have been developed to enhance the performance of both GC-MS and LC-MS.

GC-MS for Pesticides Analysis. Regarding GC-MS, different strategies have been developed to enlarge this technique to low volatile and thermolabile molecules. The derivatization by silylation, oximation, acylation, and methylation, helps the vaporization and separation of non-volatile and highly polar compounds (44,45); however, an increase in time-consuming and difference in derivatization efficiency are the main drawbacks of this approach (46). Another issue for GC-MS relies on the high instrumental limit of detection (LOD), in the range of $\mu\text{g/L}$. To overcome the problem researchers are mainly focused on sample extraction and enrichment steps optimization. Different sample enrichment techniques have been developed over the years. Among them, Solid phase extraction (SPE), and solid phase micro-extraction (SPME) are currently the most used (47). In a work, Simon et. al. (48) developed a miniaturized, simple, and sensitive method for 10 OCPs and trifluralin analysis in soil; the extraction was performed using Quick Easy Cheap Effective Rugged, and Safe (QuEChERS) procedure, followed by head-space (HD) SPME. The analyses were conducted using GC-MS and the LODs and LOQs reached were between 0.001-1.48 $\mu\text{g/kg}$ and 0.004-4.93 $\mu\text{g/kg}$ (48). In another study, Cheng et. al. (49) developed another quantification method based on GC coupled with quadrupole Time-of-Flight (Q-TOF) for 15 OCPs analysis in soil and water. For water samples, the extraction was performed using SPE. The LODs and LOQs were respectively between 0.33-1.88 and 0.89-4.16 $\mu\text{g/L}$ (49).

In addition, other strategies were developed to enhance the analytical performance of GC-MS systems, such as combining it with Atmospheric pressure chemical ionization (APCI), which is

included in API (Atmospheric pressure ionization) techniques normally coupled with LC-MS. Due to the reduced fragmentation, APCI enables a decrease in the LODs of 7-305 times, compared to the analysis conducted using EI. The comparison was performed by analyzing 15 OCPs in both EI and APCI (49). However, as in LC-MS APCI is more prone to matrix effects (ME) than EI.

Regarding the time of analysis, one of the main issues in GC separation is the time involved for a run, which is normally between 30 min and 60 min. The different strategies to reduce the run time and increase the sample throughput implemented different columns (shorter columns (50), and smaller I.D. (<0.18mm) called narrow-bore column (Nb) (51)), higher carrier gas flow rate (52), and a fast temperature program (53). Another described approach was the low-pressure chromatography (LP-GC); the use of a short wide-bore column (length 5-10 m; I.D. 0.32-0.5 mm) allows the increase of gas linear velocity, enhancing the diffusion coefficient of the analytes. Han et. al. developed a method for the separation of 234 pesticides in meats and poultry in less than 10 min (54).

LC-MS for pesticide analysis. The LC-MS is suitable for a wider variety of molecules avoiding the derivatization, which results in a longer and time-consuming process moving away from the principles of green chemistry (55). LC-MS/MS demonstrated to be the most sensitive technique, reaching LODs and LOQs required for pesticides, in the range of ng/L (47). For MS detection and quantification of pesticides, both low-resolution (LR) MS and high-resolution (HR) MS are involved. To enhance the selectivity and sensitivity, in particular for LRMS, targeted approaches are operated in selected ion monitoring (SRM), or multiple ion monitoring (MRM) (56). Because analyses of pesticides usually include several analytes, to increase the

specificity of MRM transitions, and enhance the rate of analysis, the dynamic MRM (d-MRM) has been developed; the acquisition mode involves a timetable based on the retention time (T_R) of the analytes.

Nevertheless, the ionization techniques coupled with LC are more prone to ME; the co-elution of compounds or matrix components with the compounds of interest, leads to signal enhancement or suppression (57). Despite different strategies have been developed to overcome this problem, such as the use of internal standards (IS), accurate good chromatographic separation and sample preparation steps are required (57).

Due to the greater separation efficiency, Ultra high-performance LC (UHPLC) is currently the most used separation technique (58). Coupling UHPLC to MS helps to improve the separation of complex mixtures, allowing the development of multi-class and multi-residue methods. Different additional strategies were developed to increase the separation performance (58).

A more recent strategy based on multidimensional (MD) chromatography was developed to increase the separation efficiency of complex matrices; MD-LC involves two different separation stages, which can combine different separation modes, such as NPLC (Normal-phase liquid chromatography) x RPLC (Reversed-phase liquid chromatography), RPLC x RPLC, and HILIC (Hydrophilic Interaction Liquid Chromatography) x RPLC. This strategy was widely used for pesticide analyses in food (59); Kittlaus et. al. (60) developed an automated method for analyzing 300 pesticides in foods using two-dimensional LC-MS/MS (2D-LC-MS/MS). In the first dimension, the separation was performed in HILIC mode and was used for the separation of the analytes from sample matrix components, while in the second dimension, the separation was conducted using RPLC and was used for the separation of analytes. Another method based on 2D-LC for analyses of 24 pesticides was developed by Diaz Merino et. al.

(61). In this work, a chiral column in the first dimension, and an achiral column in the second dimension, were involved in the LC separation. The separation method also increased the enantio-resolution capacity of multiple chiral pesticides, in a single analysis run, of a complex mixture containing achiral compounds.

Sample preparation in pesticide analyses. Sample preparation steps can directly affect the performance of both GC-MS and LC-MS systems. As mentioned previously, optimizing them is fundamental to reducing the ME, which can strongly affect quantitative analyses, and increasing the method sensitivity.

The extraction techniques used for pesticide analyses in complex matrices depend mainly on the matrix type. Starting from the soil, which is a complex media containing different % of organic matter (between 1% and 5% of the weight in agriculture, and till 100% in organic soil), and minerals of different chemical and physical properties (62). The main compound extraction techniques used for soil are SPE (62), QuEChERS (36), Pressurised liquid extraction (PLE) (63,64), Solid-Liquid extraction (SLE) (65), Ultrasound extraction (UAE) (66), and Microwave- assisted extraction (MAE) (67).

As for soils, the extraction of pesticides from water bodies requires accurate sample preparation steps, particularly for wastewaters, and aquaculture waters; the extraction techniques are mainly based on Liquid-liquid extraction (LLE) (68,69), SPE (70), SPME (71), Stir bar sorptive extraction (SBSE) (72), Dispersive liquid-liquid microextraction (DLLME) (73), Salting-out homogenous liquid-liquid extraction (SHLLE) (74).

Regarding food, the most employed extraction method is QuEChERS, which became rapidly the official method for the extraction of pesticides mainly from fruits and vegetables. Initially,

it was largely used for extracting pesticides from fruits (75-77) and vegetables (77-79), but over the years, it was adapted to other food matrices. Different sorbents were studied to improve the extraction efficiency in other types of food, such as beef and chicken (80), milk (81), and so on. QuEChERS however showed less recovery efficiency for the more polar compounds, therefore the Quik polar pesticide (QuPPE) procedure is preferred (82).

Other pesticide extraction techniques applied in food matrices include Soxhlet (83), SPE, dispersive solid-phase extraction (d-SPE), and DLLME. However, for the last three mentioned techniques for pesticide extraction from solid food, additional steps are required (84,85).

Pesticides are widely studied in the air as well. Airborne pesticides are generally sampled with passive air samplers (PAS), or high-volume active samplers for current-use pesticides (CUPs) analyses; active samplers collect CUPs from gaseous and particulate phases of air (86,87). The extraction of CUPs from PAS discs, usually formed by polyurethane foam (PUF), is performed using different extraction techniques, such as Soxhlet (88), PLE (89), MAE (90), and Ultrasound Assisted Microextraction (UAME). Among them, UAME shows the greenest features.

1.3. LC-MS Based on Liquid Electron Ionization (LEI)

1.3.1. The history of EI in LC-MS

The EI was developed by Dempster between 1918 and 1920 and then improved by Bleakney, Winn, and Nier (39). The great advantage of EI is that, by maintaining the analytical conditions constant and controlled, it provides highly reproducible spectra. Setting the electron energy at 70 eV and maintaining always the same temperature of the ion source, makes the EI depending only on intramolecular reactions, and not on chemical interactions, allowing for highly informative and characteristic spectra. Universal libraries containing several EI spectra, such as Wiley, the National Institute of Science and Technology (NIST), and Technology-NIST were created; comparing the experimental spectra with the libraries spectra facilitates the identification of unknown compounds (39).

The first steps in LC-MS were made by coupling EI with LC (39), since EI was the only ionization technique available. However, the main issue relies on the high quantity of gas produced by the vaporization of the liquid phase, which breaks the high vacuum in the ion source (92). Therefore, the development of API techniques solved the problem, moving the ionization process outside the vacuum zone. Among them, ESI is currently the most widely used due to its versatility, sensitivity, and ability to extend MS to a wider variety of molecules (93,94).

As carefully described by Famiglini et. al. (39), the attempts to couple LC with EI have continued, even after the development of API, trying to overcome the main limitation due the incompatibility of a liquid phase in high-vacuum conditions (**Figure 1.2**). In the 1960s Tal'roze

and coworkers (95) presented the first LC-EI-MS direct coupling, while, later, in 1974, the direct liquid introduction (DLI) coupled with CI was developed by Arpino et. al. (96). Unfortunately, both were turned down for several drawbacks.

Simultaneously, a different concept based on an offline approach in which the analytes were separated from the mobile phase before entering the ion source was developed and commercialized; this LC-MS interface was called the moving belt (MB) (97). In MB, the eluent exiting from the column is sprayed onto a belt and evaporated at atmospheric pressure, solving the problem of the high gas quantity in the ion source (97). However, in this case, the complexity, fragility, and high memory effects lead to deserting the idea (39). Still based on MB principles, different interfaces were developed over the years, such as particle beam (PB), Micro-PB (98), and Capillary-EI (Cap-EI) (99).

In 2001, a novel and simpler prototype was proposed by Cappiello et. al. The interface, named direct-EI (DEI) showed a simplified configuration, in which the eluent from the column enters directly into the ion source (100). Maintaining the mobile phase's flow rate very low (<1 $\mu\text{L}/\text{min}$), and making some ion source modifications, help to avoid mobile phase-induced CI phenomena. Different parameters were carefully evaluated, such as optimal flow rates, mobile phase composition, temperature, nebulizer positioning, and different vaporization surface materials. The DEI was used for 15 years and several applications were published, including the analysis of phenoxyacid pesticides (LC-amenable) and OCPs (GC-amenable), endocrine disrupting compounds, free fatty acid in mussels, and so on (101,102). A modified version of DEI was presented by Rigano et. al.; this new version aimed to reduce capillary clogging (103). In the meantime, a different concept of LC-EI-MS interface was developed by Amirav and co-workers; in this case, the interface is based on a pneumatic spray formation as in APCI or APPI

(104). It was coupled with a supersonic molecular beam (SBM), already used coupled with GC-MS (40). Also, this kind of interface demonstrated versatility and robustness, in addition to high identification power due to the cold-EI, which provides the same EI fragmentation with more intense molecular ions (104).

However, so far, the DEI developed by Cappiello and co-workers remained the most used technique. Despite its success, DEI was affected by some limitations, such as the early solvent vaporization, followed by the necessity to vent the instrument to replace the clogged capillary; these limitations remained unsolved, leading to the development of one of the latest LC-MS interface configurations in 2017, the liquid electron ionization interface (LEI) (105).

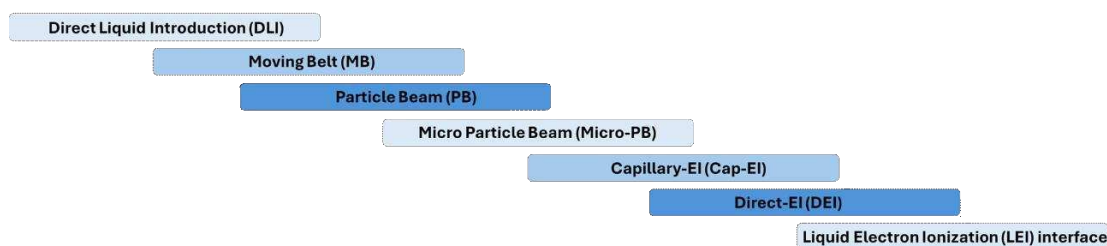


Figure 1.2. The history of electron ionization in LC-MS.

1.3.2. Liquid Electron Ionization (LEI) interface

The LEI interface is currently the most employed LC-EI-MS interface. The first configuration was proposed by Termopoli and co-workers in 2017 (105). Differently from DEI, the vaporization of solvent and analytes occurs at atmospheric pressure, in the vaporization microchannel (VMC), the core of LEI, before the eluent reaches the ion source. This helps to prevent the early solvent vaporization, reducing the risk of capillary clogging. In addition, in

the rare event that happens, the structure of VMC allows for quick capillary replacement without the need for a vent (105).

Regarding the first LEI interface configuration proposed, a tee junction is connected to three different capillaries: (1) VMC, (2) inlet capillary, and (3) He carrier capillary. The VMC consists of a fused silica capillary (O.D. 800 μm ; I.D. 400 μm), which enters inside a standard GC transfer line that can be heated up to 400°C. Its use is fundamental because it can be easily replaced, avoiding contamination, and it prevents the thermal degradation of analytes due to contact with transfer line metal walls. The liquid flow enters in the VMC flowing through the inlet capillary. The best inlet capillary dimensions were carefully evaluated, leading to finding the most suitable (O.D. 150 μm , I.D. 25 μm). The liquid flow and the analytes are released in the VMC. A Peltier unit was added before the entrance of the VMC to prevent the early vaporization of the solvent. In the VMC they meet the He flow, and they are vaporized due to the high temperature. Releasing the solvent and analytes in the VMC, which has a bigger I.D. to the inlet capillary, avoids the clogging problem; while the use of He as carrier gas speeds up their transport to the ion source.

In addition to the inlet dimensions, the liquid flow rate was carefully evaluated as well to find the compatible value; a flow rate between 300 and 800 nL/min showed the best compatibility with EI ion source (105).

In 2019, a novel version of LEI interface was presented by the same group (106). The main difference relies on the introduction of a cooling gap which replaced the Peltier unit (**Figure 1.3**). The cooling gap is a prolongation of VMC (5 cm) outside the hot zone, preventing the early solvent vaporization as well. To reduce even more the risk of inlet clogging, its position

inside the transfer line was evaluated and the ideal position was fixed at 2 mm inside the VMC hot zone (106).

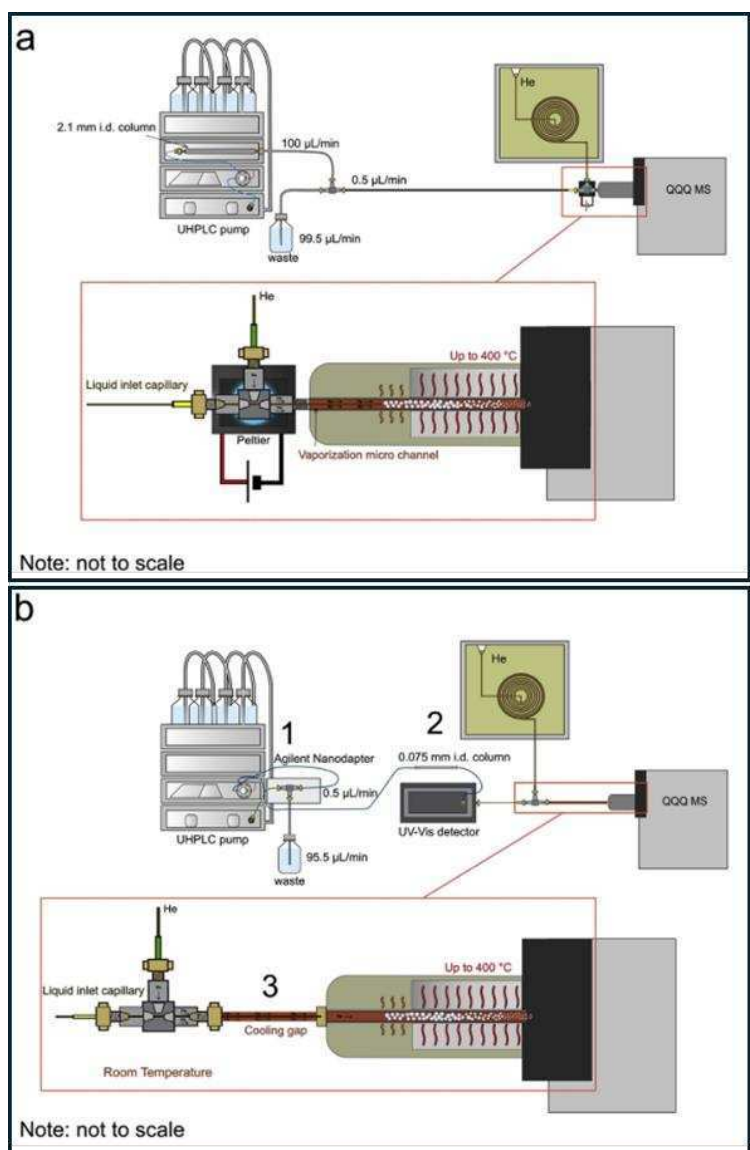


Figure 1.3. Schematic comparison of the LC–MS system equipped with the previous (a), and the new (b) LEI interface. 1: Nanodapter; 2: nano-LC column and online UV–vis detector; 3: cooling gap.

Another innovation was introduced more recently to enlarge the LEI interface to a wider variety of applications (107-112) (**Figure 1.4**). Being the ideal flow rate compatible with EI fixed between 300 and 800 nl/min, the LEI analyses were limited to nano-HPLC. To extend it to higher flow rates, a passive flow splitter (PFS) was introduced before the interface to reduce the flow rate set by the HPLC (113). The PFS is formed by a tee junction; the three ports are connected to (1) the capillary which transports the eluate from HPLC, (2) the split capillary, and (3) the inlet capillary of the LEI interface. The split capillary length and inside diameter can be modulated to change the split ratio (113).

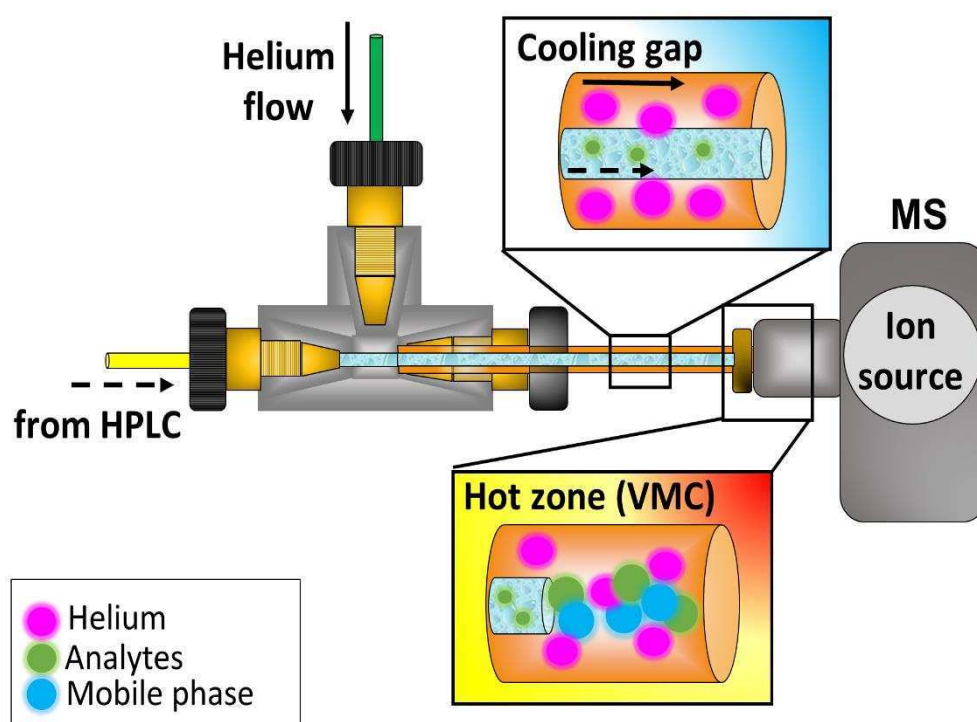


Figure 1.4. Scheme of the LC-LEI-MS/MS and LC-LEI-QTOF-HRMS systems.

More recently, a novel prototype called Extractive Liquid-Sampling Electron Ionization Mass Spectrometry (E-LEI-MS) was presented by Arigò and coworkers (114). For the first time, EI

is combined with ambient sampling, allowing to perform two-dimensional (2D) and three-dimensional (3D) analyses in real time, without chromatography. The system's main components are the sampling tip, the on/off valve, the tee junction, and the micromanipulator (**Figure 1.5**). The sampling tip consists of two coaxial tubes; it is positioned in proximity to the sample surface by using the micromanipulator. The liquid flow, pumped by a syringe pump, reaches the sample surface flowing through the external capillary of the sampling tip, and a drop is deposited on the sample surface for analyte extractions. The internal capillary, connected to the high vacuum zone in the MS, sucks and transfers the solvent and extracted analytes to the ion source. The on/off valve has the function of isolating the ion source from the ambient when the sampling is not performed (off position). The high vacuum inside the MS makes possible the transfer of the analytes and the MS signal is obtained in few minutes (114,115).

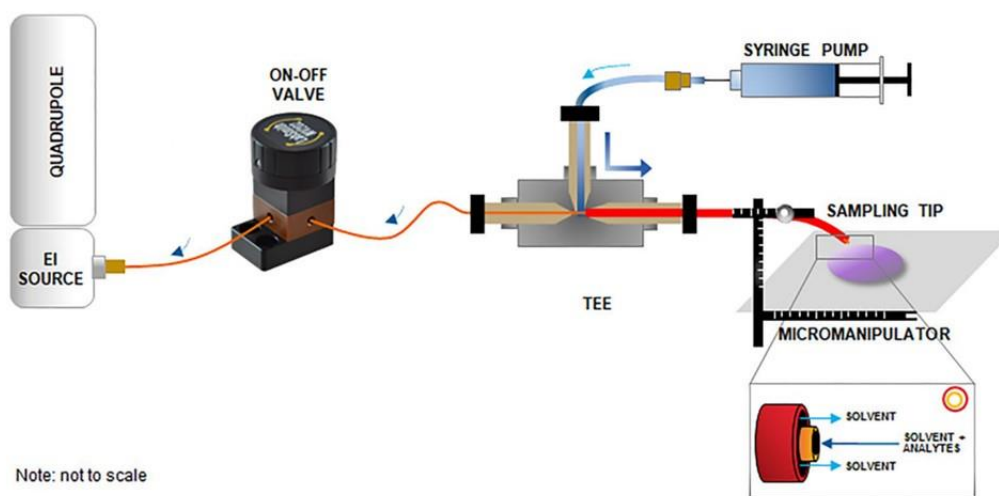


Figure 1.5. Configuration of E-LEI-MS system.

1.3.3. Non-direct and direct application of LC-LEI-MS/MS

Different non-direct and direct LC-MS methods based on LEI were developed over the years (105-108, 110-112, 116). The use of LEI showed several advantages, such as:

- I. possibility to analyze low volatile and thermolabile molecules using EI, ensuring high identification power combined with LC;
- II. possibility to analyze simultaneously compounds with different physical and chemical properties, which normally require different ionization techniques;
- III. a negligible ME in complex sample analyses.

The short time in which the analytes are maintained at high temperatures, together with the fast vaporization, allows the analysis of thermolabile and low volatile molecules that cannot be analyzed in GC, or require derivatizations (105). In the first work presented by Termopoli et. al., two high molecular weight compounds, deoxycholic acid (392.57 g/mol), and imatinib (493.61 g/mol) were successfully analyzed using LC-LEI-MS/MS system; in addition, imatinib spectrum was not present in NIST, confirming the possibility of structure elucidation in compounds not suitable for GC as well (105). Differently from the other LC-MS interfaces, LEI interface allows the simultaneous analysis of compounds that normally are analyzed with different instrumentations (110). In a study, Cappiello et. al. demonstrated the possibility of analyzing simultaneously tefluthrin, a pyrethroid pesticide commonly analyzed in GC-MS due to its non-polar nature (110), and dicamba, a highly polar pesticide analyzed in ESI-LC-MS, or GC-MS after derivatization (117). The compounds were analyzed in a complex matrix, represented by a water-based commercial formulation (CF) containing different interfering additives. In the case of ESI-LC-MS analysis, the extraction of dicamba from CF was

fundamental for reducing ME; however, its extraction from the water-base matrix is a challenge. In this case, LC-LEI-MS/MS was coupled with NCI, increasing the selectivity and sensitivity of analytes of interest; this was necessary to minimize the sample preparation step, which was limited just to filtration and dilution, but ensuring a negligible ME (110).

LC-LEI-MS/MS system demonstrated a negligible ME also when chromatographic separation is not involved. Tyrosol (Tyr) and Hydroxytyrosol (Htyr) were analyzed from olive oil in flow injection analysis (FIA); the samples were only diluted, reducing time and cost with respect to the conventional analytical techniques. The ME was close to 0 for both analytes, ensuring reliable quantitative and qualitative analyses (112).

One limitation of the LC-LEI-MS/MS system relies on the sensitivity. The use of a nano column, suitable for the low flow rate required by EI (≈ 500 nL/min), limits the injection volume to less than 100 nL (115). To overcome this limitation, different strategies have been employed, such as large volume injection (LVI) strategies, or sample enrichment processes, as SPE and SPME. Regarding the LVI strategy, a novel disposable was developed in 2020, to increase the sample injection volume by 400-fold (108). In this way, it was possible to increase the injection volume of the sample in organic solvent up to 20 μ L. The developed system, named water-assisted trap focusing (M-WATF), is based on a passive diffusion micromixer, formed by a Tee junction, which helps the water dilution of the high volume of samples in organic solvent. After the dilution, the sample's compounds are separated by a nano-LC column. Mixing the sample with water helps to focalize the sample performing a RPLC. Five pesticides (clomazone, paclobutrazol, fludiozonil, metolachor, and pirimiphos-methyl) were successfully detected and quantified, reaching LOD and LOQ in the range of ng/L. The increase in sensitivity was obtained without affecting the chromatographic separation (108).

Still improving sensitivity, another innovative device was developed for the direct coupling of an SPME fiber to LEI-MS/MS (107). The system, called Microfluidic Open Interface (MOI), allows for the direct analysis of one, or more, analytes in a complex matrix without the use of chromatographic separation (107). The first configuration was developed by Pawliszyn and co-workers and was combined with ESI (118). Rocio-Bautista et. al., after some time, proposed a novel version adapted to work with LEI-MS/MS system (107). In 2022, a second configuration coupled with LEI-MS/MS was proposed by the same group introducing the PFS (113); this permitted to increase the flow rate, from 500 nL/min to 10 μ L/min, speeding up the process and reducing the peak width. Briefly, the MOI is formed by a desorption chamber of 2,5 μ L, which allows the desorption of analytes, previously adsorbed by the SPME fiber, in a small quantity of solvent. After the desorption time, the valve switching permits the entrance of eluent in the liquid flow, and it transports to the LEI-MS/MS system. The system was applied for the analysis of fentanyl in urine and blood, allowing it to reach a LOD and LOQ between 1.5 and 13.7 μ g/L with a negligible ME (107,113).

The advantage of performing direct analysis relies on avoiding sample preparation steps and chromatographic separations, reducing time and solvent consumption (119). Vandergrift and co-workers presented the condensed phase membrane introduction mass spectrometry (CP-MIMS) system for direct analysis of different analytes in complex matrices (119). Being EI not affected by ME, quantitative analyses were performed for different analytes simultaneously (120). The system is based on the use of a hollow fiber poly-dimethyl siloxane (PDMS) membrane assembled on a direct sampling probe immersed in the stirred sample. The solvent, pumped by the HPLC, flows through the fiber and acts as an acceptor phase. The analytes move from the sample to the acceptor phase and, after splitting, reach the VMC to be vaporized.

Different studies were conducted using the CP-MIMS-LEI system: (1) quantification of different polycyclic aromatic hydrocarbons (PAHs) in soil and water [120], (2) quantification of phthalates in house dust (119), and (3) quantification of benzophenone-3 (BP-3) in water samples (122). In all studies, the analyses were conducted without any sample preparation steps, and the results were obtained in about 6 min. In particular, in the last work regarding BP-3, real-time monitoring assumes a key role allowing to obtain a geospatial assessment to correlate the number of swimmers and the quantity of BP-3 released (122). CP-MIMS-LEI was also used to monitor organic reactions in real-time. The real-time monitoring of various alkylation products formation plays a significant role in synthesis (116).

In the category of direct LC-MS systems coupled with EI is included also the new prototype previously described, E-LEI-MS (114,115). As reported in *paragraph 1.3.2*, E-LEI-MS allows real-time analysis directly on the sample surfaces; different 2D and 3D analyses were presented recently in different fields, such as pharmaceutical, forensic, food, and artwork. About pharmaceuticals, Surgamyl and Brufen tables were analyzed for the detection of tiaprofenic acid and ibuprofen. In both, the active ingredients spectra were obtained in full scan and successfully recognized by the NIST library. In the case of the detection of more than one active ingredient has to be detected, the possibility of performing SIM and MRM allows for separate simultaneous signals; in this regard, three active principles were detected in NeoNisidina tablets: acetylsalicylic acid, acetaminophen, and caffeine (114).

Regarding pesticides, E-LEI-MS was involved in the detection of chlorpyrifos and imazalil, directly on the orange peel surface, and benomyl in banana peel. Also, in this case, the analyses were conducted in full scan and the compounds were recognized by the NIST library with probability values higher than 90%. Although the system is used mainly for qualitative analysis,

a calibration curve was built to evaluate the linearity performance of the system, obtaining a R^2 value of 0.9950 (114).

In forensics, the prototype was used for the detection of cocaine in banknotes, while in the artwork field, the 2D and 3D analyses were performed to understand the best restoration approach. In the last application, the 3D analyses were obtained by prolonging the sampling time; the acetonitrile used for the extraction continued to dissolve the surface of the paint, going deeper. Herein the composition of the paint was different, and a different spectrum belonging to the isopropyl palmitate was detected (114).

Therefore, the use of LC-MS system coupled with EI demonstrated several advantages for direct and non-direct analysis in pesticides and other compounds analyses.

1.4. Objectives of the present study

The main part of this PhD project is the outcome of a collaboration among the University of Urbino, Syngenta Ltd., and Agilent Technologies, which can be divided into three parts; in addition to the collaborations with companies, the project included also a forensic case:

1. The first part of the project was aimed at establishing an innovative, rapid, and sustainable approach for pesticide analyses in a complex matrix. The two pesticides, dicamba and tefluthrin, and the sample matrix, which is a water-based CF, were provided by Syngenta Ltd. The quantification method was developed using MOI-PFS-LEI-QqQ/Q-TOF systems.
2. The second part was focused on the improvement of the performance and the demonstration of the versatility and advantages of the LC-LEI-MS system. The LEI proved to be compatible with different kinds of solvent, allowing to couple normal phase liquid chromatography (NPLC) with MS that is not suitable with the most common LC-MS ionization techniques.
3. In the third part of the project LC-LEI-QTOF system was used for targeted and untargeted analysis in a forensic case, and to identify an impurity in a formulation that had to be commercialized. A novel workflow for untargeted analysis was developed by using HRMS, allowing for the identification of different compounds in complex mixtures. The system was implicated in solving a forensic problem; Carbofuran (CBF), a carbamate pesticide banned by the EU, was involved in an animal poisoning case. The system LC-LEI-QTOF was able to detect and quantify CBF. The use of CBF was confirmed, and the

concentration of pesticide found in animal samples was compatible with the lethal dose (LD50).

1.5. Work done and structure of the thesis

Chapter 1 contains a brief overview of the benefits and issues concerning the use of pesticides, their classifications, and the EU regulations and limits in the main matrices. In addition, it carefully describes the role of the MS in pesticide analyses. The most used sample preparation and analytical techniques are explored and described. A particular focus has been given to the LEI interface as an innovative tool for pesticides and other compounds analysis, highlighting the advantages with respect to the conventional MS interfaces. In the end, the aims and motivations of the works carried out during my PhD are thoroughly listed.

In *Chapter 2*, all materials and instrumentations involved in all studies are listed.

Chapter 3 is about the development of an innovative, rapid, and green method for quantitative analyses of two pesticides provided by Syngenta Ltd., dicamba and tefluthrin in a complex water-based matrix, the CF. The method is based on the use of an SPME fiber for direct immersion (DI) extraction of the two analytes from the matrix. The analysis was performed using the MOI-PFS-LEI-QqQ/QTOF. The system allows for direct coupling of an SPME fiber with MS, ensuring rapid analysis (≈ 5 min) from the sampling step to the signal, with satisfactory system performance, also in the absence of chromatographic separation.

In *Chapter 4* one published study concerning the first aim of my PhD project is reported. In the work, different model compounds were chosen and analyzed using normal-phase liquid chromatography (NPLC) coupled with LEI-QqQ/QTOF. The analyses were performed to demonstrate the compatibility of NPLC with LEI. This application wants to enhance the versatility of the LEI interface with the respect to the conventional ionization techniques used with LC.

Chapter 5 regards the last part of my PhD project, concerning the use of LEI interface coupled with QTOF for untargeted analysis. The first work has been carried out in collaboration with the Charles University of Prague and is aimed at the development of a novel workflow for the characterization of carbofuran formulations, with different proveniences, to understand the one used for an animal poisoning case. In this work, it was developed a quantitative method for the carbofuran in three real samples from poisoned animals, also. A second application was developed in collaboration with Syngenta Ltd. and presents an application of LC-LEI-QTOF aimed at the identification of an impurity that exceeded the concentration admitted in commercialized products, in a formulation that had to be commercialized.

Chapter 6 includes additional activities, outside the PhD project, carried out during the PhD.

Chapter 7 discusses the results obtained during these three years of my PhD program, highlighting the advantages of using the LEI interface in targeted and untargeted analyses, with respect to the most used conventional ionization techniques, its limits, and the future prospects aimed to enhance its performance.

References

- (1) United Nations Department of Economic and Social Affairs, Population Division (2024). World Population Prospects 2024: Summary of Results (UN DESA/POP/2024/TR/NO. 9).
- (2) Oerke, E. C.; Dehne, H. W. Safeguarding production - Losses in major crops and the role of crop protection. *Crop Prot.* 2004, 23, 275–285.
- (3) Silver, W. L.; Perez, T.; Mayer, A.; Jones, A. R. The role of soil in the contribution of food and feed. *Philos. Trans. R. Soc. B Biol. Sci.* 2021, 376, 20200181.
- (4) Aktar, W.; Sengupta, D.; Chowdhury, A. Impact of pesticides use in agriculture: Their benefits and hazards. *Interdiscip. Toxicol.* 2009, 2, 1–12.
- (5) Godoy, D., Dewbre, J., PIN, Amegnaglo, C. J., Soglo, Y. Y., Akpa, A. F., Bickel, M., Sanyang, S., Ly, S., Kuiseu, J., Ama, S., Gautier, B. P., Officer, E. S., Officer, E. S., Eberlin, R., Officer, P., Branch, P. A., Oduro-ofori, E., Aboagye Anokye, P., Acquaye, N. E. A., Dandelar, V. M., Mineo, J., FAO, F. and A. O. of the U., IFAD, WFP, Fadipe A.E.A, *Amolegbe K.B. and Afun O.O., Ganiev, I., Maguire, C., Hoover, T. S., Scanlon, D. C., FAO, F. and A. O. of the U., Singh, K. M., Meena, M. S., Swanson, B. E., *The Future of Food and Agriculture: Trends and Challenges.* 2014.
- (6) Tudi, M.; Ruan, H. D.; Wang, L.; Lyu, J.; Sadler, R.; Connell, D.; Chu, C; Phung, D.T. Agriculture Development, Pesticide Application and Its Impact on the Environment. *J. Environ. Res. Public Heal.* 2021, 18, 1–23.

- (7) Lamichhane, J. R. Pesticide use and risk reduction in European farming systems with IPM: An introduction to the special issue. *Crop Prot.* 2017, 97, 1–6.
- (8) U.S. Department of Health and Human Services and U.S. Department of Agriculture. Dietary Guidelines for Americans, 2005. 6th Edition, Washington, DC: U.S. Government Printing Office, January 2005.
- (9) Ross, G. Risks and benefits of DDT. *Lancet.* 2005, 366, 1771–1772.
- (10) Kaur, R.; Mavi, G. K.; Raghav, S.; Khan, I. Pesticides Classification and its Impact on Environment. *Int. J. Curr. Microbiol. Appl. Sci.* 2019, 8, 1889–1897.
- (11) Hassaan, M. A.; El Nemr, A.; Pesticides pollution: Classifications, human health impact, extraction and treatment techniques. *Egypt. J. Aquat. Res.* 2020, 46, 207–220.
- (12) Taiwo, A. M. A review of environmental and health effects of organochlorine pesticide residues in Africa. *Chemosphere.* 2019, 220, 1126–1140.
- (13) Das, B.; Khan, Y. S. A.; Das, P.; Shaheen, S. M. Organochlorine pesticide residues in catfish, *Tachysurus thalassinus* (Ruppell, 1835), from the South Patches of the Bay of Bengal. *Environ. Pollut.* 2002, 120, 255–259.
- (14) Ore, O. T.; Adeola, A. O.; Bayode, A. A.; Adedipe, D. T.; Nomngongo, P. N. Organophosphate pesticide residues in environmental and biological matrices: Occurrence, distribution and potential remedial approaches. *Environ. Chem. Ecotoxicol.* 2023, 5, 9–23.
- (15) Fosu-Mensah, B. Y.; Okoffo, E. D.; Darko, G.; Gordon, C. Organophosphorus pesticide residues in soils and drinking water sources from cocoa producing areas in Ghana. *Environ. Syst. Res.* 2016, 5.

- (16) Sánchez-Santed, F., Colomina, M. T., Herrero Hernández, E., Organophosphate pesticide exposure and neurodegeneration. *Cortex* 2016, 74, 417–426.
- (17) Needham, L. L. Assessing exposure to organophosphorus pesticides by biomonitoring in epidemiologic studies of birth outcomes. *Environ. Health Perspect.* 2005, 113, 494–498.
- (18) Carazo-Rojas, E.; Pérez-Rojas, G.; Pérez-Villanueva, M.; Chinchilla-Soto, C.; Chin-Pampillo, J. S.; Aguilar-Mora, P.; Alpízar-Marín, M.; Masís-Mora, M.; Rodríguez-Rodríguez, C. E.; Vryzas, Z. Pesticide monitoring and ecotoxicological risk assessment in surface water bodies and sediments of a tropical agro-ecosystem. *Environ. Pollut.* 2018, 241, 800–809.
- (19) Carvalho, F. P. Pesticides, environment, and food safety. *Food Energy Secur.* 2017, 6, 48–60.
- (20) Pivariu, D.; Oros, A.; Tabaran, F.; Gal, A.; Martonos, C.; Nagy, A. Intentional Carbofuran poisoning in 7 dogs. *BMC Vet. Res.* 2020, 1–9.
- (21) Kwon, J.T.; Park, J.E.; Son, K.; Kim, J.; Lee, D.H.; Kim, Y.K.; Kim, Y.; Jeong, H.; Kil, J.; Jheong, W.H.; Kim, M.S. Characterization Analysis Of Wild Bird Poisoning Cases Through Spraying Seeds Artificially. *Appl. ecol. environ. res.* 22, 2, 1467–1477.
- (22) Kumar, S.; Baggi, T. R.; Al-Zughaibi, T. Forensic toxicological and analytical aspects of carbamate poisoning – A review. *J. Forensic Leg. Med.* 2022, 92, 102450.
- (23) Kernalléguen, A.; Le Daré, B.; Pelletier, R.; Ferron, P. J.; Maamar, A.; Bouvet, R.; Baert, A.; Morel, I.; Gicquel, T. Carbofuran self-poisoning: forensic and analytic investigations in twins and literature review. *Int. J. Legal Med.* 2022, 136, 1585–1596.

- (24) Noghrehchi, F.; Dawson, A. H.; Raubenheimer, J.; Mohamed, F.; Gawarammana, I. B.; Eddleston, M.; Buckley, N. A. Restrictions on Pesticides and Deliberate Self-Poisoning in Sri Lanka. *JAMA Netw. Open.* 2024, 7, 2426209.
- (25) Tang, W.; Wang, D.; Wang, J.; Wu, Z.; Li, L.; Huang, M.; Xu, S.; Yan, D. Pyrethroid pesticide residues in the global environment: An overview. *Chemosphere* 2018, 191, 990–1007.
- (26) Sabzevari, S.; Hofman, J. A worldwide review of currently used pesticides' monitoring in agricultural soils. *Sci. Total Environ.* 2022, 812, 152344.
- (27) Antwi, F. B.; Reddy, G. V. P. Toxicological effects of pyrethroids on non-target aquatic insects. *Environ. Toxicol. Pharmacol.* 2015, 40, 915–923.
- (28) Aznar, R.; Moreno-Ramón, H.; Albero, B.; Sánchez-Brunete, C.; Tadeo, J. L.; Spatio-temporal distribution of pyrethroids in soil in Mediterranean paddy fields. *J.S.S.* 2017, 17, 1503–1513.
- (29) Morgan, M. K. Children's exposures to pyrethroid insecticides at home: a review of data collected in published exposure measurement studies conducted in the United States. *Int. J. Environ. Res. Public Health* 2012, 9, 2964–2985.
- (30) Khatun, P.; Islam, A.; Sachi, S.; Islam, M. Z.; Islam, P. Pesticides in vegetable production in Bangladesh: A systemic review of contamination levels and associated health risks in the last decade. *Toxicol. Rep.* 2023, 11, 199–211.
- (31) Ambrus, Á.; Yang, Y. Z. Global Harmonization of Maximum Residue Limits for Pesticides. *J. Agric. Food Chem.* 2016, 64, 30–35.
- (32) FAO; WHO. *Codex Alimentarius - Understanding Codex.* 2018.

- (33) Leong, W. H.; Teh, S. Y.; Hossain, M. M.; Nadarajaw, T.; Zabidi-Hussin, Z.; Chin, S. Y.; Lai, K. S.; Lim, S. H. E. Application, monitoring and adverse effects in pesticide use: The importance of reinforcement of Good Agricultural Practices (GAPs). *J. Environ. Manage.* 2020, 260, 109987.
- (34) FAO. Submission and Evaluation of Pesticide Residues Data for the Estimation of Maximum Residue Levels in Food and Feed. 2016.
- (35) DIRECTIVE (EU) 2020/2184 OF THE COUNCIL of 16 December 2020 on the quality of water intended for human consumption (recast). *Off. J. Eur. Union* 2020, L435, 1–62.
- (36) Silva, V.; Mol, H. G. J.; Zomer, P.; Tienstra, M.; Ritsema, C. J.; Geissen, V. Pesticide residues in European agricultural soils – A hidden reality unfolded. *Sci. Total Environ.* 2019, 653, 1532–1545.
- (37) Arias-Estévez, M.; López-Periago, E.; Martínez-Carballo, E.; Simal-Gándara, J.; Mejuto, J. C.; García-Río, L. The mobility and degradation of pesticides in soils and the pollution of groundwater resources. *Agric. Ecosyst. Environ.* 2008, 123, 247–260.
- (38) SANTE/2020/12830. Guidance Document on Pesticide Analytical Methods for Risk Assessment and Post-approval Control and Monitoring Purposes. 2021, 1–50.
- (39) Famigliani, G.; Palma, P.; Termopoli, V.; Cappiello, A. The history of electron ionization in LC-MS, from the early days to modern technologies: A review. *Anal. Chim. Acta.* 2021, 1167, 338350.
- (40) Fialkov, A. B.; Steiner, U.; Jones, L.; Amirav, A. A new type of GC-MS with advanced capabilities. *Int. J. Mass Spectrom.* 2006, 251, 47–58.

- (41) Lee, T. D. Introduction to Modern Liquid Chromatography, Third Edition. J. Am. Soc. Mass Spectrom. 2011, 22, 196.
- (42) Pico, Y.; Alfaran, A. H.; Barcelo, D. How recent innovations in gas chromatography-mass spectrometry have improved pesticide residue determination: An alternative technique to be in your radar. TrAC - Trends Anal. Chem. 2020, 122, 115720.
- (43) Ccancapa-Cartagena, A.; Masiá, A.; Picó, Y. Simultaneous determination of pyrethroids and pyrethrins by dispersive liquid-liquid microextraction and liquid chromatography triple quadrupole mass spectrometry in environmental samples. Anal. Bioanal. Chem. 2017, 409, 4787–4799.
- (44) Alder, L.; Greulich, K.; Kempe, G.; Barbel, V. Residue analysis of 500 high priority pesticides: better by GC–MS or LC-MS/MS? Mass. Spectrom. Rev. 2006, 25, 838-865.
- (45) Fodor, B.; Molnár-Perl, I. The role of derivatization techniques in the analysis of plant cannabinoids by gas chromatography-mass spectrometry. TrAC - Trends Anal. Chem. 2017, 95, 149–158.
- (46) Hernández-Mesa, M.; Moreno-González, D. Current Role of Mass Spectrometry in the Determination of Pesticide Residues in Food. Separations. 2022, 9, 148.
- (47) Campanale, C.; Massarelli, C.; Losacco, D.; Bisaccia, D.; Triozzi, M.; Uricchio, V. F. The monitoring of pesticides in water matrices and the analytical criticalities: A review. TrAC - Trends Anal. Chem. 2021, 144, 116423.

- (48) Simon, M. P.; Knuth, D.; Böhm, L.; Wiltshka, K.; Schatz, M.; Düring, R. A. A miniaturized method for fast, simple, and sensitive pesticide analysis in soils. *J. Soils Sediments*. 2022, 22, 496–508.
- (49) Cheng, Z.; Dong, F.; Xu, J.; Liu, X.; Wu, X.; Chen, Z.; Pan, X.; Zheng, Y. Atmospheric pressure gas chromatography quadrupole-time-of-flight mass spectrometry for simultaneous determination of fifteen organochlorine pesticides in soil and water. *J. Chromatogr. A*. 2016, 1435, 115–124.
- (50) Cagliero, C.; Bicchi, C.; Cordero, C.; Rubiolo, P.; Sgorbini, B.; Liberto, E. Fast headspace-enantioselective GC-mass spectrometric-multivariate statistical method for routine authentication of flavoured fruit foods. *Food Chem*. 2012, 132, 1071–1079.
- (51) Zoccali, M.; Purcaro, G.; Schepis, A.; Tranchida, P. Q.; Mondello, L. Miniaturization of the QuEChERS Method in the Fast Gas Chromatography-Tandem Mass Spectrometry Analysis of Pesticide Residues in Vegetables. *Food Anal. Methods*. 2017, 10, 2636–2645.
- (52) Maštovská, K.; Lehotay, S. J.; Hajšlová, J. Optimization and evaluation of low-pressure gas chromatography-mass spectrometry for the fast analysis of multiple pesticide residues in a food commodity. *J. Chromatogr. A*. 2001, 926, 291–308.
- (53) Blumberg, L. M., Klee, M. S., Method Translation and Retention Time Locking in Partition GC. *Anal. Chem*. 1998, 70, 3828–3839.
- (54) Han, L.; Sapozhnikova, Y.; Lehotay, S. J. Method validation for 243 pesticides and environmental contaminants in meats and poultry by tandem mass spectrometry coupled to low-pressure gas chromatography and ultrahigh-performance liquid chromatography. *Food Control*. 2016, 66, 270–282.

- (55) Schummer, C.; Delhomme, O.; Appenzeller, B. M. R.; Wennig, R.; Millet, M.; Comparison of MTBSTFA and BSTFA in derivatization reactions of polar compounds prior to GC/MS analysis. *Talanta*. 2009, 77, 1473–1482.
- (56) Brandi, J.; Siragusa, G.; Robotti, E.; Marengo, E.; Cecconi, D. Analysis of veterinary drugs and pesticides in food using liquid chromatography-mass spectrometry. *TrAC - Trends Anal. Chem.* 2024, 179, 117888.
- (57) Cappiello, A.; Famiglini, G.; Palma, P.; Pierini, E.; Termopoli, V.; Trufelli, H. Overcoming matrix effects in liquid chromatography-mass spectrometry. *Anal. Chem.* 2008, 80, 9343–9348.
- (58) Stachniuk, A.; Fornal, E. Liquid Chromatography-Mass Spectrometry in the Analysis of Pesticide Residues in Food. *Food Anal. Methods*. 2016, 9, 1654–1665.
- (59) Wu, F.; Yang, S.; Wang, L.; Wei, W.; Ding, C. F. Simultaneous enrichment and analysis of tobacco alkaloids by microextraction coupled with mass spectrometry using a poly (N-isopropyl-acrylamide-co-divinyl-benzene-co-N, N'-methylene diacrylamide) monolithic column. *Talanta*. 2019, 198, 118–127.
- (60) Kittlaus, S.; Schimanke, J.; Kempe, G.; Speer, K. Development and validation of an efficient automated method for the analysis of 300 pesticides in foods using two-dimensional liquid chromatography-tandem mass spectrometry. *J. Chromatogr. A*. 2013, 1283, 98–109.
- (61) Díaz Merino, M. E.; Acquaviva, A.; Padró, J. M.; Castells, C. B. Comprehensive two-dimensional liquid chromatographic method (Chiral × Achiral) for the simultaneous resolution of pesticides. *J. Chromatogr. A* 2022, 1673, 463126.
- (62) Brinco, J.; Guedes, P.; Gomes da Silva, M.; Mateus, E. P.; Ribeiro, A. B.

Analysis of pesticide residues in soil: A review and comparison of methodologies. *Microchem. J.* 2023, 195, 109465.

- (63) Chiaia-Hernandez, A. C.; Keller, A.; Wächter, D.; Steinlin, C.; Camenzuli, L.; Hollender, J.; Krauss, M. Long-Term Persistence of Pesticides and TPs in Archived Agricultural Soil Samples and Comparison with Pesticide Application. *Environ. Sci. Technol.* 2017, 51, 10642–10651
- (64) Cui, S.; Hough, R.; Yates, K.; Osprey, M.; Kerr, C.; Cooper, P.; Coull, M.; Zhang, Z. Effects of season and sediment-water exchange processes on the partitioning of pesticides in the catchment environment: Implications for pesticides monitoring. *Sci. Total Environ.* 2020, 698, 134228.
- (65) Kemmerich, M.; Bernardi, G.; Adaime, M. B.; Zanella, R.; Prestes, O. D. A simple and efficient method for imidazolinone herbicides determination in soil by ultra-high performance liquid chromatography-tandem mass spectrometry. *J. Chromatogr. A.* 2015, 1412, 82–89.
- (66) García Valverde, M.; Martínez Bueno, M. J.; Gómez-Ramos, M. M.; Aguilera, A.; Gil García, M. D.; Fernández-Alba, A. R. Determination study of contaminants of emerging concern at trace levels in agricultural soil. A pilot study. *Sci. Total Environ.* 2021, 782, 146759.
- (67) Fuentes, E.; Báez, M. E.; Labra, R. Parameters affecting microwave-assisted extraction of organophosphorus pesticides from agricultural soil. *J. Chromatogr. A* 2007, 1169, 40–46.

- (68) Alemayehu, Y.; Tolcha, T.; Megersa, N. Salting-Out Assisted Liquid-Liquid Extraction Combined with HPLC for Quantitative Extraction of Trace Multiclass Pesticide Residues from Environmental Waters. *Am. J. Anal. Chem.* 2017, 8, 433–448.
- (69) Anderson, B. S.; Phillips, B. M.; Voorhees, J. P.; Siegler, K.; Tjeerdema, R. Bioswales reduce contaminants associated with toxicity in urban storm water. *Environ. Toxicol. Chem.* 2016, 35, 3124–3134.
- (70) Gil García, M. D.; Dahane, S.; Arrabal-Campos, F. M.; SocíasVicianá, M. M.; García, M. A.; Fernández, I.; Martínez Galera, M. MCM-41 as novel solid phase sorbent for the pre-concentration of pesticides in environmental waters and determination by microflow liquid chromatography-quadrupole linear ion trap mass spectrometry. *Microchem. J.* 2017, 134, 181–190.
- (71) Yao, W.; Fan, Z.; Zhang, S. Preparation of metal-organic framework UiO-66-incorporated polymer monolith for the extraction of trace levels of fungicides in environmental water and soil samples. *J. Sep. Sci.* 2019, 42, 2679–2686.
- (72) Décuq, C.; Bourdat-Deschamps, M.; Benoit, P.; Bertrand, C.; Benabdallah, R.; Esnault, B.; Durand, B.; Loubet, B.; Fritsch, C.; Pelosi, C.; Gaba, S.; Bretagnolle, V.; Bedos, C., A multiresidue analytical method on air and rainwater for assessing pesticide atmospheric contamination in untreated areas. *Sci. Total Environ.* 2022, 823, 153582.
- (73) Caldas, S. S.; Rombaldi, C.; De Oliveira Arias, J. L.; Marube, L. C.; Primel, E. G. Multi-residue method for determination of 58 pesticides, pharmaceuticals and personal care products in water using solvent demulsification dispersive liquid-liquid microextraction combined with liquid chromatography-tandem mass spectrometry. *Talanta.* 2016, 146, 676–688.

- (74) Farajzadeh, M. A.; Feriduni, B.; Afshar Mogaddam, M. R. Development of counter current salting-out homogenous liquid-liquid extraction for isolation and preconcentration of some pesticides from aqueous samples. *Anal. Chim. Acta* 2015, 885, 122–131.
- (75) Gormez, E.; Golge, O.; González-Curbelo, M. Á.; Kabak, B. Pesticide Residues in Mandarins: Three-Year Monitoring Results. *Molecules*. 2023, 28, 1–15.
- (76) Morsi, R.; Ghoudi, K.; Meetani, M. A. Determination and health risk assessment of carbamate pesticide residues in date palm fruits (*Phoenix dactylifera*) using QuEChERS method and UHPLC-MS/MS. *Sci. Rep.* 2024, 14, 1–13.
- (77) Odabas, E.; Keklik, M.; Golge, O.; González-Curbelo, M. Á.; Kabak, B. Monitoring and Risk Assessment of Multi-Pesticide Residues in Apples: A Focus on Consumer Safety. *Foods*. 2024, 13, 3186.
- (78) Han, S. G.; Nam, T. G. Simultaneous determination of sulfonylurea herbicides in tomatoes using the QuEChERS method coupled with HPLC. *Appl. Biol. Chem.* 2024, 67.
- (79) Abdallah, O. I.; Abd El-Hamid, R. M.; Ahmed, N. S.; Alhewairini, S. S.; Abdel Ghani, S. B. Development of Green and Facile Sample Preparation Method for Determination of Seven Neonicotinoids in Fresh Vegetables, and Dissipation and Risk Assessment of Imidacloprid and Dinotefuran. *Foods*. 2024, 13, 1106.
- (80) Wang, H.; Tian, H.; Lian-feng, A.; Shu-Xuana, L. Screening and quantification of 146 veterinary drug residues in beef and chicken using QuEChERS combined with high performance liquid chromatography-quadrupole orbitrap mass spectrometry. *Food Chem.* 2023, 408, 135207.

- (81) Kaufmann, A.; Butcher, P.; Maden, K.; Walker, S.; Widmer, M. Improving the QuEChERS Liquid/Liquid Extraction of Analytes With Widely Varying Physicochemical Properties: Example of 201 Veterinary Drugs in Milk. *J. AOAC Int.* 2022, 105, 1030–1042.
- (82) Liao, W. R.; Wu, K. L.; Chiang, K. H.; Teng, C. E.; Chen, S. F. Analysis of highly polar pesticides in foods by LC-MS/MS. *J. Food Drug Anal.* 2022, 30, 538–548.
- (83) Zhong, G.; Tang, J.; Xie, Z.; Mi, W.; Chen, Y.; Möller, A.; Sturm, R.; Zhang, G.; Ebinghaus, R. Selected current-use pesticides (CUPs) in coastal and offshore sediments of Bohai and Yellow seas. *Environ. Sci. Pollut. Res.* 2015, 22, 1653–1661.
- (84) Di Muccio, A.; Fidente, P.; Barbini, D. A.; Dommarco, R.; Seccia, S.; Morrica, P. Application of solid-phase extraction and liquid chromatography-mass spectrometry to the determination of neonicotinoid pesticide residues in fruit and vegetables. *J. Chromatogr. A* 2006, 1108, 1–6.
- (85) Gebreyohannes, B. E.; Dube, S.; Nindi, M. M. Simultaneous Determination of Multiple Contaminants in Chicken Liver Using Dispersive Liquid-Liquid Microextraction (DLLME) Detected by LC-HRMS/MS. *Foods* 2023, 12, 2594.
- (86) Wang, S.; Steiniche, T.; Romanak, K. A.; Johnson, E.; Quirós, R.; Mutegeki, R.; Wasserman, M. D.; Venier, M. Atmospheric Occurrence of Legacy Pesticides, Current Use Pesticides, and Flame Retardants in and around Protected Areas in Costa Rica and Uganda. *Environ. Sci. Technol.* 2019, 53, 6171–6181.
- (87) De Souza Guida, Y.; Meire, R. O.; Torres, J. P. M.; Malm, O. Air contamination by legacy and current-use pesticides in Brazilian mountains: An overview of national

- regulations by monitoring pollutant presence in pristine areas. *Environ. Pollut.* 2018, 242, 19–30.
- (88) Luque de Castro, M. D.; García-Ayuso, L. E. Soxhlet extraction of solid materials: An outdated technique with a promising innovative future. *Anal. Chim. Acta* 1998, 369, 1–10.
- (89) Lesueur, C.; Gartner, M.; Mentler, A.; Fuerhacker, M. Comparison of four extraction methods for the analysis of 24 pesticides in soil samples with gas chromatography-mass spectrometry and liquid chromatography-ion trap-mass spectrometry. *Talanta* 2008, 75, 284–293.
- (90) Climent, M. J.; Coscollà, C.; López, A.; Barra, R.; Urrutia, R. Legacy and current-use pesticides (CUPs) in the atmosphere of a rural area in central Chile, using passive air samplers. *Sci. Total Environ.* 2019, 662, 646–654.
- (91) Nascimento, M. M.; Da Rocha, G. O.; De Andrade, J. B. Pesticides in fine airborne particles: From a green analysis method to atmospheric characterization and risk assessment. *Sci. Rep.* 2017, 7, 1–11.
- (92) Niessen, W. M. A. Progress in liquid chromatography-mass spectrometry instrumentation and its impact on high-throughput screening. *J. Chromatogr. A* 2003, 1000, 413–436.
- (93) Yamashita, M.; Fenn, J. B. Electrospray ion source. Another variation on the free-jet theme. *J. Phys. Chem.* 1984, 88, 4451–4459.
- (94) Whitehouse, C. M.; Dreyer, R. N.; Yamashita, M.; Fenn, J. B. Electrospray Interface for Liquid Chromatographs and Mass Spectrometers. *Anal. Chem.* 1985, 57, 675–679.

- (95) Tal'roze, V. L.; Karpov, G. V.; Gordetskii, I. G.; Skurat, V. E. Capillary Systems for the Introduction of Liquid Mixtures into an Analytical Mass Spectrometer, *Russ. J. Phys. Chem.* 1968, 42, 1658–1664.
- (96) Arpino, P.; Baldwin, M. A.; McLafferty, F. W. Liquid Chromatography/Mass Spectrometry. II: Continuous Monitoring. *Biomed. Mass Spectrom.* 1974, 1, 80–82.
- (97) Hayes, M. J.; Lankmayer, E. P.; Vouros, P.; Karger, B. L.; McGuire, J. M. Moving Belt Interface with Spray Deposition for Liquid Chromatography/Mass Spectrometry. *Anal. Chem.* 1983, 55, 1745–1752.
- (98) Cappiello, A.; Bruner, F. Micro Flow Rate Particle Beam Interface for Capillary Liquid Chromatography/Mass Spectrometry. *Anal. Chem.* 1993, 65, 1281–1287.
- (99) Cappiello, A.; Balogh, M.; Famiglini, G.; Mangani, F.; Palma, P. An efficient liquid chromatography - Mass spectrometry interface for the generation of electron ionization spectra. *Anal. Chem.* 2000, 72, 3841–3846.
- (100) Cappiello, A.; Famiglini, G.; Mangani, F.; Palma, P. New trends in the application of electron ionization to liquid chromatography-mass spectrometry interfacing. *Mass Spectrom. Rev.* 2001, 20, 88–104.
- (101) Palma, P.; Famiglini, G.; Trufelli, H.; Pierini, E.; Termopoli, V.; Cappiello, A. Electron ionization in LC-MS: Recent developments and applications of the direct-EI LC-MS interface. *Anal. Bioanal. Chem.* 2011, 399, 2683–2693.
- (102) Cappiello, A.; Famiglini, G.; Palma, P.; Siviero, A. Liquid Chromatography-Electron Ionization Mass Spectrometry: Fields Of Application And Evaluation Of The Performance Of A Direct-EI Interface. *Mass Spectrom. Rev.* 2005, 24, 978–989.

- (103) Rigano, F.; Albergamo, A.; Sciarrone, D.; Beccaria, M.; Purcaro, G.; Mondello, L. Nano Liquid Chromatography Directly Coupled to Electron Ionization Mass Spectrometry for Free Fatty Acid Elucidation in Mussel. *Anal. Chem.* 2016, 88, 4021–4028.
- (104) Seemann, B.; Alon, T.; Tsizin, S.; Fialkov, A. B.; Amirav, A. Electron ionization LC-MS with supersonic molecular beams - The new concept, benefits and applications. *J. Mass Spectrom.* 2015, 50, 1252–1263.
- (105) Termopoli, V.; Famiglini, G.; Palma, P.; Piergiovanni, M.; Cappiello, A. Atmospheric pressure vaporization mechanism for coupling a liquid phase with electron ionization mass spectrometry. *Anal. Chem.* 2017, 89, 2049–2056.
- (106) Termopoli, V.; Famiglini, G.; Palma, P.; Piergiovanni, M.; Rocio-Bautista, P.; Ottaviani, M. F.; Cappiello, A.; Saeed, M.; Perry, S. Evaluation of a liquid electron ionization liquid chromatography–mass spectrometry interface. *J. Chromatogr. A* 2019, 1591, 120–130.
- (107) Rocío-Bautista, P.; Famiglini, G.; Termopoli, V.; Palma, P.; Nazdrajić, E.; Pawliszyn, J.; Cappiello, A. Direct Coupling of Bio-SPME to Liquid Electron Ionization-MS/MS via a Modified Microfluidic Open Interface. *J. Am. Soc. Mass Spectrom.* 2021, 32, 262–269.
- (108) Termopoli, V.; Famiglini, G.; Vocale, P.; Morini, G. L.; Palma, P.; Rocío-Bautista, P.; Saeed, M.; Perry, S.; Cappiello, A. Microfluidic water-assisted trap focusing method for ultra-large volume injection in reversed-phase nano-liquid chromatography coupled to electron ionization tandem-mass spectrometry. *J. Chromatogr. A* 2020, 1627, 461421.

- (109) Marittimo, N.; Grasselli, G.; Arigò, A.; Famiglini, G.; Agostini, M.; Renzoni, C.; Palma, P.; Cappiello, A. Liquid electron ionization-mass spectrometry as a novel strategy for integrating normal-phase liquid chromatography with low and high-resolution mass spectrometry. *Analyst*. 2024, 2664–2670.
- (110) Cappiello, A.; Termopoli, V.; Palma, P.; Famiglini, G.; Saeed, M.; Perry, S.; Navarro, P. Liquid Chromatography–Electron Capture Negative Ionization–Tandem Mass Spectrometry Detection of Pesticides in a Commercial Formulation. *J. Am. Soc. Mass Spectrom.* 2022, 33, 141–148.
- (111) Dutt, M.; Arigò, A.; Famiglini, G.; Zappia, G.; Palma, P.; Cappiello, A. Exploring Negative Chemical Ionization of Per- and Polyfluoroalkyl Substances via a Liquid Electron Ionization LC-MS Interface. *J. Am. Soc. Mass Spectrom.* 2024, 35.
- (112) Termopoli, V.; Piergiovanni, M.; Cappiello, A.; Palma, P.; Famiglini, G. Tyrosol and hydroxytyrosol determination in extra virgin olive oil with direct liquid electron ionization–tandem mass spectrometry. *Separations*. 2021, 8, 173.
- (113) Marittimo, N.; Famiglini, G.; Palma, P.; Arigò, A.; Cappiello, A. Enhanced microfluidic open interface for the direct coupling of solid phase microextraction with liquid electron ionization-tandem mass spectrometry. *J. Chromatogr. A* 2022, 1681, 463479.
- (114) Arigò, A.; Famiglini, G.; Marittimo, N.; Agostini, M.; Renzoni, C.; Palma, P.; Cappiello, A. Extractive-liquid sampling electron ionization-mass spectrometry (E-LEI-MS): a new powerful combination for direct analysis. *Sci. Rep.* 2023, 13, 1–10.

- (115) Grasselli, G.; Arigò, A.; Palma, P.; Famiglini, G.; Cappiello, A. Latest Developments in Direct and Non-Direct LC-MS Methods Based on Liquid Electron Ionization (LEI). *Crit. Rev. Anal. Chem.* 2024, 1–18.
- (116) Termopoli, V.; Torrisi, E.; Famiglini, G.; Palma, P.; Zappia, G.; Cappiello, A.; Vandergrift, G. W.; Zvekic, M.; Krogh, E. T.; Gill, C. G. Mass Spectrometry Based Approach for Organic Synthesis Monitoring. *Anal. Chem.* 2019, 91, 11916–11922.
- (117) Guo, H.; Riter, L. S.; Wujcik, C. E.; Armstrong, D. W. Quantitative analysis of dicamba residues in raw agricultural commodities with the use of ion-pairing reagents in LC – ESI – MS / MS. *Talanta.* 2016, 149, 103–109.
- (118) Tascon, M.; Pawliszyn, J. Development of a Microfluidic Open Interface with Flow Isolated Desorption Volume for the Direct Coupling of SPME Devices to Mass Spectrometry. 2018, 90, 2631-2638.
- (119) Vandergrift, G. W.; Lattanzio-Battle, W.; Krogh, E. T.; Gill, C. G. Condensed Phase Membrane Introduction Mass Spectrometry with in Situ Liquid Reagent Chemical Ionization in a Liquid Electron Ionization Source (CP-MIMS-LEI/CI). *J. Am. Soc. Mass Spectrom.* 2020, 31, 908–916.
- (120) Vandergrift, G. W.; Krogh, E. T.; Gill, C. G. Direct, Isomer-Specific Quantitation of Polycyclic Aromatic Hydrocarbons in Soils Using Membrane Introduction Mass Spectrometry and Chemical Ionization. *Anal. Chem.* 2020, 92, 15480–15488.
- (121) Vandergrift, G. W.; Monaghan, J.; Krogh, E. T.; Gill, C. G. Direct Analysis of Polyaromatic Hydrocarbons in Soil and Aqueous Samples Using Condensed Phase Membrane Introduction Tandem Mass Spectrometry with Low-Energy Liquid Electron Ionization. *Anal. Chem.* 2019, 91, 1587–1594.

- (122) Vandergrift, G. W.; Lattanzio-Battle, W.; Rodgers, T. R.; Atkinson, J. B.; Krogh, E. T.; Gill, C. G. Geospatial Assessment of Trace-Level Benzophenone-3 in a Fish-Bearing River Using Direct Mass Spectrometry. *ACS Environ. Sci. Technol. Water.* 2022, 2, 262-267.

Chapter 2

2. Materials, Methods, and Equipment

This chapter lists and describes materials, instrumentations, and methods used in all studies.

2.1. Materials

The following tables list all materials and equipment. **Table 2.1** lists all solvents used in all studies and publications. All solvents are LC-MS grade unless otherwise stated. **Table 2.2** lists all gases used in all studies and publications. **Table 2.3** and **Table 2.4** list all samples, and sample matrices used in all studies and publications. In the right columns of each table are specified in which result chapters the materials were used.

Table 2.1. List of solvents.

Solvent	Manufacturer/Supplier	Chapter
Acetonitrile	VWR International, part of Avantor (Milan, Italy)	3
Ultrapure Water	Direct-Q3 UV water purification system from Merck Millipore Co. (Milan, Italy)	3
Hexane	VWR International, part of Avantor (Milan, Italy)	4
Isopropanol	VWR International, part of Avantor (Milan, Italy)	4
Ethyl Acetate	VWR International, part of Avantor (Milan, Italy)	4
Ethanol	VWR International, part of Avantor (Milan, Italy)	4
Phosphoric Acid	Merck (Milan, Italy)	3
Formic Acid	VWR International, part of Avantor (Milan, Italy)	5

Table 2.2. List of gas used in all studies.

Gas	Manufacturer/Supplier	Chapter
Helium (He)	Air Liquid, Italy	3,4,5
Nitrogen (N)	Air Liquid, Italy	3,4,5
Methane (CH ₄) (grade 6.0, purity 99.9%)	Nippon Gases, Italy	3

Table 2.3. List of standard compounds used in all studies.

Standard	Manufacturer/Supplier	Molecular Formula	Chapter
Dicamba	Syngenta Ltd. (Bracknell, UK)	C ₈ H ₆ Cl ₂ O ₃	3
Tefluthrin	Syngenta Ltd. (Bracknell, UK)	C ₁₇ H ₁₄ ClF ₇ O ₂	3
(+)- α -tocopherol	Sigma-Aldrich (Milan, Italy)	C ₂₉ H ₅₀ O ₂	4
(+)- δ -tocopherol	Sigma-Aldrich (Milan, Italy)	C ₂₇ H ₄₆ O ₂	4
(+)- β -tocopherol	Extrasynthese (Genay, France)	C ₂₈ H ₄₈ O ₂	4
(-)-delta9-trans- tetrahydrocannabinol	Toxicology Laboratory facilities A.S.T. AV1, (Pesaro, Italy)	C ₂₁ H ₃₀ O ₂	4
Cannabidiol	Toxicology Laboratory facilities A.S.T. AV1, (Pesaro, Italy)	C ₂₁ H ₃₀ O ₂	4
Diisodecyl phthalate	Department of Biomolecular Sciences of the University of Urbino	C ₂₆ H ₄₆ O ₄	4
Diethyl phthalate	Department of Biomolecular Sciences of the University of Urbino	C ₁₂ H ₁₄ O ₄	4
Dimethyl phthalate	Department of Biomolecular Sciences of the University of Urbino	C ₁₀ H ₁₀ O ₄	4
Phenol	Department of Biomolecular Sciences of the University of Urbino	C ₆ H ₆ O	4

Catechol	Department of Biomolecular Sciences of the University of Urbino	C ₆ H ₆ O ₂	4
Resorcinol	Department of Biomolecular Sciences of the University of Urbino	C ₆ H ₆ O ₂	4
Hydroquinone	Department of Biomolecular Sciences of the University of Urbino	C ₆ H ₆ O ₂	4
Carbofuran	Sigma-Aldrich (Milan, Italy)	C ₁₂ H ₁₅ NO ₃	5

Table 2.4. List of sample matrices of all studies.

Sample Matrix	Manufacturer/Supplier	Analysis	Chapter
Commercial Formulation 1	Syngenta Ltd. (Bracknell, UK)	Targeted	3
Fox Liver	Charles University, Prague	Targeted	5
Eagle Gastric Content	Charles University, Prague	Targeted	5
Red Kite Gastric Content	Charles University, Prague	Targeted	5
CBF Formulation (UA_3)	Charles University, Prague	Untargeted	5
CBF Formulation (BV_PF_B)	Charles University, Prague	Untargeted	5
CBF Formulation (CG_23_B)	Charles University, Prague	Untargeted	5
CBF Formulation (F0)	Charles University, Prague	Untargeted	5

CBF Formulation (F1)	Charles University, Prague	Untargeted	5
CBF Formulation (F2)	Charles University, Prague	Untargeted	5
CBF Formulation (F3)	Charles University, Prague	Untargeted	5
CBF Formulation (F4)	Charles University, Prague	Untargeted	5
CBF Formulation (F5)	Charles University, Prague	Untargeted	5
CBF Formulation (F6)	Charles University, Prague	Untargeted	5
CBF Formulation (F7)	Charles University, Prague	Untargeted	5
CBF Formulation (F8)	Charles University, Prague	Untargeted	5
CBF Formulation (F9)	Charles University, Prague	Untargeted	5
Commercial Formulation 2	/	Untargeted	5

2.2. Instruments and Equipment

The analyses were performed using LEI interface coupled with different mass spectrometers and HPLC, listed in **Table 2.5**. The latest LEI interface and MOI configurations are carefully described in *paragraphs 2.2.1 and 2.3.1.3*.

Table 2.5. Instrumentation used in all studies.

Instrumentation	Manufacturer/Supplier	Chapter
Agilent 1290 Infinity II binary pump	Agilent Technologies Inc., Santa Clara, CA, USA	3,4,5
GC Agilent 7890B	Agilent Technologies Inc., Santa Clara, CA, USA	3,4,5
Triple quadrupole Agilent 7010 GC/MS (QqQ)	Agilent Technologies Inc., Santa Clara, CA, USA	3,4
Quadrupole Time-Of-Flight Agilent 7250 (QTOF)	Agilent Technologies Inc., Santa Clara, CA, USA	3,4,5
1290 Infinity Valve Drive	Agilent Technologies Inc., Santa Clara, CA, USA	3
Ultrahigh-pressure valve head	Agilent Technologies Inc., Santa Clara, CA, USA	3

2.2.1. Liquid Electron Ionization (LEI) interface

The LEI interface converts a liquid effluent to a gas-phase mixture of analytes and mobile phase solvents addressed to a conventional EI source. An overview of the different configurations of the LEI interface was given in *Chapter 1, paragraph 1.3.2*. Herein, the last configuration used in all studies reported in this thesis is explained in detail. In the LEI system (**Figure 2.1**), a tee junction is connected to three different capillaries: (1) VMC, (2) inlet capillary, and (3) He carrier capillary. The VMC is the core of the LEI, where the conversion in the gas phase occurs. The VMC is formed by a fused silica capillary (800 μm O.D. 400 μm I.D.), which enters on the MS, passing through the transfer line, and protrudes 1 mm inside the ion source. The VMC can be divided into 2 different regions: (I) the cooling gap, which is the zone outside the transfer line and is kept at ambient temperature; (II) the hot zone, which can be warmed up to 400 °C.

The carrier capillary releases the gas in the cooling gap, while the inlet capillary (flexible stainless-steel capillary, 150 μm O.D. 30 μm I.D.) allows the entrance of analytes and mobile phase solvents in the LEI interface, releasing them at the beginning of the VMC hot zone. Herein, the vaporized eluate is mixed with the He and reaches the ion source.

2.2.2. Passive-flow-splitter (PFS)

The compatible liquid flow rate with the LEI interface was established between 400 nl/min and 800 nl/min, with an ideal value of 500 nl/min. The PSF (**Figure 2.1**) was introduced to couple the LEI interface with higher flow rates; this allowed to extend LEI to a wider variety of applications. PFS is formed by a tee junction with an internal volume of 0.57 μL . The tee junction is positioned between the HPLC and the LEI interface and is connected in the following way: (I) outlet capillary of the HPLC; (II) waste capillary; (III) inlet capillary of LEI. The analytes and the mobile phase solvents reach the PFS, and they are split with different split ratios controlled by changing the length and diameter of the waste capillary. The split flow reaches then the LEI interface flowing through the inlet capillary. A Sensirion Liquid Flowmeter (Sensirion SLG64-0075, Sensirion AG, Switzerland) is used to measure the flow rate that reaches the LEI, allowing the assessment of the suitable split ratio.

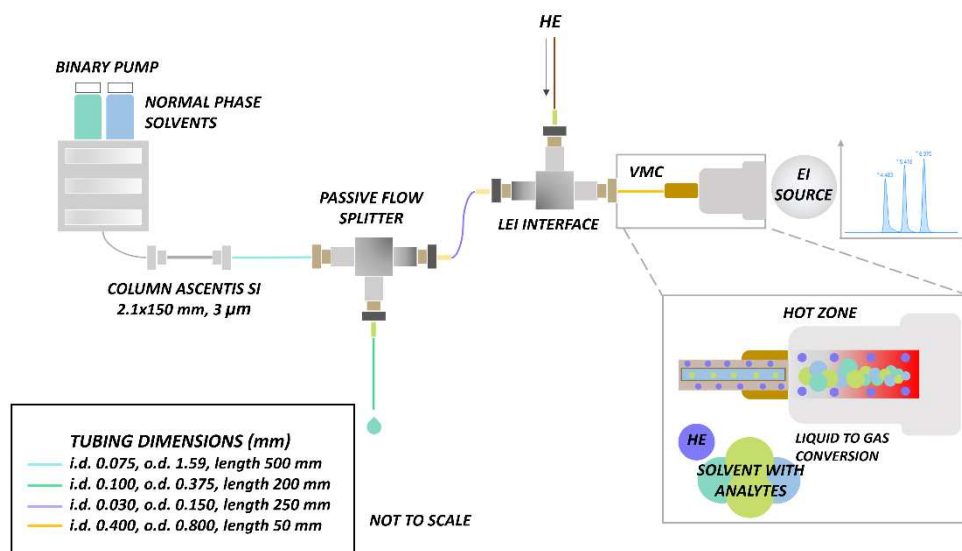


Figure 2.1. Scheme of PFS-LEI-QqQ/QTOF.

2.3.Methods

In this section are grouped and described all methods used in all studies and publications of this PhD project. Methods are divided into three sections, corresponding to the three results chapters.

2.3.1. Methods of *Chapter 3*

Sustainable and Rapid Determination of Two Halogenated Pesticides in a Commercial Formulation by Solid Phase Microextraction and Liquid Phase Chemical Ionization Mass Spectrometry

2.3.1.1. Standard Solution Preparation

Commercial Formulation (CF) stock solution was prepared by weighing 150 mg and diluting in 30 mL of water acidified with 0.2% PA ($\text{pH} > 2$). The diluted CF solution was vortexed for 5 min and divided into 1 mL aliquots for low- and high-resolution experiments. For calibration experiments and matrix effects (ME) evaluation, those aliquots were fortified with 1 μL of working standard solutions of dicamba and tefluthrin to obtain the following concentrations: 0.5, 2.5, 5, 25, 50 and 100 ng/mL. Aliquots (1 mL) of water acidified with 0.2% PA, without CF, were fortified with 1 μL of working standard solutions of dicamba and tefluthrin for calibration experiments in water. For the direct immersion-solid phase micro extraction (DI-SPME) method optimization, repeatability test, and LODs and LOQs evaluation, 1 mL aliquots of diluted CF were fortified with the two pesticides at 100 ng/mL. All measurements were performed in triplicate, and the relative standard deviation was calculated. LODs and LOQs

were calculated as the minimum concentration with an S/N ratio equal to or higher than 3 and 10, respectively.

2.3.1.2. Equipment and Analytical Method

The analyses were performed using the systems MOI-PFS-LEI-QqQ and MOI-PFS-LEI-QTOF.

An Agilent Zorbax Eclipse XDB C18 HPLC column (0.5 x 150 mm, 1.7 μ m particle size) was used for stabilizing the flow rate, no chromatographic separation was performed. VMC and quadrupole temperatures were 260 °C and 150 °C, respectively. The analyses were conducted in Negative Chemical Ionization (NCI) because it enabled the ionization of compounds containing electronegative atoms, increasing the signal-to-noise ratio (S/N). The ion source temperature was 150 °C for providing low-kinetic energy electrons. Methane was introduced into the ion source at $\sim$ 2 mL/min (40%) as a reagent gas to promote dicamba and tefluthrin chemical ionization. The percentage of methane (40%) was chosen according to previous work, in which it gave the most intense signal for both pesticides. Dicamba and tefluthrin data acquisitions were carried out in MRM, using the following transitions and collision energies: Q = 149 $\rightarrow$ 105 (10 eV) and q = 184 $\rightarrow$ 104 (5 eV) for dicamba, Q = 241 $\rightarrow$ 205 (10 eV) and q = 243 $\rightarrow$ 205 (10 eV) for tefluthrin. The setup described above was also employed for high-resolution experiments using a Q-TOF. Dicamba and tefluthrin data acquisitions were carried out in full scan. Acquisition range and data extraction windows were set from m/z 80 to m/z 500 and 25 mg L⁻¹ respectively. Q-TOF mass calibration was performed after each analysis. During calibration, no mobile phase was admitted into the ion source.

2.3.1.3. Microfluidic Open Interface (MOI) - Description and Operation

MOI allows the direct coupling of SPME with PFS-LEI-MS (**Figure 2.2**). The core of this device is represented by a desorption chamber, formed by a 5 cm of 500 μm *i.d.* PEEK tubing. The internal flow isolated volume is 9.8 μL , while when the SPME fiber is inserted into the chamber the internal volume is approximately 2.5 μL . The MOI chamber dimensions must be as small as possible, to obtain a narrow peak width, but sufficiently large to allow fiber insertion promoting the desorption of the desired analyte. The use of a 6 ports-2 positions valve (Agilent G1170A 1290 Infinity Valve Drive and Agilent G4231B ultrahigh-pressure valve head) allows the analysis procedure. The system is connected to the valve in the following way: the ACN flow, coming from the HPLC, is connected to the valve by port 4 via a 50 cm PEEKsil capillary (75 μm *i.d.* and 1.59 mm *o.d.*); the MOI chamber is connected to port 3 (entrance) and port 6 (exit) by 30 cm of flexible stainless-steel capillaries (175 μm *i.d.* and 1.59 mm *o.d.*); the PFS-LEI-MS is connected to the valve by the port 5 via 20 cm PEEKsil capillary (50 μm *i.d.* and 1.59 mm *o.d.*). The valve has 2 positions (A) Stand-by and Injection; (B) Desorption. The whole procedure can be divided into 2 steps:

- I. Step 1: When the valve is in the *desorption position*, port 4 is connected to port 5, therefore the ACN pumped by the HPLC reaches directly the PFS-LEI-MS. In this step, the MOI chamber is isolated and filled with ACN, and the desorption of the fiber is performed in static conditions. The fiber can be immersed in the MOI chamber by removing the plug.

II. Step 2: After desorption the fiber is removed, the plug is inserted again, and the valve is switched to the *Stand-By and Injection position*. At this moment, port 4 is connected to port 3; hence the ACN flow passes through the MOI chamber and moves the eluate from the MOI to the PSF-LEI-MS system.

The HPLC column is used only for backpressure; no chromatographic separation is performed.

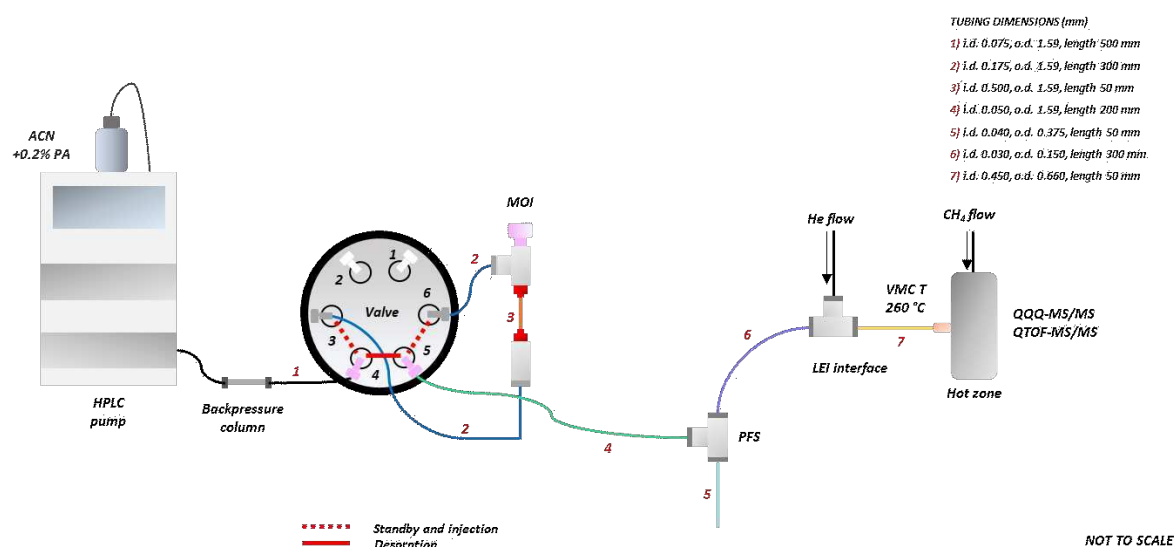


Figure 2.2. Scheme of MOI-PFS-LEI-QqQ/QTOF.

2.3.1.4. Direct Immersion-SPME Method Optimization

SPME was used in direct immersion mode (DI-SPME) for dicamba and tefluthrin sampling in CF and ultra-pure water. Several parameters were optimized for improving extraction and desorption efficiency and process speeding, such as the amount of organic solvent in the sample, sampling and desorption time, and agitation methods. Since it is independent of the MS technique, DI-SPME method optimization was carried out with the low-resolution

instrumentation (MOI-PFS-LEI-QQQ), using 1 mL aliquots of CF and water fortified with 100 ng/mL of dicamba and tefluthrin. The optimized parameters were selected considering the highest integrated peak area values of the most intense transitions (Q). The DI-SPME optimized procedure was then applied to HR experiments. Before use, the SPME fiber was preconditioned in H₂O/ACN (50/50, v/v) for 10 min using a magnetic stir bar. Sampling was conducted by completely immersing the fiber in 1 mL of the sample. Before desorption, a 5s rinsing step in water was performed using a vortex to clean the fiber from any matrix components adhering to the coating surface. Desorption was performed by inserting the fiber inside the MOI chamber, filled with ACN acidified with 0.2% PA.

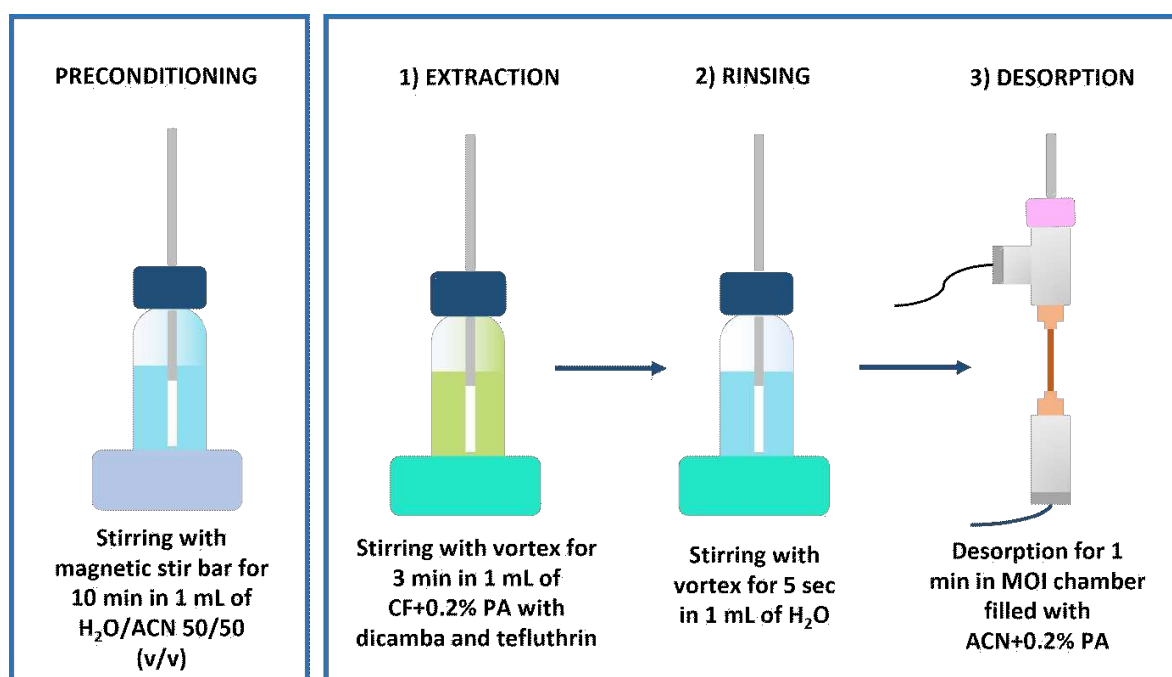


Figure 2.3. DI-SPME optimized workflow. The procedure consists of (1) extraction, (2) rinsing and (3) desorption. The C18 fiber was preconditioned before use.

2.3.2. Methods of *Chapter 4*

Liquid electron ionization-mass spectrometry as a novel strategy for integrating normal-phase liquid chromatography with low and high-resolution mass spectrometry

2.3.2.1. Standard Solutions Preparation

The stock solutions of tocopherols and phthalates were prepared at 1000 mg L⁻¹ in HEX, while stock solutions of phenols were prepared at the same concentration in EtOH. For cannabinoids, the stock solutions were prepared at the Toxicology Laboratory facilities A.S.T. AV1 (Pesaro, Italy). Cannabidiol (CBD) was provided at a concentration of 1000 mg L⁻¹, while (-)-delta9-trans-Tetrahydrocannabinol (THC) was provided at a concentration of 100 mg L⁻¹, both in EtOH. Working standard solutions of tocopherols were prepared at a concentration of 333 mg L⁻¹ each in HEX (100 µL of α-tocopherol at 1000 mg L⁻¹, 100 µL of β-tocopherol at 1000 mg L⁻¹, and 100 µL of δ-tocopherol at 1000 mg L⁻¹). Working standard solutions of phthalates were prepared at a concentration of 333 mg L⁻¹ each in HEX (100 µL of diisodecyl phthalate at 1000 mg L⁻¹, 100 µL of diethyl phthalate at 1000 mg L⁻¹, and 100 µL of dimethyl phthalate at 1000 mg L⁻¹). Working standard solutions of phenols were prepared at a concentration of 250 mg L⁻¹ each in EtOH (100 µL of phenol at 1000 mg L⁻¹, 100 µL of catechol at 1000 mg L⁻¹, 100 µL of resorcinol at 1000 mg L⁻¹, and 100 µL of hydroquinone at 1000 mg L⁻¹). Working standard solutions of CBD and THC were prepared at a final concentration of 333 mg L⁻¹ and 66 mg L⁻¹ respectively in EtOH (200 µL of THC at 100 mg L⁻¹, and 100 µL of CBD at 1000 mg L⁻¹).

2.3.2.2. Analytical Methods

The system LC-LEI-QqQ-MS/MS for low-resolution experiments is carefully described in *Paragraph 2.2.1*. The analyses were conducted using an Ascentis silica column (2.1 i.d. x 150 mm, 3 μm particle size). The flow rate was set at 100 $\mu\text{L min}^{-1}$. The injection volume was 1 μL for cannabinoids and phenols and 4 μL for tocopherols and phthalates. The analyses were conducted in isocratic mode, and the mobile phases used are listed in **Table 2.6**. Agilent OpenLab CDS ChemStation software was used for UHPLC instrument control. Different VMC temperatures (250 $^{\circ}\text{C}$, 300 $^{\circ}\text{C}$, 350 $^{\circ}\text{C}$ and 400 $^{\circ}\text{C}$) were tested for the different analytes to ensure rapid and efficient compound vaporization, preventing thermal degradation. VMC temperatures chosen for each group of analytes are listed in **Table 2.6** as well.

The analyses were carried out first in *full scan* mode for all analytes to optimize the MRM transitions and selected ions. The analyses in *full scan* mode were performed in flow injection analysis (FIA) using an external manual injector with an internal loop of 0.1 μL . After the optimization, the data acquisition was performed in MRM for tocopherols, cannabinoids, and phthalates, while in SIM for phenols; phenols response in MRM was not satisfactory due to the lack of product ions, therefore SIM mode was chosen. The m/z ions transitions monitored are listed in **Table 2.6**. Agilent Mass Hunter GC/MS acquisition software (Version B.07.04.2260) and Agilent Mass Hunter Qualitative Analysis software (Version B.07.00) were employed for data acquisition and processing.

Regarding HRMS analyses, the LC-LEI-QTOF-HRMS system used is described in *Paragraph 2.2.1*. Unlike low-resolution analyses, the analyses were conducted in full scan mode for all analytes; the acquisition range was set from m/z 90 to m/z 500 to avoid the HEX interfering ion

(MW 86.18 g mol⁻¹). The data extraction window was set at 25 mg L⁻¹. All operational parameters are listed in **Table 2.7**.

After each analysis, QTOF mass calibration was performed, during which no mobile phase was admitted into the ion source. Data were acquired with the Agilent Mass Hunter GC/ MS acquisition software (Version 10.1.49.0) and processed with the Agilent Mass Hunter Qualitative Analysis software (Version 10.0).

Table 2.6. Operational parameters for NPLC-LEI-QQQ-MS (low-resolution) separations of tocopherols, cannabinoids, phthalates, and phenols.

Compound name	Mobile phase	Injection volume	Acquisition mode	m/z MRM transitions/selected ions (collision energies)	VMC T °C
<i>α</i>-Tocopherol	HEX:IPA 98:2 (v:v)	4 μL	MRM	430–165 (15 eV) 430–205 (5 eV)	250
<i>β</i>-Tocopherol				416–151 (20 eV) 191–135 (10 eV)	
<i>δ</i>-Tocopherol				402–137 (15 eV) 402–177 (10 eV)	
THC	HEX:EtOH 95:5 (v:v)	1 μL	MRM	314–299 (5 eV) 231–174 (20 eV)	400
CBD				246–174 (25 eV) 231–174 (20 eV)	
Diisodecyl phthalate	HEX:IPA 90:10 (v:v)	4 μL	MRM	149–93 (15 eV) 149–121 (10 eV)	350
Diethyl phthalate				177–149 (5 eV) 222–149 (5 eV)	
Dimethyl phthalate				163–133 (5 eV)	
Phenol	HEX:EtOH 80:20 (v:v)	1 μL	SIM	94	350
Catechol				110	
Resorcinol				110	
Hydroquinone				110	
Flow rate: 100 μL min ^{−1} split at 500 nL min ^{−1} (1:200 split ratio)					
Column: Ascentis Si 2.1 x 150 mm, 3 μm particle size					
Ion source temperature: 280 °C					
Quadrupole Temperature: 150 °C					

Table 2.7. Operational parameters for NPLC-LEI-QTOF separations of tocopherols, cannabinoids, phthalates, and phenols.

Compound name	Mobile phase	Injection volume	VMC T °C
α -Tocopherol			
β -Tocopherol	HEX:IPA 98:2 (v:v)	4 μ L	250
δ -Tocopherol			
THC			
CBD	HEX:EtOH 95:5 (v:v)	1 μ L	400
Diisodecyl phthalate			
Diethyl phthalate	HEX:IPA 90:10 (v:v)	4 μ L	350
Dimethyl phthalate			
Phenol			
Catechol			
Resorcinol	HEX:EtOH 80:20 (v:v)	1 μ L	350
Hydroquinone			
Flow rate: 100 μ L min ⁻¹ split at 500 nL min ⁻¹ (1:200 split ratio)			
Column: Ascentis Si 2.1 x 150 mm, 3 μ m particle size			
Ion source temperature: 260 °C			
Acquisition mode: full scan from m/z 90 to m/z 500			
Quadrupole Temperature: 150 °C			

2.3.3. Methods of *Chapter 5*

As reported in *Chapter 1*, *Chapter 5* describes two different studies. The first study regards a submitted paper titled “Targeted and Non-Targeted Analysis Workflow in Animal Poisoning Cases: Challenges in Source Tracing”, and was aimed to develop targeted and non-targeted methods for detecting, identify, and quantify poisoning substances used to poison animals. Because was confirmed the use of a suspected provenience carbofuran (CBF) formulation, the CBF standard was used to build the calibration curve. The second work reported was performed in collaboration with Syngenta Ltd. and is named “LEI for non-targeted analyses (NTA): the Impurity Y case”. Hence, the methods are divided into two parts concerning the first and the second work, respectively.

2.3.3.1. Standard solutions, formulations, and real samples preparation

Targeted and Non-Targeted Analysis Workflow in Animal Poisoning Cases: Challenges in Source Tracing”. CBF stock solution, the only standard used in this study, was prepared at the concentration of 500 mg/L in MeOH/H₂O 80:20 (v/v). The working solutions were prepared daily by diluting the stock solution with ultrapure H₂O. 13 CBF formulations were provided in granular and liquid form. The 10 granular formulations were extracted by adding 600 µL of two different solvents, ultrapure H₂O and MeOH, to about 0.06 gr of formulation granules. MeOH did not extract additional compounds compared to ultrapure H₂O. Therefore, ultrapure H₂O was preferred as it also allows for higher injection volumes. Granules were sonicated for 10 min and filtered using 4 mm syringe filters PTFE 0.2 µm (Thermo Fisher Scientific, USA). For the 3

liquid formulations, the samples were diluted 1:100 v/v using both MeOH and ultrapure H₂O and filtered using 4 mm syringe with PTFE 0.2 µm filters. In this case, analyzing the diluted formulations with both solvents, MeOH was preferred because in one formulation (UA_3) different compounds were detected in addition to CBF; these compounds resulted soluble in MeOH but not in H₂O. Regarding the real samples, the biological specimens were freeze-dried, homogenized, and extracted using pressurized liquid extraction (PLE) before analysis. An already validated PLE procedure, used for extraction of pesticides from animal foods, was performed using the Dionex ASE system (Thermo Fisher Scientific, USA). The focus of this work was the detection of characteristic compounds in real samples corresponding to those found in each formulation. Therefore, the extraction procedure was designed to be as non-selective as possible. MeOH was used as the extraction solvent. The samples were mixed with 10 g of sea sand, used as in-cell adsorbent to minimize interference, and put in stainless steel cells. Extractions were performed using MeOH at 80 °C and 1500 psi for 3 cycles, with 5-minute static periods. The program also included 5-minute of preheat, 5 minutes of heating, and 60-second purge. Methanolic extracts were collected under a nitrogen stream. To remove lipids, a two-step sample cleanup was applied to each extract. The purification was performed using Captiva EMR lipid columns (Agilent Technologies, USA). Before lipid cartridges loading, 2 mL of each sample were transferred in Eppendorf tubes and centrifuged for 10 min at 14,000 rpm. Supernatants were then transferred to the cartridges. 10 minutes after loading, 800 µL of MeOH:H₂O 80:20 solution was added to elute the extracts. For qualitative and quantitative analysis, real samples were injected both undiluted and diluted with H₂O. The water content in the real samples and standard solution was carefully evaluated as it significantly influenced peak width. Higher water content yielded better chromatographic peak shapes, even with larger

injection volume (up to 20 µL), enabling higher S/N compared to undiluted samples. The best results were obtained by injecting samples with more than 74% of water content.

LEI for non-targeted analyses (NTA): the Impurity Y case. As explained previously, additional work in the field of NTA was performed using LC-LEI-QTOF. In this case, the study aimed to detect and identify an impurity in a new commercial formulation 2, named CF2; the CF2 was injected as a pure sample due to the low concentration of the impurity.

2.3.3.2. Quantitative method validation

Targeted and Non-Targeted Analysis Workflow in Animal Poisoning Cases: Challenges in Source Tracing". The calibration curves were constructed by analyzing standard solutions prepared in ultrapure H₂O and in the sample matrix at six concentrations: 0.3, 0.5, 0.75, 1, 1.5, and 2 mg/L. For the calibration curve in water, a standard solution at a concentration of 5 mg/L was diluted with ultrapure H₂O. For the calibration curve in the sample matrix, the CBF-free gastric content of an eagle was used as matrix, and 20 µL of the matrix was spiked with 80 µL of the standard solutions. Also, for calibration curves in both solvent and sample matrix, the solutions were prepared keeping the water content higher than 74%. Each concentration was injected four times. The two calibration curves were compared for matrix effects (ME) evaluation; the ME was calculated using the following formula:

$$ME(\%) = \frac{\text{Slope matrix}}{\text{Slope solvent}} \times 100$$

The limit of detection (LOD) and limit of quantification (LOQ) were determined by injecting five replicates at concentrations corresponding to S/N of 3 and 10 respectively. Intra-day repeatability was assessed by performing 10 injections on the same day, while inter-day repeatability was determined with five injections for five consecutive days. Both repeatability assessments were performed at two concentrations: 0.75 and 1.5 mg/L.

LEI for non-targeted analyses (NTA): the Impurity Y case. Quantitative analyses were not performed because the aim was only to detect and identify the impurity Y.

2.3.3.3. LC-LEI-QTOF-MS/MS analysis

Targeted and Non-Targeted Analysis Workflow in Animal Poisoning Cases: Challenges in Source Tracing''. The analyses were conducted using the system LC-LEI-QTOF-MS/MS. The LC separations were performed in RPLC using a Kinetex 1.7 μ m XB-C18 150x2.1 mm column. The following elution gradient was applied (mobile phase A: H₂O + 0.1% FA; mobile phase B: ACN + 0.1% FA): 5% B for 1 min; from 5% B to 100% B in 6 min, 100% B for 7 min. The flow rate was set at 200 μ L/min and the injection volume at 20 μ L. MS conditions were as follows: VMC 350 °C; ion source temperature 260 °C; quadrupole temperature was set at 150 °C; LEI helium flow 1.2 mL/min; quenching flow (helium) 4 mL/min; collision flow (nitrogen) 1 mL/min. TOF calibration was performed before each analysis. Agilent Mass Hunter GC/MS acquisition software (Version 10.1.49.0) was employed to acquire all data. Mass spectra were acquired in *full-scan* mode using 70 eV, 15 eV, and 9 eV electron energies, and in targeted MS/MS, using the molecular ion as precursor ion. Regarding *full-scan* analysis, the mass range was set from 85 to 700 m/z to eliminate low-mass background noise given by the mobile phases.

The emission current was set at 4.4 μ A for 70 eV, and at 0.6 μ A for 15 eV and 9 eV. The acquisition rate was set at 8.00 spectra/s for all analyses.

LEI for non-targeted analyses (NTA): the Impurity Y case. The analyses were conducted using the system LC-LEI-QTOF-MS/MS. The LC separation was performed in NPLC using an Agilent Ascentis SIL 3.0 μ m 150x2.1 mm column. The following elution gradient was applied (mobile phase A: Hexane; mobile phase B: Ethyl Acetate): from 1% to 40% B in 25 min; from 40% B to 90% B in 1 min, 100% B hold for 10 min. The flow rate was 100 μ L/min and the injection volume was 1 μ L. MS conditions were as follows: vaporization microchannel (VMC) 250 °C; ion source temperature 260 °C; quadrupole temperature was set at 150 °C; LEI helium flow 1.2 mL/min; quenching flow (helium) 4 mL/min; collision flow (nitrogen) 1 mL/min. The other conditions regarding MS were the same as described in the carbofuran work.

2.3.3.4. Software and database

Agilent MassHunter Qualitative Analysis software (Version 10.0) was employed for data processing using a 5 ppm extraction window (**Figure 2.4**). Using the “Find Compounds by Integration” algorithm, identified compounds through MS/MS peak integration, after which the software generated possible formulas for each compound. The resulting .csv files, generated by Agilent Mass Hunter Qualitative Analysis software, containing MS/MS fragmentation data obtained at different collision energies and the corresponding molecular formula information, were exported to Agilent MassHunter Molecular Structure Correlator software (Version 8.2) for formula confirmation and possible structures determination. The software compares the compound formulas with the ChemSpider database giving a score for each possible structure. The scores are assigned considering each MS/MS fragment ion possible structures; three factors

contribute to the definitive score: the “systematic bond-breaking” approach, the mass accuracy of each fragment, and the match of the ion intensities with the candidate structures.

The same procedure was applied for both studies described in *Chapter 5*.

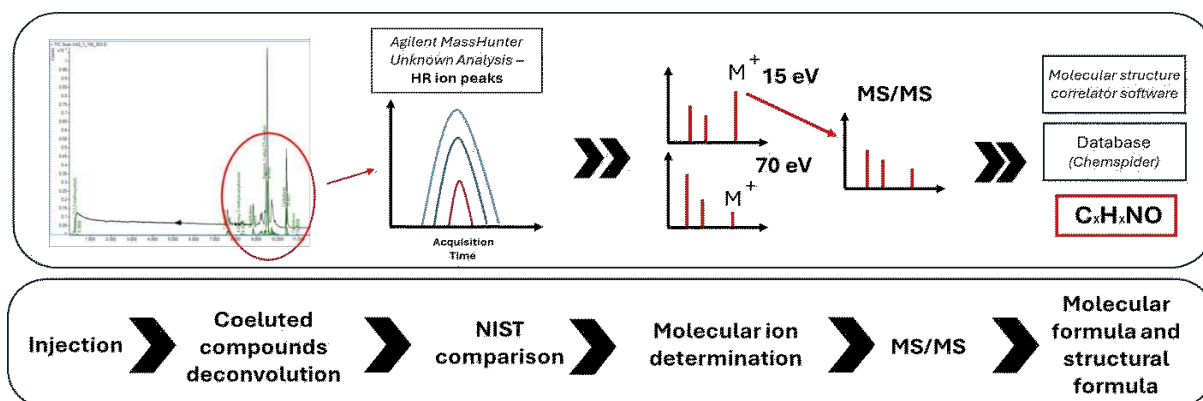


Figure 2.4. The workflow developed for compound identifications was used to characterize formulations and structure determination of Impurity Y.

Chapter 3

Sustainable and Rapid Determination of Two Halogenated Pesticides in a Commercial Formulation by Solid Phase Microextraction and Liquid Phase Chemical Ionization Mass Spectrometry

3.1. Introduction

As reported in *Chapter 1*, the LEI interface shows negligible ME in the absence of LC separation as well. This enables the development of methods that minimize both time and solvent usage, aligning with the principles of Green Analytical Chemistry (GAC). Consequently, this chapter presents an innovative and sustainable quantification approach designed utilizing the LEI interface.

GAC mainly follows the principles of decreasing the use of toxic chemicals and reagents, using energy-efficient equipment, and minimizing waste production (1). Direct analyses match those principles perfectly, also limiting the quantities of samples used and reducing the time involved. Different extraction techniques were developed to enhance the green aspect of analytical chemistry; among them, SPME is one of the preferred due to its affordability, quickness, and low organic solvent consumption (2,3). Different extraction and desorption modes have been developed using this extraction technique; direct immersion (DI-SPME) and headspace are the most common extraction modes. In DI-SPME the fiber is immersed in the sample, and the analytes move from the sample matrix to the active phase. In headspace extraction, instead, the fiber is inserted in the headspace above the sample matrix, and only volatile analytes are moving in the extraction phase (4). Regarding desorption, the use of GC is correlated mainly with thermal desorption; in this case, the fiber is exposed to high temperatures in the GC injector,

and the analytes are released from the fiber. If the SPME fiber is used with HPLC, usually the analytes are desorbed plunging the fiber in organic solvents (4). Therefore, SPME represents a versatile extraction technique perfectly compatible with different chromatographic separations, such as LC (5,6), GC (7,8), and capillary electrophoresis (CE) (9,10), allowing the extraction of a wide variety of molecules in different complex matrices (11-15).

Recently, Pawliszyn et. al. (16) developed a device called MOI that allows coupling directly the SPME with MS equipped with ESI. This MOI employed a flow-isolated desorption chamber of 7 μ L connected to the ionization source, and the analytes reached the ion source by self-aspiration (16). A novel MOI configuration was proposed later by Rocio-Bautista and co-workers (5); the device, differently from the previous one, presents a reduced desorption chamber volume (2.5 μ L) and was coupled with the LEI-QqQ system. The performance of the MOI-LEI-QqQ system was improved afterward by Marittimo et. al. (6), with the introduction of a Passive Flow Splitter (PFS). Its introduction allows for an increase in the flow rate for the eluate transfer into the LEI interface, reducing the peak width with consequently increasing the signal-to-noise (S/N) ratio.

The detection and quantification of pesticides in complex matrices, as already reported in *Chapter 1*, represent a very important challenge for the scientific community; sensitive and reliable analytical methods are required due to strict regulations (17,18). Because most pesticides are not GC amenable, LC-MS/MS systems are preferred for multiple residue analyses unless derivatization is performed (19). These last ensure high sensitivity and selectivity, working mainly in multiple reaction monitoring (MRM). Poor informative spectra and the ME are the main drawbacks of API techniques coupled with LC; these motivations have driven our research group to the development of the LEI interface, allowing the extension of high vacuum

ionization techniques, EI and CI, to LC (20). The use of the LEI interface already demonstrated the possibility of analyzing simultaneously polar/nonpolar and thermolabile/thermostable compounds, which normally require different instrumentation, due to the fast analytes' vaporization inside VMC (21). Maintaining stable ionization conditions significantly reduces the ME.

In a previous work involving the LC-LEI-QqQ system, tefluthrin and dicamba were analyzed in a complex water-based matrix, the commercial formulation (CF) (22). The simultaneous analysis of the two halogenated pesticides represented a challenge due to their different chemical/physical properties. Dicamba is a highly polar compound analyzed in LC-ESI-MS, or GC after the derivatization step, and its extraction from water-based matrices represents a challenge (23), while tefluthrin is commonly analyzed in GC-MS (22).

Because of the complex matrix, the two pesticides were analyzed coupling the LEI interface with the negative chemical ionization (NCI) (22). In NCI, a buffer gas generates low-kinetic energy electrons after the impact with the 70 eV electrons coming from the filament; these thermal electrons react with the sample molecules to form negative ions. NCI ensures the ionization of compounds containing electronegative atoms, increasing the S/N and allowing it to reach lower LODs and LOQs (24). The separation of compounds was conducted in reverse-phase liquid chromatography (RPLC), and the samples were injected after only dilution and filtration.

In this work, a green and rapid method, based on MOI-PFS-LEI coupled with QqQ and QTOF, both equipped with NCI ion source, was established for the simultaneous analysis of dicamba and tefluthrin in CF. The extraction was performed by DI-SPME using a C18 fiber; different parameters affecting the extraction efficiency were optimized. In addition, a proof-of-concept

of the MOI-PFS-LEI system in NCI mode coupled with a Q-TOF instrument was presented for the first time. Because the system is not affected by ME, no chromatographic separation was involved in the analyses, unlike the study already reported. The method proved suitable for trace-level analysis of dicamba and tefluthrin in a complex matrix, showing satisfactory linearity and repeatability, demonstrating accuracy, sensitivity, greenness, and speed of analysis in low and high-resolution experiments.

Materials and Methods used in the study are carefully described in *Chapter 2*. The study was published in the *Separation* journal and presented at XXIX Congresso della Divisione di Chimica Analitica in Milazzo (ME), 11-15 September 2022.

3.2.Results and Discussion

3.2.1. Sampling and Desorption Steps

To optimize the extraction step, four parameters that play a significant role in DI-SPME were optimized for both CF and water: percentage of organic modifier in the sample, extraction and desorption time, and agitation method. Concerning the percentage of the organic modifier in the sample, because it can affect the extraction efficiency it should be kept as low as possible (25). However, small amounts of organic solvent must be present because of the spiking process with the standard solutions. In this work, ACN was chosen as the organic modifier, and it was tested at the following percentages in the sample: 0.1, 0.25, 1.25, 2.25, 5, and 10%. As shown in **Figure 3.1 (A-D)**, the best extraction efficiency was obtained with low percentages of ACN.

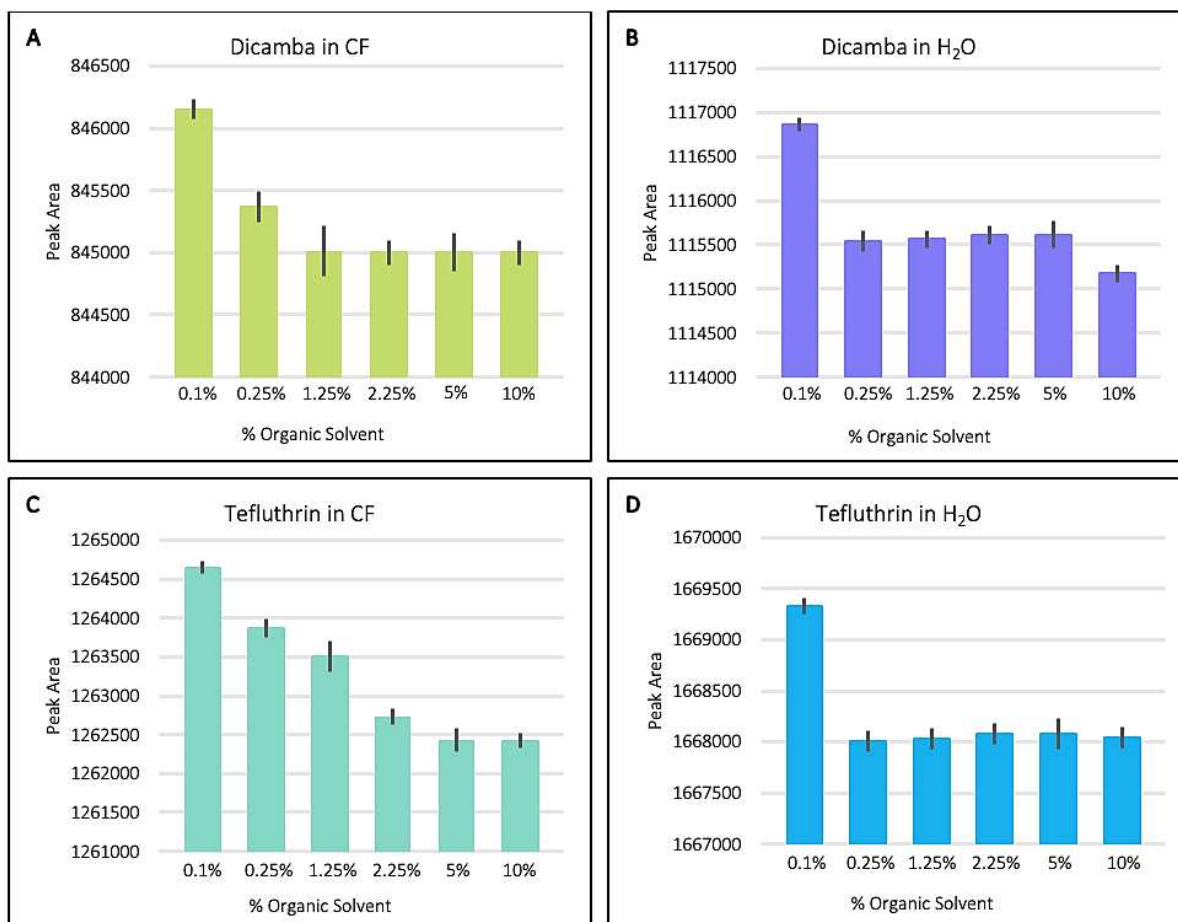


Figure 3.1 (A-D). Peak area values at different percentages of ACN in (A) dicamba in CF, (B) dicamba in H₂O, (C) tefluthrin in CF, (D) tefluthrin in H₂O. Analytes concentration: 100 ng/mL.

As for the percentage of organic modifiers, different extraction times were evaluated using two different agitation methods, magnetic stir bar, and vortex, to obtain the best extraction efficiency. According to the literature (26), the time required to reach the equilibrium depends on different features, such as molecular size, coating thickness, and distribution constant (K). Using the magnetic stir bar, the extraction times evaluated were: 15, 20, 25, and 30 min. In this case, the equilibrium was reached at 25 min. Differently, using vortex the times to reach the equilibrium were greatly lower; the times tested were: 1, 2, 3, and 4 min. As shown in **Figure**

3.2 (A-D), the equilibrium for dicamba and tefluthrin in CF and water was achieved after 3 min, after which the signal remained constant with area values comparable to the ones obtained with the magnetic stir bar. Hence, 3 min of vortex agitation was chosen as the extraction time step.

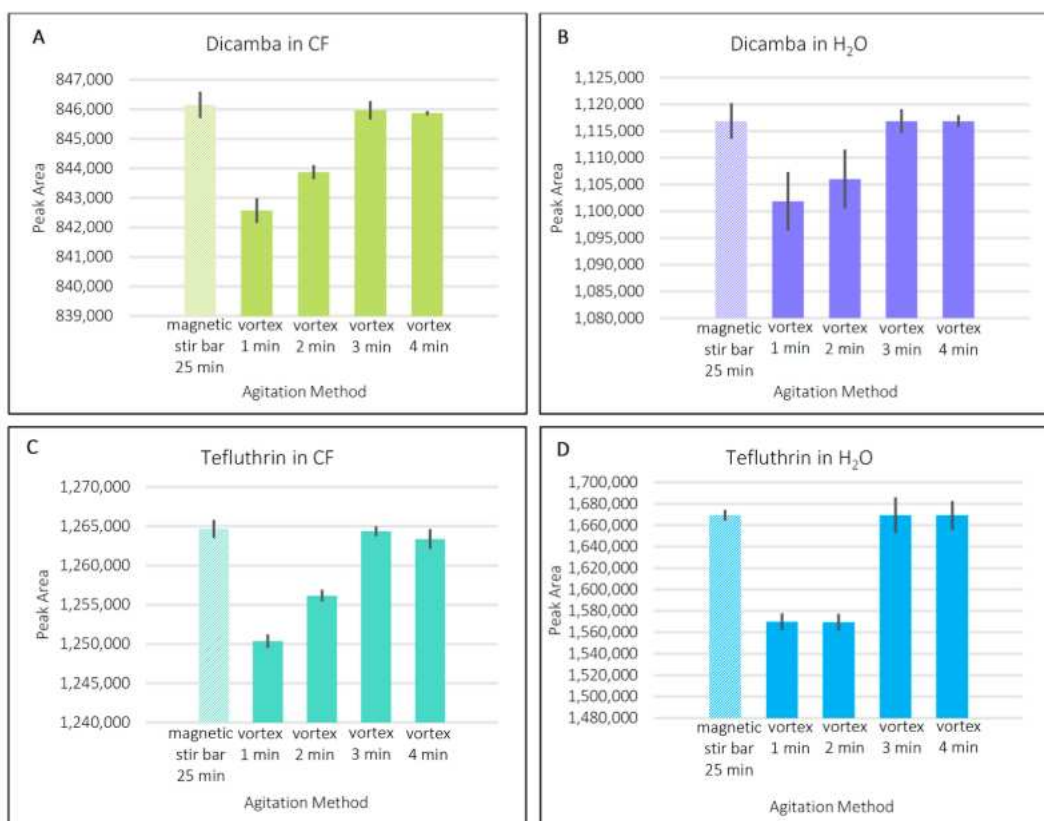


Figure 3.2 (A-D). Effects of different extraction times on the integrated peaks area values of dicamba in (A) CF and (B) H₂O and tefluthrin in (C) CF and (D) H₂O using vortex as agitation method compared with magnetic stir bar. Analytes concentration: 100 ng/mL.

The desorption occurs in static conditions inside the MOI chamber filled with ACN acidified with 0.2% PA. Its efficiency is determined by desorption time; the following times were considered: 30 s, 1, 2, and 3 min. The effect of desorption times on peak areas is shown in

Figure 3.3 (A, B); 1 min was selected as the best time for the desorption. Therefore, the overall optimized procedure is shown in **Figure 2.3**.

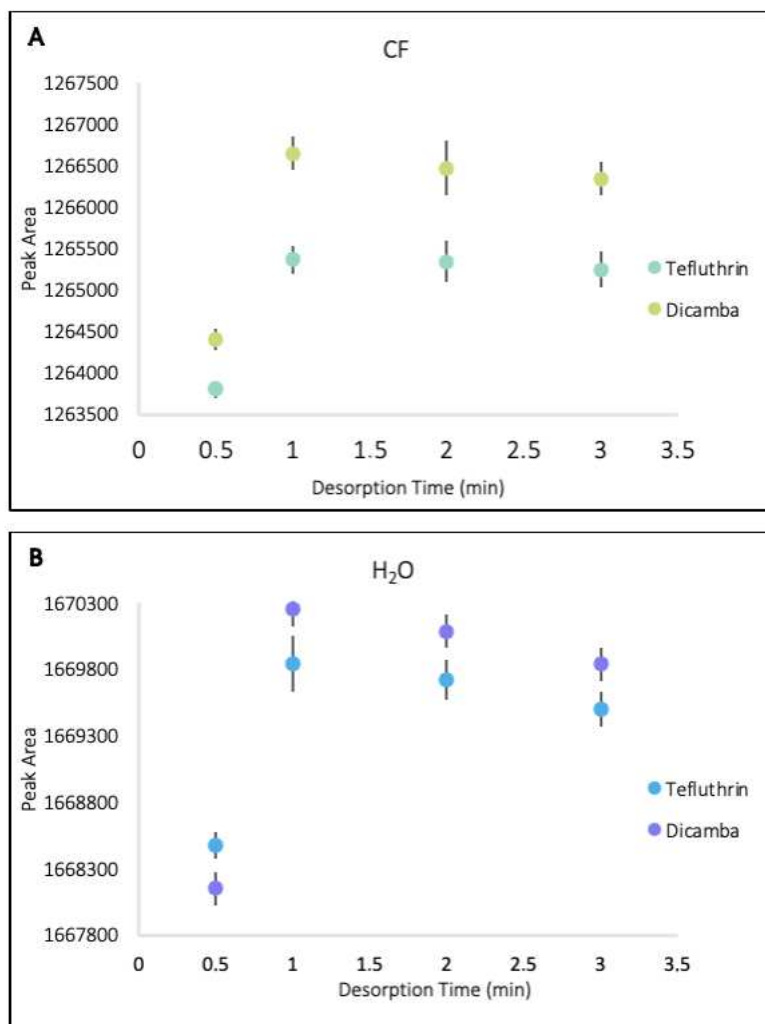


Figure 3.3 (A, B). Effects of different desorption times on the integrated peaks area values of dicamba and tefluthrin at 100 ng/mL using magnetic stir bar as agitation method in (A) CF and (B) H₂O.

3.2.2. Low-Resolution Experiments: MOI-PFS-LEI-QqQ

Method validation was performed by evaluating the intraday and interday repeatability, limits of detection (LOD) and quantification (LOQ), and linearity range of dicamba and tefluthrin in CF, as reported in **Table 3.1**. For repeatability tests and calibration curves, the integrated peak area values of the most intense transitions (Q) were considered, while for calculating the LODs and LOQs, the less intense transitions (q) were used.

The intraday precisions were obtained by performing 10 analyses on the same day, while the interday precisions were assessed by performing 5 analyses per day for 5 days. The intraday RSD% values for dicamba and tefluthrin were 20% and 8%, while the interday RSD% were 21% and 12%, respectively.

The LODs and LOQs calculated in CF were 0.05 ng/mL, and 0.5 ng/mL for both; these values were in line with the previous LC-LEI-MS/MS work (22), and comparable with those reported in the literature (23,27,28).

The calibration curves for both compounds showed good linearity, with R^2 values of 0.9925 for dicamba, and 0.9958 for tefluthrin (**Figure 3.4 A, B**).

Table 3.1. Method validation data for dicamba and tefluthrin in CF, obtained with the MOI-PFS-LEI- QQQ system. Intraday and interday tests were conducted using 100 ng/mL solutions.

Compound	Matrix	Linearity Range (ng/mL)	Levels	R^2	LOD (ng/mL)	LOQ (ng/mL)	RSD% Intraday	RSD% Interday
Dicamba	CF	0.5-100	6	0.9925	0.05	0.5	20%	21%
Tefluthrin	CF	0.5-100	6	0.9958	0.05	0.5	8%	12%

3.2.3. Matrix Effects (ME) Evaluation

The major drawback of the analytical performance is the ME. It is given when ion suppression, or signal enhancement, phenomena occur during the ionization process. ME is evaluated by comparing the slopes of calibration curves in solvent and matrix, and several methods were reported to calculate it (29,30). MEs for dicamba and tefluthrin in CF were calculated by comparing the slopes in water and CF using the following formula:

$$ME (\%) = \frac{Slope\ CF}{Slope\ H_2O} \times 100$$

The results obtained were 76.80% for dicamba and 79.09% for tefluthrin; considering a result of 100% as no matrix effect, a variance of 23.2% and 20.91%, respectively, can be considered acceptable (**Figure 3.5 A, B**).

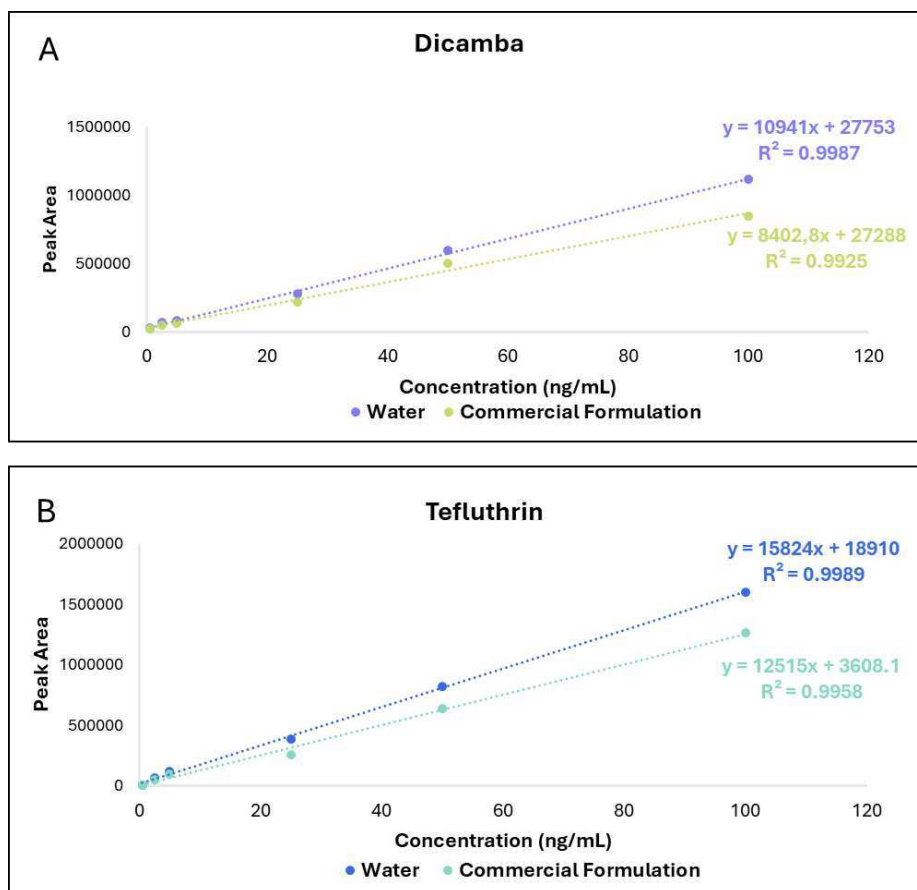


Figure 3.4 (A, B). 6 points-calibration curves of dicamba (A), and tefluthrin (B) in H₂O and CF.

3.2.4. High-Resolution Experiment: MOI-PFS-LEI-QTOF

In this study, for the first time, the system MOI-PFS-LEI was coupled with a high-resolution mass spectrometer, the QTOF. The system MOI-PFS-LEI-QTOF-MS/MS operated in NCI mode for the HR mass spectra acquisition of dicamba and tefluthrin. The full scan spectra were acquired first by analyzing the two compounds at 100 ng/mL in water; the NCI fragmentation

pathways are shown in **Figure 3.5 (A, B)**. Then, the analytical performance was evaluated in the same way as low-resolution experiments. The only difference with low-resolution experiments relying on the acquisition mode; in fact, differently from QqQ, the Q-TOF cannot acquire in MRM. Therefore, the intraday and interday repeatability were obtained by integrating the peak area of the most intense extracted ions (EIC), which were for dicamba and tefluthrin m/z 149.0255 and m/z 241.0252, respectively, while for LODs and LOQs, being the sample matrix very complex, two less intense ions were used (m/z 183.9960 for dicamba; m/z 205.0481 for tefluthrin).

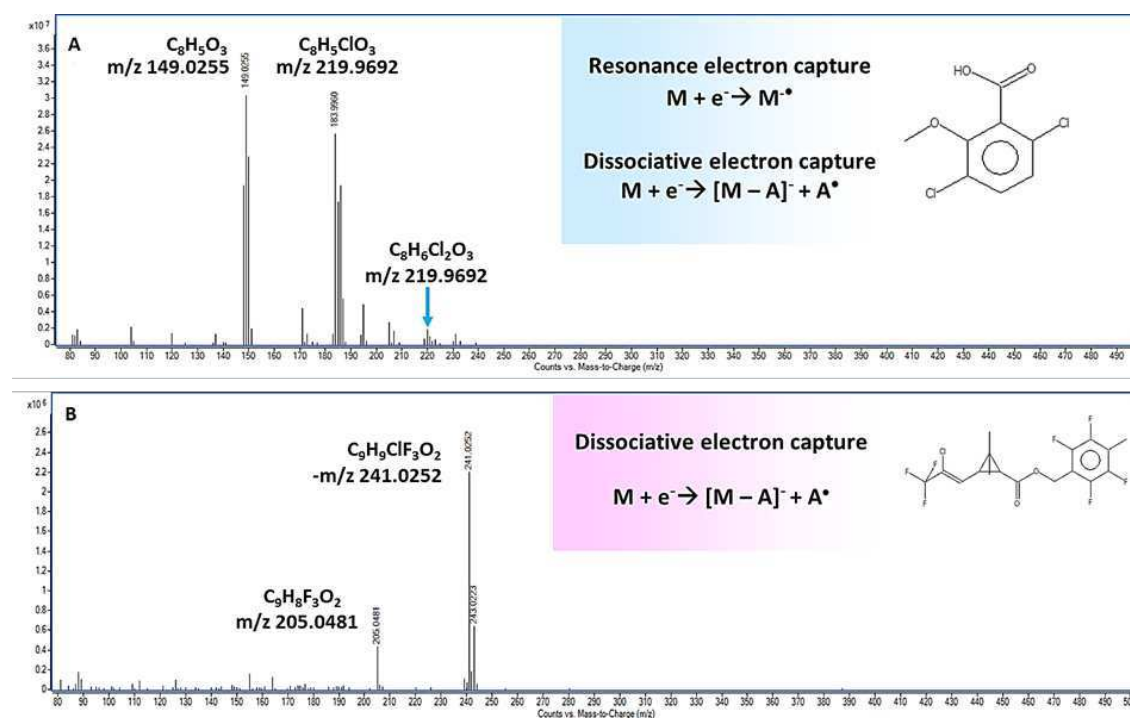


Figure 3.5 (A, B). High-resolution mass spectra and NCI fragmentation pathways of (A) dicamba and (B) tefluthrin at 100 ng/mL in H_2O recorded with the MOI-PFS-LEI-QTOF-MS/MS system.

Regarding repeatability, the RSD% of intraday precision for dicamba and tefluthrin was 26% and 24%, respectively, while the LODs and LOQs values were 0.05 ng/mL and 0.5 ng/mL for both compounds. Using the same linearity range of low-resolution analysis, the R^2 values calculated were 0.9752 for dicamba and 0.9397 for tefluthrin; the values were slightly lower than the low-resolution, probably due to the absence of chromatographic separation and the acquisition in scan mode (**Table 3.2**).

Table 3.2. Method validation data for dicamba and tefluthrin in CF, obtained with the MOI-PFS-LEI- QTOF system. Intraday test was conducted using 100 ng/mL solutions.

Compound	Matrix	Linearity Range (ng/mL)	Levels	R^2	LOD (ng/mL)	LOQ (ng/mL)	RSD% Intraday
Dicamba	CF	0.5-100	6	0.9751	0.05	0.5	26%
Tefluthrin	CF	0.5-100	6	0.9397	0.05	0.5	24%

3.2.5. Greenness Evaluation

Despite different methods for greenness evaluation being developed (31-35), in this work, the Analytical GREENess Metric Approach (AGREE) was selected due to its easiness (32). Free downloadable software is used for the evaluation of 12 fundamental principles concerning GAC, providing a visual output regarding the assessment of the methods' eco-sustainability.

The 12 principles are shown in **Figure 3.6 (A, B)**. The total score is between 0 and 1; 1 is the high ecological method footprint. Herein, the AGREE score was calculated for the study based on LC-LEI-QqQ proposed by Cappiello and co-workers (score 0.49) (35), and for the current method based on MOI-PFS-LEI-QqQ developed in this work (score 0.67).

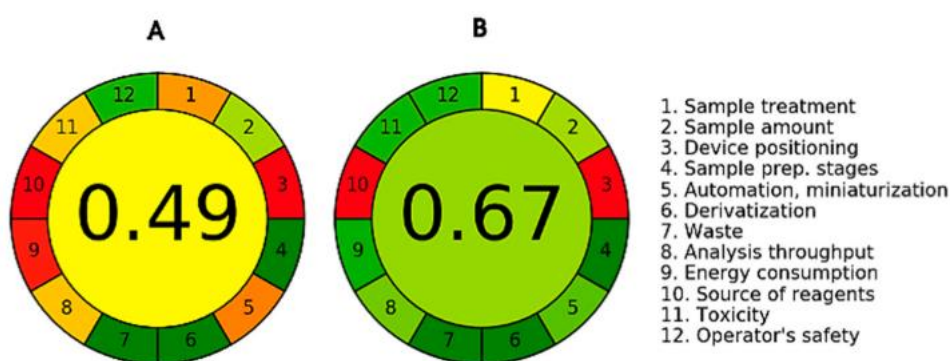


Figure 3.6 (A, B). AGREE comparison between LC-LEI-QQQ (A) and MOI-PFS-LEI-QQQ (B) methods for detecting dicamba and tefluthrin in CF.

The method developed using the MOI-PFS-LEI-QqQ system shows higher scores for principles 1,5,8,9 and 11. Principle 1 regards the sample treatment; the use of SPME and MOI allows coupling directly the sample treatment with MS. In addition, SPME reduces the use of toxic solvents, reagents, and energy consumption, allowing the assignment of a higher score to principle 5, which regards automatization and miniaturization. In addition, the method involving MOI-PFS-LEI-QqQ is greatly rapid, about 5 min, with respect to the method involving chromatographic separation (20 min); this increases the score of principle 8 concerning the throughput of analyses. Consequently, less time involved in the analysis leads to less consumption of energy, allowing to obtain a better score for principle 9. As concerns principle 11, both systems involved toxic solvents; however, the system MOI-PFS-LEI-QqQ,

although it works at 0.01 mL/min, permits to consume less amount of solvent due to the short time required for analysis.

Hence, following the AGREE evaluation system, the proposed MOI-PFS-LEI-QqQ method demonstrated to be aligned more closely to GAC principles with respect to the conventional analytical methods based on chromatographic separation.

3.3. Conclusions

A novel rapid and green method was established for the simultaneous analysis of two compounds with different chemical/physical properties, dicamba, and tefluthrin, in CF; although the system MOI-PFS-LEI-QqQ was already tested, in this work for the first time was coupled also with a Q-TOF in NCI mode. According to the literature, different parameters that can affect the SPME procedure were optimized to reach the best extraction efficiency. Because the chromatographic separation was not involved, the time of analysis depends mainly on the time required for the sampling and desorption step. The overall procedure takes about 5 min, increasing the throughput of the analysis. The method performance was acceptable, and the ME was negligible, demonstrating that working in MRM, or in full scan mode for the HRMS, can overcome the absence of chromatographic separation. Regarding the green evaluation, the method proposed was compared with a previous method developed for dicamba and tefluthrin in CF using the LC-LEI-QqQ system; the novel method demonstrated higher compliance with CAG principles, emphasizing its green character.

References

- (1) Anastas, P.; Eghbali, N. Green Chemistry: Principles and Practice. *Chem. Soc. Rev.* 2010, 39, 301–312.
- (2) Bojko, B.; Cudjoe, E.; Gómez-Ríos, G. A.; Gorynski, K.; Jiang, R.; Reyes-Garcés, N.; Risticvic, S.; Silva, É. A. S.; Togunde, O.; Vuckovic, D.; Pawliszyn, J. SPME - Quo vadis? *Anal. Chim. Acta.* 2012, 750, 132–151.
- (3) Bessonneau, V.; Bojko, B.; Pawliszyn, J. Analysis of human saliva metabolome by direct immersion solid-phase microextraction LC and benchtop orbitrap MS. *Bioanalysis.* 2013, 5, 783–792.
- (4) Siouffi, A. M., High performance liquid chromatography. *Handb. Food Sci. Technol. Eng.* 2005, 4, 859–900.
- (5) Rocío-Bautista, P.; Famiglini, G.; Termopoli, V.; Palma, P.; Nazdrajić, E.; Pawliszyn, J.; Cappiello, A. Direct Coupling of Bio-SPME to Liquid Electron Ionization-MS/MS via a Modified Microfluidic Open Interface. *J. Am. Soc. Mass Spectrom.* 2021, 32, 262–269.
- (6) Marittimo, N.; Famiglini, G.; Palma, P.; Arigò, A.; Cappiello, A. Enhanced microfluidic open interface for the direct coupling of solid-phase microextraction with liquid electron ionization-tandem mass spectrometry. *J. Chromatogr. A.* 2022, 1681, 463479.
- (7) Arcoleo, A.; Bianchi, F.; Careri, M. Helical multi-walled carbon nanotube-coated fibers for solid-phase microextraction determination of polycyclic aromatic hydrocarbons at ultra-trace levels in ice and snow samples. *J. Chromatogr. A.* 2020, 1631, 461589.
- (8) Arthur, C. L.; Pawliszyn, J. Solid Phase Microextraction with Thermal Desorption Using Fused Silica Optical Fibers. *Anal. Chem.* 1990, 62, 2145–2148.

- (9) Merkle, S.; Kleeberg, K.; Fritsche, J. Recent Developments and Applications of Solid Phase Microextraction (SPME) in Food and Environmental Analysis—A Review. *Chromatography*. 2015, 2, 293–381.
- (10) Whang, C. W.; Pawliszyn, J. Solid phase microextraction coupled to capillary electrophoresis. *Anal. Commun.* 1998, 35, 353–356.
- (11) Rodrigues, C.; Alves, C.; Santos-neto, A. J.; Fernandes, C.; Lan, F. M. Analysis of tricyclic antidepressant drugs in plasma by means of solid-phase microextraction-liquid chromatography-mass spectrometry. *J. mass Spectrom.* 2007, 1342–1347.
- (12) Vuckovic, D.; de Lannoy, I.; Gien, B.; Yang, Y.; Musteata, F. M.; Shirey, R.; Sidisky, L.; Pawliszyn, J. In vivo solid-phase microextraction for single rodent pharmacokinetics studies of carbamazepine and carbamazepine-10,11-epoxide in mice. *J. Chromatogr. A*, 2011, 1218, 3367–3375.
- (13) Jiang, R.; Pawliszyn, J. Thin-film microextraction offers another geometry for solid-phase microextraction. *TrAC - Trends Anal. Chem.* 2012, 39, 245–253.
- (14) Wu, J.; Tragas, C.; Lord, H.; Pawliszyn, J. Analysis of polar pesticides in water and wine samples by automated in-tube solid-phase microextraction coupled with high-performance liquid chromatography-mass spectrometry. *J. Chromatogr. A*. 2002, 976, 357–367.
- (15) Cagliero, C.; Bicchi, C.; Cordero, C.; Rubiolo, P.; Sgorbini, B.; Liberto, E. Fast headspace-enantioselective GC-mass spectrometric-multivariate statistical method for routine authentication of flavoured fruit foods. *Food Chem.* 2012, 132, 1071–1079.
- (16) Tascon, M.; Pawliszyn, J. Development of a Microfluidic Open Interface with Flow Isolated Desorption Volume for the Direct Coupling of SPME Devices to Mass

- Spectrometry. 2018, 90, 2631-2638.
- (17) Silva, V.; Mol, H. G. J.; Zomer, P.; Tienstra, M.; Ritsema, C. J.; Geissen, V. Pesticide residues in European agricultural soils – A hidden reality unfolded. *Sci. Total Environ.* 2019, 653, 1532–1545.
- (18) Kaur, R.; Mavi, G. K.; Raghav, S.; Khan, I. Pesticides Classification and its Impact on Environment. *Int. J. Curr. Microbiol. Appl. Sci.* 2019, 8, 1889–1897.
- (19) Brandi, J.; Siragusa, G.; Robotti, E.; Marengo, E.; Cecconi, D. “Analysis of veterinary drugs and pesticides in food using liquid chromatography-mass spectrometry.” *TrAC - Trends Anal. Chem.* 2024, 179, 117888.
- (20) Famiglini, G.; Palma, P.; Termopoli, V.; Cappiello, A. The history of electron ionization in LC-MS, from the early days to modern technologies: A review. *Anal. Chim. Acta.* 2021, 1167, 338350.
- (21) Termopoli, V.; Famiglini, G.; Palma, P.; Piergiovanni, M.; Cappiello, A. Atmospheric pressure vaporization mechanism for coupling a liquid phase with electron ionization mass spectrometry. *Anal. Chem.* 2017, 89, 2049–2056.
- (22) Cappiello, A.; Termopoli, V.; Palma, P.; Famiglini, G.; Saeed, M.; Perry, S.; Navarro, P. Liquid Chromatography–Electron Capture Negative Ionization–Tandem Mass Spectrometry Detection of Pesticides in a Commercial Formulation. *J. Am. Soc. Mass Spectrom.* 2022, 33, 141–148.
- (23) Shin, E. H.; Choi, J. H.; Abd El-Aty, A. M.; Khay, S.; Kim, S. J.; Im, M. H.; Kwon, C. H.; Shim, J. H. Simultaneous determination of three acidic herbicide residues in food crops using HPLC and confirmation via LC-MS/MS. *Biomed. Chromatogr.* 2011, 25, 124–135.

- (24) Dutt, M.; Arigò, A.; Famiglini, G.; Zappia, G.; Palma, P.; Cappiello, A. Exploring Negative Chemical Ionization of Per- and Polyfluoroalkyl Substances via a Liquid Electron Ionization LC-MS Interface. *J. Am. Soc. Mass Spectrom.* 2023, 35.
- (25) Gionfriddo, E.; Gruszecka, D.; Li, X.; Pawliszyn, J. Direct-immersion SPME in soy milk for pesticide analysis at trace levels by means of a matrix-compatible coating. *Talanta.* 2020, 211, 120746.
- (26) Górecki, T.; Yu, X.; Pawliszyn, J. Theory of analyte extraction by selected porous polymer SPME fibers. *Analyst.* 1999, 124, 643–649.
- (27) Guo, H.; Riter, L. S.; Wujcik, C. E.; Armstrong, D. W. Quantitative analysis of dicamba residues in raw agricultural commodities with the use of ion-pairing reagents in LC-ESI-MS/MS. *Talanta.* 2016, 149, 103–109.
- (28) Ccanccapa-Cartagena, A.; Masiá, A.; Picó, Y. Simultaneous determination of pyrethroids and pyrethrins by dispersive liquid-liquid microextraction and liquid chromatography triple quadrupole mass spectrometry in environmental samples. *Anal. Bioanal. Chem.* 2017, 409, 4787–4799.
- (29) Gosetti, F.; Mazzucco, E.; Zampieri, D.; Gennaro, M. C. Signal suppression/enhancement in high-performance liquid chromatography tandem mass spectrometry. *J. Chromatogr. A.* 2010, 1217, 3929–3937.
- (30) Bruce-Vanderpuije, P.; Megson, D.; Ryu, S. H.; Choi, G. H.; Park, S. W.; Kim, B. S.; Kim, J. H.; Lee, H. S. A comparison of the effectiveness of QuEChERS, FaPEX and a modified QuEChERS method on the determination of organochlorine pesticides in ginseng. *PLoS One.* 2021, 16, 1–18.
- (31) Sajid, M.; Płotka-Wasyłka, J. Green analytical chemistry metrics: A review. *Talanta.*

2022, 238, 123046.

- (32) Gomes, J. V. D.; Tosta, C. L.; Da Cunha Neto, Á.; Fagg, C. W.; Silva, C. A. G.; Gomes-Copeland, K. K. P.; Magalhães, P. O.; Fonseca-Bazzo, Y. M.; Jamal, C. M.; Silveira, D. Chemical profile and biological activity of *Crinum americanum* L. (Amaryllidaceae). *South African J. Bot.* 2022, 146, 25–35.
- (33) Płotka-Wasyłka, J. A new tool for the evaluation of the analytical procedure: Green Analytical Procedure Index. *Talanta*. 2018, 181, 204–209.
- (34) Gałuszka, A.; Migaszewski, Z. M.; Konieczka, P.; Namieśnik, J. Analytical Eco-Scale for assessing the greenness of analytical procedures. *TrAC - Trends Anal. Chem.* 2012, 37, 61–72.
- (35) Termopoli, V.; Famiglini, G.; Palma, P.; Piergiovanni, M.; Rocio-Bautista, P.; Ottaviani, M. F.; Cappiello, A.; Saeed, M.; Perry, S. Evaluation of a liquid electron ionization liquid chromatography–mass spectrometry interface. *J. Chromatogr. A* 2019, 1591, 120–130.

Chapter 4

Liquid electron ionization-mass spectrometry as a novel strategy for integrating normal-phase liquid chromatography with low and high-resolution mass spectrometry

4.1. Introduction

As reported in *Chapter 1*, the LEI interface has shown great advantages. In addition to extending the EI to low volatile and thermolabile molecules, it allows for solving different analytical problems, such as analyzing simultaneously compounds with different chemical/physical properties, which normally require different instrumentations. LEI proved to be versatile, working perfectly with different MS detectors, such as single quadrupole (Q), QqQ, and Q-TOF, without making any changes to the instrument, and, because of the negligible ME, it allows the development of rapid direct and non-direct methods avoiding sample preparation steps and chromatographic separations. Over the years, also the configuration of the LEI interface was modified to make it easier to use and more reliable.

A part of this PhD project was focused on the improvement of LEI performances and the expansion of LEI applications. Concerning the novel applications, recently, a study was published regarding the coupling of the LEI interface with another LC technique. So far, LEI has been coupled with only RPLC (1-4). RPLC is the most used chromatographic separation technique due to its wide range of applications; in this case, a non-polar stationary phase is used in combination with mobile phases with different polarities. Although a large variety of RP stationary phases are available, a limited number of applications require different retention mechanisms with superior selectivity. To fill the gap, NPLC can be one option in case analytes

are poorly separated in RPLC, or when hydrophobic impurities, strongly retained in RPLC, have to be rapidly eluted to focus on target analytes. In NPLC, polar stationary phases, such as silica-based, alumina, zirconia, or polar-embedded, are used in combination with slightly polar or non-polar solvents, such as hexane, heptane, ethyl acetate, and dichloromethane (5-8). NPLC is mainly performed in isocratic mode; avoiding column conditioning reduces the time between two analyses and solvents consumed (5). In addition, another advantage of NPLC is that non-polar solvents help complex sample solubilization, enabling higher loading capacity and reducing the back pressure (9).

Currently, the most used detector coupled with NPLC is the UV absorbance spectrophotometric system; however, it has low selectivity and sensibility compared to MS. Non-polar solvents involved in NPLC are not compatible with the common ionization techniques normally coupled with LC, such as ESI, in particular when n-hexane, hexane, benzene, and toluene are involved, due to their low conductivity, surface tension, and dielectric constant (10-14). However, different approaches were developed in trying to couple NPLC with MS, such as hyphenation, off-line mode, post-column solvent addition, sheath liquid, and ambient ionization (14). However, as already discussed in *Chapter 1*, API techniques are less informative and reproducible with respect to high vacuum ionization techniques, EI, and CI (15).

In order to extend NPLC to more informative and reproducible ionization techniques, in this study, NPLC was coupled with the LEI interface for the first time; different from ESI, EI ionization is not affected by the polarity of solvents, allowing its direct coupling with non-polar solvents. Herein, the advantages of using NPLC coupled with EI, such as fast compound separation, isomer separation, and the generation of EI spectra are demonstrated; different analytes, including cannabinoids, tocopherols, phenols, and phthalates, were used as model

compounds due to their optimal response in NPLC and analytical relevance (16-21). The analyses were conducted using a silica column in isocratic mode and as detectors both low-resolution MS (QqQ) and high-resolution MS (Q-TOF).

To the best of our knowledge, for the first time, nonpolar LC solvents were used with EI-MS, emphasizing the versatility of the LEI interface. The results obtained open new possibilities in coupling NPLC with MS for the analysis of real samples in the presence of highly hydrophobic impurities.

The methods and materials used to carry out experiments are described in *Chapter 2*. The study was published in the *Analyst* journal and presented at the 71st Conference in Mass Spectrometry and Allied Topics in Houston, Texas, organized by the American Society for Mass Spectrometry, 4-8 June 2023.

4.2.Results and discussion

4.2.1. Low-resolution experiments

The possibility of carrying out analyses in SIM and MRM modes allows us to reach a higher sensitivity; however, to choose the most suitable selected ions and transitions, the analyses must be performed before in *full scan* mode. The latter were performed by injecting each analyte in FIA. The analytes were injected at the following concentrations: α -, β -, and δ -tocopherol at 1000 mg L⁻¹ in HEX; THC and CBD in MeOH at 100 mg L⁻¹, and 1000 mg L⁻¹, respectively; diisodecyl-, diethyl, and dimethyl phthalate at 1000 mg L⁻¹ in HEX; phenol, catechol, resorcinol, and hydroquinone at 1000 mg L⁻¹ in EtOH. In addition, for each group of analytes, the best VMC temperatures were chosen among 250 °C, 300 °C, 350 °C, and 400 °C. The most

suitable temperatures chosen were: 250 °C for tocopherols, 400 °C for cannabinoids, and 350 °C for phthalates and phenols.

Full scan spectra of all analytes were compared with NIST library spectra. As is shown in **Figure 4.1 (A-C)** the experimental spectra of tocopherols show satisfactory matches with reference spectra present in the library. α -tocopherol exhibited a matching factor of 740, which indicates how similar the unknown spectrum is with the library spectrum with a scale of 0-1000, and reverse match of 870, in which the score is assigned ignoring the presence of ions not belonging to the compound of interest. β -tocopherol showed a matching factor of 910, and reverse match of 950. δ -tocopherol demonstrated a matching factor of 880, and reverse match of 900. Unlike tocopherols, the comparison between experimental and NIST library spectra of cannabinoids, phthalates, and phenols showed slightly lower match and reverse match values. One of the main limitations relies on using aprotic solvents, such as HEX. The latter requires to set a narrow scan interval starting from m/z 90. This poses a problem when analytes such as phenols show the most abundant ions below m/z 90, leading to a decrease in match and reverse match factor values proposed by NIST libraries.

However, the high similarity between experimental and NIST library spectra demonstrated that typical NPLC solvents do not affect spectral quality.

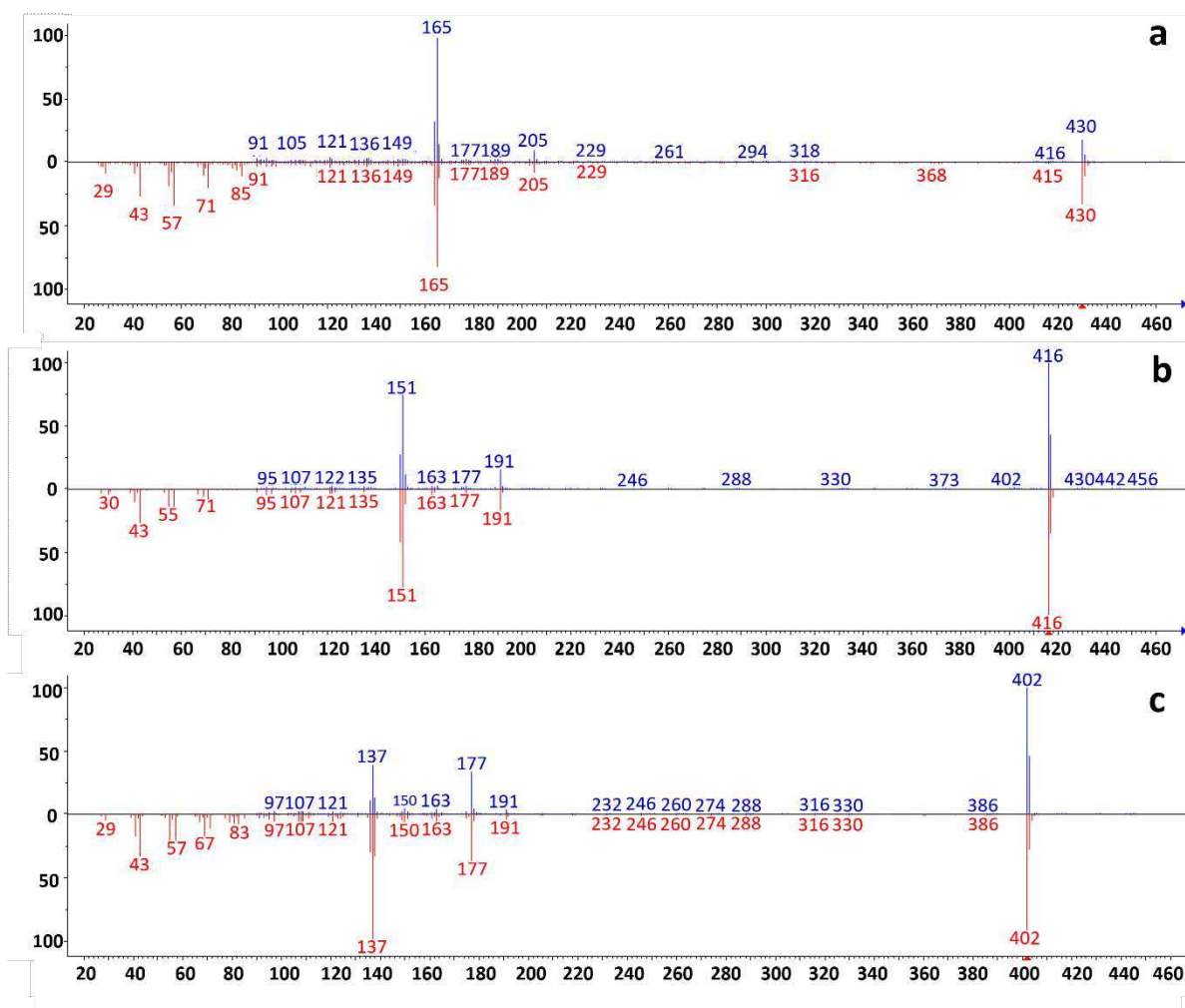


Figure 4.1 (A-C). Low-resolution experiments. Comparison between the recorded and the NIST library reference spectra of (a) α -tocopherol, (b) β -tocopherol, and (c) δ -tocopherol. Top: experimental spectrum; Bottom: NIST reference spectrum.

After recording *full scan* spectra for all compounds, MRM and SIM methods were developed and applied to analyze mixtures. Although MRM is the analytical mode of choice, due to its specificity and sensitivity, SIM mode was chosen for phenols because the lack of product ions led to an unsatisfactory response in MRM. The NPLC separations are shown in **Figure 4.2 (A-D)** using the conditions listed in *Chapter 2, Table 2.6*.

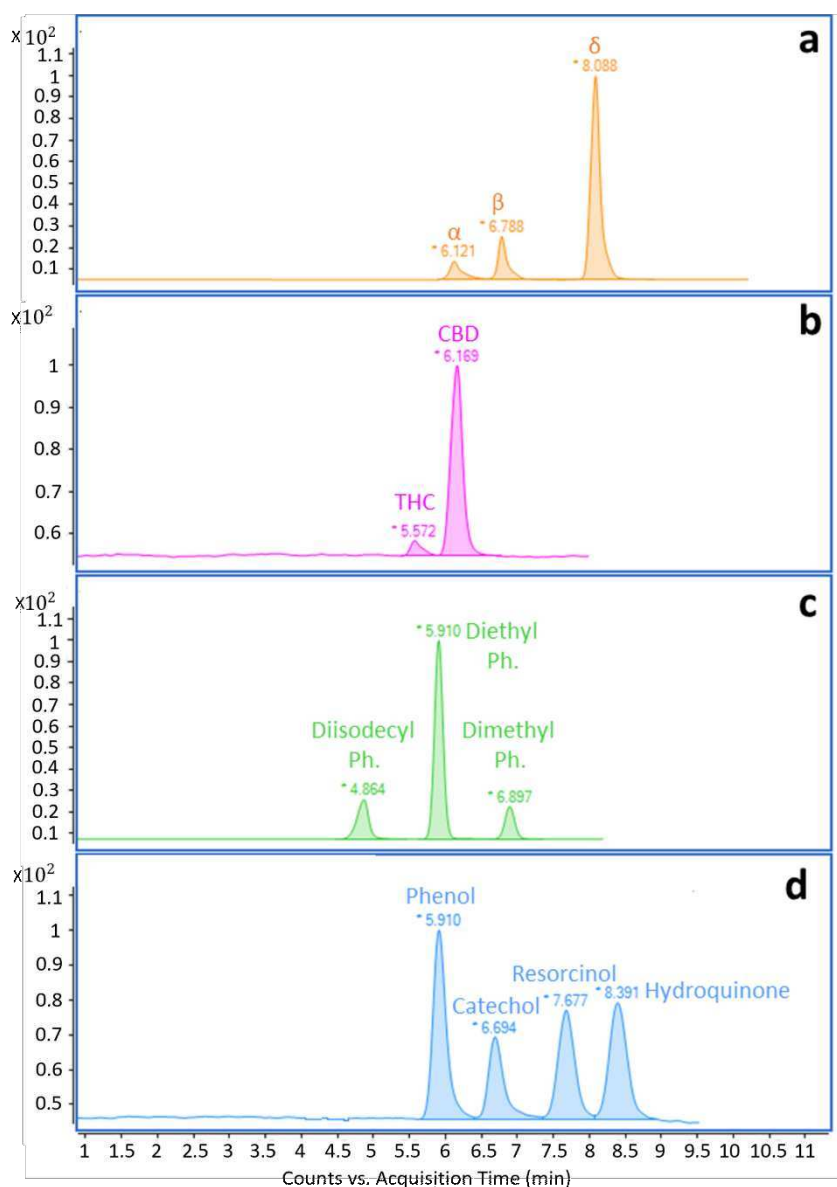


Figure 4.2 (A-D) NPLC MRM and SIM reconstructed ion chromatograms of the selected model compounds: (a) tocopherols; (b) cannabinoids; (c) phthalates; (d) phenols. Analytical conditions are reported in Table 4.1.

4.2.2. High-resolution experiments

The analyses were conducted using NPLC-LEI coupled with QTOF-HRMS as well. The analytical conditions used were the same used for low-resolution experiments, which have been optimized. The main difference relies on the geometry of the ion source of the HR instrument, ensuring results repeatability for superior performance, and the acquisition mode that was in *full scan*. Performing the analysis in full scan using a QTOF-HRMS instrument ensures higher sensitivity with respect to the *full scan* acquisition mode of the low-resolution instrument; this allows the acquisition of the analyte's full scan spectra directly performing the chromatographic separation without the need for FIA. The separations of all groups of analytes are reported in **Fig. 4.3 (A-D)**.

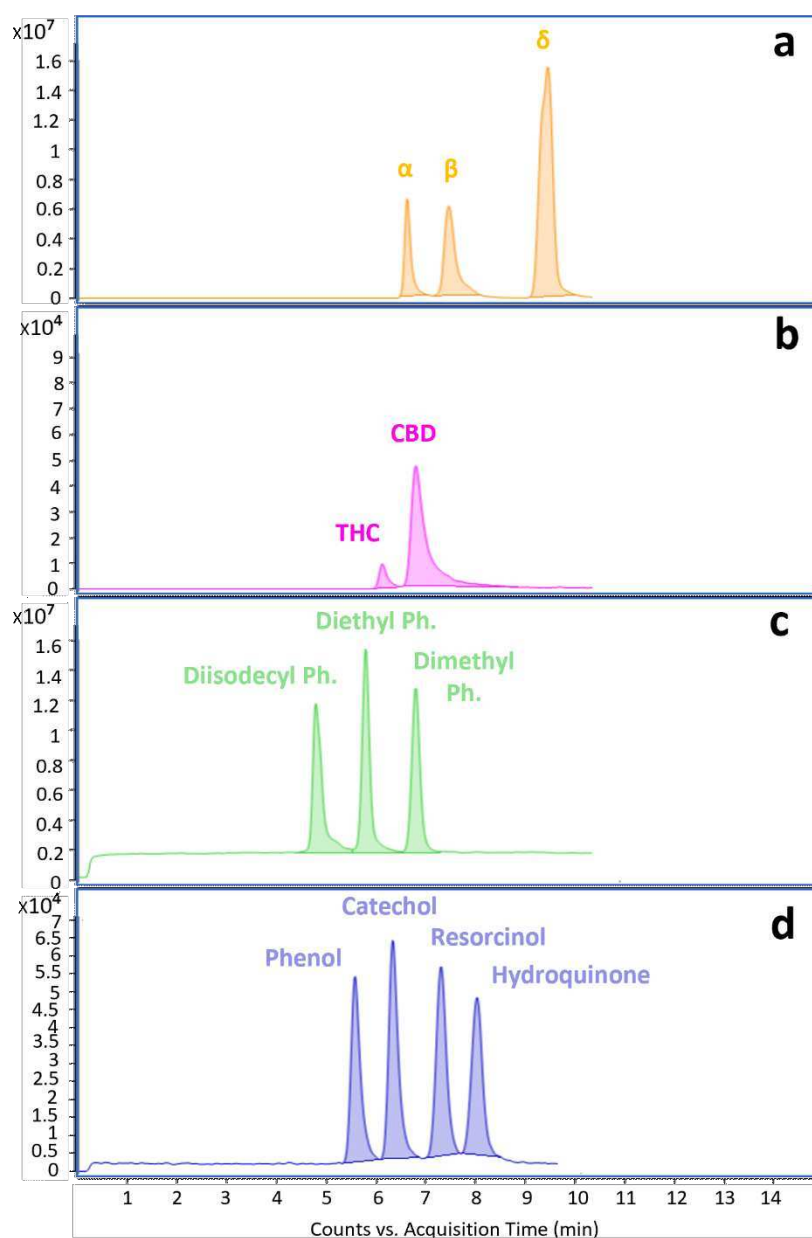


Figure 4.3 (A-D). NPLC full scan chromatograms of the selected model compounds: (a) tocopherols; (b) cannabinoids; (c) phthalates; (d) phenols. Analytical conditions are reported in Table 4.2.

The differences between the EI ion sources of QTOF and QqQ rely on different volumes. This difference could obstacle solvent vapors evacuation, leading to chemical ionization phenomena. The latter has been studied and solved in the past using RPLC solvents; however, this phenomenon was not observed using NPLC in both QqQ and QTOF, highlighting the advantages of coupling NPLC with the LEI interface.

The HRMS spectra of tocopherols are shown in **Figure 4.4 (A-C)**; molecular formulas and structures have been assigned to the main fragments using the MS interpreter utility developed by NIST, facilitating unambiguous molecular identification. The HRMS spectra of the other model compounds are reported in **Figure 4.5 (A, B)**, **Figure 4.6 (A-C)**, and **Figure 4.7 (A-D)**.

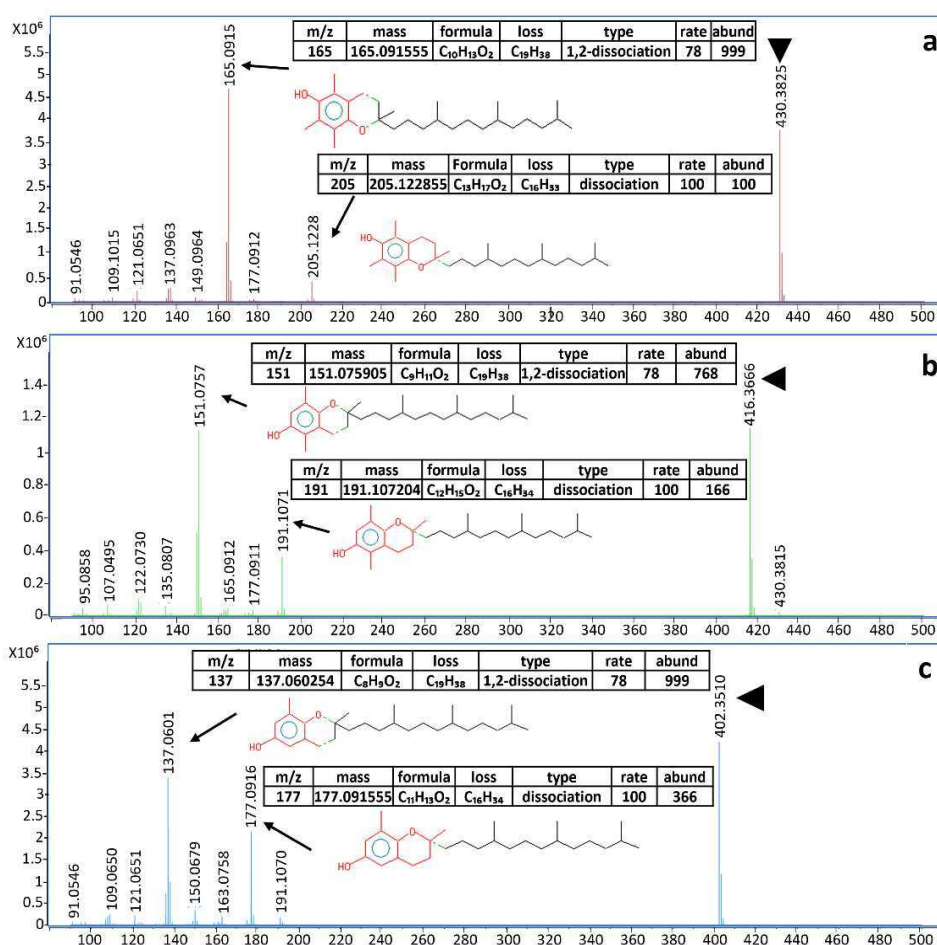


Figure 4.4 (A-C). Recorded EI high-resolution full scan spectra of (A) α-tocopherol, (B) β-tocopherol, and (C) δ-tocopherol and assignment of the main fragments according to the MS interpreter utility developed by NIST. Molecular ions are indicated by the green triangle.

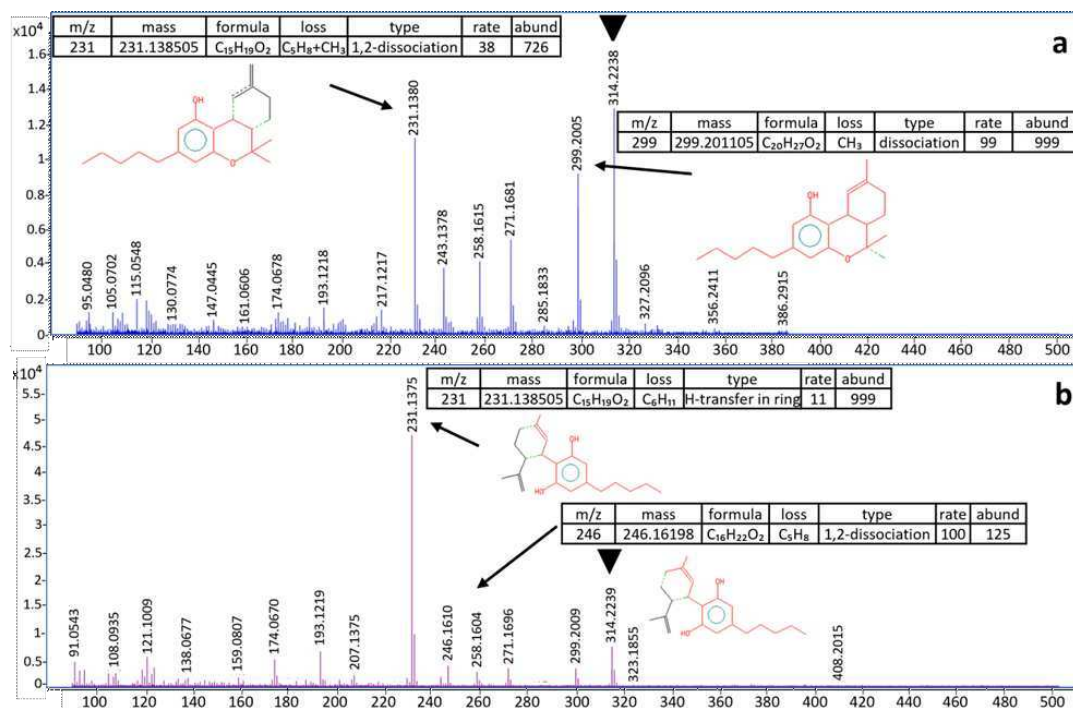


Figure 4.5 (A, B). Recorded EI high-resolution full scan spectra of a) THC, b) CBD and assignment of the main fragments according to the MS Interpreter utility developed by NIST. Molecular ions are indicated by the green triangle.

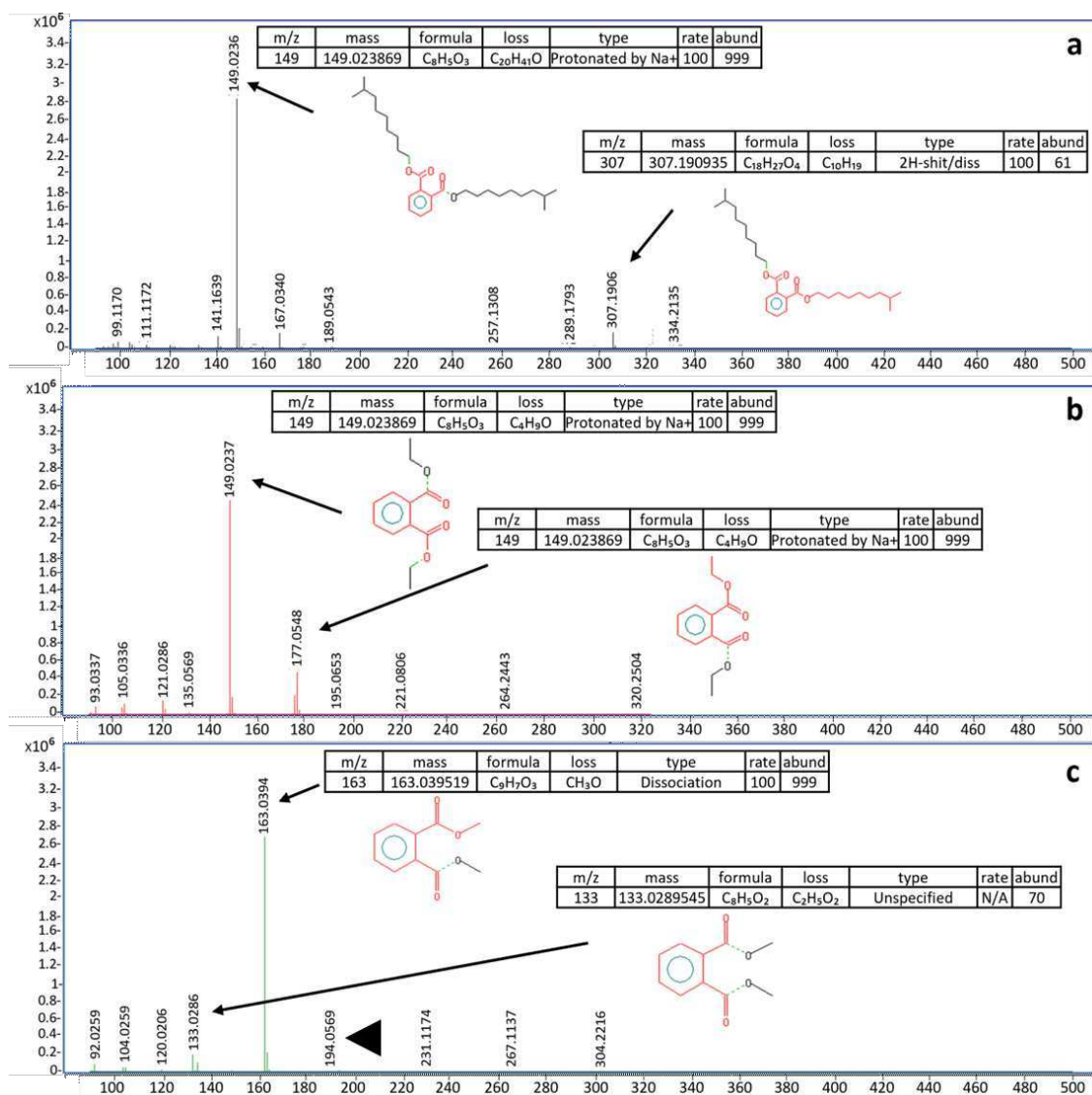


Figure 4.6 (A-C). Recorded EI high-resolution full scan spectra of a) diisodecyl phthalate; b) diethyl phthalate; c) dimethyl phthalate and assignment of the main fragments according to the MS Interpreter utility developed by NIST.

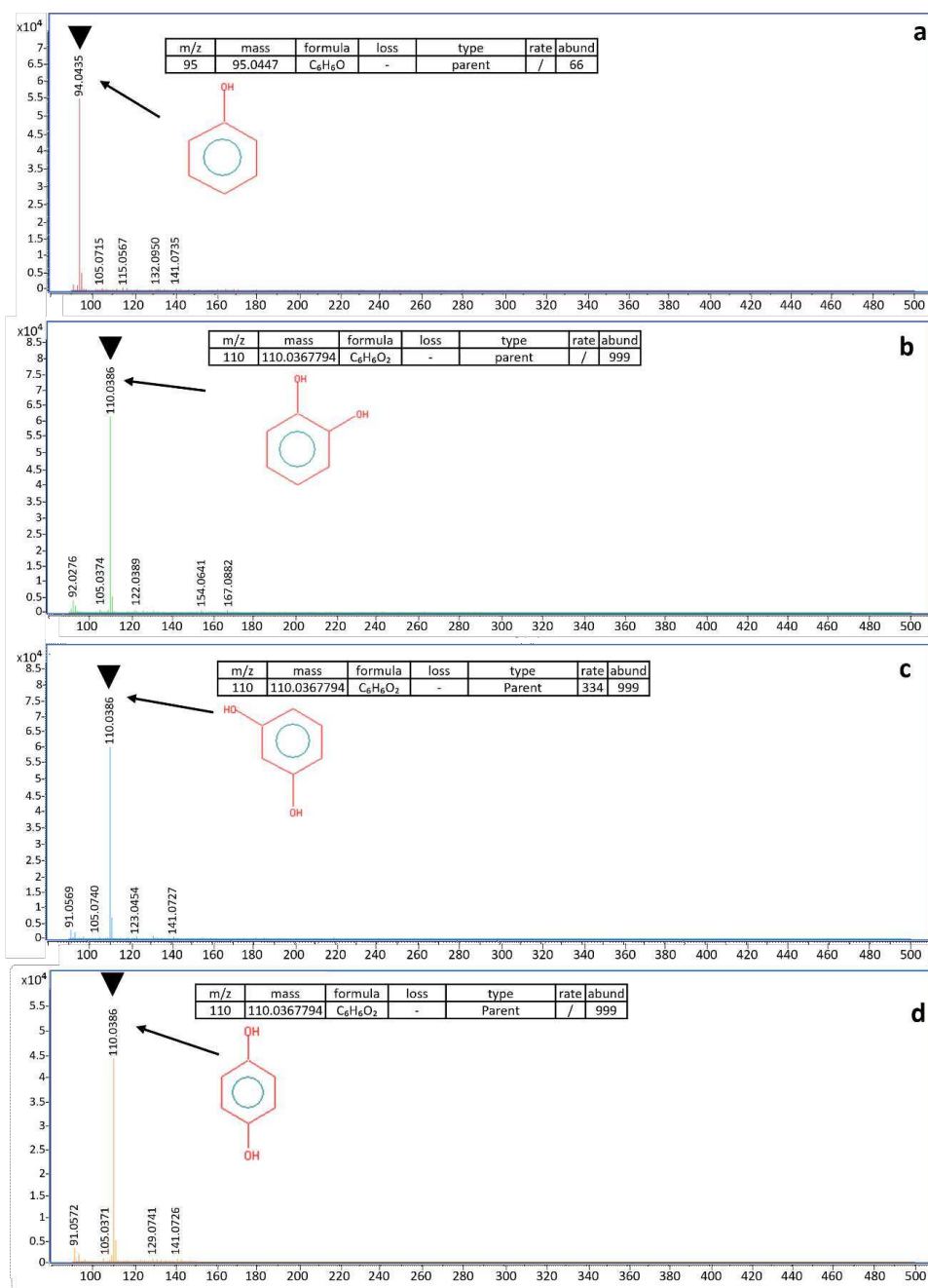


Figure 4.7 (A-D). Recorded EI high-resolution full scan spectra of a) phenol; b) catechol; c) resorcinol; d) hydroquinone and assignment of the main fragments according to the MS Interpreter utility developed by NIST. Molecular ions are indicated by the green triangle.

As already mentioned, NPLC gains an advantage by reducing the elution times of highly hydrophobic and highly retained RPLC interferences. In **Figure 4.8 (A, B)** are reported two chromatograms acquired in RPLC and NPLC of a non-disclosable commercial mixture where secondary components required characterization. In the RPLC chromatogram, the two components show elution time between 45 and 50 min; in the NPLC chromatogram, the same two components eluted between 4 and 6 min before the main compound of the mixture. Therefore, the two separation mechanisms keep the same separation, but the use of NPLC significantly reduces the time of analysis.

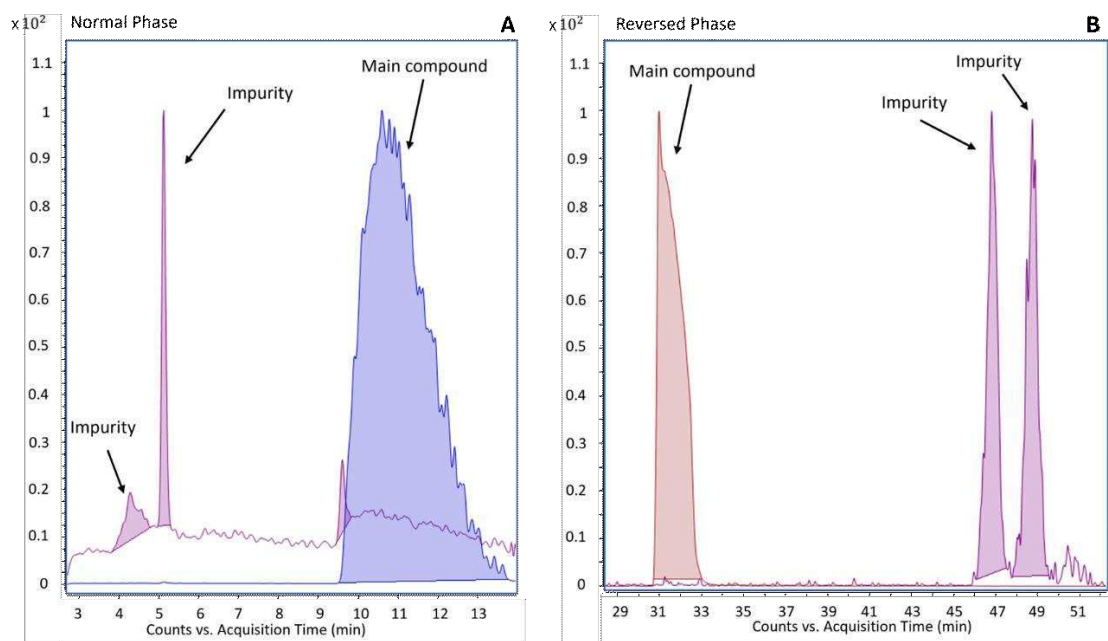


Figure 4.8 (A, B). Extracted ion chromatograms of secondary components and main compound present in a commercial mixture obtained using the following separation conditions: (A) NPLC. A: HEX, B: ethyl acetate; gradient: 1–40% B in 25 min, 40–90% in 1 min, 90% held for 10 min; column: Ascentis Si 2.1×150 mm, 3 μm particle size; flow rate: $100 \mu\text{L min}^{-1}$; (B) RPLC. A: water + 0.3% formic acid; B: ACN + 0.3% formic acid; gradient: 0–100% B in 30 min, 100% B held for 20 min; column: Waters Acquity UHPLC C18 1.7 μm ; flow rate: $100 \mu\text{L min}^{-1}$.

4.3. Conclusions

This preliminary study demonstrated the feasibility and the advantages of developing innovative solutions, such as the LEI interface, to couple MS with NPLC. NPLC, although not the most used separation technique, is the most suitable for some applications, such as when

compounds are strongly retained in RPLC. So far, the most used detector combined with NPLC is the UV; although it shows less sensitivity and specificity, coupling NPLC with MS is a challenge due to the non-compatibility of non-polar solvents with ESI. LEI interface has proven to be a great alternative technique to couple NPLC with MS; differently from ESI, it is compatible with non-polar solvents commonly involved in NPLC. The system NPLC-LEI-MS demonstrated to keep unaltered the MS spectra of model compounds in both low-resolution and HRMS instrumentations. In addition, the reliability of analyses performed in MRM and SIM modes demonstrated that the system could be involved in quantitative analysis in future works.

The next phase of the research will focus on developing methods for analyzing a variety of samples, taking advantage of the potential of NPLC-LEI-MS.

References

- (1) Termopoli, V.; Famiglini, G.; Vocale, P.; Morini, G. L.; Palma, P.; Rocío-Bautista, P.; Saeed, M.; Perry, S.; Cappiello, A. Microfluidic water-assisted trap focusing method for ultra-large volume injection in reversed-phase nano-liquid chromatography coupled to electron ionization tandem-mass spectrometry. *J. Chromatogr. A*. 2020, 1627, 461421.
- (2) Termopoli, V.; Piergiovanni, M.; Cappiello, A.; Palma, P.; Famiglini, G. Tyrosol and hydroxytyrosol determination in extra virgin olive oil with direct liquid electron ionization–tandem mass spectrometry. *Separations* 2021, 8, 173.
- (3) Cappiello, A.; Termopoli, V.; Palma, P.; Famiglini, G.; Saeed, M.; Perry, S.; Navarro, P. Liquid Chromatography–Electron Capture Negative Ionization–Tandem Mass Spectrometry Detection of Pesticides in a Commercial Formulation. *J. Am. Soc. Mass Spectrom.* 2022, 33, 141–148.
- (4) Dutt, M.; Arigò, A.; Famiglini, G.; Zappia, G.; Palma, P.; Cappiello, A. Exploring Negative Chemical Ionization of Per- and Polyfluoroalkyl Substances via a Liquid Electron Ionization LC-MS Interface. *J. Am. Soc. Mass Spectrom.* 2023, 35.
- (5) Cooper, W. T. Normal-Phase Liquid Chromatography. *Encycl. Anal. Chem.* 2000.
- (6) L. R. Snyder. High-performance liquid chromatography - Advances and perspectives, ed. C. S. Horvath. Academic Press, New York. 1980, 207-316.
- (7) Cooper, W.T. Encyclopedia of Analytical Chemistry: Application, Theory, and Instrumentation, ed. R. A. Meyers and J. G. Dorsey, Wiley Online Library. 2006.

- (8) Younes, A. A.; Galea, C.; Mangelings, D.; Vander Heyden, Y. *Analytical Separation Science*, ed. J. L. Anderson, A. Berthod, V. Pino and A. M. Stalcup, Wiley-VCH, Weinheim. 2015, 227–244.
- (9) Lee, T. D. *Introduction to Modern Liquid Chromatography*, Third Edition. J. Am. Soc. Mass Spectrom. 2011, 22, 196–196.
- (10) Jiahao, L.; Xiyue, X.; Ming, M.; Bo, C.; Yingzhuang, C; Oliver, J. S. Enhancing the compatibility of normal-phase chromatography x reversed-phase chromatography by combination of low-temperature sensitive aqueous-phase compatible normal- phase chromatography and at-column dilution modulation. *J. Chromatogr. A*, 2023, 1691, 463821.
- (11) Zhengyue, M.; Panpan, S.; Delu, L.; Yangyang, N.; Yanli, L.; Xinyuan, G.; Benben, W.; Ligai, B.; Xiaoqiang, Q. Preparation and chromatographic performance of chiral peptide-based stationary phases for enantiomeric separation. *Chirality*. 2023, 35, 636–644.
- (12) You-Ping, Z.; Kuan, L.; Ling-Xiao, X.; Bang-Jin, W.; Sheng- Ming, X.; Jun-Hui, Z.; Li-Ming, Y. “Click” preparation of a chiral macrocycle-based stationary phase for both normal-phase and reversed-phase high performance liquid chromatography enantioseparation. *J. Chromatogr. A*, 2022, 1683, 463551.
- (13) Boyd-Boland, A. A.; Pawliszyn, J. B. New solvent-free sample preparation techniques. *Environ Sci Technol*. 1994, 28, 569-574.
- (14) Jiang, P.; Lucy, C. A. Coupling normal phase liquid chromatography with electrospray ionization mass spectrometry: Strategies and applications. *Anal. Methods*. 2016, 8, 6478–6488.

- (15) Grasselli, G., Arigò, A., Palma, P., Famiglini, G., Cappiello, A., Latest Developments in Direct and Non-Direct LC-MS Methods Based on Liquid Electron Ionization (LEI). *Crit. Rev. Anal. Chem.* 2024, 1–18.
- (16) Stefkov, G.; Cvetkovikj Karanfilova, I.; Stoilkovska Gjorgievska, V.; Trajkovska, A.; Geskovski, N.; Karapandzova, M.; Kulevanova, S. Analytical Techniques for Phytocannabinoid Profiling of Cannabis and Cannabis-Based Products—A Comprehensive Review. *Molecules.* 2022, 27, 975.
- (17) De Luca, C.; Buratti, A.; Krauke, Y.; Stephan, S.; Monks, K.; Brighenti, V.; Pellati, F.; Cavazzini, A.; Catani, M.; Felletti, S. Investigating the effect of polarity of stationary and mobile phases on retention of cannabinoids in normal phase liquid chromatography. *Anal. Bioanal. Chem.* 2022, 414, 5385–5395
- (18) Huang, L.; Li, J.; Bi, Y.; Xu, Y.; Wang, Y. et al. Simultaneous determination of α -tocopherol, β -tocopherol, γ -tocopherol, δ -tocopherol, sesamin, sesamol, and asaridin in sesame oil by normal-phase high performance liquid chromatography. *J. Food Compos. Anal.* 104, 104132 (2021).
- (19) Barros, L.; Correia, D. M.; Ferreira, I. C. F. R.; Baptista, P.; Santos-Buelga, C. Optimization of the determination of tocopherols in *Agaricus* sp. Edible mushrooms by a normal phase liquid chromatographic method. *Food Chem.* 2008, 110, 1046–1050.
- (20) Bouymajane, A.; El Majdoub, Y. O.; Cacciola, F.; Russo, M.; Salafia, F.; Trozzi, A.; Filali, F. R.; Dugo, P.; Mondello, L. Characterization of Phenolic Compounds, Vitamin E and Fatty Acids from Monovarietal Virgin Olive Oils of “Picholine marocaine” Cultivar. *Molecules*, 2020, 25, 5428.

- (21) Lechhab, T.; Salmoun, F.; Lechhab, W.; El Majdoub, Y. O.; Russo, M.; Testa Camillo, M. R.; Trovato, E.; Dugo, P.; Mondello, L.; Cacciola, F. Determination of bioactive compounds in extra virgin olive oils from 19 Moroccan areas using liquid chromatography coupled to mass spectrometry: a study over two successive years. *Eur. Food Res. Technol.* 2021, 247, 2993–3012.

Chapter 5

The Liquid Electron Ionization (LEI) interface coupled with a High-Resolution Mass Spectrometer (HRMS) for Targeted and Non-Targeted Analysis.

5. Introduction

Non-targeted analysis (NTA) represents a challenge for the scientific community. NTA can be divided into semi-target and completely non-target analyses (1). The first group includes the analysis of compounds expected to be in, such as metabolites, while the second group compounds are completely unknown, without any prior information, which can include compounds that can show also different chemical and physical properties (1,2). Hence, for the second group, different sample preparation, separation, and ionization techniques can be required (1). Sample preparation and separation have to be optimized in order to increase the sensitivity of the detection method and to remove the endogenous compounds, but, at the same time, should be as unspecific as possible concerning the analytes of interest (1,3). MS is the most used technique for NTA due to its high identification power; in particular, LC-MS equipped with ESI became predominantly, offering high sensitivity, and allowing the ionization of thermolabile, high-molecular-weight, and non-volatile compounds. However, ESI produces mainly molecular ions, providing less structural information compared to EI, hence making more difficult the determination of unknown structures (4). To enhance specificity, analyses are usually conducted in MS/MS mode (4). Despite their widespread use, no universal spectral library has been created for unknown compound identifications in LC-MS due to the variability

of ionization conditions, which are influenced by different instrument types, polarity modes, and mobile phase composition (5,6).

High-resolution mass spectrometry (HRMS), combined with different chemical databases, currently represents the best technique for unknown compound identification. Instruments such as Orbitrap and Q-TOF enable HRMS in both LC and GC applications (7-11). Both instruments achieve the required mass accuracy (below 5 ppm) and absolute isotope ratio deviation (within 5%) for accurate structure elucidation. However, factors like ion count levels, complex sample matrices, and compound co-elution in LC can sometimes compromise accuracy (12-14).

As already explained in *Chapter 1*, LEI interface was developed to combine the advantages of EI with those of LC (15). LEI interface extends the EI searchable library spectra to compounds not amenable to GC (16-18). Compared to typical LC ionization techniques like ESI (19) the LEI interface demonstrates reduced ME (4,20).

Recent studies have shown that LEI is compatible with QTOF, making it a versatile tool (16). While EI spectra commonly lack measurable molecular ions, the QTOF's low-energy EI capabilities allow the acquisition of high-informative spectra under standard EI conditions and softer fragmentation at lower energies (15 and 9 eV), which yields molecular ions for most compounds. Switching between high and low energies is straightforward, enabling qualitative analyses of unknowns in complex matrices using EI-HRMS.

Therefore, because so far, the advantages of the LEI interface were demonstrated only for targeted and quantitative analyses, in this chapter two different studies were conducted to demonstrate the advantages of using LEI for non-targeted analysis. A novel workflow for NTA, described in detail in *Paragraph 2.3.3.4*, based on deconvolution, low-energies analyses, and MS/MS was developed and applied in both cases.

In the first study, the system LC-LEI-QTOF was used in an animal poisoning case, as a complementary tool for source tracing. The study, conducted in collaboration with the Charles University of Prague, Czech Republic, was submitted to the “Environmental Science and Technology” journal with the following title “Targeted and Non-Targeted Analysis Workflow in Animal Poisoning Cases: Challenges in Source Tracing”. In addition, it was presented at the 28th International Symposium on Separation Sciences in Messina, Italy on 22-25 September 2024.

The second study included in this chapter was conducted in collaboration with Syngenta Ltd. The LC-LEI-QTOF-MS/MS system, in this case, was used for the detection and identification of non-polar impurity, which was not detectable using conventional LC-HRMS systems. The work was included in the published review “Latest Developments in Direct and Non-Direct LC-MS Methods Based on Liquid Electron Ionization (LEI)” as a novel application, and presented at the 71st Conference of American Society Mass Spectrometry and Allied Topics in Houston, Texas on 4-8 June 2023.

5.1. Targeted and Non-Targeted Analysis Workflow in Animal Poisoning Cases: Challenges in Source Tracing

5.1.1. Introduction

Pesticides play a crucial role in agriculture management, improving crop yield, quality, and safety, by acting against pests (21). Despite these benefits, pesticides often exhibit toxicity

toward non-target organisms (22). Among pesticides, the carbamate class is largely used in agriculture, gardening, forestry, and therapeutic pharmaceuticals. Carbamates are replacing other pesticide classes, such as organophosphate pesticides, because of their shorter half-life and, lower persistence and bioaccumulation in the environment (23). Despite their positive effects, carbamate pesticides show high toxicity for non-target organisms. They bind to the active site of the acetylcholinesterase, inhibiting the enzyme and causing an accumulation of acetylcholine at the nerve synapse. The inhibition of acetylcholine hydrolysis leads to increased cholinergic stimulation culminating in poisoning symptoms (24). In addition, they are suspected carcinogens and mutagens (25). Carbofuran (CBF) is a carbamate systemic insecticide, nematicide, and acaricide largely used for its broad spectrum of activity. Because of its high mobility in different soils and high solubility in water, CBF has been detected in high concentrations in rivers, food, and soils worldwide (23). The European Union banned carbofuran in 2008 due to its toxicity (26), yet it remains available in various countries (27). Different accidental and intentional animal poisonings have been documented over the years in different countries, such as Spain (28), Romania (27), South Korea (29), and so on. In addition, CF was used to commit human suicide (30). Cases of intoxication and fatalities caused by carbamate pesticides were mainly determined by GC and LC coupled with MS (31-35).

Recently, a case of animal poisoning occurred in the Czech Republic, where poisoned food was used to kill multiple animals. The National Veterinary Institute of Prague performed autopsies, finding morphological signs consistent with carbamate pesticide poisoning. The Faculty of Sciences at Charles University in Prague suspected the use of certain CBF formulations. Given that CBF is banned in the Czech Republic, CBF formulations from various countries were provided for analysis using LC-LEI-QTOF-MS/MS. This approach aimed to characterize

volatile and low-volatile fractions to identify the specific CBF formulation used in the poisonings. A novel targeted and non-targeted analysis (NTA) method was developed to characterize formulations and detect and quantify CBF in three poisoned animal samples. The characterized formulations were compared to real biological samples to trace the source of the CBF used in the poisonings. By combining EI with HRMS in LC-LEI-QTOF-MS/MS, nearly all compounds in the formulations were identified, establishing a reliable identification method. Due to EI's reduced susceptibility to matrix effects (ME), deconvolution allowed the identification of coeluted compounds. In the analysis of the three biological samples, carbofuran poisoning was confirmed in two cases. The method demonstrated satisfactory repeatability, linearity, and negligible ME without using an internal standard.

5.1.2. Results

LC-LEI-QTOF/MSMS system represents a powerful tool for unknown structure elucidation and identification, as already demonstrated in a previous work (36). Its ability to generate EI spectra comparable to the NIST library enables rapid and straightforward compound identification. However, when unknown concentrations are too low or coeluting compounds are present, the NIST match can become less reliable. The fast scan speed of the Q-TOF combined with the possibility of acquiring HRMS and HR-MS/MS spectra enhances the deconvolution of coeluted compound spectra. This process improves background subtraction, permitting a more accurate identification using the NIST library (38).

5.1.2.1. Characterization of Carbofuran formulations

In 7 of the 13 formulations, different compounds other than carbofuran were identified using the software Agilent MassHunter unknown analysis. In the UA_3 formulation, 12 peaks were detected after deconvolution, as shown in **Figure 5.1** and summarized in **Table 5.1**. Nine were readily identified by matching their spectra with the NIST library (peaks 2, 3, 4, 6, 7, 8, 11). Two compounds were identified with a probability of 57.21% (peak 1), and 76.74% (peak 10). Two compounds gave incorrect matches (peaks 5 and 9), while no matches were proposed for the remaining two (peaks 10 and 12).

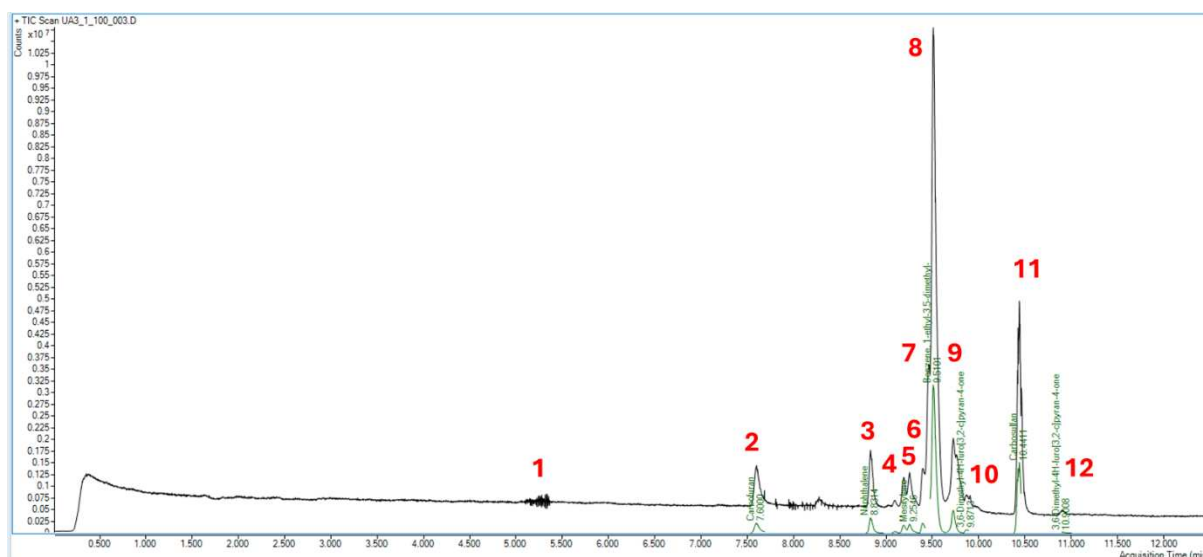


Figure 5.1. Deconvoluted chromatogram of UA_3 formulation.

Table 5.1. Peaks list of UA_3 formulation.

<i>Formulation UA_3</i>						
Peak	RT	Molecular ion (m/z)	Suspected compound	dM (PPM)	NIST Match (%)	Classification
1	5.35	129.1507	Dibutylamine	-2.3	57.21	Metabolite
2	7.69	221.1043	Carbofuran	-1.6	95.68	Pesticide
3	8.83	128.0618	Naphtalene	1.2	99.38	PAH
4	9.09	118.0772	Indane	-4.2	92.40	Petrochemical
5	9.19	142.0773	2-Methylnaphthalene	3.5	/	PAH
6	9.25	120.0931	Mestylene	1.3	96.48	Contaminant
7	9.39	132.0931	Indane-1Methyl	2.7	94.66	Petrochemical
8	9.51	134.1089	Cumene	0	98.28	Petrochemical
9	9.72	170.1082	2,3,6-Trimethylnaphtalene	4.7	/	PAH
10	9.87	504.1408	C ₂₄ H ₂₈ N ₂ O ₆ S ₂	-2.2	/	Impurity
11	10.46	381.2166 [M+H]	Carbosulfan	1.9	76.74	Pesticide
12	11.18	/	/	/	/	/

For the first group of compounds, CBF, Naphtalene, Indane, Mestylene, Indane-1Methyl, Cumene, and Benzene1-ethyl-3,5-dimethyl were identified with probabilities ranging between 92.40 and 99.38%. Dibutylamine and Carbosulfan were the only compounds identified with a probability below 80%. To confirm the identity of Carbosulfan, and to assign structures to the unknown compounds 5, 10, and 12, low energies analyses were conducted at 15 and 9 eV to establish suspected molecular ions. For Carbosulfan, as shown in **Figure 5.2**, the ion at m/z 381.2166 showed an increment in relative abundance from 0.19% at 70 eV to 5.73% at 15 eV to, indicating it as suspected molecular ion. The same ion was selected as the precursor ion for MS/MS analysis, and its high-resolution fragmentation pattern was compared with the ChemSpider database. Using protonation as the default ionization, the compound was confirmed as Carbosulfan, confirming the previous identification by NIST.

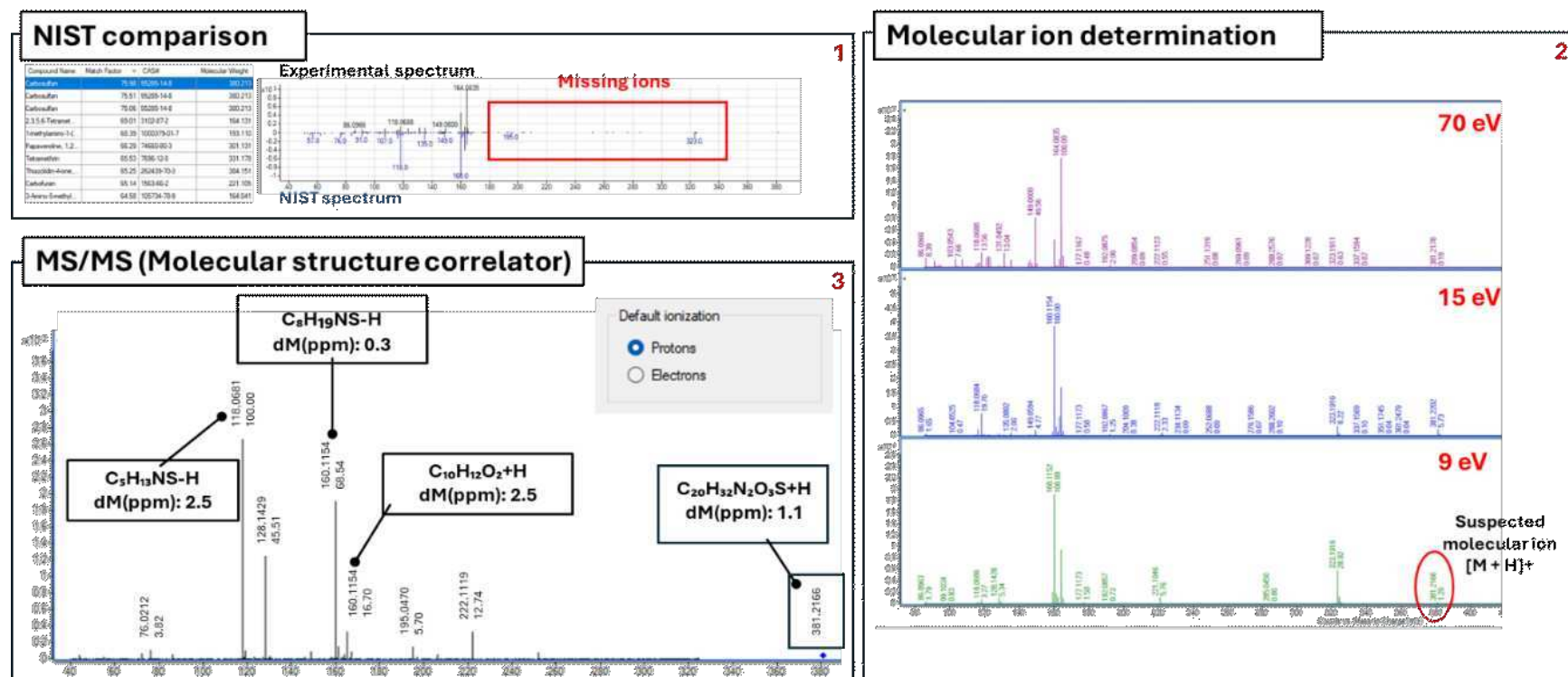


Figure 5.2. (1) Comparison between deconvoluted spectrum and NIST spectrum of Carbosulfan (peak 11). (2) Molecular ion determination comparing the spectra obtained at 70, 15 and 9 eV. (3) MS/MS analysis of suspected (M+H)⁺ (m/z 381.2166) and determination of fragment structures with their corresponding accuracy.

The ion at m/z 381.2166 corresponds to the $(M+H)^+$ ion of Carbosulfan, as the compound readily gains a proton from the mobile phase, forming the protonated ion under chemical ionization (CI) conditions. In fact, for certain compounds, CI can occur even in an EI source showing the appearance of the $(M+H)^+$ ion. Comparing the experimental mass with the accurate mass of $C_{20}H_{32}N_2S+H^+$, the accuracy results in 1.9 ppm, confirming this assumption. At 9 eV a decrement in the relative abundance of the ion at m/z 381.2166 may be explained by a reduced CI efficiency. The formation of the two competing ions at m/z 381.2166 and m/z 380.2133 is not controlled into an EI source, and the lower energy disfavour CI. The presence of Carbosulfan is particularly significant because it was banned by the EU as well (38). The injection of a less diluted UA_3 formulation (1:10 v/v instead of 1:100 v/v), enabled the detection of also Dibutylamine (m/z 129.1507), peak 1, at RT 5.35, a well-known Carbosulfan degradation product and highly toxic compound (39,40). The low probability of the match proposed by the NIST library can be ascribed to the compound's low concentration and because most ions fall outside the acquisition range; however, the exact mass of the molecular ion confirms its identity (**Figure 5.3**).

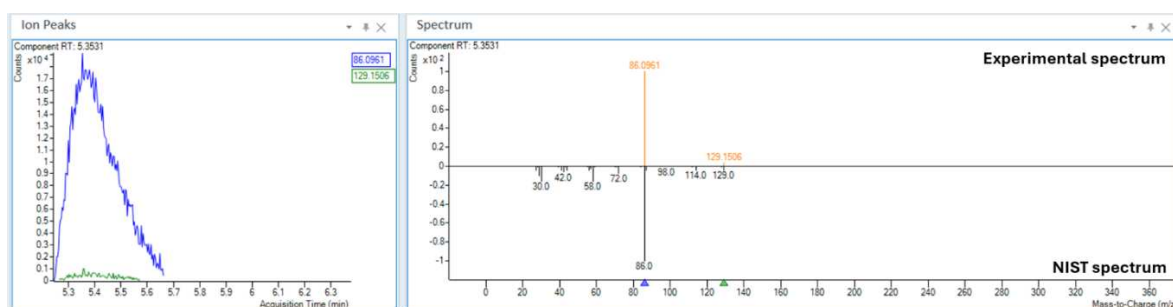


Figure 5.3. Comparison between experimental deconvoluted spectrum and NIST library spectrum of Dibutylamine (Peak 1).

Carbofuran is considered a photolytic and hydrolysis product of carbosulfan (39,40). The same procedure adopted for Carbosulfan was applied to peaks 5 and 9 which were not properly identified using the NIST library. NIST proposed two incorrect structures with different molecular ions. Using low energies analysis, the suspected molecular ions were determined. The MSC software proposed 2-Methylnaphalente for peak 5, and 2,3,6-Trimethylnaphtalene for peak 9.

The same procedure was applied for the identification of peak 10 where the comparison with NIST was unsatisfactory. Low energy analysis showed the ion at m/z 504.1138 as a suspected molecular ion. In this case, the proposed formula was $C_{24}H_{28}N_2O_6S_2$ with an accuracy of -2.24 ppm. Unfortunately, MS/MS analysis could not be performed due to the ion's low abundance. The structure of the base peak m/z 192.0867, present in the $C_{24}H_{28}N_2O_6S_2$ spectrum, was also established as $C_8H_{18}NS_2$ with good accuracy (-4.26 dMppm), confirming once again the proposed formula.

For peak 12, establishing the molecular ion was impossible due to its low concentration.

The second formulation containing another compound beside carbofuran was BV_PF_B (**Table 5.2**). In this case, the additional compound was identified as N,N-diethyl-m-toluamide (DEET), a common mosquito repellent, with a NIST library match probability of 96.48%. The compound was also confirmed with the RT injecting a pure DEET standard solution.

Table 5.2. Peaks list of BV_PF_B formulation.

<i>Formulation BV_PF_B</i>						
Peak	RT	Molecular ion (m/z)	Suspected compound	dM (PPM)	NIST Match (%)	Classification
1	7.49	191.1271	DEET	3.7	96.48	Mosquito repellent
2	7.69	221.1043	Carbofuran	-1.6	95.68	Pesticide

The third formulation containing a compound other than carbofuran was CG_23_B (**Table 5.3**). In this case, the comparison with NIST library gave a low recognition (probability 57%). In addition, the suspected molecular ion (m/z 278.1258) (**Figure 5.4**), established by performing the low energies analysis, differed from the compound suggested by the NIST library. Therefore, MS/MS analysis using the m/z 278.1238 ion as precursor ion was performed. The MSC software identified the formula $C_{14}H_{18}N_2O_4$, with an accuracy of 1.1 ppm.

Table 5.3. Peaks list of CG_23_B formulation.

<i>Formulation CG_23_B</i>						
Peak	RT	Molecular ion (m/z)	Suspected compound	dM (PPM)	NIST Match (%)	Classification
1	7.52	221.1043	Carbofuran	-1.6	95.68	Pesticide
2	7.76	278.1258	$C_{14}H_{18}N_2O_4$	1.1	/	/

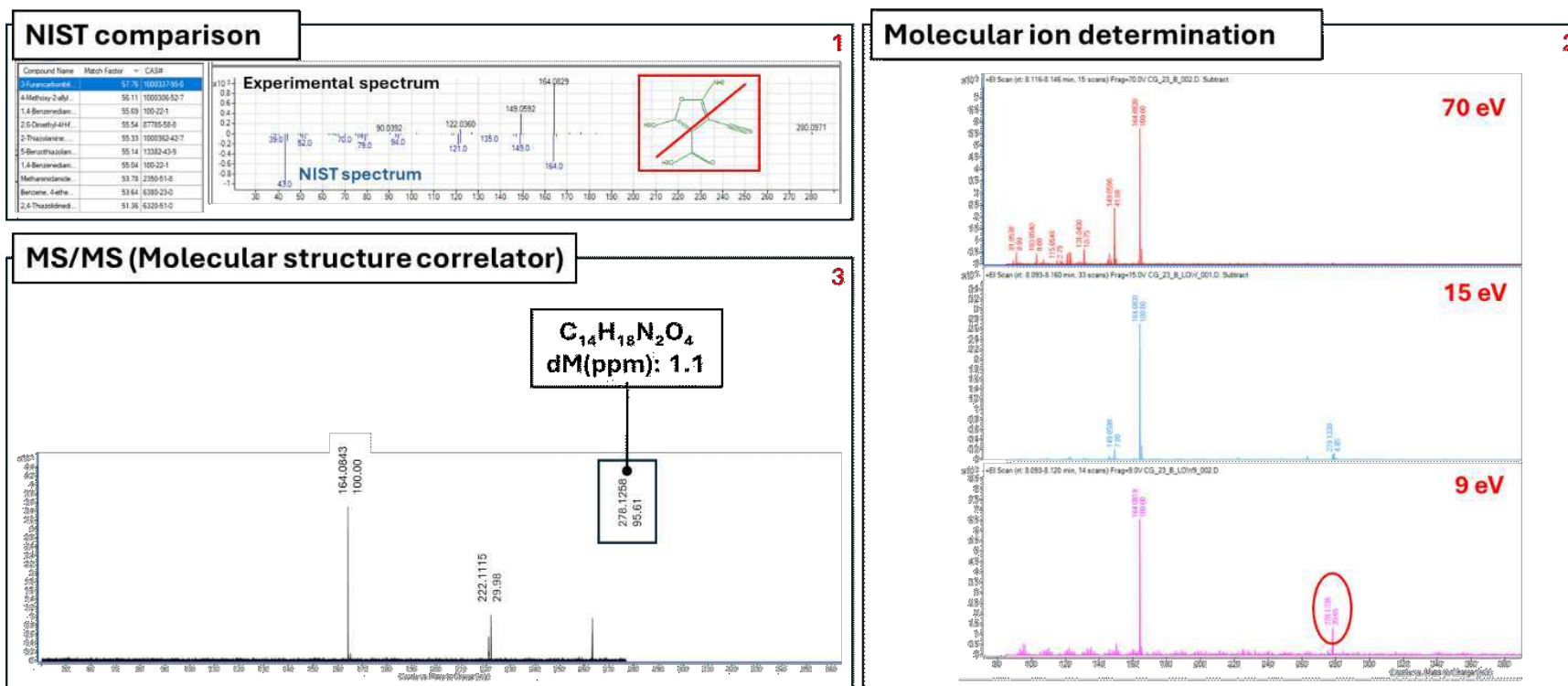


Figure 5.4. (1) Peak 2 molecular formula determination of the CG_23_B formulation. (2) Molecular ion determination comparing the spectra obtained at 70, 15, and 9 eV. (3) MS/MS of the suspected molecular ion, and its structure and accuracy.

In the last four formulations, F0, F2, F8, and F9, a well-known carbofuran metabolite, 3-keto-carbofuran, was detected (**Table 5.4**). The compound concentration was low in each formulation, which explains the low match value proposed by NIST. Due to the low concentration, it was impossible to determine the molecular ion or perform MS/MS analysis. The metabolite is given by the neutral loss of carbamic acid and water (41). In the 6 remaining formulations, only carbofuran was found.

Table 5.4. Peaks list of F0, F2, F8 and F9 formulations.

<i>Formulations F0, F2, F8, F9</i>						
Peak	RT	Molecular ion (m/z)	Suspected compound	dM (PPM)	NIST Match (%)	Classification
1	6.80	235.0952	3-keto-carbofuran	-0.9	68.40	Metabolite
2	7.69	221.1043	Carbofuran	-1.6	95.68	Pesticide

5.1.2.2. Method validation

The method for CBF was validated by assessing limits of detection (LOD) and quantification (LOQ), intraday and interday repeatability, and linearity range in both solvent and matrix. ME was evaluated by comparing the slope of the two calibration curves.

The LOD and LOQ for CBF were 0.09 and 0.30 $\mu\text{g/mL}$ respectively. Regarding the linearity, R^2 values of calibration curves in solvent and matrix were 0.9975 and 0.9993 respectively (**Figure 5.5**). The RSD% intraday and interday calculated at 0.75 $\mu\text{g/mL}$ were 3% and 10%, and at 1.50 $\mu\text{g/mL}$ were 1% and 9% respectively.

While previous studies have shown that the LEI interface exhibits negligible ME, even with compound coelutions (17,42,43), this work involved analyzing complex real-world samples, necessitating ME evaluation. ME was calculated as reported in section 2.3. The ideal ME value is 100%, indicating no ME, with values within $\pm 20\%$ considered acceptable. In this work, the

ME value was 107%; hence it can be considered negligible, demonstrating the reliability of the method.

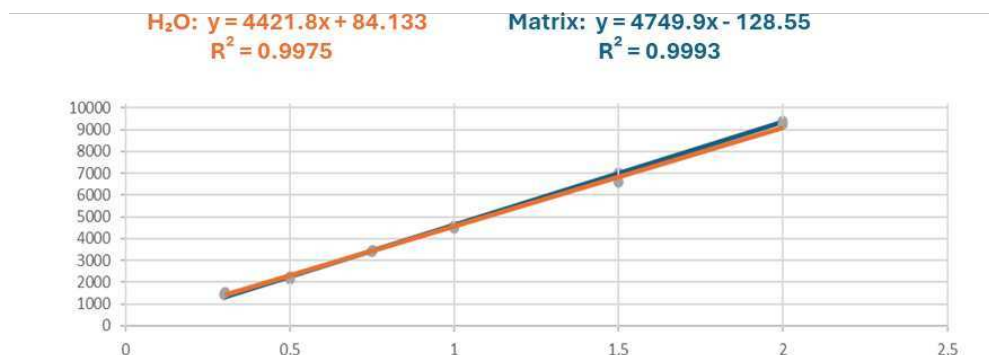


Figure 5.5. CBF Calibration curve in water (orange) and in matrix (blue).

5.1.2.3. Real samples analyses

LC-LEI-QTOF-MS/MS analysis confirmed the presence of CBF in two out of the three real samples (**Figure 5.6**). The analyses of the three samples were performed by diluting them with water 1:5 (v/v). The concentrations of CF found in the extract were: 5.5 mg/kg in sample 544/2 (fox liver), 4.2 mg/kg in sample 18894 (red kite gastric content), and below the limit of detection in sample 237 (eagle gastric content). To determine if one of the 13 characterized formulations was used in the poisoning cases, the peak spectra of real samples were compared with our HR laboratory-created library by using Agilent MassHunter Unknown software. In the real samples, the sole compound found in sample 544/2 was the 3-keto-carbofuran, which unfortunately cannot be used as a discriminatory marker to trace the specific formulation used (**Figure 5.7**).

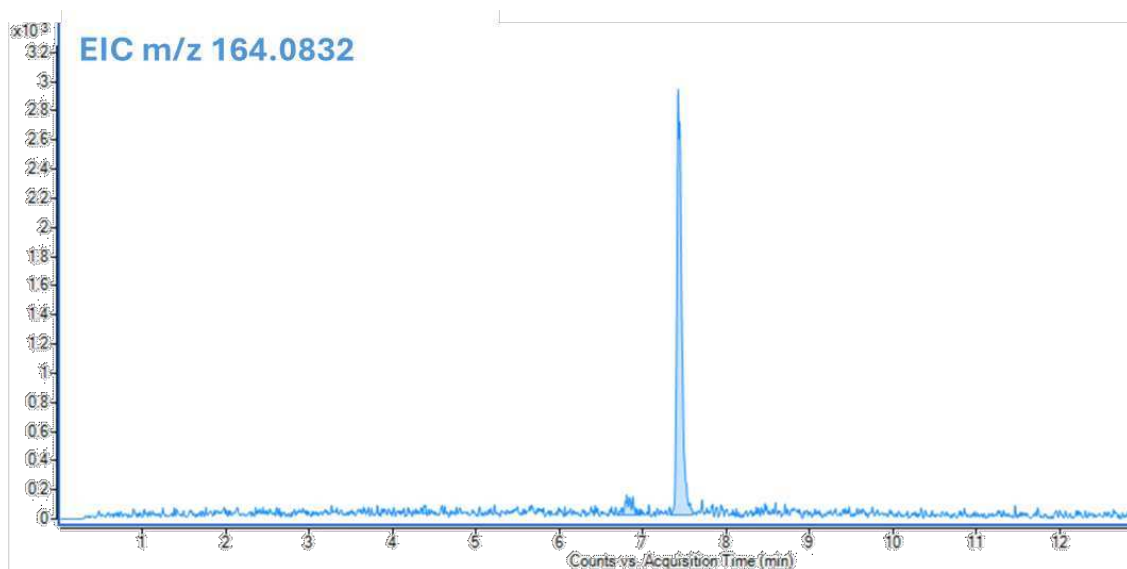


Figure 5.6. EIC chromatogram of m/z 164.0832 in sample 544/2.

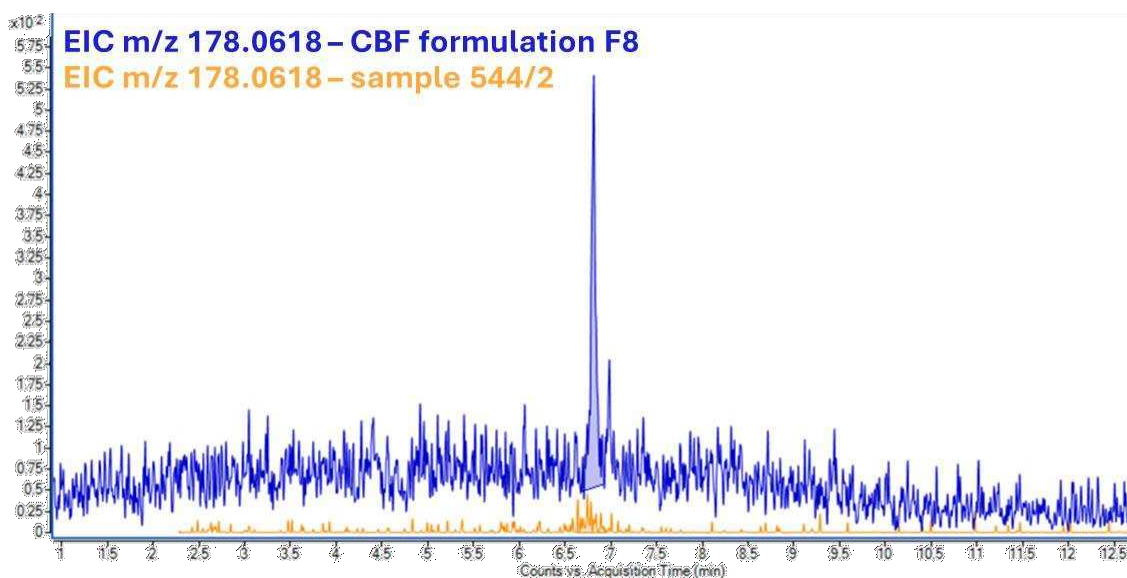


Figure 5.7. EIC chromatogram of m/z 178.0618 (3-Keto-Carbofuran) in F8 formulation (blue) and sample 544/2 (orange).

5.1.3. Discussion

The use of EI as ionization technique makes the identification process easier with respect to the conventional ionization techniques used with LC-MS/MS. However, being usually coupled with GC, the chemical coverage is limited to volatile compounds. Coupling LC with EI allows to extend this ionization technique to a wider variety of compounds. In this work, a novel identification method was developed using a LC-LEI-QTOF-MS/MS system, adding the HRMS capabilities to EI identification power. This approach facilitated the characterization of volatile, low volatile, and thermolabile fractions of 13 CBF formulations. For most compounds, the identification was easily performed by comparing the experimental spectra with the NIST library. Additionally, low energies analysis confirmed compound identities determining molecular ions without the need for other instruments. In the UA_3 formulation, various contaminants were identified, including unexpected such as the banned pesticide Carbosulfan, its toxic metabolite Dibutylamine, and its impurity $C_{24}H_{28}N_2O_6S_2$. Other notable findings across formulations included DEET, a common mosquito repellent, $C_{14}H_{18}N_2O_4$, and 3-keto-carbofuran, a primary metabolite of carbofuran. A custom high-resolution spectral library simplified the detection of these compounds in real samples, even in cases of low concentrations or matrix co-elution. 3-keto-carbofuran, present in four different formulations (F0, F2, F8, F9), was detected alongside with CBF in the fox liver sample (544/2). However, as a metabolite of carbofuran, 3-keto-carbofuran cannot serve as a marker for a specific CBF formulations. Regarding the quantitative analysis, the method demonstrated excellent performance. As expected, a negligible matrix effect was confirmed also without the use of an internal standard, confirming that the LC-LEI-QTOF-MS/MS system is a good alternative for qualitative and quantitative analysis of volatile and low volatile fractions of complex samples.

However, recovery experiments were not performed, so the concentrations in real samples were assumed to have 100% recovery. As a result, the concentrations found in CBF-positive samples could be underestimated, bringing their real values well above the LD50 values in rats (5 mg/kg) and birds (0.7-8.0 mg/kg), and comparable with values reported in human suicide cases (7,33) confirming the suspected intentional poisoning.

5.2. LEI for non-targeted analyses (NTA): the Impurity Y case

5.2.1. Introduction

LC not only allows for the separation of compounds that have to be analyzed but it is used also for the elaboration of isolation strategies. Hence, the detection of impurities using a detector coupled with LC sometimes is mandatory to develop an isolation strategy. Despite the LC-MS equipped with ESI being the most used technique, it does not allow the detection of nonpolar compounds.

For this peculiarity, the LEI-QTOF, which allows the detection of compounds with different chemical and physical properties, was fundamental to develop an LC-based workflow, described in the *paragraph 2.3.3.4*, to detect and identify an impurity (called impurity Y) present in a complex CF. Due to the high non-polar nature of impurity Y, its detection with the conventional atmospheric pressure ionization techniques was a challenge. Not knowing the nature of the substances present in the sample and considering the low concentration of impurity Y, compared to the main components of the sample, the CF itself was injected as is, without any preparation step.

5.2.2. Results

The analyses were performed in standard conditions at 70 eV and lower electron energies (15 eV and 9 eV) to determine the molecular ion (**Figure 5.8**). Molecular ion was necessary to reconstruct the molecular formula of the unknown compound. The suspected molecular ion (m/z 362.3940) reached 36.33% at 15 eV and 100% of relative abundance at 9 eV. The progressive increase in the presumed molecular ion's intensity as the electrons' energy decreases confirmed the choice. This ion was then fragmented in the collision cell to produce daughter ions. The high-resolution MS/MS fragmentation was used for determining the unknown compound structure using a molecular structure correlation software, which enables the assignment of the formula and structure starting from the molecular ion fragmentation and the exact mass of each fragment, using an approach described by Alastair W. Hill and Russell J. Mortishire (44). MS/MS spectrum was compared with the structures in ChemSpider and PubChem databases, which assigned possible formulas and structures based on each compound's molecular ion fragmentation and exact masses. The assigned formula for the impurity Y was $C_{26}H_{50}$ (**Figure 5.9**).

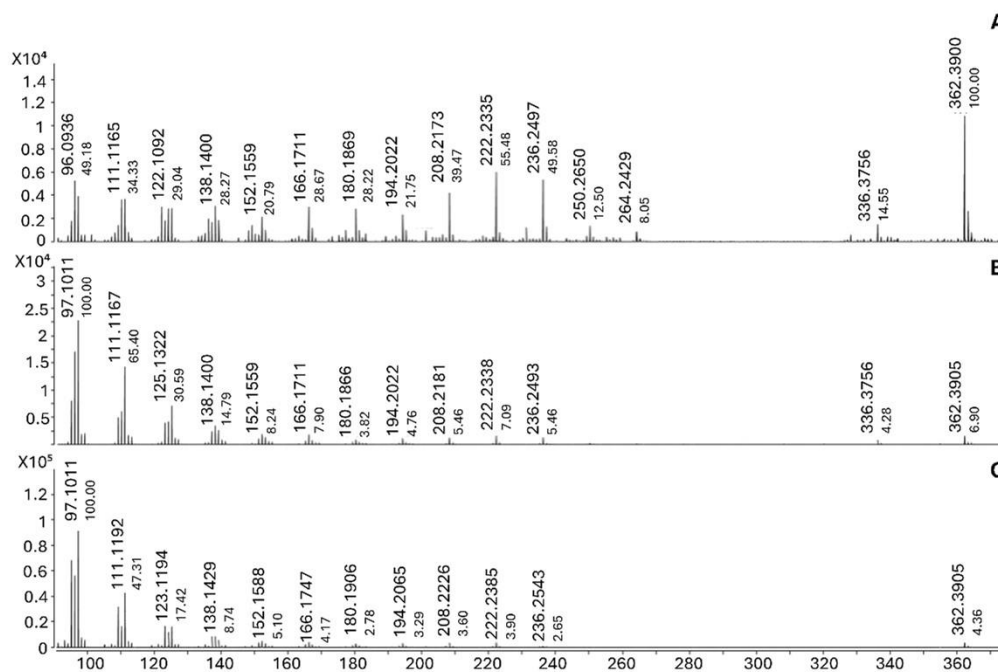


Figure 5.8. Impurity Y spectra were acquired at (A) 9 eV, (B) 15 eV, and (C) 70 eV.

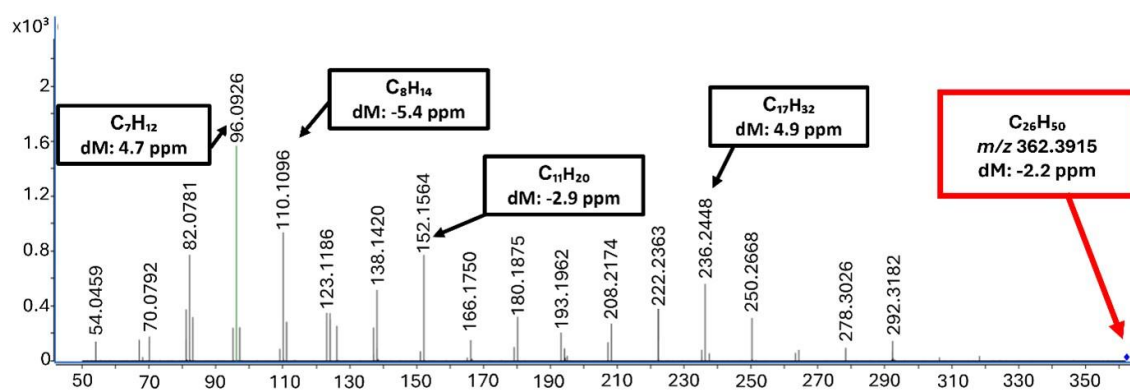


Figure 5.9. HR MS/MS spectrum of Impurity Y with the molecular formula and the accuracy of each fragment.

5.2.3. Conclusions

The system LC-LEI-QTOF-MS/MS allowed for the detection and identification of a non-polar impurity in a CF that had to be commercialized. Due to its non-polar nature, its detection using ESI techniques was a challenge. Because the LEI interface shows negligible ME, the sample was injected without any sample preparation step, reducing the risk of loss of analyte present in trace level in the CF. The determination of the molecular ion, performed by using different electron energies, was fundamental for the MS/MS analysis. Indeed, it was used as the precursor ion and, due to the HRMS fragmentation, it was possible to determine the compounds' molecular formula.

References

- (1) Milman, B.L.; Zhurkovich, I.K. The chemical space for non-target analysis. *TrAC - Trends Anal Chem.* 2017, 97, 179–187.
- (2) Krauss, M.; Singer, H.; Hollender J. LC-high resolution MS in environmental analysis: From target screening to the identification of unknowns. *Anal Bioanal Chem.* 2010, 397, 943–951.
- (3) Hajeb, P.; Zhu, L.; Bossi, R.; Vorkamp, K. Sample preparation techniques for suspect and non-target screening of emerging contaminants. *Chemosphere.* 2022, 132306.
- (4) Famiglini, G.; Palma, P.; Termopoli, V.; Cappiello, A. The history of electron ionization in LC-MS, from the early days to modern technologies: A review. *Anal. Chim. Acta.* 2021, 1167, 338350.
- (5) Lee, S.; Hwang, S.; Seo, M.; Shin, K. B.; Kim, K. H.; Park, G. W.; Kim, J.Y.; Yoo, J. S.; No, K.T. BMDMS-NP: A comprehensive ESI-MS/MS spectral library of natural compounds. *Phytochemistry.* 2020, 177, 112427.
- (6) Volna, K; Holcapek, M.; Kolarova, L.; Lemr, K.; Caslavsky, J.; Kacer, P.; Poustka, J.; Hubalek, M. Comparison of negative ion electrospray mass spectra measured by seven tandem mass analyzers towards library formation. *Rapid Commun. Mass Spectrom.* 2008, 22, 101-108.
- (7) Kernalléguen, A.; Le Daré, B.; Pelletier, R.; Ferron, P. J.; Maamar, A.; Bouvet, R.; Baert, A.; Morel, I.; Gicquel, T. Carbofuran self-poisoning: forensic and analytic investigations in twins and literature review. *Int. J. Legal Med.* 2022, 136, 1585–1596.
- (8) Hardy, E. M.; Dereumeaux, C.; Guldner, L.; Briand, O.; Vandentorren, S.; Oleko, A.; Zaros, C.; Appenzeller, B. M. R. Hair versus urine for the biomonitoring of

pesticide exposure: Results from a pilot cohort study on pregnant women. *Environ. Int.* 2021, 152, 106481

- (9) Milman, B. L.; Zhurkovich, I. K. The chemical space for non-target analysis. *TrAC - Trends Anal. Chem.* 2017, 97, 179–187.
- (10) Tozzi Wurzler, G.; da Silva Antonio, A.; de Andrade Bhering, C.; Machado Pereira Ajuz, A.; Rissi Carvalhosa, D.; Celso Jardim, A.; Yves Chalom, M.; de Aquino Neto, F. R.; Vanini, G. Fast high-resolution mass spectrometry in the triage investigation of poisonings by pesticides in unusual samples - a case study. *Forensic Chem.* 2024, 37, 100555.
- (11) Vitoria, M.; Vargas, M.; Rosalía, L.; Oliveira, S.; Teixeira, H.; Garrido, A.; Romero-gonz, R. Comprehensive analysis of contaminants in Brazilian infant formulas : Application of QuEChERS coupled with UHPLC-QqQ-MS / MS and suspect screening-unknown analysis by UHPLC-Q-Orbitrap-MS. 2024, 1726, 464967.
- (12) Knolhoff, A. M.; Callahan, J. H.; Croley, T. R. Mass accuracy and isotopic abundance measurements for HR-MS instrumentation: Capabilities for non-targeted analyses. *J. Am. Soc. Mass Spectrom.* 2014, 25, 1285–1294.
- (13) Portolés, T.; Mol, J. G. J.; Sancho, J. V.; Hernández, F. Use of electron ionization and atmospheric pressure chemical ionization in gas chromatography coupled to time-of-flight mass spectrometry for screening and identification of organic pollutants in waters. *J. Chromatogr. A.* 2014, 1339, 145–153.
- (14) Schymanski, E. L.; Singer, H. P.; Longrée, P.; Loos, M.; Ruff, M.; Stravs, M. A.; Ripollés Vidal, C.; Hollender, J. Strategies to characterize polar organic contamination in wastewater: Exploring the capability of high resolution mass spectrometry. *Environ. Sci. Technol.* 2014, 48, 1811–1818.

- (15) Termopoli, V.; Famiglini, G.; Palma, P.; Piergiovanni, M.; Cappiello, A. Atmospheric pressure vaporization mechanism for coupling a liquid phase with electron ionization mass spectrometry. *Anal. Chem.* 2017, 89, 2049–2056.
- (16) Marittimo, N.; Grasselli, G.; Arigò, A.; Famiglini, G.; Palma, P.; Saeed, M.; Perry, S.; Navarro, P.; Clarke, P.; Brittin, M.; Cappiello, A. Sustainable and Rapid Determination of Two Halogenated Pesticides in a Commercial Formulation by Solid Phase Microextraction and Liquid Phase Chemical Ionization Mass Spectrometry. *Separation.* 2023, 10, 325.
- (17) Cappiello, A.; Termopoli, V.; Palma, P.; Famiglini, G.; Saeed, M.; Perry, S.; Navarro, P. Liquid Chromatography–Electron Capture Negative Ionization–Tandem Mass Spectrometry Detection of Pesticides in a Commercial Formulation. *J. Am. Soc. Mass Spectrom.* 2022, 33, 141–148.
- (18) Grasselli, G., Arigò, A., Palma, P., Famiglini, G., Cappiello, A., Latest Developments in Direct and Non-Direct LC-MS Methods Based on Liquid Electron Ionization (LEI). *Crit. Rev. Anal. Chem.* 2024, 1–18.
- (19) Rentsch, K. M. Trends in Analytical Chemistry Knowing the unknown – State of the art of LCMS in toxicology. *Trends Anal. Chem.* 2016, 84, 88–93.
- (20) Termopoli, V.; Famiglini, G.; Palma, P.; Piergiovanni, M.; Rocio-Bautista, P.; Ottaviani, M. F.; Cappiello, A.; Saeed, M.; Perry, S. Evaluation of a liquid electron ionization liquid chromatography–mass spectrometry interface. *J. Chromatogr. A* 2019, 1591, 120–130.
- (21) Damalas, C. A.; Eleftherohorinos, I. G. Pesticide exposure, safety issues, and risk assessment indicators. *Int. J. Environ. Res. Public Health.* 2011, 8, 1402–1419.

- (22) Oliveira, G. R.; Gallas-Lopes, M.; Chitolina, R.; Bastos, L. M.; Portela, S. M.; Stahlhofer-Buss, T.; Gusso, D.; Gomez, R.; Wyse, A. T. S.; Herrmann, A. P.; Piato, A. Evaluation of behavioral and neurochemical changes induced by carbofuran in zebrafish (*Danio rerio*). *Comp. Biochem. Physiol. Part.* 2024, 283, 109969.
- (23) Mishra, S.; Zhang, W.; Lin, Z.; Pang, S.; Huang, Y.; Bhatt, P.; Chen, S. Carbofuran toxicity and its microbial degradation in contaminated environments. *Chemosphere.* 2020, 259, 127419.
- (24) Kernalléguen, A.; Le Daré, B.; Pelletier, R.; Ferron, P. J.; Maamar, A.; Bouvet, R.; Baert, A.; Morel, I.; Gicquel, T. Carbofuran self-poisoning: forensic and analytic investigations in twins and literature review. *Int. J. Legal Med.* 2022, 136, 1585–1596.
- (25) Mdeni, N. L.; Adeniji, A. O.; Okoh, A. I.; Okoh, O. O. Analytical Evaluation of Carbamate and Organophosphate Pesticides in Human and Environmental Matrices: A Review. *Molecules.* 2022, 27, 1–21.
- (26) UNEP/FAO/RC/CRC/11/6. CRC-11 / 3 : Carbofuran notifications of final regulatory action submitted by the European Union, Rotterdam Convention. 2015.
- (27) Pivariu, D.; Oros, A.; Tabaran, F.; Gal, A.; Martonos, C.; Nagy, A. Intentional Carbofuran poisoning in 7 dogs. *BMC Vet. Res.* 2020, 16, 318.
- (28) Ruiz-suárez, N.; Boada, L. D.; Henríquez-hernández, L. A.; González-moreo, F.; Suárez-pérez, A.; Camacho, M.; Zumbado, M.; Almeida-gonzález, M.; Travieso-aja, M.; Luzardo, O. P. Science of the Total Environment Continued implication of the banned pesticides carbofuran and aldicarb in the poisoning of domestic and wild animals of the Canary Islands (Spain). *Sci. Total Environ.* 2015, 505, 1093–1099.
- (29) Kwon, J.T.; Park, J.E.; Son, K.; Kim, J.; Lee, D.H.; Kim, Y.K.; Kim, Y.; Jeong, H.; Kil, J.; Jheong, W.H.; Kim, M.S. Characterization Analysis Of Wild Bird Poisoning

Cases Through Spraying Seeds Artificially. *Appl. ecol. environ. res.*, 2023, 22, 1467–1477.

- (30) Kumar, S.; Baggi, T. R.; Al-Zughaibi, T. Forensic toxicological and analytical aspects of carbamate poisoning – A review. *J. Forensic Leg. Med.* 2022, 92, 102450.
- (31) Hardy, E. M.; Dereumeaux, C.; Guldner, L.; Briand, O.; Vandentorren, S.; Oleko, A.; Zaros, C.; Appenzeller, B. M. R. Hair versus urine for the biomonitoring of pesticide exposure: Results from a pilot cohort study on pregnant women. *Environ. Int.* 2021, 152, 106481
- (32) Petropoulou, S. S. E.; Tsarbopoulos, A.; Siskos, P. A. Determination of carbofuran, carbaryl and their main metabolites in plasma samples of agricultural populations using gas chromatography-tandem mass spectrometry. *Anal. Bioanal. Chem.* 2006, 385, 1444–1456.
- (33) Tennakoon, D. A. S. S.; Karunarathna, W. D. V.; Udugampala, U. S. S. Carbofuran concentrations in blood, bile and tissues in fatal cases of homicide and suicide. *Forensic Sci. Int.* 2013, 227, 106–110.
- (34) Oenning, A. L.; Merib, J.; Carasek, E. An effective and high-throughput analytical methodology for pesticide screening in human urine by disposable pipette extraction and gas chromatography – mass spectrometry. *J. Chromatogr. B.* 2018, 1092, 459–465.
- (35) Tracqui, A.; Flesch, F.; Sauder, P.; Raul, J.S.; Geraut, A.; Ludes, B.; Jaeger, A. Repeated measurements of aldicarb in blood and urine in a case of nonfatal poisoning. *Hum. Exp. Toxicol.* 2001, 20, 657–660.
- (36) Grasselli, G., Arigò, A., Palma, P., Famiglini, G., Cappiello, A., Latest Developments in Direct and Non-Direct LC-MS Methods Based on Liquid Electron

Ionization (LEI). *Crit. Rev. Anal. Chem.* 2024, 1–18.

- (37) Agilent Technologies. 7200 Accurate-Mass Time-of-Flight GC / MS System. Concepts Guide.
- (38) Kyprianou, M. Concerning the non-inclusion of carbosulfan in Annex I to Council Directive 91/414/EEC and the withdrawal of authorisations for plant protection products containing that substance. *Eur. Union.* 2007, 4, 267–268.
- (39) Soler, C.; Mañes, J.; Picó, Y. Determination of carbosulfan and its metabolites in oranges by liquid chromatography ion-trap triple-stage mass spectrometry. *J. Chromatogr. A.* 2006, 1109, 228–241.
- (40) Conclusion on the peer review of the pesticide risk assessment of the active substance carbosulfan. *EFSA J.* 2009, 310, 7–132.
- (41) Soler, C.; Hamilton, B.; Furey, A.; James, K. J.; Mañes, J.; Picó, Y. Liquid chromatography quadrupole time-of-flight mass spectrometry analysis of carbosulfan, carbofuran, 3-hydroxycarbofuran, and other metabolites in food. *Anal. Chem.* 2007, 79, 1492–1501.
- (42) Rocío-Bautista, P.; Famiglini, G.; Termopoli, V.; Palma, P.; Nazdrajić, E.; Pawliszyn, J.; Cappiello, A. Direct Coupling of Bio-SPME to Liquid Electron Ionization-MS/MS via a Modified Microfluidic Open Interface. *J. Am. Soc. Mass Spectrom.* 2021, 32, 262–269.
- (43) Termopoli, V.; Piergiovanni, M.; Cappiello, A.; Palma, P.; Famiglini, G. Tyrosol and hydroxytyrosol determination in extra virgin olive oil with direct liquid electron ionization–tandem mass spectrometry. *Sep.* 2021, 8, 173.
- (44) Hill, A.W.; Mortishire-Smith, R.J. Automated assignment of high-resolution collisionally activated dissociation mass spectra using a systematic bond disconnection

approach. *Rapid Commun. Mass Spectrom.* 2005, 19, 3111–3118.

Chapter 6

Additional activities

6.1. Review: “Latest Developments in Direct and Non-Direct LC-MS Methods Based on Liquid Electron Ionization (LEI)”

Being a part of my PhD project based on the improvement and capabilities demonstration of the LEI interface, a novel review titled “Latest Developments in Direct and Non-Direct LC-MS Methods Base on Liquid Electron Ionization” was published in July 2024 by our group. In brief, the review starts with a quick overview of the traditional MS ionization techniques, followed by an explanation of the advantages of using LEI coupled with MS, and an overview of the novel applications.

MS is a powerful tool for accurately identifying and quantifying molecules, particularly when paired with chromatography. The introduction of API techniques enabled the effective integration of liquid chromatography with MS (LC-MS), allowing the analysis of non-volatile and thermolabile compounds (1). However, API methods face several limitations, such as poor reproducibility and low information yield in mass spectra, which contribute to increase instrument complexity and cost. Other challenges include the analysis of low-polarity molecules and matrix effects (ME), which can interfere with quantitative accuracy, often requiring expensive labeled standards or extensive sample preparation (2, 3). These limitations have driven the exploration of alternative solutions, culminating in the development of the LEI interface. This system has proven to be highly reliable and reproducible (4). LEI has been successfully applied to a wide range of substances, including pesticides, drugs of abuse, phenols, polycyclic aromatic hydrocarbons (PAHs), phthalates, and more (5-8). Its flexibility

has been confirmed using different types of detectors, such as Q (9), QqQ (7, 8), and Q-TOF (10), in EI and CI modes, as well as in combination with RPLC and NPLC (10). LEI has also been effectively integrated with the MOI (11,12), Membrane Introduction Mass Spectrometry (MIMS) (8), and Microfluidic Water-Assisted Trap Focusing (M-WATF) (7), extending its potential applications and consistently showing excellent results in terms of sensitivity and compound identification. The latest innovation in LEI technology is the development of E-LEI-MS, a technique that enables surface sampling and real-time analysis (9). This review provides a thorough and up-to-date overview of the promising capabilities of LEI.

6.2. Book chapter: “Alkaloids’ wide world (extraction, quantification, biological activities)”

During my PhD, I was a co-author of a book chapter titled "Alkaloids’ wide world (extraction, quantification, biological activities)." It is a chapter in Elsevier's book “Phytochemicals for Health” (in press). The first part of the chapter focuses on the biological activity of different alkaloid groups, while the second part is dedicated to summarizing the main extraction and analytical techniques for alkaloid quantification in different matrices.

Alkaloids are a class of secondary metabolites chemically identified as nitrogen-containing compounds that, usually, possess one or more nitrogen atoms incorporated in a heterocyclic ring (13). Different studies demonstrated that those compounds show different effects on human and animal health due to their alkaline properties given by nitrogen atoms (13). Based on the starting molecules, they can be divided into six classes (14): (I) tropane and pyrrolizidine alkaloids, derived from ornithine; (II) purine alkaloids, derived from purine; (III) isoquinoline, and phenylethylamine alkaloids, derived from tyrosine; (IV) piperidine, indolizidine,

quinolizidine alkaloids, derived from lysine; (V) indole and quinoline alkaloids, derived from tryptophan; (VI) pyridine alkaloids, derived from L-aspartate. Alkaloids show several beneficial/non-beneficial biological activities; for example, they can act as antiviral, anti-inflammatory, analgesic, anticancer, and antioxidant, but they can also have cancerogenic and hallucinogenic effects (15-17). Because of the variety of alkaloid biological activities, analytical methods for trace level detection, metabolite identification, and isomer separation are necessary. In addition, the development of green analytical strategies is nowadays mandatory, without losing analytical performance. The second part of the chapter describes carefully the techniques applied for alkaloids extraction from different matrices, such as biological fluids and tissues, plant parts, and foods, and the analytical techniques, mainly based on HPLC and GC coupled with MS (18-20) used for their analysis.

References

- (1) Watson, J. T.; Sparkman, O. D. Introduction to Mass Spectrometry: Instrumentation, Applications, and Strategies for Data Interpretation, 4th ed.; John Wiley & Sons, Ltd. England, 2007.
- (2) Horning, E. C.; Horning, M. G.; Carroll, D. I.; Dzidic, I.; Stillwell, R. N. New Picogram Detection System Base on Mass Spectrometer with an External Ionization Source at Atmospheric Pressure. *Anal. Chem.* 1973, 45, 936–943
- (3) Sun, W.; Wu, S.; Wang, X.; Zheng, D.; Gao, Y. An Analysis of Protein Abundance Suppression in Data Dependent Liquid Chromatography and Tandem Mass Spectrometry with Tryptic Peptide Mixtures of Five Known Proteins. *Eur. J. Mass Spectrom.* 2005, 11, 575–580.
- (4) Termopoli, V.; Famiglini, G.; Palma, P.; Piergiovanni, M.; Cappiello, A. Atmospheric Pressure Vaporization Mechanism for Coupling a Liquid Phase with Electron Ionization Mass Spectrometry. *Anal. Chem.* 2017, 89, 2049–2056.
- (5) Cappiello, A.; Termopoli, V.; Palma, P.; Famiglini, G.; Saeed, M.; Perry, S.; Navarro, P. Liquid Chromatography–Electron Capture Negative Ionization–Tandem Mass Spectrometry Detection of Pesticides in a Commercial Formulation. *J. Am. Soc. Mass Spectrom.* 2022, 33, 141–148.
- (6) Termopoli, V.; Piergiovanni, M.; Cappiello, A.; Palma, P.; Famiglini, G. Tyrosol and Hydroxytyrosol Determination in Extra Virgin Olive Oil with Direct Liquid Electron Ionization– Tandem Mass Spectrometry. *Separations* 2021, 8, 173.
- (7) Termopoli, V.; Famiglini, G.; Vocale, P.; Morini, G. L.; Palma, P.; Rocío-Bautista, P.; Saeed, M.; Perry, S.; Cappiello, A. Microfluidic Water-Assisted Trap Focusing Method for Ultra- Large Volume Injection in Reversed-Phase Nano-Liquid

- Chromatography Coupled to Electron Ionization Tandem-Mass Spectrometry. *J. Chromatogr. A* 2020, 1627, 461421.
- (8) Vandergrift, G. W.; Monaghan, J.; Krogh, E. T.; Gill, C. G. Direct Analysis of Polyaromatic Hydrocarbons in Soil and Aqueous Samples Using Condensed Phase Membrane Introduction Tandem Mass Spectrometry with Low-Energy Liquid Electron Ionization. *Anal. Chem.* 2019, 91, 1587–1594.
- (9) Arigò, A.; Famiglini, G.; Marittimo, N.; Agostini, M.; Renzoni, C.; Palma, P.; Cappiello, A. Extractive-liquid sampling electron ionization-mass spectrometry (E-LEI-MS): a new powerful combination for direct analysis. *Sci. Rep.* 2023, 13, 6429.
- (10) Marittimo, N.; Grasselli, G.; Arigò, A.; Famiglini, G.; Agostini, M.; Renzoni, C.; Palma, P.; Cappiello, A. Liquid Electron Ionization-Mass Spectrometry as a Novel Strategy for Integrating Normal-Phase Liquid Chromatography with Low and High-Resolution Mass Spectrometry. *Analyst* 2024, 149, 2664–2670.
- (11) Marittimo, N.; Grasselli, G.; Arigò, A.; Famiglini, G.; Palma, P.; Saeed, M.; Perry, S.; Navarro, P.; Clarke, P.; Brittin, M.; Cappiello, A. Sustainable and Rapid Determination of Two Halogenated Pesticides in a Commercial Formulation by Solid Phase Microextraction and Liquid Phase Chemical Ionization Mass Spectrometry. *Separations* 2023, 10, 325.
- (12) Rocio-Bautista, P.; Famiglini, G.; Termopoli, V.; Palma, P.; Nazdrajic, E.; Pawliszyn, J.; Cappiello, A. Direct Coupling of Bio-SPME to Liquid Electron Ionization-MS/MS via a Modified Microfluidic Open Interface. *J. Am. Soc. Mass Spectrom.* 2021, 32, 262–269.
- (13) Debnath, B.; Singh, W. S.; Das, M.; Goswami, S.; Singh, M. K.; Maiti, D.; Manna, K. Role of plant alkaloids on human health: A review of biological activities. *Mate. Today Chemistry.* 2018, 9, 56-72.

- (14)Ganan, J.; Martínez-García, G.; Morante-Zarcero, S.; Pérez-Quintanilla, D.; Sierra, I. Nanomaterials-modified electrochemical sensors for sensitive determination of alkaloids: Recent trends in the application to biological, pharmaceutical and agri-food samples. *Microchem. Journal*. 2023, 184, 108136.
- (15)Kohnen-Johannsen, K.L.; Kayser, O. Tropane Alkaloids: Chemistry, Pharmacology, Biosynthesis and Production. *Mol*. 2019, 24, 796.
- (16)Chou, M.W.; Yan, J.; Nichols, J.; Xia, Q.; Beland, F.A.; Chan, P.C.; Peter, P.F. Correlation of DNA adduct formation and riddelliine-induced liver tumorigenesis in F344 rats and B6C3F1 mice. *Cancer Lett*. 2003, 193, 119–125.
- (17)Elbein, A.D.; Molyneux, R.J. Alkaloid Glycosidase Inhibitors, *Comprehensive Natural Products*. Chem. 1999, 129–160.
- (18)Song, J.; Guo, T.; Wu, W.; Liao, M.; Xiao, Q.; Qiao, B.; Wan, Y.; Dai, Q.; Zhou, Y. Simultaneous determination of five poppy husk alkaloids and three main components of acetaminophen-caffeine-aspirin tablets in hot pot broth by ultra-high-performance liquid chromatography–tandem mass spectrometry. *Sep. Sci. Plus*. 2022, 5, 616-625.
- (19)Kaczyński, P.; Łozowicka, B. A novel approach for fast and simple determination pyrrolizidine alkaloids in herbs by ultrasound-assisted dispersive solid phase extraction method coupled to liquid chromatography-tandem mass spectrometry. *JPBA*. 2020, 187, 113351.
- (20) Pereira, A.S.; Amaral, A.C.F.; Barnes, R.A.; Cardoso, J.N.; Aquino Neto, F.R. Identification of isoquinoline alkaloids in crude extracts by high temperature gas chromatography-mass spectrometry. *Phytochem. Anal*. 1999, 10, 254–258.

Chapter 7

7.1. Conclusions and future perspectives

Pesticides are largely used due to their fundamental role in increasing agricultural yield; however, their awareness is continuously increasing due to their negative impact on the environment and non-targeted organisms, including humans. Therefore, the development of selective and sensitive analytical methods is fundamental to meet the requirements. In 2017, after several attempts, a novel LC-EI-MS interface, called the LEI interface, was developed as an alternative to the conventional GC-MS and LC-MS interfaces. LEI interface permits the advantages of LC to be combined with those of EI. Despite its reliability and robustness were widely demonstrated over the years, this dissertation was focused on demonstrating the great additional capabilities of the LEI interface for targeted and untargeted analyses of pesticides, and other analytes. *Chapter 3* reported a sensitive and reliable MOI-PFS-LEI-QqQ method for the simultaneous analysis of two pesticides with different chemical/physical properties, which generally require two different instruments for their analysis and long sample preparation procedures. Each analysis was performed in about 5 min (from the extraction to the signal acquisition). In addition, the method demonstrated acceptable performance, with LOD and LOQ in the range of ng L^{-1} , and negligible ME also in absence of chromatographic separation. Due to the use of MOI, the SPME fiber was directly coupled with MS, increasing the Greenness evaluation score from 0.49 of the conventional method involving chromatographic separation to 0.67. The LEI interface was also coupled for the first time with an HR mass spectrometer, the Q-TOF, demonstrating remarkable versatility.

In *Chapter 4*, the LEI interface was successfully coupled with NPLC. The coupling of NPLC with API MS techniques is a challenge due to the non-compatibility of non-polar solvents with the ionization step. In addition, although NPLC is not the most used separation technique, it is the most suitable in specific applications, such as for isomer separation or non-polar compounds strongly retained with RPLC. The system NPLC-LEI-MS performance was tested using different model compounds and, despite the use of non-polar solvents, it demonstrated to keep unaltered the EI spectra of model compounds in both low-resolution and HRMS instrumentations. The results of this study hence demonstrated the feasibility and advantage of using MS with NPLC, enhancing both sensitivity and specificity to the UV detector commonly coupled with NPLC.

In *Chapter 5*, an innovative workflow developed for NTA, based on LC-LEI-QTOF, has been reported for the first time. Due to the reliability and high fragmentation of EI, the workflow developed allowed for the identification of unknown compounds in complex matrices also when their spectra were not recognized by the NIST library; in addition, due to the use of LEI interface, both volatile and low-volatile fractions of mixtures could be analyzed simultaneously. The workflow was successfully applied in two different applications. In the first study, it was employed to understand the provenience of CBF formulations, banned in the EU, used in a case of animal poisoning. Different formulations were characterized, and the identified characteristic compounds were searched in the animal samples. In the second work, the workflow was applied to solve a real case in a company; a non-polar impurity, called impurity Y, was successfully detected and identified in a complex mixture, called CF2.

Regarding future prospects, since the LEI interface demonstrated its robustness, the next step will focus on enhancing instrumental sensitivity. Although the interface has shown significant advantages in qualitative analyses, sensitivity remains a key limitation of LEI. To address it,

the initial approach will involve exploring alternative materials for the VMC. Another objective is to improve the user-friendliness of the interface. While the latest configuration reduced capillary clogging and simplified capillary replacement, further refinements are planned. Given its proven utility across different fields, including forensic and industrial applications, achieving these objectives will expand the applicability of the LEI interface. This advancement could establish it as an essential alternative for addressing specific analytical challenges.

Appendix 1

- I.** Publication 1
- II.** Supporting Information - Publication 1
- III.** Publication 2
- IV.** Supporting Information - Publication 2
- V.** Publication 3

Article

Sustainable and Rapid Determination of Two Halogenated Pesticides in a Commercial Formulation by Solid Phase Microextraction and Liquid Phase Chemical Ionization Mass Spectrometry

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Abstract: This work presents a sustainable and rapid method for halogenated pesticide analysis without chromatographic separation. The system is composed of a microfluidic open interface (MOI) for solid-phase microextraction (SPME) liquid phase desorption, connected to a liquid electron ionization mass spectrometry interface (LEI-MS). Either a triple quadrupole mass spectrometer (QQQ-MS/MS, (low-resolution) or a quadrupole-time-of-flight tandem MS (QTOF-MS/MS, high-resolution) were employed, each operating in negative chemical ionization (NCI) conditions. The flow rate used (100 μ L/min) to rapidly empty the MOI chamber (approximately 2.5 μ L) is reduced to the working flow rate of the LEI interface (500 nL/min) by a passive flow splitter (PFS). NCI is an appropriate ionization technique for electrophilic compounds, increasing specificity and reducing background noise. Two halogenated pesticides, dicamba and tefluthrin, were extracted simultaneously from a commercial formulation matrix (CF) using a C18 fiber by direct immersion (3 min under vortex agitation). Analyte desorption occurred in static conditions inside MOI filled with acidified acetonitrile (ACN) (0.2% phosphoric acid, PA). Extraction and desorption steps were optimized to increase efficiency and accelerate the process. No chromatographic separation was involved; therefore, the system fully exploited MS/MS selectivity and HRMS accuracy demonstrating good linearity, repeatability and limits of detection (LODs) and limits of quantification (LOQs) in the pg/mL range (50 and 500 pg/mL, respectively). Low-resolution experiments showed that matrix effects (ME) did not affect the results. The fast workflow (5 min) makes the system suitable for high-throughput analysis observing the principles of green analytical chemistry (GAC).

Keywords: liquid electron ionization (LEI); microfluidic open interface (MOI); negative chemical ionization (NCI); halogenated pesticides; mass spectrometry; solid-phase microextraction (SPME); green analytical chemistry (GAC)

1. Introduction

Green analytical chemistry (GAC) represents a source of inspiration to develop new research undertaking the challenge of reaching a good compromise between the data quality and environmental friendliness. Method optimization following GAC criteria is mandatory in modern analytical procedures [1]. Direct analyses are preferable, reducing solvent and energy use as per green chemistry requirements. In addition, the possibility of avoiding

sample preparation steps limits the risk of sample loss and reduces the analysis time. Several extraction techniques were developed to increase the green character and speed of the original sample preparation, reducing or eliminating solvent consumption. Solid phase microextraction (SPME) is a technique of choice for the extraction of a large variety of analytes, even in complex matrices, due to the affordability, quickness and low organic solvent consumption. In addition, because of its versatility of use with GC and LC, the number of molecules analyzed using SPME is increasing steadily [2–4]. Pawliszyn et al. [5] developed an innovative device named microfluidic open interface (MOI) that allows the direct coupling of SPME with a triple quadrupole MS/MS via ESI. MOI is based on the concept of a flow-isolated desorption chamber with a volume of 7 μL connected to an ionization source. Analytes are conveyed to the ionization source through self-aspiration. Recently, a modified MOI configuration in which the desorption chamber was reduced in volume to approximately 2.5 μL was proposed and coupled with an LEI-QQQ system [6].

Detecting and quantifying pesticides in complex matrices is a challenge for the scientific community, given the many laws and regulations safeguarding human health and the environment [7–9]. The most popular analytical methods for pesticide analysis are based on gas chromatography (GC) or liquid chromatography (LC), coupled with various detection methods, including MS instruments. However, considering the wide variety of pesticide classes and to accomplish the changing legislation, developing more specific and sensitive analytical methods is mandatory [10–14].

In GC-MS, electron ionization (EI), performed in standard ionization conditions (70 eV), generates a library-matchable spectra of pesticides, allowing a reliable identification [15]. However, unless derivatized, the types of molecules amenable to GC are characterized by low molecular weight, volatility and thermostability. Alternatively, several classes of pesticides are analyzed by LC-MS/MS methods, mainly in multiple reaction monitoring mode (MRM), which allows multiple residue determination and low limits of quantification (LOQs) [8]. The type of molecules analyzable and the structural information obtainable in the same analysis depend on the selected atmospheric pressure ionization (API) technique. In addition, detecting substances with different chemical/physical properties may require different instrumental approaches. Soft ionization techniques provide less informative spectra and high matrix effects (MEs) in complex sample analysis [16]. Compared to GC, LC extends MS to high molecular weight, non-volatile and thermolabile analytes. Our research group recently presented LEI, an LC-MS interface that extends to LC hard ionization techniques, such as electron ionization (EI) and chemical ionization (CI). LEI allows the simultaneous detection of polar/nonpolar and thermolabile/thermostable molecules [17]. Furthermore, because the ionization occurs under a high vacuum environment, MEs are significantly reduced [18]. The core of LEI is the vaporization microchannel (VMC), where the liquid flow from the LC is released from the inlet capillary and vaporized by the synergistic action of high temperature and constant helium flow. The hot zone is preceded by a non-heated zone, called the “cooling gap,” which helps prevent the solvent’s early vaporization, avoiding analytes’ precipitation and inlet capillary obstruction.

The LEI interface allows the analysis of low volatile and thermolabile molecules simultaneously. No compound degradation is observed because of the fast analytes’ vaporization inside the VMC, as demonstrated in several applications [17,19–22].

In a previous work [23], our research group proposed an LC-LEI-QQQ method using a reversed-phase column to detect dicamba and tefluthrin in a commercial formulation (CF). In this complex matrix, due to active ingredients and additives, and with that method, LODs and LOQs of 0.08 and 0.3 ng/mL for dicamba and 0.05 and 0.2 ng/mL for tefluthrin were obtained. The simultaneous analysis of dicamba and tefluthrin, two halogenated pesticides, is challenging because they show opposite physico-chemical properties. Indeed, dicamba is a highly polar compound usually analyzed with LC-ESI-MS, whereas a derivatization step is required for GC-MS analysis. Tefluthrin is a nonpolar compound, hence hardly ionized with ESI, and is usually detected with GC-MS [23,24]. NCI is typically used in GC-MS for ionizing compounds containing electronegative atoms, increasing the signal-to-noise ratio

(S/N) and showing better sensitivity than EI. In NCI, a buffer gas generates low-kinetic energy electrons after the impact with the 70 eV electrons coming from the filament. These thermal electrons react with the sample molecules to form negative ions [25,26]. In the previous analytical approach [23], the analyses were performed using chromatographic separations, and the CF, fortified with dicamba and tefluthrin standards, was injected after dilution, filtration and pH adjustment.

Herein, a green and fast method based on MOI-PFS-LEI coupled to QQQ (low-resolution analysis) or QTOF (high-resolution analysis) instruments in liquid-phase NCI mode for the simultaneous analysis of dicamba and tefluthrin in a CF is presented for the first time. The QQQ instrument is demonstrated to be a very sensitive and specific detector in MRM mode, even without chromatographic separation [6,27]. In this work, we also present the proof-of-concept of the MOI-PFS-LEI system in NCI mode coupled with a Q-TOF instrument in MS mode for high-resolution experiments in full scan MS mode. The high acquisition rate of Q-TOF improves deconvolution, supporting the simultaneous identification and quantification of different compounds without chromatographic separation. Both methods proved suitable for trace-level analysis of dicamba and tefluthrin in a complex matrix, showing negligible MEs and satisfactory linearity and repeatability, demonstrating accuracy, sensitivity, greenness and speed of analysis in low and high-resolution experiments.

2. Materials and Methods

2.1. Materials and Supplies

LC-MS grade acetonitrile (ACN) was purchased from VWR International, part of Avantor (Milan, Italy). Ultrapure water was obtained from a Direct-Q3 UV water purification system from Merck Millipore Co. (Milan, Italy). Methane (grade 6.0, purity 99.9%) was provided by Nippon Gases, Italy.

Phosphoric acid (PA, 85%) was purchased from Merck (Milan, Italy). Standards of dicamba and tefluthrin (purity > 99%) and CF were provided by Syngenta Ltd. (Bracknell, UK). Stock solutions of the two pesticides were prepared gravimetrically at a concentration of 2 mg/mL in ACN and stored at 4 °C. Working standard solutions of the two-pesticide mixture was prepared volumetrically at concentrations of 0.5, 2.5, 5, 25, 50 and 100 µg/mL in ACN. VWR International (Milan, Italy) provided 1.5 mL vials and slit septa screw caps for fiber insertion. SPME fibers were kindly provided by Professor Janusz Pawliszyn (University of Waterloo, Canada) and were assembled using nitinol wires (length 50 mm, diameter 200 µm) coated with a mixture of polyacrylonitrile (PAN) and C18 particles. Coating thickness and fiber length were 20 µm and 10 mm, respectively. A detailed description of the fibers' manufacturing process is reported elsewhere [27]. PEEK-coated fused silica capillaries were purchased from IDEX (Oak Harbor, WA, USA); fused silica capillaries were from Molex Polymicro (Lisle, IL, USA); flexible stainless-steel tubing was from Agilent Technologies, Inc. (Santa Clara, CA, USA). The dimensions of the capillaries are reported in Figure 1.

2.2. Standard Solution Preparation

CF stock solution was prepared by weighing 150 mg and diluting it in 30 mL of water acidified with 0.2% PA (pH > 2) to obtain a 5 mg/mL solution. The diluted CF solution was vortexed for 5 min and divided into 1 mL aliquots for low- and high-resolution experiments. For calibration experiments and ME evaluation, those aliquots were fortified with 1 µL of working standard solutions of dicamba and tefluthrin to obtain the following concentrations: 0.5, 2.5, 5, 25, 50 and 100 ng/mL. Aliquots (1 mL) of water acidified with 0.2% PA, without CF, were fortified with 1 µL of working standard solutions of dicamba and tefluthrin for calibration experiments in water. For the DI-SPME method optimization, repeatability test, and LODs and LOQs evaluation, 1 mL aliquots of diluted CF were fortified with the two pesticides at 100 ng/mL. All measurements were performed in triplicate, and the relative standard deviation was calculated. LODs and LOQs were calculated as the minimum concentration with an S/N ratio equal to or higher than 3 and 10, respectively.

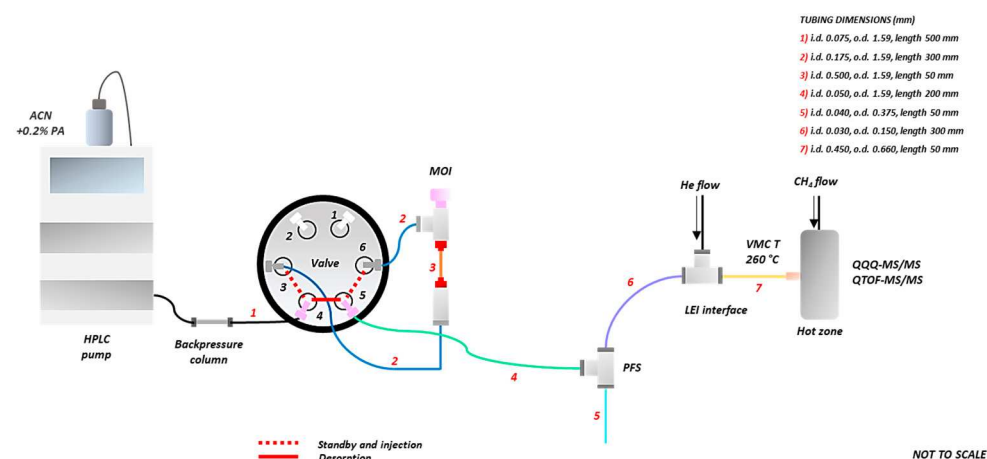


Figure 1. Scheme and operation of the MOI-PFS-LEI-QQQ and MOI-PFS-LEI-QTOF systems. Step 1: MOI is filled in the standby position. The solvent flows from the pump to port 4 of the valve at 10 $\mu\text{L}/\text{min}$, which is in line with port 3 (MOI inlet). Once the MOI chamber is filled, the liquid flows to port 6 (MOI outlet) and then to PFS through port 5. The flow is split inside the PFS: part of the flow goes to waste, and only 500 nL/min are allowed into the MS via LEI. Step 2: The valve is switched to the desorption position. The solvent flows through PFS directly to LEI and MS. The MOI chamber, filled with an organic solvent, is isolated and ready for fiber introduction and analyte desorption. Step 3: The valve is switched back to the injection position for MOI draining and MS analysis.

2.3. Instruments and Equipment

2.3.1. Microfluidic Open Interface (MOI) and Passive Flow Splitter (PFS)

MOI allows the direct coupling of SPME with LEI-QQQ and LEI-QTOF. The core of this device is represented by a desorption chamber with an internal volume of $\sim 2.5 \mu\text{L}$. The MOI chamber dimensions must be as small as possible but sufficiently large to allow fiber insertion promoting the desorption of the desired analyte. During the desorption step, which occurs in static conditions, the chamber is filled with organic solvent and the analytes can be partitioned between the fiber and the solvent. MOI is comprehensively described elsewhere [6,26]. PFS reduces the flow rate from 10 $\mu\text{L}/\text{min}$ (for fast emptying of MOI) to 500 nL/min (flow rate required for the proper LEI functioning), providing a 1:20 mobile phase split ratio [6]. PFS consists of a stainless-steel tee junction connected to a fused silica capillary (50 mm length, 40 μm i.d. and 375 μm o.d.) in which part of the flow is conveyed to waste. The length and diameter of the splitter fused silica capillary can be adjusted according to the split ratio needed. Additional information about the PFS is reported elsewhere [6].

2.3.2. MOI-PFS-LEI-QQQ and MOI-PFS-LEI-QTOF Systems

LEI interface coupled with a conventional EI-based MS can operate in EI and CI modes. A detailed description of the LEI interface is reported elsewhere [17,19]. An Agilent 1290 Infinity II binary pump (Agilent Technologies Inc., Santa Clara, CA, USA) was used to deliver ACN acidified with 0.2% PA through the system, and an Agilent Zorbax Eclipse XDB C18 backpressure column (4.6 $\times$ 150 mm, 5 μm particle size) was employed for stabilizing the flow rate, and it did not interact with the analytes at all. As reported in Figure 1, a six-port valve (Agilent G1170A 1290 Infinity valve drive and Agilent G4231B ultrahigh-pressure valve head) was used to connect the pump and column (port 4) via a 500 mm PEEK-coated fused silica capillary (75 μm i.d., 1.59 mm o.d.). Inlet and outlet MOI flexible stainless-steel capillaries (175 μm i.d., 1.59 mm o.d., 300 mm length) were connected to ports 3 and 6, respectively. PFS was connected to port 5 and MOI exit via a 200 mm PEEK-coated fused silica capillary (50 μm i.d., 1.59 mm o.d.) and to the LEI fused silica inlet capillary (300 mm length, 30 μm i.d., 150 μm o.d.). VMC and quadrupole temperatures were 260 $^{\circ}\text{C}$ and 150 $^{\circ}\text{C}$, respectively. The NCI ion source temperature was

150 °C for providing low-kinetic energy electrons [23]. Methane was introduced into the ion source at ~2 mL/min (40%) as a reagent gas to promote dicamba and tefluthrin chemical ionization. The percentage of methane (40%) was chosen according to previous work, in which it gave the most intense signal for both pesticides [23]. Dicamba and tefluthrin data acquisitions were carried out in MRM, using the following transitions and collision energies: $Q = 149 \rightarrow 105$ (10 eV) and $q = 184 \rightarrow 104$ (5 eV) for dicamba, $Q = 241 \rightarrow 205$ (10 eV) and $q = 243 \rightarrow 205$ (10 eV) for tefluthrin. The setup described above was also employed for high-resolution experiments using a Q-TOF Agilent 7250 MS (Agilent Technologies Inc., Santa Clara, CA, USA). Dicamba and tefluthrin data acquisitions were carried out in full scan. Acquisition range and data extraction windows were set from m/z 80 to m/z 500 and 25 ppm, respectively. Q-TOF mass calibration was performed after each analysis. During calibration, no mobile phase was admitted into the ion source.

2.4. Direct Immersion-SPME Method Optimization

SPME was used in direct immersion mode (DI-SPME) for dicamba and tefluthrin sampling in CF and ultra-pure water. Several parameters were optimized for improving extraction and desorption efficiency and process speeding, such as the amount of organic solvent in the sample, sampling and desorption time, and agitation methods. Since it is independent of the MS technique, DI-SPME method optimization was carried out with the low-resolution instrumentation (MOI-PFS-LEI-QQQ), using 1 mL aliquots of CF and water fortified with 100 ng/mL of dicamba and tefluthrin. The optimized parameters were selected considering the highest integrated peak area values of the most intense transitions (Q). The DI-SPME optimized procedure was then applied to high-resolution experiments. Before use, the SPME fiber was preconditioned in H₂O/ACN (50/50, v/v) for 10 min using a magnetic stir bar. Sampling was conducted by completely immersing the fiber in 1 mL of the sample. Before desorption, a 5-s rinsing step in water was performed using a vortex to clean the fiber from any matrix components adhering to the coating surface. Desorption was performed by inserting the fiber inside the MOI chamber, filled with ACN acidified with 0.2% PA.

3. Results and Discussion

3.1. Sampling and Desorption Steps

In this work, four parameters that play a significant role in DI-SPME were optimized in CF and water: percentage of organic modifier in the sample, extraction and desorption time and agitation method. According to the literature [28], the amount of organic solvent in the samples should be kept very low because it may alter DI-SPME extraction efficiency. However, the concentration of organic solvent cannot be zero because of the spiking process with ACN standard solutions. It is worth pointing out that, in some cases, a certain percentage of organic modifier can positively affect the extraction yield [28–31]. ACN was selected because it is the best performing solvent used with LEI. The percentages of ACN in the sample tested were: 0.1, 0.25, 1.25, 2.25, 5 and 10%. Using a C18 fiber, it was observed that extraction efficiencies were higher at low percentages of ACN for both compounds and matrices, as shown in the graphics in Figure S1A–D.

Concerning extraction time, several factors, such as molecular size, coating thickness and distribution constant (K), can affect the time required to reach equilibrium [32]. Different extraction times (15, 20, 25 and 30 min) using a magnetic stir bar as an agitation method were initially considered. The differences in area values for dicamba and tefluthrin in CF and water are reported in Figure S2A,B, which shows that the highest response was obtained at 25 and 30 min for both matrices.

The use of vortex was also tested as an alternative to magnetic stir bar agitation to reduce the extraction time whilst maintaining the same efficiency. According to Pawliszyn and co-workers [33], vibration reduces sampling time, ensuring the same extraction efficiency and good repeatability. Different extraction times were evaluated: 1, 2, 3 and 4 min. As demonstrated in Figure 2A–D, the equilibrium for dicamba and tefluthrin in CF and

water was achieved after 3 min, after which the signal remained constant with area values comparable to the ones obtained with the magnetic stir bar. Hence, 3 min of vortex agitation was chosen as the extraction time step.

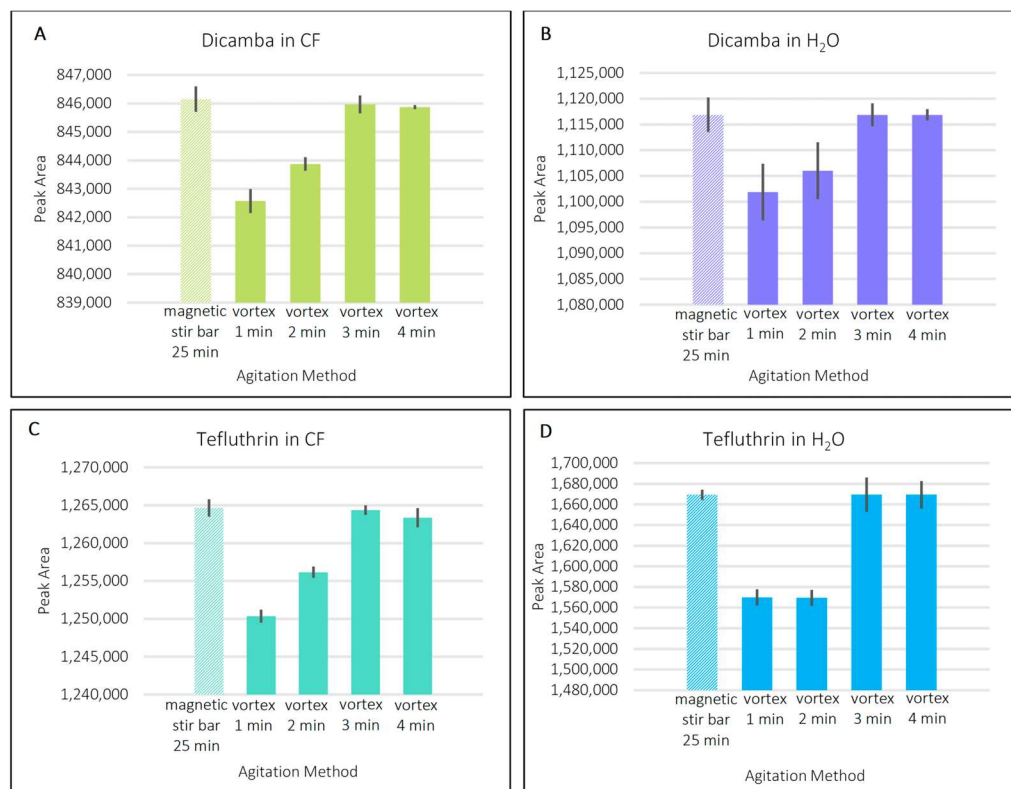


Figure 2. Effects of different extraction times on the integrated peaks area values of dicamba in (A) CF and (B) H₂O and tefluthrin in (C) CF and (D) H₂O using vortex as agitation method compared with magnetic stir bar. Analytes concentration: 100 ng/mL.

Desorption occurs in static conditions by inserting the SPME fiber inside the MOI chamber filled with ACN acidified with 0.2% PA. The static desorption efficiency is determined by desorption time. The following desorption times were considered: 30 s, 1, 2 and 3 min. Figure S3A,B shows the effects of different desorption times on peak areas in both matrices; 1 min was selected as the optimal desorption time.

To summarize, the optimized DI-SPME method consists of stirring the fiber for 3 min with vortex, using a sample solution with 0.1% ACN, and desorbing the fiber for 1 min in the MOI chamber filled with ACN acidified with 0.2% PA. The DI-SPME workflow is shown in Figure 3.

3.2. Low-Resolution Experiments: MOI-PFS-LEI-QQQ

Method validation was performed by evaluating the intraday and interday repeatability, limits of detection (LOD) and quantification (LOQ) and linearity range of dicamba and tefluthrin in CF, as reported in Table 1. The integrated peak area values of the more intense transitions (Q) were considered for repeatability tests and calibration curves. The least intense transitions were used (q) for LOD and LOQ calculations. Ten consecutive analyses of 1 mL of CF fortified with dicamba and tefluthrin at 100 ng/mL were performed to establish the intraday precision, whereas the interday repeatability was assessed by performing five analyses for five consecutive days. Regarding intraday measurements, the RSD% values for dicamba and tefluthrin in CF were 20% and 8%, respectively, showing good intraday repeatability. RSD% values of 21% and 12% for dicamba and tefluthrin were obtained for the interday repeatability test. LODs and LOQs were calculated in CF: both dicamba and tefluthrin showed LODs of 0.05 ng/mL and LOQs of 0.5 ng/mL, confirming

the limits obtained in previous work with LC-LEI-MS/MS [23] and comparable to those reported in the literature [34–41]. Dicamba and tefluthrin calibration curves showed good linearity in CF with an R^2 of 0.9925 and 0.9958, respectively.

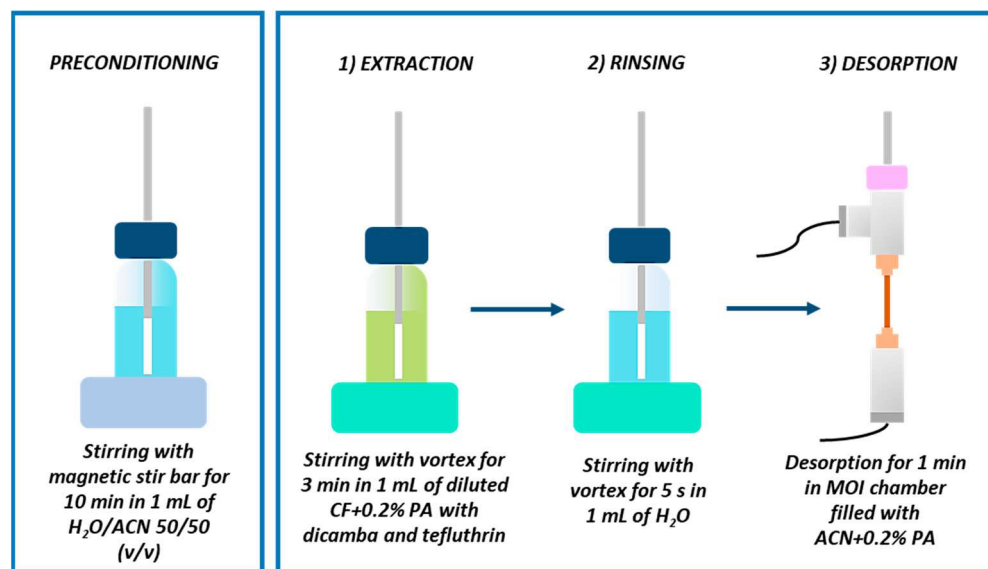


Figure 3. DI-SPME optimized workflow. The procedure consists of (1) extraction, (2) rinsing and (3) desorption. The C18 fiber was preconditioned before use.

Table 1. Method validation data for dicamba and tefluthrin in CF obtained with the MOI-PFS-LEI-QQQ system. Intraday and interday tests were conducted using 100 ng/mL solutions.

Compound	Matrix	Linearity Range (ng/mL)	Levels	R^2	LOD (ng/mL)	LOQ (ng/mL)	RSD% Intraday	RSD% Interday
Dicamba	CF	0.5–100	6	0.9925	0.05	0.5	20%	21%
Tefluthrin	CF	0.5–100	6	0.9958	0.05	0.5	8%	12%

Matrix Effects (ME) Evaluation

Ion suppression and signal enhancement are the major drawbacks affecting the analytical performance when matrix components compete with the analytes of interest during the ionization process. Different methods can be exploited to calculate MEs [42–44]. MEs evaluation was performed by comparing the slopes of calibration curves for dicamba and tefluthrin analyzed in CF and water (Figure S4A,B) using the following formula:

$$ME (\%) = \frac{\text{Slope CF}}{\text{Slope } H_2O} \times 100$$

A result of 100% indicates no MEs. The results for dicamba and tefluthrin were 76.80% and 79.09%, respectively (with a variance of 23.2% and 20.91%). The suppression effect indicated by the percentages obtained for the two compounds is limited and acceptable, demonstrating the system’s suitability for trace-level analysis in a complex matrix.

3.3. High-Resolution Experiments: MOI-PFS-LEI-QTOF

An additional novel aspect of this work is based on coupling MOI-PFS-LEI with a QTOF-MS/MS system using NCI. Preliminary experiments were dedicated to acquiring the high-resolution mass spectra of dicamba and tefluthrin using the optimized DI-SPME procedure on different solutions of the two compounds at 100 ng/mL in water. The full scan mass spectra and NCI fragmentation pathways are shown in Figure 4A,B. The results are consistent with those reported in the literature [23], demonstrating the applicability

of the system and the potential advantages of using HRMS to provide accurate masses in complex matrices, combined with NCI, which ionizes exclusively electrophilic compounds, thereby reducing the background noise.

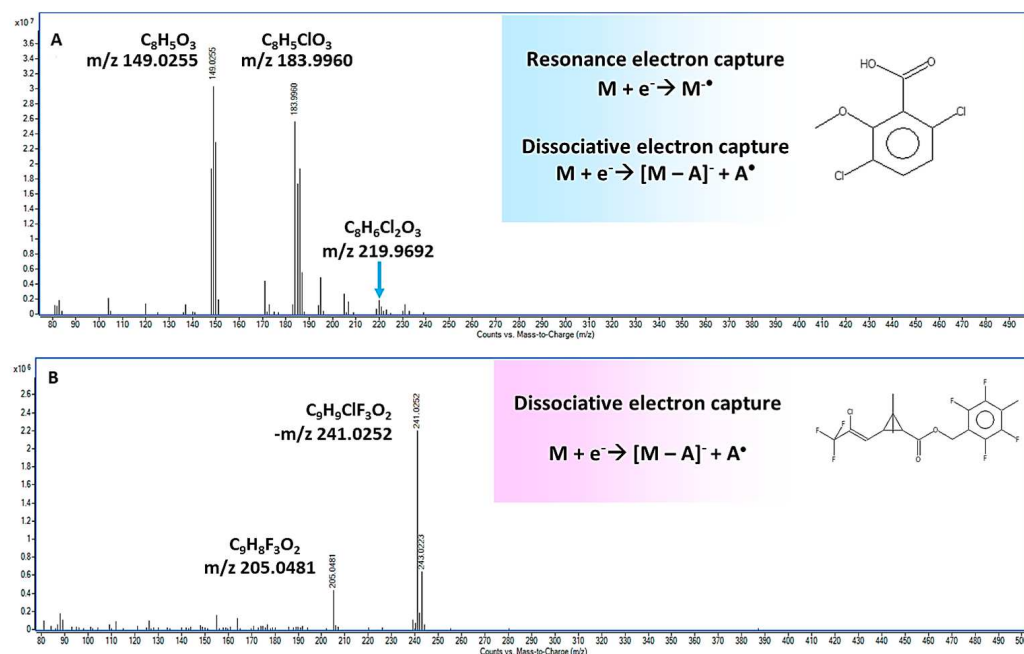


Figure 4. High-resolution mass spectra and NCI fragmentation pathways of (A) dicamba and (B) tefluthrin at 100 ng/mL in H₂O recorded with the MOI-PFS-LEI-QTOF-MS/MS system.

The performance of the system was evaluated considering intraday repeatability and assessing by performing 10 consecutive analyses of 1 mL aliquots of CF fortified with dicamba and tefluthrin at 100 ng/mL, LODs, LOQs and linearity. The integrated peak area values of the most intense extracted ions (EIC) were considered for repeatability and calibration curves (m/z 149.0255 for dicamba and m/z 241.0252 for tefluthrin). Considering the sample complexity and the absence of chromatographic separation, two exact masses for each compound have been considered: The least intense ions were used for LODs and LOQs calculations (m/z 183.9960 for dicamba; m/z 205.0481 for tefluthrin) (Figure 5).

As shown in Table 2, the RSD% values of intraday precision for dicamba and tefluthrin in CF were 26% and 24%, respectively, slightly higher than the results obtained with low-resolution experiments. LODs and LOQs were 0.05 ng/mL and 0.5 ng/mL for both compounds, equal to the limits obtained in full scan mode with MOI-PFS-QQQ. Dicamba and tefluthrin showed sufficient linearity in CF, with an R^2 of 0.9752 for dicamba and an R^2 of 0.9397 for tefluthrin. The R^2 values are slightly lower than those obtained in low-resolution. However, it must be considered that no chromatographic separation was involved, and, unlike the low-resolution, the analyses were performed in scan mode.

Table 2. Method validation data for dicamba and tefluthrin in CF obtained with the MOI-PFS-LEI-QTOF system. Intraday and interday tests were conducted using 100 ng/mL solutions.

Compound	Matrix	Linearity Range (ng/mL)	Levels	R^2	LOD (ng/mL)	LOQ (ng/mL)	RSD% Intraday
Dicamba	CF	0.5–100	6	0.9752	0.05	0.5	26%
Tefluthrin	CF	0.5–100	6	0.9397	0.05	0.5	24%

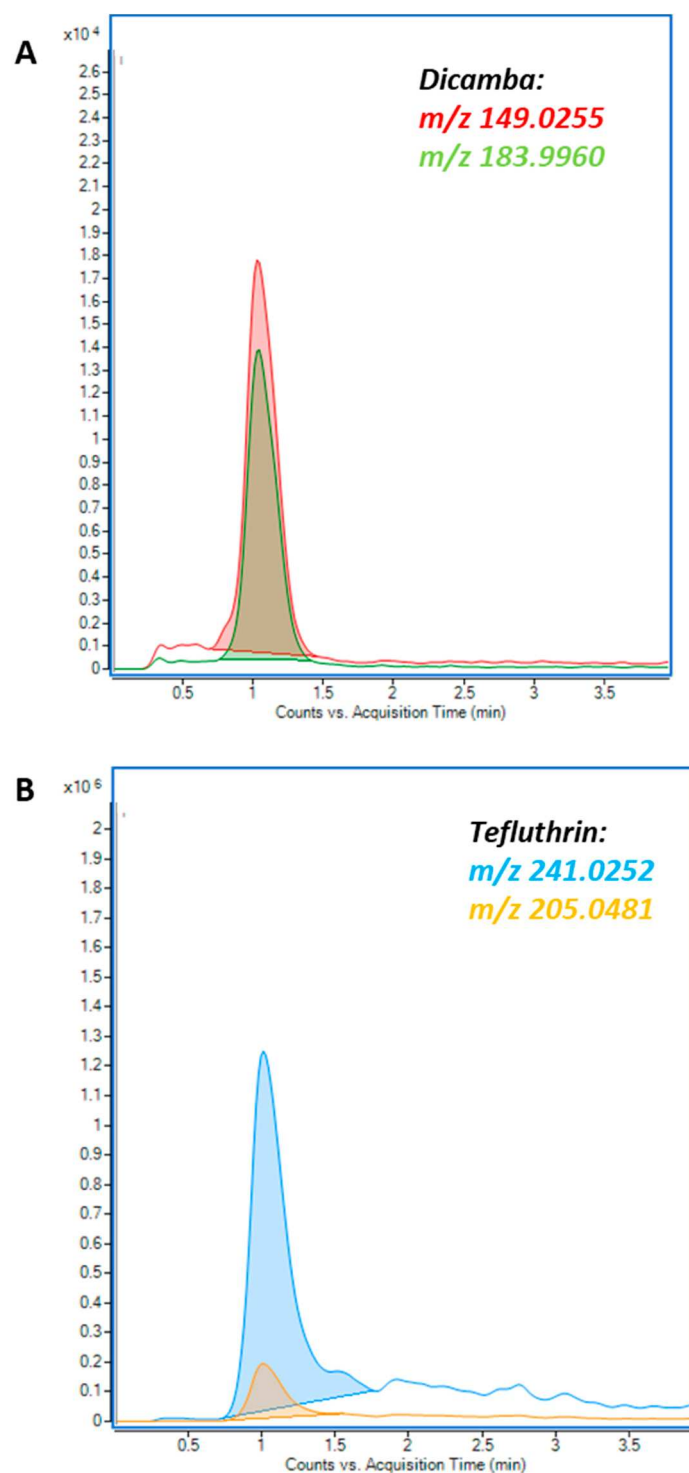


Figure 5. EIC chromatogram of dicamba (A) and tefluthrin (B) at 100 ng/mL in CF obtained with the MOI-PFS-LEI-QTOF system in NCI. Dicamba peaks: m/z 149.0255 (red), m/z 183.9960 (green). Tefluthrin peaks: m/z 241.0252 (blue), m/z 205.0481 (orange). Since chromatography has not been used, the signal obtained consists of a single peak from which the ions of the two compounds with accurate mass can be extracted and distinguished.

3.4. Greenness Evaluation

According to Sajid and Płotka-Wasyłka [45], different methods such as the Analytical GREENness Metric Approach (AGREE) [46], the Green Analytical Procedure Index (GAPI) [47] and the Analytical Eco-Scale [48] can be used to evaluate the environmental

friendliness of analytical procedures. In this work, AGREE was selected because of its effectiveness and simplicity. AGREE consists in free downloadable software that offers instant visual feedback on the eco-sustainability of the method, evaluating the 12 fundamental principles of GAC. The result is a circular pictogram with an outer crown of 12 areas colored in a range between green and red according to the given answers. The final score can vary between 0 and 1 (1 corresponds to a high ecological method footprint), and the sustainability of the overall method can be deduced from the color of the circle and the number reported within it. Figure 6A,B shows the AGREE comparison between the LC-LEI-QQQ method (score 0.49) for the simultaneous detection of dicamba and tefluthrin in CF presented in our previous work [23] and the MOI-PFS-LEI-QQQ procedure (score 0.67) herein proposed. Both methods have two limitations: They do not provide the possibility of performing in situ analyses (principle 3) and do not use reagents from renewable sources (point 10).

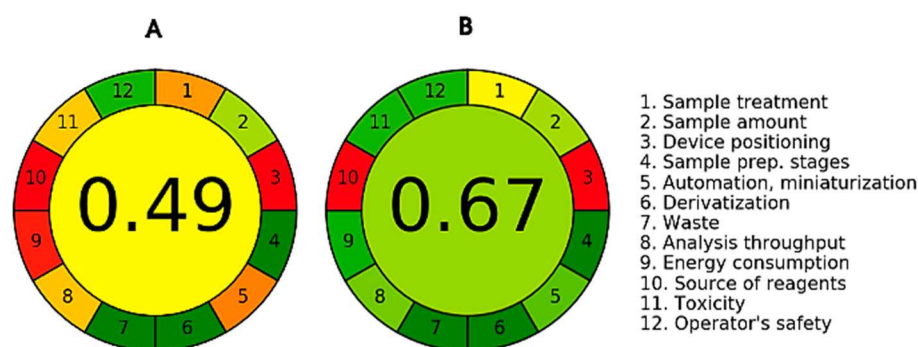


Figure 6. AGREE comparison between LC-LEI-QQQ (A) and MOI-PFS-LEI-QQQ (B) methods for detecting dicamba and tefluthrin in CF.

The method involving chromatographic separation and sample pretreatment showed a lower score. Regarding principle 1, using MOI and SPME allowed sampling and sample preparation to integrate efficiently with the direct introduction to MS [4]. A higher score is attributed to using SPME, which reduces toxic solvents, reagents and energy consumption (principle 5). According to principle 8, another drawback of the previous method lies in the low sample throughput with two analytes determined in a single chromatographic analysis of 20 min and three analyses/hour). The proposed approach allows a higher sample throughput avoiding chromatographic separations and fully exploiting MS/MS selectivity. The procedure takes approximately 5 min; two compounds are detected in a single analysis; and sample throughput is 12 analyses/hour. The LC-MS method is penalized by the total power consumption (kWh), which is high considering the 20 min analysis time. This work significantly reduced energy consumption due to the overall procedure speed (principle 9). Concerning principle 11, in both methods, toxic reagents were employed, but in the LC-MS one, the amount is higher considering that the HPLC flow rate was set at 100 $\mu\text{L}/\text{min}$ (2 mL of organic solvent/analysis), whereas in the proposed method, minimal solvent quantities are employed (the flow rate 0.01 mL/min and only 0.05 mL in 5 min were used).

The AGREE evaluation of the MOI-PFS-LEI-Q-TOF gave the same score obtained with the QQQ system (0.67), demonstrating once again that the proposed approach is in accordance with the principles of green chemistry.

4. Conclusions

Rapid, reliable and green methods for the determination of halogenated pesticides in complex matrices are required to meet current law regulations. Novel approaches should be investigated to facilitate sample preparation and fast throughput analysis. This work exploits for the first time an SPME/MOI combination coupled with low- and high-resolution MS equipped with an LEI interface for the extraction of two halogenated pesticides from a commercial formulation. Overall, the entire procedure (sample extraction, desorption and analysis) takes approximately five minutes and does not involve a chromatographic

separation. When the lack of chromatography results in the loss of specificity, this limitation can be overcome by working either in MRM mode (low-resolution) or in HRMS in the analysis of particularly complex samples, as demonstrated in this work.

Future developments will be dedicated, such as investigating the use of more sustainable and non-toxic solvents to enhance the green character of this approach.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/separations10060325/s1>, Sustainable and Rapid Determination of Two Halogenated Pesticides in a Commercial Formulation by Solid Phase Microextraction and Liquid Phase Chemical Ionization Mass Spectrometry. Figure S1, A–D Peak area values at different percentages of ACN in (A) dicamba in CF, (B) dicamba in H₂O, (C) tefluthrin in CF, (D) tefluthrin in H₂O. Analytes concentration: 100 ng/mL; Figure S2, A,B Effects of different extraction times on the integrated peaks area values of dicamba and tefluthrin at 100 ng/mL using magnetic stir bar as agitation method in (A) CF and (B) H₂O; Figure S3, A,B Effects of different desorption times on the integrated peaks area values of dicamba and tefluthrin at 100 ng/mL using magnetic stir bar as agitation method in (A) CF and (B) H₂O; Figure S4, A,B Calibration curves (0.5, 2.5, 5, 25, 50, and 100 ng/mL) of (A) dicamba and (B) tefluthrin in CF and H₂O.

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References

1. Anastas, P.; Eghbali, N. Green Chemistry: Principles and Practice. *Chem. Soc. Rev.* **2010**, *39*, 301–312. [\[CrossRef\]](#) [\[PubMed\]](#)
2. Byliński, H.; Gębicki, J.; Dymerski, T.; Namieśnik, J. Direct Analysis of Samples of Various Origin and Composition Using Specific Types of Mass Spectrometry. *Crit. Rev. Anal. Chem.* **2017**, *47*, 340–358. [\[CrossRef\]](#) [\[PubMed\]](#)
3. Arthur, C.L.; Pawliszyn, J. Solid Phase Microextraction with Thermal Desorption Using Fused Silica Optical Fibers. *Anal. Chem.* **1990**, *62*, 2145–2148. [\[CrossRef\]](#)
4. Kissoudi, M.; Samanidou, V. Recent Advances in Applications of Ionic Liquids in Miniaturized Microextraction Techniques. *Molecules* **2018**, *23*, 1437. [\[CrossRef\]](#) [\[PubMed\]](#)
5. Tascon, M.; Alam, M.N.; Gómez-Ríos, G.A.; Pawliszyn, J. Development of a Microfluidic Open Interface with Flow Isolated Desorption Volume for the Direct Coupling of SPME Devices to Mass Spectrometry. *Anal. Chem.* **2018**, *90*, 2631–2638. [\[CrossRef\]](#) [\[PubMed\]](#)
6. Marittimo, N.; Famigliini, G.; Palma, P.; Arigò, A.; Cappiello, A. Enhanced Microfluidic Open Interface for the Direct Coupling of Solid Phase Microextraction with Liquid Electron Ionization-Tandem Mass Spectrometry. *J. Chromatogr. A* **2022**, *1681*, 463479. [\[CrossRef\]](#)
7. Cervera, M.I.; Medina, C.; Portolés, T.; Pitarch, E.; Beltrán, J.; Serrahima, E.; Pineda, L.; Muñoz, G.; Centrich, F.; Hernández, F. Multi-Residue Determination of 130 Multiclass Pesticides in Fruits and Vegetables by Gas Chromatography Coupled to Triple Quadrupole Tandem Mass Spectrometry. *Anal. Bioanal. Chem.* **2010**, *397*, 2873–2891. [\[CrossRef\]](#)
8. Paíga, P.; Sousa, S.; Vera, J.; Bitencourt, L.; Vieira, J.; Jorge, S.; Silva, J.G.; Correia, M.; Domingues, V.F.; Delerue-Matos, C. Multi-Residue Analysis of Fifty Pesticides in River Waters and in Wastewaters. *Environ. Sci. Pollut. Res.* **2021**, *28*, 66787–66803. [\[CrossRef\]](#)
9. European Commission. *EU Policy for a Sustainable Use of Pesticides. The Story Behind the Strategy*; European Commission: Brussels, Belgium, 2007; ISBN 92-79-03221-6.

10. Parra-Arroyo, L.; González-González, R.B.; Castillo-Zacarias, C.; Melchor Martínez, E.M.; Sosa-Hernández, J.E.; Bilal, M.; Iqbal, H.M.N.; Barceló, D.; Parra-Saldívar, R. Highly Hazardous Pesticides and Related Pollutants: Toxicological, Regulatory, and Analytical Aspects. *Sci. Total Environ.* **2022**, *807*, 18–26. [\[CrossRef\]](#)
11. Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on Maximum Residue Levels of Pesticides in or on Food and Feed of Plant and Animal Origin and Amending Council Directive 91/414/EEC Text with EEA Relevance. OJ L 70, 16.3.2005, p. 1–16. Available online: <http://data.europa.eu/eli/reg/2005/396/oj> (accessed on 18 May 2023).
12. Li, C.; Begum, A.; Xue, J. Analytical Methods to Analyze Pesticides and Herbicides. *Water Environ. Res.* **2020**, *92*, 1770–1785. [\[CrossRef\]](#)
13. Maragou, N.C.; Balayiannis, G.; Karanasios, E.; Markellou, E.; Liapis, K. Targeted Multiresidue Method for the Analysis of Different Classes of Pesticides in Agro-Food Industrial Sludge by Liquid Chromatography Tandem Mass Spectrometry. *Molecules* **2021**, *26*, 6888. [\[CrossRef\]](#)
14. Al-Nasir, F.M.; Jiries, A.G.; Al-Rabadi, G.J.; Alu'datt, M.H.; Tranchant, C.C.; Al-Dalain, S.A.; Alrabadi, N.; Madanat, O.Y.; Rasha, S.; Al-Dmour, R.S. Determination of pesticide residues in selected citrus fruits and vegetables cultivated in the Jordan Valley. *LWT Food Sci. Technol.* **2020**, *123*, 109005. [\[CrossRef\]](#)
15. Gross, J.H. *Mass Spectrometry. A Text Book*, 3rd ed.; Springer: Cham, Switzerland, 2017; ISBN 978-3-319-54397-0. [\[CrossRef\]](#)
16. Trufelli, H.; Palma, P.; Famiglini, G.; Cappiello, A. An Overview of Matrix Effects in Liquid Chromatography-Mass Spectrometry. *Mass Spectrom. Rev.* **2011**, *30*, 491–509. [\[CrossRef\]](#) [\[PubMed\]](#)
17. Termopoli, V.; Famiglini, G.; Palma, P.; Piergiovanni, M.; Cappiello, A. Atmospheric Pressure Vaporization Mechanism for Coupling a Liquid Phase with Electron Ionization Mass Spectrometry. *Anal. Chem.* **2017**, *89*, 2049–2056. [\[CrossRef\]](#) [\[PubMed\]](#)
18. Famiglini, G.; Palma, P.; Termopoli, V.; Cappiello, A. The History of Electron Ionization in LC-MS, from the Early Days to Modern Technologies: A Review. *Anal. Chim. Acta* **2021**, *1167*, 338350. [\[CrossRef\]](#)
19. Termopoli, V.; Famiglini, G.; Palma, P.; Piergiovanni, M.; Rocio-Bautista, P.; Ottaviani, M.F.; Cappiello, A.; Saeed, M.; Perry, S. Evaluation of a liquid electron ionization liquid chromatography–mass spectrometry interface. *J. Chromatogr. A* **2019**, *1591*, 120–130. [\[CrossRef\]](#)
20. Termopoli, V.; Torrisi, E.; Famiglini, G.; Palma, P.; Zappia, G.; Cappiello, A.; Vandergrift, G.W.; Zvekcic, M.; Krogh, E.T.; Gill, C.G. Mass Spectrometry Based Approach for Organic Synthesis Monitoring. *Anal. Chem.* **2019**, *91*, 11916–11922. [\[CrossRef\]](#)
21. Termopoli, V.; Famiglini, G.; Vocale, P.; Morini, G.L.; Palma, P.; Rocio-Bautista, P.; Saeed, M.; Perry, S.; Cappiello, A. Microfluidic water-assisted trap focusing method for ultra-large volume injection in reversed-phase nano-liquid chromatography coupled to electron ionization tandem-mass spectrometry. *J. Chromatogr. A* **2020**, *1627*, 461421. [\[CrossRef\]](#)
22. Termopoli, V.; Piergiovanni, M.; Cappiello, A.; Palma, P.; Famiglini, G. Tyrosol and Hydroxytyrosol Determination in Extra Virgin Olive Oil with Direct Liquid Electron Ionization–Tandem Mass Spectrometry. *Separations* **2021**, *8*, 173. [\[CrossRef\]](#)
23. Cappiello, A.; Termopoli, V.; Palma, P.; Famiglini, G.; Saeed, M.; Perry, S.; Navarro, P. Liquid Chromatography–Electron Capture Negative Ionization–Tandem Mass Spectrometry Detection of Pesticides in a Commercial Formulation. *J. Am. Soc. Mass Spectrom.* **2022**, *33*, 141–148. [\[CrossRef\]](#)
24. Shin, E.H.; Choi, J.H.; Abd El-Aty, A.M.; Khay, S.; Kim, S.J.; Im, M.H.; Kwon, C.H.; Shim, J.H. Simultaneous Determination of Three Acidic Herbicide Residues in Food Crops Using HPLC and Confirmation via LC-MS/MS. *Biomed. Chromatogr.* **2011**, *25*, 124–135. [\[CrossRef\]](#)
25. Dougherty, R.C. Negative Chemical Ionization Mass Spectrometry. *Anal. Chem.* **1981**, *53*, 625–636. [\[CrossRef\]](#)
26. Marsol-Vall, A.; Ainsa, S.; Lopez, R.; Ferreira, V. Development and Validation of a Method for the Analysis of Halophenols and Haloanisoles in Cork Bark Macerates by Stir Bar Sorptive Extraction Heart-Cutting Two-Dimensional Gas Chromatography Negative Chemical Ionization Mass Spectrometry. *J. Chromatogr. A* **2022**, *1673*, 463186. [\[CrossRef\]](#) [\[PubMed\]](#)
27. Rocio-Bautista, P.; Famiglini, G.; Termopoli, V.; Palma, P.; Nazdrajić, E.; Pawliszyn, J.; Cappiello, A. Direct Coupling of Bio-SPME to Liquid Electron Ionization-MS/MS via a Modified Microfluidic Open Interface. *J. Am. Soc. Mass Spectrom.* **2021**, *32*, 262–269. [\[CrossRef\]](#) [\[PubMed\]](#)
28. Gionfriddo, E.; Gruszecka, D.; Li, X.; Pawliszyn, J. Direct-Immersion SPME in Soy Milk for Pesticide Analysis at Trace Levels by Means of a Matrix-Compatible Coating. *Talanta* **2020**, *211*, 120746. [\[CrossRef\]](#) [\[PubMed\]](#)
29. Zhang, Z.; Yang, M.J.; Pawliszyn, J. Solid-Phase Microextraction. A Solvent-Free Alternative for Sample Preparation. *Anal. Chem.* **1994**, *66*, 844A–853A. [\[CrossRef\]](#)
30. Vuckovic, D.; Shirey, R.; Chen, Y.; Sidisky, L.; Aurand, C.; Stenerson, K.; Pawliszyn, J. In Vitro Evaluation of New Biocompatible Coatings for Solid-Phase Microextraction: Implications for Drug Analysis and in Vivo Sampling Applications. *Anal. Chim. Acta* **2009**, *638*, 175–185. [\[CrossRef\]](#)
31. Pawliszyn, J. *Handbook of Solid Phase Microextraction*; Elsevier: Amsterdam, The Netherlands, 2012; ISBN 9780124160170. [\[CrossRef\]](#)
32. Górecki, T.; Yu, X.; Pawliszyn, J. Theory of Analyte Extraction by Selected Porous Polymer SPME Fibres. *Analyst* **1999**, *124*, 643–649. [\[CrossRef\]](#)
33. Eisert, R.; Pawliszyn, J. Design of Automated Solid-Phase Microextraction for Trace Analysis of Organic Compounds in Aqueous Samples. *J. Chromatogr. A* **1997**, *776*, 293–303. [\[CrossRef\]](#)
34. Guo, H.; Riter, L.S.; Wujcik, C.E.; Armstrong, D.W. Talanta Quantitative Analysis of Dicamba Residues in Raw Agricultural Commodities with the Use of Ion-Pairing Reagents in LC–ESI–MS/MS. *Talanta* **2016**, *149*, 103–109. [\[CrossRef\]](#)

35. Ccancapa-Cartagena, A.; Masiá, A.; Picó, Y. Simultaneous Determination of Pyrethroids and Pyrethrins by Dispersive Liquid-Liquid Microextraction and Liquid Chromatography Triple Quadrupole Mass Spectrometry in Environmental Samples. *Anal. Bioanal. Chem.* **2017**, *409*, 4787–4799. [[CrossRef](#)] [[PubMed](#)]
36. Koesukwiwat, U.; Sanguankaew, K.; Leepipatpiboon, N. Rapid Determination of Phenoxy Acid Residues in Rice by Modified QuEChERS Extraction and Liquid Chromatography-Tandem Mass Spectrometry. *Anal. Chim. Acta* **2008**, *626*, 10–20. [[CrossRef](#)]
37. McManus, S.L.; Moloney, M.; Richards, K.G.; Coxon, C.E.; Danaher, M. Determination and Occurrence of Phenoxyacetic Acid Herbicides and Their Transformation Products in Groundwater Using Ultra High Performance Liquid Chromatography Coupled to Tandem Mass Spectrometry. *Molecules* **2014**, *19*, 20627–20649. [[CrossRef](#)] [[PubMed](#)]
38. Xiong, W.; Tao, X.; Pang, S.; Yang, X.; Tang, G.L.; Bian, Z. Separation and Quantitation of Three Acidic Herbicide Residues in Tobacco and Soil by Dispersive Solid-Phase Extraction and UPLC-MS/MS. *J. Chromatogr. Sci.* **2014**, *52*, 1326–1331. [[CrossRef](#)] [[PubMed](#)]
39. Shen, C.Y.; Cao, X.W.; Shen, W.J.; Jiang, Y.; Zhao, Z.Y.; Wu, B.; Yu, K.Y.; Liu, H.; Lian, H.Z. Determination of 17 Pyrethroid Residues in Troublesome Matrices by Gas Chromatography/Mass Spectrometry with Negative Chemical Ionization. *Talanta* **2011**, *84*, 141–147. [[CrossRef](#)]
40. Besil, N.; Uclés, S.; Mezcuía, M.; Heinzen, H.; Fernández-Alba, A.R. Negative Chemical Ionization Gas Chromatography Coupled to Hybrid Quadrupole Time-of-Flight Mass Spectrometry and Automated Accurate Mass Data Processing for Determination of Pesticides in Fruit and Vegetables. *Anal. Bioanal. Chem.* **2015**, *407*, 6327–6343. [[CrossRef](#)]
41. Belmonte Valles, N.; Retamal, M.; Mezcuía, M.; Fernández-Alba, A.R. A Sensitive and Selective Method for the Determination of Selected Pesticides in Fruit by Gas Chromatography/Mass Spectrometry with Negative Chemical Ionization. *J. Chromatogr. A* **2012**, *1264*, 110–116. [[CrossRef](#)]
42. Gosetti, F.; Mazzucco, E.; Zampieri, D.; Gennaro, M.C. Signal Suppression/Enhancement in High-Performance Liquid Chromatography Tandem Mass Spectrometry. *J. Chromatogr. A* **2010**, *1217*, 3929–3937. [[CrossRef](#)]
43. Zhou, W.; Yang, S.; Wang, P.G. Matrix Effects and Application of Matrix Effect Factor. *Bioanalysis* **2017**, *9*, 1839–1844. [[CrossRef](#)] [[PubMed](#)]
44. Bruce-Vanderpuije, P.; Megson, D.; Ryu, S.H.; Choi, G.H.; Park, S.W.; Kim, B.S.; Kim, J.H.; Lee, H.S. A Comparison of the Effectiveness of QuEChERS, FaPEX and a Modified QuEChERS Method on the Determination of Organochlorine Pesticides in Ginseng. *PLoS ONE* **2021**, *16*, e0246108. [[CrossRef](#)]
45. Sajid, M.; Plotka-Wasyłka, J. Green Analytical Chemistry Metrics: A Review. *Talanta* **2022**, *238*, 123046. [[CrossRef](#)] [[PubMed](#)]
46. Gomes, J.V.D.; Tosta, C.L.; da Cunha Neto, Á.; Fagg, C.W.; Silva, C.A.G.; Gomes-Copeland, K.K.P.; Magalhães, P.O.; Fonseca-Bazzo, Y.M.; Jamal, C.M.; Silveira, D. Chemical Profile and Biological Activity of *Crinum americanum* L. (Amaryllidaceae). *S. Afr. J. Bot.* **2022**, *146*, 25–35. [[CrossRef](#)]
47. Plotka-Wasyłka, J. A New Tool for the Evaluation of the Analytical Procedure: Green Analytical Procedure Index. *Talanta* **2018**, *181*, 204–209. [[CrossRef](#)] [[PubMed](#)]
48. Gałuszka, A.; Migaszkowski, Z.M.; Konieczka, P.; Namieśnik, J. Analytical Eco-Scale for Assessing the Greenness of Analytical Procedures. *TrAC Trends Anal. Chem.* **2012**, *37*, 61–72. [[CrossRef](#)]

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Supplementary materials

Sustainable and Rapid Determination of Two Halogenated Pesticides in a Commercial Formulation by Solid Phase Microextraction and Liquid Phase Chemical Ionization Mass Spectrometry

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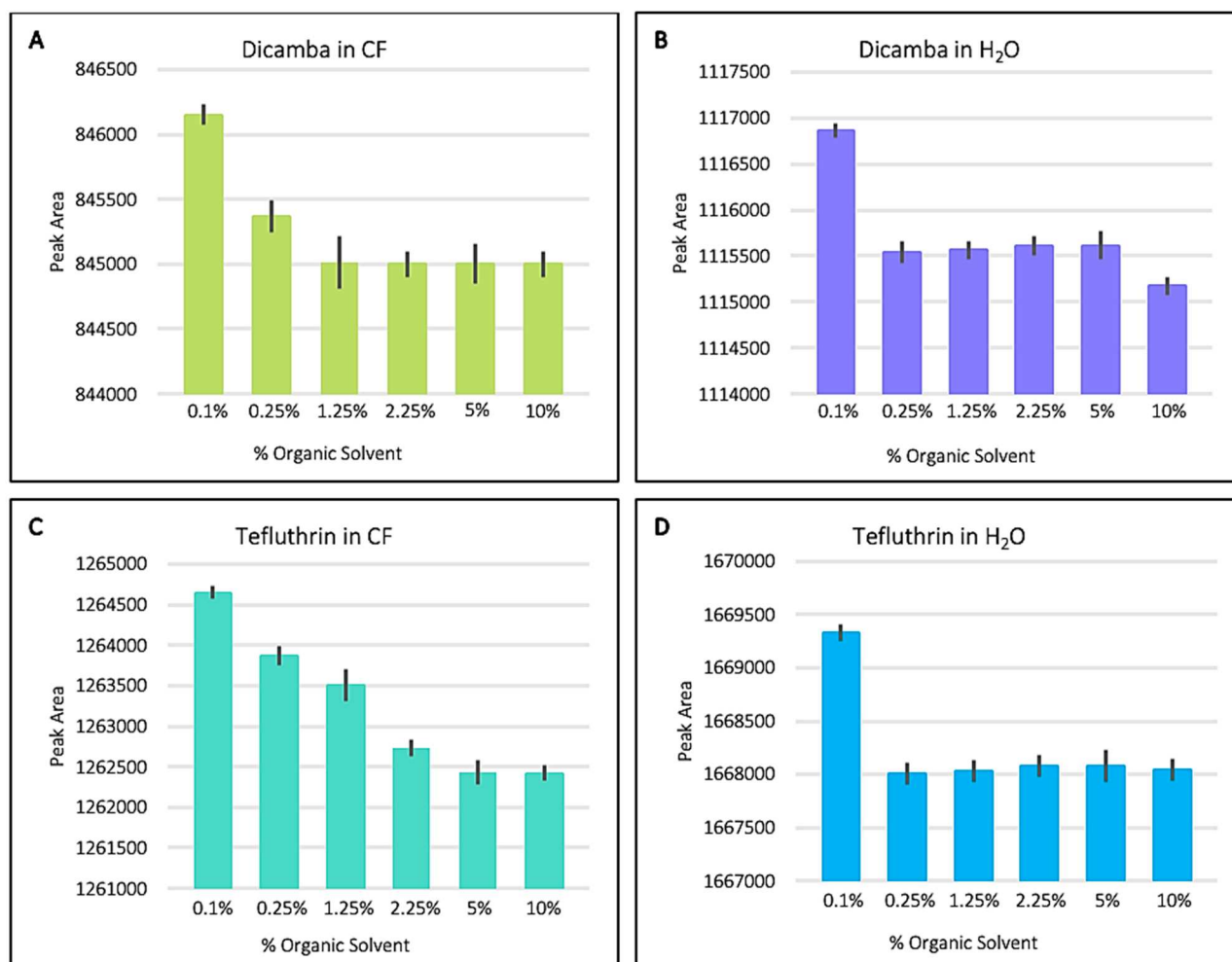


Figure S1. A–D. Peak area values at different percentages of ACN in (A) dicamba in CF, (B) dicamba in H₂O, (C) tefluthrin in CF, (D) tefluthrin in H₂O. Analytes concentration: 100 ng/mL.

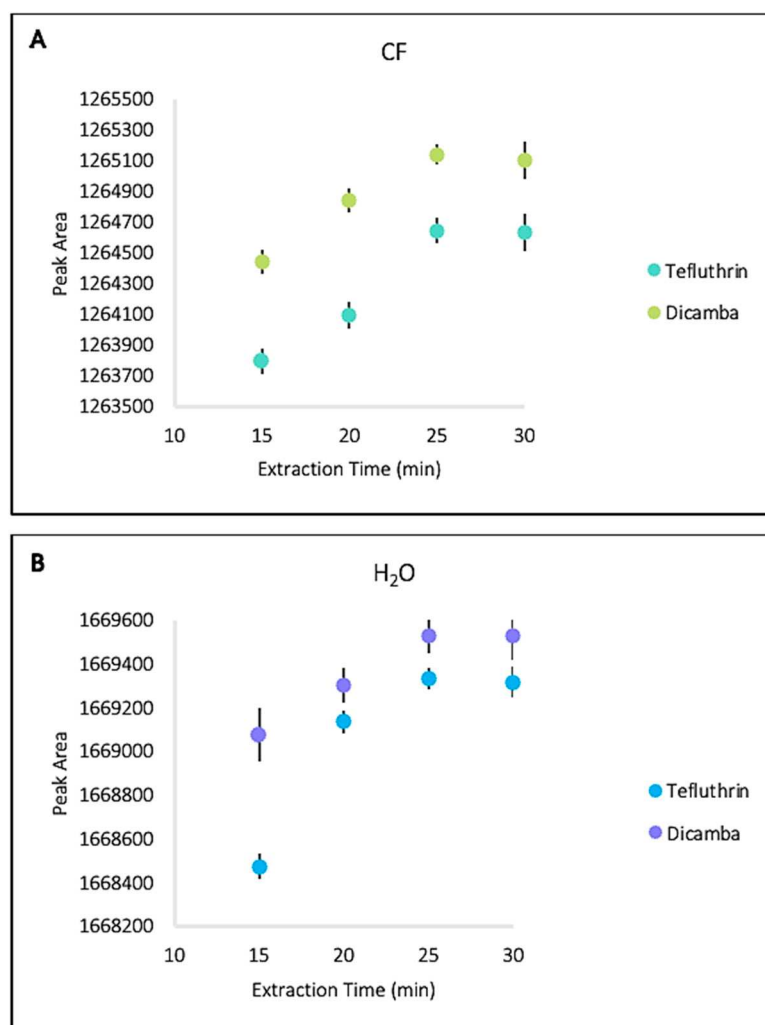


Figure S2. A,B. Effects of different extraction times on the integrated peaks area values of dicamba and tefluthrin at 100 ng/mL using magnetic stir bar as agitation method in (A) CF and (B) H₂O.

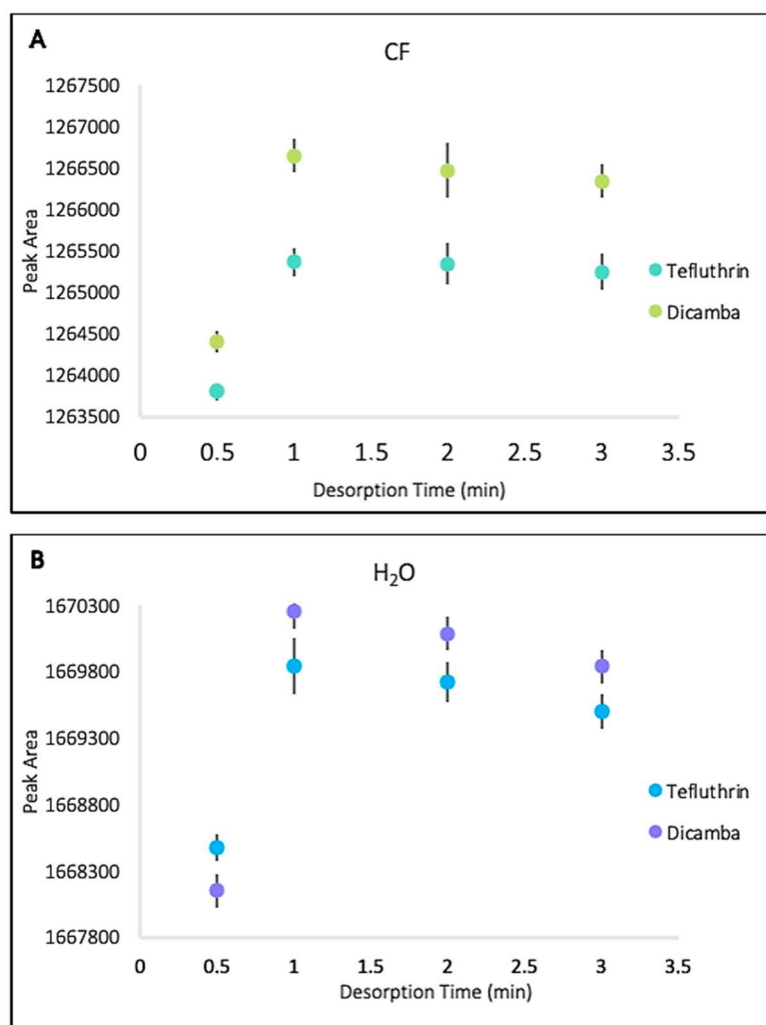


Figure S3. A,B. Effects of different desorption times on the integrated peaks area values of dicamba and tefluthrin at 100 ng/mL using magnetic stir bar as agitation method in (A) CF and (B) H₂O.

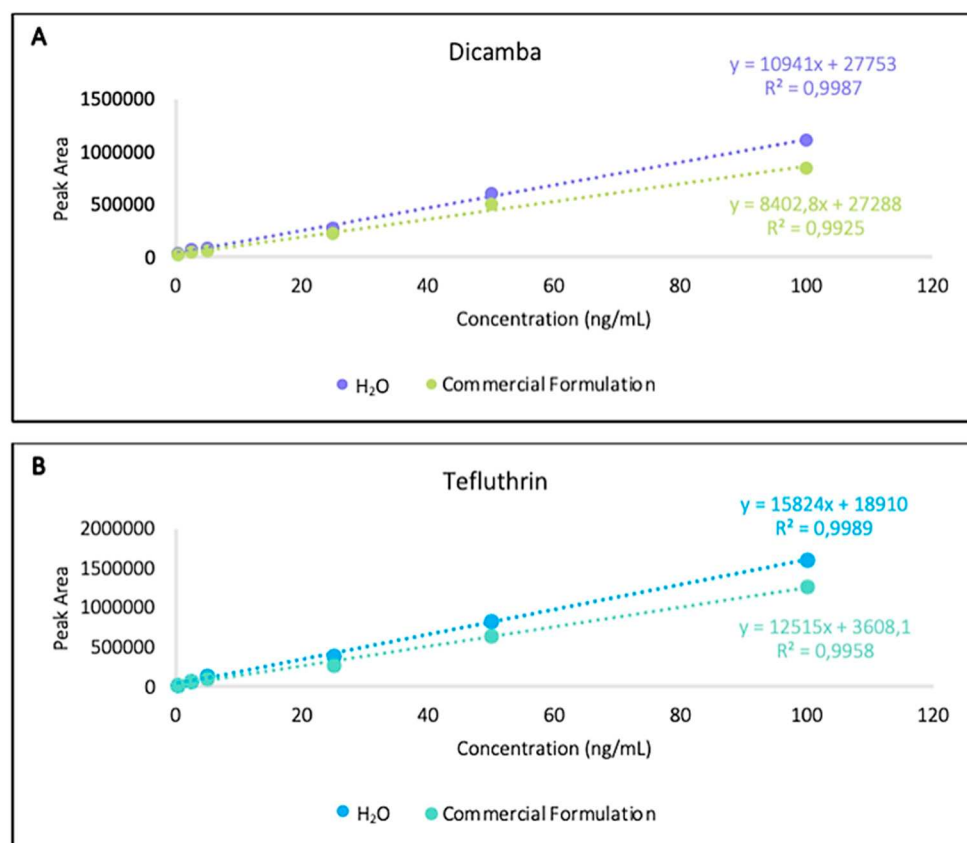


Figure S4. A,B. Calibration curves (0.5, 2.5, 5, 25, 50, and 100 ng/mL) of (A) dicamba and (B) tefluthrin in CF and H₂O.

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Liquid electron ionization-mass spectrometry as a novel strategy for integrating normal-phase liquid chromatography with low and high-resolution mass spectrometry†

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Normal-phase liquid chromatography (NPLC) plays a pivotal role in the rapid separation of non-polar compounds, facilitating isomer separation and finding applications in various crucial areas where aprotic solvents are necessary. Similar to reversed-phase liquid chromatography (RPLC), NPLC requires a robust and sensitive detector to unequivocally identify the analytes, such as a mass spectrometer. However, coupling NPLC with mass spectrometry (MS) poses challenges due to the incompatibility between the non-polar solvents used as the mobile phase and the primary ionization techniques employed in MS. Several analytical methods have been developed to combine NPLC with electrospray ionization (ESI), but these methods are restricted to the analysis of polar compounds. In most cases, atmospheric pressure chemical ionization (APCI) becomes necessary to expand the range of analysis applications. To overcome these limitations and fully realize the potential of NPLC-MS coupling, a technique termed liquid electron ionization-mass spectrometry (LEI-MS) can be used. LEI-MS offers a straightforward solution by enabling the effective coupling of NPLC with both low and high-resolution MS. LEI allows for the comprehensive analysis of non-polar compounds and provides a powerful tool for isomer separation and precise identification of analytes. Optimal separations, mass spectral qualities, and matches with the NIST library were obtained in both configurations, demonstrating the potential of the proposed approach.

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Introduction

Liquid chromatography represents one of the most versatile analytical techniques for the separation and identification of analytes, even with vastly different chemical-physical characteristics. This versatility stems from the various combinations that can be utilized with different stationary and mobile phases. Among the different types, reversed-phase liquid chromatography (RPLC) has gained immense popularity due to its ability to address a wide range of applications. In RPLC, the stationary phase is predominantly non-polar while the mobile phase exhibits varying degrees of polarity. Despite being the earliest developed technique, normal-phase liquid

chromatography (NPLC) has lost some of its analytical appeal compared to RPLC. However, NPLC, which relies on distinct retention mechanisms, can offer superior selectivity and resolution in specific applications.¹ This makes it particularly advantageous when analytes are poorly separated by RPLC or when hydrophobic impurities, retained strongly in RPLC, need to be swiftly eluted to focus on the target analytes.² In some cases, NPLC and RPLC can be combined, such as in the two-dimensional liquid chromatography approach.^{3,4} To achieve optimal separation of the target compounds, NPLC employs polar stationary phases such as silica-based, alumina, zirconia, or polar-embedded stationary phases. The mobile phases consist of a combination of slightly polar and non-polar solvents.^{5–7} Commonly used mobile phases in NPLC include hexane, heptane, ethyl acetate, and dichloromethane, which are often mixed and used in isocratic mode. This approach saves time and solvents by eliminating the need for column conditioning after consecutive analyses. Moreover, the use of non-polar solvents facilitates the solubilization of complex real samples. This enhances the loading capacity and generates low back pressure, enabling high flow rates for fast analysis time. One limitation of NPLC-based analytical methods is the

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inability to take full advantage of sensitive and specific detectors. UV absorbance spectrophotometric systems are commonly used in conjunction with NPLC, especially when highly non-polar solvents are employed as the mobile phase.^{8–11} However, these devices have limited identification capabilities and lower sensitivity compared to mass spectrometric detectors. To address these limitations, the integration of NPLC and MS (NPLC-MS) has provided high sensitivity, selectivity, and capability to ionize a diverse array of compounds.^{12–15} Among the various ionization techniques available, electrospray ionization (ESI) is commonly used to generate gas-phase ions from solution-phase samples. However, ESI can be challenging when low-polarity mobile phases are used, such as those typically employed in NPLC.¹⁶ As a result, atmospheric pressure chemical ionization (APCI)^{17–22} and atmospheric pressure photoionization (APPI)²³ are preferred techniques. The use of NPLC-MS has significantly increased in various fields, including environmental, food, pharmaceutical analyses, and metabolomics. However, in terms of identification, there are currently no LC-MS spectral libraries comparable to those available for GC-MS based on electron ionization (EI). This poses limitations on qualitative analysis. This study aims to explore the possibility of coupling NPLC with EI-MS (NPLC-EI-MS). The advantages of NPLC-EI-MS can include fast separation of compounds, isomer separation, and the generation of EI spectra. Low- and high-resolution instrumentation (HRMS) were tested to assess selected ion monitoring (SIM), multiple reaction monitoring (MRM), and high-resolution full scan detection. The study utilizes an LC-MS interface called the liquid electron ionization interface (LEI), which converts the liquid phase into a gas phase for analysis in a mass spectrometer equipped with an EI ion source. The LEI interface enables targeted and untargeted identification by extending access to EI libraries. A more detailed description of the LEI concept has been discussed elsewhere.^{24–26} To test the proof-of-concept of NPLC-LEI-MS coupling, a selection of cannabinoids, tocopherols, phenols, and phthalates were used as model compounds due to their optimal response in NPLC and analytical relevance.^{27–39} Encouraging preliminary data demonstrate that NPLC-LEI-MS is a reliable alternative approach, providing high selectivity, resolution, and structural information of analytes. Preliminary experiments focused on acquiring full scan spectra and MRM transitions of the selected compounds using a triple quadrupole mass spectrometer. The compound mixtures were separated using selected ion monitoring (SIM), MRM, and HRMS modes, employing a UHPLC pump and a silica column in isocratic mode to increase sample throughput. Two different low- and high-resolution instruments were utilized to test the performance of NPLC-LEI-MS with two EI sources of different geometry. To the best of our knowledge, the experiments described in this study represent the first attempt to use non-polar LC mobile phases with EI-MS. The obtained results open new possibilities in coupling NPLC with MS and offer the potential for developing alternative methods to RPLC in the analysis of real samples, particularly in cases involving poor analyte resolution or highly hydrophobic impurities.

Material and methods

Standards and reagents

LC-MS grade hexane (HEX), isopropanol (IPA), ethyl acetate, ethanol (EtOH) and formic acid were purchased from VWR International, part of Avantor (Milan, Italy). Standards of (+)- α -tocopherol and (+)- δ -tocopherol (purity $\geq 98\%$) were purchased from Sigma-Aldrich (Milan, Italy), (+)- β -tocopherol (purity $\geq 98\%$) was supplied by Extrasynthese (Genay, France). Solutions of cannabinoids ((-)-delta9-*trans*-tetrahydrocannabinol 100 mg L⁻¹ in methanol (MeOH); cannabidiol 1000 mg L⁻¹ in MeOH) were provided by and manipulated at the Toxicology Laboratory facilities A.S.T. AV1, (Pesaro, Italy). Phthalates (diisodecyl phthalate; diethyl phthalate; dimethyl phthalate, purity $\geq 99\%$), and phenols (phenol; catechol; resorcinol; hydroquinone, purity $\geq 99\%$) were a gift of the Department of Biomolecular Sciences of the University of Urbino. Pure standards were stored at $-20\text{ }^{\circ}\text{C}$.

Standard solution preparation

Stock solutions of tocopherols and phthalates were volumetrically prepared at a concentration of 1000 mg L⁻¹ in HEX and stored in dark vials (VWR International, part of Avantor Milan, Italy) at $4\text{ }^{\circ}\text{C}$. Stock solutions of phenols were gravimetrically prepared at a concentration of 1000 mg L⁻¹ in EtOH and stored in dark vials at $4\text{ }^{\circ}\text{C}$. Working standard solutions of tocopherols were volumetrically prepared as combined suites (100 μL of α -tocopherol at 1000 mg L⁻¹, 100 μL of β -tocopherol at 1000 mg L⁻¹, and 100 μL of δ -tocopherol at 1000 mg L⁻¹) at a concentration of 333 mg L⁻¹ each in HEX. Working standard solutions of cannabinoids were volumetrically prepared as combined suites (200 μL of THC at 100 mg L⁻¹ and 100 μL of CBD at 1000 mg L⁻¹) at a concentration of 67 mg L⁻¹ for THC and 333 mg L⁻¹ for CBD in MeOH. The cannabinoid solutions were prepared at the Toxicology Laboratory facilities A.S.T. AV1, (Pesaro, Italy). Working standard solutions of phthalates were volumetrically prepared as combined suites (100 μL of diisodecyl phthalate at 1000 mg L⁻¹, 100 μL of diethyl phthalate at 1000 mg L⁻¹, and 100 μL of dimethyl phthalate at 1000 mg L⁻¹) at a concentration of 333 mg L⁻¹ each in HEX. Working standard solutions of phenols were volumetrically prepared as combined suites (100 μL of phenol at 1000 mg L⁻¹, 100 μL of catechol at 1000 mg L⁻¹, 100 μL of resorcinol at 1000 mg L⁻¹, and 100 μL of hydroquinone at 1000 mg L⁻¹) at a concentration of 250 mg L⁻¹ each in EtOH.

LC-LEI-QQQ-MS/MS system for low-resolution experiments

Low-resolution experiments were performed with an Agilent 1290 Infinity II UHPLC (Agilent Technologies, Santa Clara, CA, USA) with an Ascentis silica column (2.1 i.d. $\times$ 150 mm, 3 μm particle size) (Sigma-Aldrich-Supelco, Milan, Italy) coupled with an Agilent triple quadrupole (QQQ) 7010 B mass spectrometer (Agilent Technologies, Santa Clara, CA, USA) equipped with an LEI interface. The LC pump flow rate was set at 100 $\mu\text{L min}^{-1}$, but a post-column passive flow splitter (PFS) was used for decreasing the flow rate to 500 nL min⁻¹ (1 : 200 split ratio)



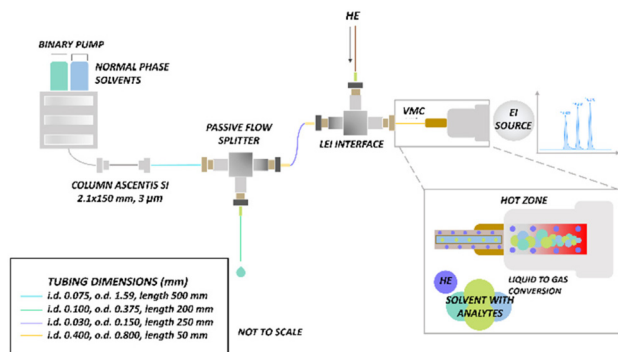


Fig. 1 Scheme of the LC-LEI-MS/MS and LC-LEI-QTOF-HRMS systems.

to accommodate LEI requirements. A detailed description of the post-column PFS is provided elsewhere.⁴⁰ Fig. 1 shows the schematic of the system for low- and high-resolution experiments. The injection volume was 1 μL for cannabinoids and phenols, and 4 μL for tocopherols and phthalates. The analyses were carried out in isocratic mode using different mobile phase compositions as indicated in Table 1. Agilent OpenLab CDS ChemStation software was used for UHPLC instrument control. The core of LEI is the vaporization microchannel (VMC) where the liquid phase is mixed with a helium flow, heated, and converted into the gas phase. VMC was constructed modifying the standard GC transfer line, totally lined with a 0.4 mm i.d., 0.8 mm o.d. removable fused silica capillary. The mobile phase and the analytes flow into the VMC through the inlet capillary, a narrower 150 μm o.d., 30 μm i.d. fused silica capillary that penetrates in the first portion of the VMC. A detailed description of the interface is provided elsewhere.^{24–26} The ion source and quadrupoles were maintained at temperatures of 280 $^{\circ}\text{C}$ and 150 $^{\circ}\text{C}$, respectively. To ensure the rapid and efficient vaporization of the analytes without thermal degradation, different VMC temperatures were tested (250, 300, 350, and 400 $^{\circ}\text{C}$). Specific VMC tempera-

tures were set for the different classes of compounds to promote rapid and efficient vaporization, while avoiding thermal degradation. Data acquisitions of tocopherols, cannabinoids, and phthalates were carried out in MRM. For phenols, data acquisition was performed in SIM (Table 1). Flow injection analyses (FIA) were conducted using an external manual injector with an internal loop of 0.1 μL (Vici, Switzerland). Agilent Mass Hunter GC/MS acquisition software (Version B.07.04.2260) and Agilent Mass Hunter Qualitative Analysis software (Version B.07.00) were employed for data acquisition and processing.

LC-LEI-QTOF-HRMS system

An Agilent quadrupole-time of flight (QTOF) 7250 mass spectrometer (Agilent Technologies Inc., Santa Clara, CA, USA) equipped with an LEI interface was used for the detection of the analytes in high-resolution experiments. Also in this case, the UHPLC instrument was an Agilent 1290 Infinity II (Agilent Technologies Inc., Santa Clara, CA, USA). To facilitate the comparison with the low-resolution setup, the same conditions were applied in terms of column, flow rate, injection volume, mobile phase composition, and VMC temperature. The ion source temperature was kept at 260 $^{\circ}\text{C}$ for all high-resolution experiments. Data acquisition was performed in full scan mode for each compounds' class. The acquisition range was set from m/z 90 to m/z 500 to avoid detection of interfering ions originating from HEX (MW 86.18 g mol^{-1}). The data extraction window was set at 25 ppm. Operational parameters used for high-resolution experiments are presented in Table 2. After each analysis, QTOF mass calibration was performed, during which no mobile phase was admitted into the ion source. Data were acquired with the Agilent Mass Hunter GC/MS acquisition software (Version 10.1.49.0) and processed with the Agilent Mass Hunter Qualitative Analysis software (Version 10.0).

Table 1 Operational parameters for NPLC-LEI-QQQ-MS (low-resolution) separations of tocopherols, cannabinoids, phthalates, and phenols

Compound name	Mobile phase	Injection volume	Acquisition mode	<i>m/z</i> MRM transitions/selected ions (collision energies)	VMC <i>T</i> °C
α-Tocopherol	HEX : IPA 98 : 2 (v : v)	4 μL	MRM	430–165 (15 eV) 430–205 (5 eV)	250
β-Tocopherol				416–151 (20 eV) 191–135 (10 eV)	
δ-Tocopherol				402–137 (15 eV) 402–177 (10 eV)	
THC	HEX : EtOH 95 : 5 (v : v)	1 μL	MRM	314–299 (5 eV) 231–174 (20 eV)	400
CBD				246–174 (25 eV) 231–174 (20 eV)	
Diisodecyl phthalate	HEX : IPA 90 : 10 (v : v)	4 μL	MRM	149–93 (15 eV) 149–121 (10 eV)	350
Diethyl phthalate				177–149 (5 eV) 222–149 (5 eV)	
Dimethyl phthalate	HEX : EtOH 80 : 20 (v : v)	1 μL	SIM	163–133 (5 eV)	350
Phenol				94	
Catechol				110	
Resorcinol				110	
Hydroquinone				110	
Flow rate: 100 μL min ^{−1} split to 500 nL min ^{−1} (1 : 200 split ratio)					
Column: Ascentis Si 2.1 × 150 mm, 3 μm particle size					
Ion source temperature: 280 °C					
Quadrupoles temperature: 150 °C					



Table 2 Operational parameters for NPLC-LEI-QTOF-HRMS (high-resolution) separations of tocopherols, cannabinoids, phthalates, and phenols

Compound name	Mobile phase	Injection volume	VMC $T^{\circ}\text{C}$
α -Tocopherol	HEX : IPA 98 : 2 (v : v)	4 μL	250
β -Tocopherol			
δ -Tocopherol			
THC	HEX : EtOH 95 : 5 (v : v)	1 μL	400
CBD			
Diisodecyl phthalate	HEX : IPA 90 : 10 (v : v)	4 μL	350
Diethyl phthalate			
Dimethyl phthalate			
Phenol	HEX : EtOH 80 : 20 (v : v)	1 μL	350
Catechol			
Resorcinol			
Hydroquinone			
Flow rate: 100 $\mu\text{L min}^{-1}$ split at 500 nL min^{-1} (1 : 200 split ratio)			
Column: Ascentis Si 2.1 $\times$ 150 mm, 3 μm particle size			
Ion source temperature: 260 $^{\circ}\text{C}$			
Acquisition mode: full scan from m/z 90 to m/z 500			

Results and discussion

Low-resolution experiments

The initial experiments focused on acquiring low-resolution EI full scan mass spectra of all targeted analytes. The results were obtained using FIA. Each compound was injected and analyzed separately using the following solutions: α -, β -, and δ -tocopherol at 1000 mg L^{-1} in HEX; THC and CBD in MeOH at 100 mg L^{-1} and 1000 mg L^{-1} , respectively; diisodecyl-, diethyl-, and dimethyl phthalate at 1000 mg L^{-1} in HEX; phenol, catechol, resorcinol, and hydroquinone at 1000 mg L^{-1} in EtOH. The LC pump flow rate was set at 100 $\mu\text{L min}^{-1}$, split at 500 nL min^{-1} (1 : 200 split ratio), using different mobile phase compositions in isocratic mode. The scan range was set from m/z 90 to m/z 500, and the VMC temperature was set to 250 $^{\circ}\text{C}$ for tocopherols, 400 $^{\circ}\text{C}$ for cannabinoids, and 350 $^{\circ}\text{C}$ for phthalates and phenols. The ion source temperature was kept at 280 $^{\circ}\text{C}$. In Fig. 2a–c, low-resolution spectra of tocopherols were compared with the reference spectra present in the NIST library. α -tocopherol exhibited a matching factor of 740, and reverse match of 870; β -tocopherol showed a matching factor of 910, and reverse match of 950; δ -tocopherol demonstrated a matching factor of 880, and reverse match of 900. Fig. S1–S3† present the low-resolution spectra of cannabinoids, phthalates, and phenols along with NIST library spectra and matching data. An evident drawback of the use of aprotic solvents like HEX is the requirement to start the scan interval from m/z 90. This poses a challenge when dealing with compounds that exhibit intense and characteristic ions below this threshold, as it negatively impacts the automatic search in the database, resulting in slightly lower match and reverse match values. Experimental and library spectra are comparable, demonstrating that the typical NPLC mobile phases do not affect spectral quality. Based on the recorded spectra, MRM and SIM methods were developed and applied to separate the selected mixtures. Although MRM is more specific and sensitive than

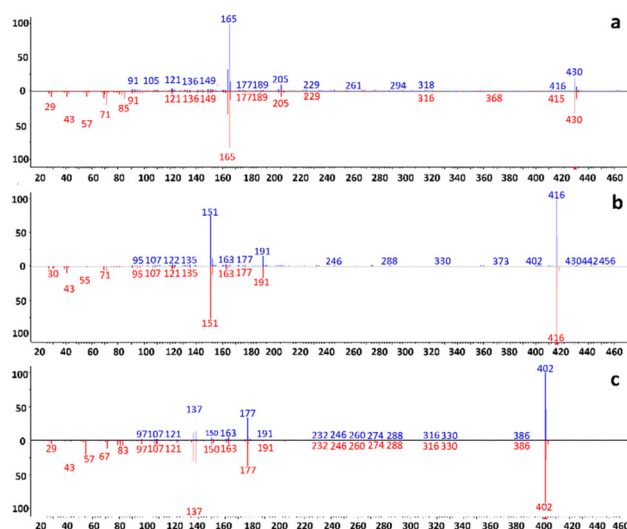


Fig. 2 (a–c). Low-resolution experiments. Comparison between the recorded and the NIST library reference spectra of (a) α -tocopherol, (b) β -tocopherol, and (c) δ -tocopherol. Top: experimental spectrum; Bottom: NIST reference spectrum.

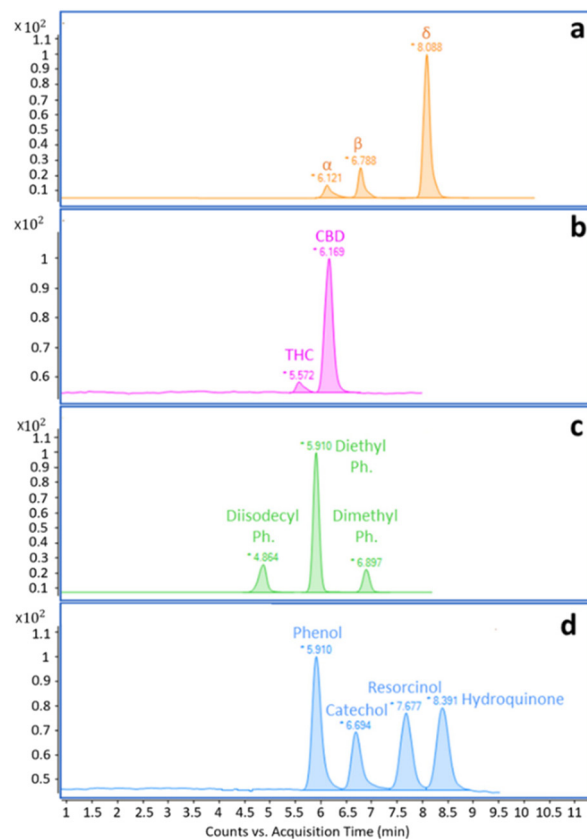


Fig. 3 (a–d). NPLC MRM and SIM reconstructed ion chromatograms of the selected model compounds: (a) tocopherols; (b) cannabinoids; (c) phthalates; (d) phenols. Analytical conditions are reported in Table 1.

SIM, in the case of phenols, the response in MRM was not satisfactory due to the lack of product ions, and SIM mode was therefore chosen. The NPLC separations obtained using the conditions described in Table 1 are shown in Fig. 3a–d. All separations were performed in isocratic mode, as it often occurs in NPLC. Isocratic elution, avoiding column re-conditioning, offers a clear advantage when analyzing a large number of consecutive samples, saving both time and solvents.

High-resolution experiments

The NPLC-LEI system was installed on a QTOF-HRMS to replicate the sets of experiments on a high-resolution instrument with a different ion source geometry, ensuring results repeatability for superior performance. The separations of the same model compounds were carried out without modifying the previously optimized chromatographic and mass spectrometric parameters, but the acquisitions were performed in full scan mode. This was possible because the QTOF-HRMS offers greater sensitivity in this mode, eliminating the need for FIA to acquire mass spectra. The separations are reported in Fig. 4a–d. The use of a source with a different geometry, especially with a different volume, could impede the evacuation of the solvent vapors used in NPLC, leading to chemical

ionization phenomena. However, this phenomenon, which has been previously studied and solved with the typical RPLC solvents, was not observed in either the QQQ or QTOF-HRMS source, as evidenced by the perfectly comparable mass spectra. The recorded high-resolution mass spectra and main fragments assignment of tocopherols are presented in Fig. 5a–c. High-resolution mass spectra of the other model compounds can be found in Fig. S4–S6.† The software supplied with the QTOF-HRMS automatically identifies the exact masses of the

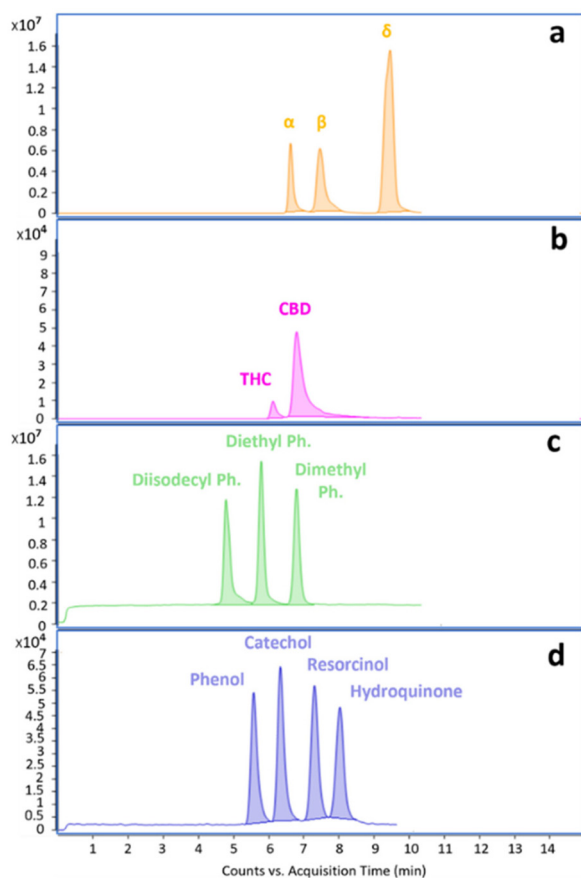


Fig. 4 (a–d). NPLC full scan chromatograms of the selected model compounds: (a) tocopherols; (b) cannabinoids; (c) phthalates; (d) phenols. Analytical conditions are reported in Table 2.

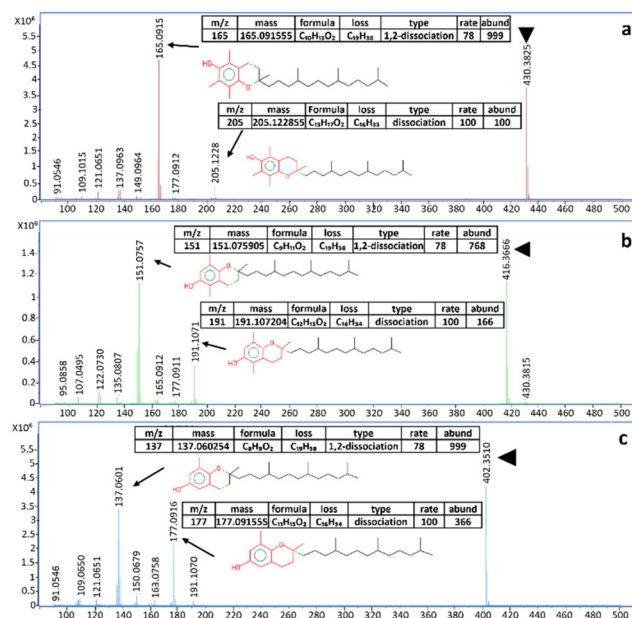


Fig. 5 (a–c). Recorded EI high-resolution full scan spectra of (a) α -tocopherol, (b) β -tocopherol, and (c) δ -tocopherol and assignment of the main fragments according to the MS interpreter utility developed by NIST. Molecular ions are indicated by the green triangle.

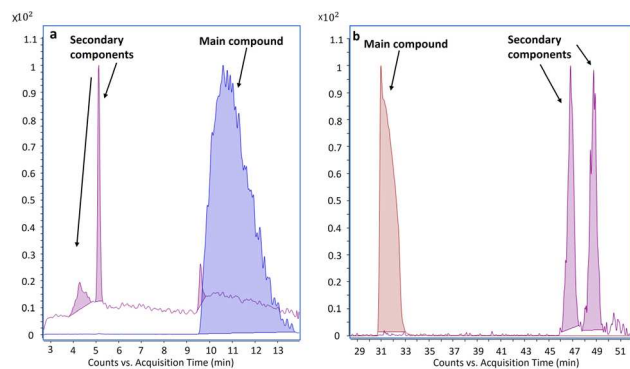


Fig. 6 (a and b). Extracted ion chromatograms of secondary components and main compound present in a commercial mixture obtained using the following separation conditions: (a) NPLC. A: HEX, B: ethyl acetate; gradient: 1–40% B in 25 min, 40–90% in 1 min, 90% held for 10 min; column: Ascentis Si 2.1 $\times$ 150 mm, 3 μ m particle size; flow rate: 100 μ L min⁻¹; (b) RPLC. A: water + 0.3% formic acid; B: ACN + 0.3% formic acid; gradient: 0–100% B in 30 min, 100% B held for 20 min; column: Waters Acquity UHPLC C18 1.7 μ m; flow rate: 100 μ L min⁻¹.



main fragments by considering the possible cleavages of the molecular bonds, facilitating unambiguous molecule identification. As described in the introduction, NPLC can also serve as a rapid elution method for highly hydrophobic and highly retained interferences in RPLC, effectively separating them from the analytes of interest. Fig. 6a and b shows the chromatograms obtained from the analysis of a non-disclosable commercial mixture where secondary components required characterization. In RPLC, these components elute after the main compound, with a retention time of approximately 50 min, making their identification a time-consuming and challenging process. However, in NPLC, the same separation demonstrates a completely different trend, as the secondary components swiftly exit the chromatographic column in less than 6 min and before the elution of the main compound. The intensity of the two peaks appears different, but their areas are comparable.

Conclusions

The results of this preliminary study demonstrate, thanks of the LEI interface, the feasibility and advantage of using MS with NPLC. By combining the unique separation capabilities of NPLC with a high-performance detector like MS with an EI source, both sensitivity and specificity are enhanced compared to traditional spectrophotometric detectors. These characteristics show the potential for future applications in targeted and untargeted analysis of complex matrices, especially with a high-resolution analyzer. NPLC-LEI-MS has proven to be a promising technique that offers an alternative approach to RPLC-MS based on soft ionization techniques. The application of NPLC-LEI-MS to the analysis of selected model compounds and a real sample has shown that the mass spectrometric results remain unaltered, as evidenced by the comparison of the experimental EI spectra with those recorded in the NIST library. The presented separations also indicate that SIM or MRM analyses can be performed without particular precautions. The next phase of the research will focus on developing methods for analyzing a variety of samples, taking advantage of the potential of NPLC-LEI-MS.

Author contributions

N. M.: Investigation, formal analysis. G. G.: Investigation. A. A.: Validation, methodology, project administration. G. F.: Methodology, writing—original draft, writing—review & editing, project administration. M. A.: Drug of abuse—sample preparation. C. R.: Drug of abuse—sample preparation. P. P.: Methodology, Writing—review & editing, project administration. A. C.: Supervision, writing—review & editing, funding acquisition, project administration, conceptualization.

Conflicts of interest

There are no conflicts to declare.

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References

- 1 A. W. Salotto, E. L. Weiser, K. P. Caffey, R. L. Carty, S. C. Racine and L. R. Snyder, *J. Chromatogr.*, 1990, **498**, 55–65.
- 2 L. R. Snyder, J. J. Kirkland and J. W. Dolan, *Introduction to modern liquid chromatography*, Wiley, Hoboken, 3rd edn, 2010.
- 3 P. Dugo, O. Favoino, R. Luppino, G. Dugo and L. Mondello, *Anal. Chem.*, 2004, **76**(9), 2525–2530.
- 4 K. Arena, E. Trovato, F. Cacciola, L. Spagnuolo, E. Pannucci, P. Guarnaccia, L. Santi, P. Dugo, L. Mondello and L. Dugo, *Molecules*, 2022, **27**(5), 1727.
- 5 L. R. Snyder, *High-performance liquid chromatography - Advances and perspectives*, ed. C. S. Horvath, Academic Press, New York, 1980, p. 207.
- 6 W. T. Cooper, *Encyclopedia of Analytical Chemistry: Application, Theory, and Instrumentation*, ed. R. A. Meyers and J. G. Dorsey, Wiley Online Library, 2006.
- 7 A. A. Younes, C. Galea, D. Mangelings and Y. Vander Heyden, *Analytical Separation Science*, ed. J. L. Anderson, A. Berthod, V. Pino and A. M. Stalcup, Wiley-VCH, Weinheim, 2015, pp. 227–244.
- 8 L. Jiahao, X. Xiyue, M. Ming, C. Bo, C. Yingzhuang and J. S. Oliver, *J. Chromatogr. A*, 2023, **1691**, 463821.
- 9 M. Zhengyue, S. Panpan, L. Delu, N. Yangyang, L. Yanli, G. Xinyuan, W. Benben, B. Ligai and Q. Xiaoqiang, *Chirality*, 2023, **35**(9), 636–644.
- 10 Z. You-Ping, L. Kuan, X. Ling-Xiao, W. Bang-Jin, X. Sheng-Ming, Z. Jun-Hui and Y. Li-Ming, *J. Chromatogr. A*, 2022, **1683**, 463551.
- 11 A. A. Boyd-Boland and J. B. Pawliszyn, *Anal. Chem.*, 1996, **68**(9), 1521–1529.
- 12 S. Lababidi, S. K. Panda, J. T. Andersson and W. Schrader, *Anal. Chem.*, 2013, **85**(20), 9478–9485.
- 13 S. Lababidi and W. Schrader, *Rapid Commun. Mass Spectrom.*, 2014, **28**(12), 1345–1352.
- 14 E. V. S. Maciel, A. L. de Toffoli, E. Sobieski, C. E. D. Nazario and F. M. Lanças, *Anal. Chim. Acta*, 2020, **1103**, 11–31.
- 15 K. Mejía-Carmona, J. S. da Silva Burato, J. V. Basolli Borsatto, A. L. de Toffoli and F. M. Lanças, *TrAC, Trends Anal. Chem.*, 2020, **122**, 115735.
- 16 P. Kalo, A. Kemppinen and V. Ollilainen, *Lipids*, 2009, **44**(2), 169–195.
- 17 N. Strobel, S. Buddhadasa, P. Adorno, K. Stockham and H. Greenfield, *Food Chem.*, 2013, **138**(2–3), 1042–1047.
- 18 M. Mejean, A. Brunelle and D. Touboul, *Anal. Bioanal. Chem.*, 2015, **407**, 5133–5142.
- 19 T. Saldanha, A. C. Sawaya, M. N. Eberlin and N. Bragagnolo, *J. Agric. Food Chem.*, 2006, **54**(12), 4107–4113.



- 20 R. K. Khajuria, V. Bhardwaj, R. K. Gupta, P. Sharma, P. Somal, P. Mehta and G. N. Qazi, *J. Chromatogr. Sci.*, 2007, **45**(8), 519–523.
- 21 S. Kemmo, V. Ollilainen, A. M. Lampi and V. Piironen, *Food Chem.*, 2007, **101**(4), 1438–1445.
- 22 R. Schex, V. M. Lieb, V. M. Jiménez, P. Esquivel, R. M. Schweiggert, R. Carle and C. B. Steingass, *Food Res. Int.*, 2018, **105**, 645–653.
- 23 C. H. Grün and S. Besseau, *J. Chromatogr. A*, 2016, **1439**, 74–81.
- 24 V. Termopoli, G. Famiglini, P. Palma, M. Piergiovanni and A. Cappiello, *Anal. Chem.*, 2017, **89**(3), 2049–2056.
- 25 V. Termopoli, G. Famiglini, P. Palma, M. Piergiovanni, P. Rocio-Bautista, M. F. Ottaviani, A. Cappiello, M. Saeed and S. Perry, *J. Chromatogr. A*, 2019, **1591**, 120–130.
- 26 A. Cappiello, G. Famiglini, M. Piergiovanni and V. Termopoli, Machine for Chemical Analysis Comprising the Combination of Electron Ionization Mass Spectrometry with Liquid Chromatography, *International Application Patent No. PCT/IB2018/057970, US Application Patent No 16/755*, 2020, 332.
- 27 G. Stefkov, I. Cvetkovikj, Karanfilova, V. Stoilkovska Gjorgievska, A. Trajkovska, N. Geskovski, M. Karapandzova and S. Kulevanova, *Molecules*, 2022, **27**(3), 975.
- 28 C. De Luca, A. Buratti, Y. Krauke, S. Stephan, K. Monks, V. Brighenti, F. Pellati, A. Cavazzini, M. Catani and S. Felletti, *Anal. Bioanal. Chem.*, 2022, **414**, 5385–5395.
- 29 R. F. X. Klein, *Microgram J.*, 2015, **12**, 1–4.
- 30 T. Onishi and W. J. Umstead, *Pharmaceuticals*, 2021, **14**(12), 1250.
- 31 L. Huang, J. Li, Y. Bi, Y. Xu, Y. Wang, J. Wang and D. Peng, *J. Food Compos. Anal.*, 2021, **104**, 104132.
- 32 L. Barros, D. M. Correia, I. C. F. R. Ferreira, P. Baptista and C. Santos-Buelga, *Food Chem.*, 2008, **110**(4), 1046–1050.
- 33 A. Bouymajane, Y. O. El Majdoub, F. Cacciola, M. Russo, F. Salafia, A. Trozzi, F. R. Filali, P. Dugo and L. Mondello, *Molecules*, 2020, **25**(22), 5428.
- 34 P. D. Boes, S. R. Elleman and N. D. Danielson, *Separations*, 2023, **10**(2), 70.
- 35 M. Zhang, A. K. Mallik, M. Takafuji, H. Ihara and H. Qiu, *Anal. Chim. Acta*, 2015, **887**, 1–16.
- 36 E. Guerrero-Hurtado, A. Gutiérrez-Docio, R. Fiedorowicz, E. Mollá, G. Reglero and M. Prodanov, *J. Chromatogr. A*, 2023, **1696**, 463957.
- 37 W. Cuijie, Z. Ling, L. Xinglin, Y. Ajuan and Z. Shusheng, *Talanta*, 2020, **218**, 121155.
- 38 C. L. Flakelar, P. D. Prenzler, D. J. Luckett, J. A. Howitt and G. Doran, *Food Chem.*, 2017, **214**, 147–155.
- 39 T. Lechhab, F. Salmoun, W. Lechhab, Y. O. El Majdoub, M. Russo, M. R. Testa Camillo, E. Trovato, P. Dugo, L. Mondello and F. Cacciola, *Eur. Food Res. Technol.*, 2021, **247**(12), 2993–3012.
- 40 N. Marittimo, G. Famiglini, P. Palma, A. Arigò and A. Cappiello, *J. Chromatogr. A*, 2022, **1681**, 463479.



Liquid Electron Ionization-Mass Spectrometry as a Novel Strategy for integrating Normal-Phase Liquid Chromatography with Low and High-Resolution Mass Spectrometry

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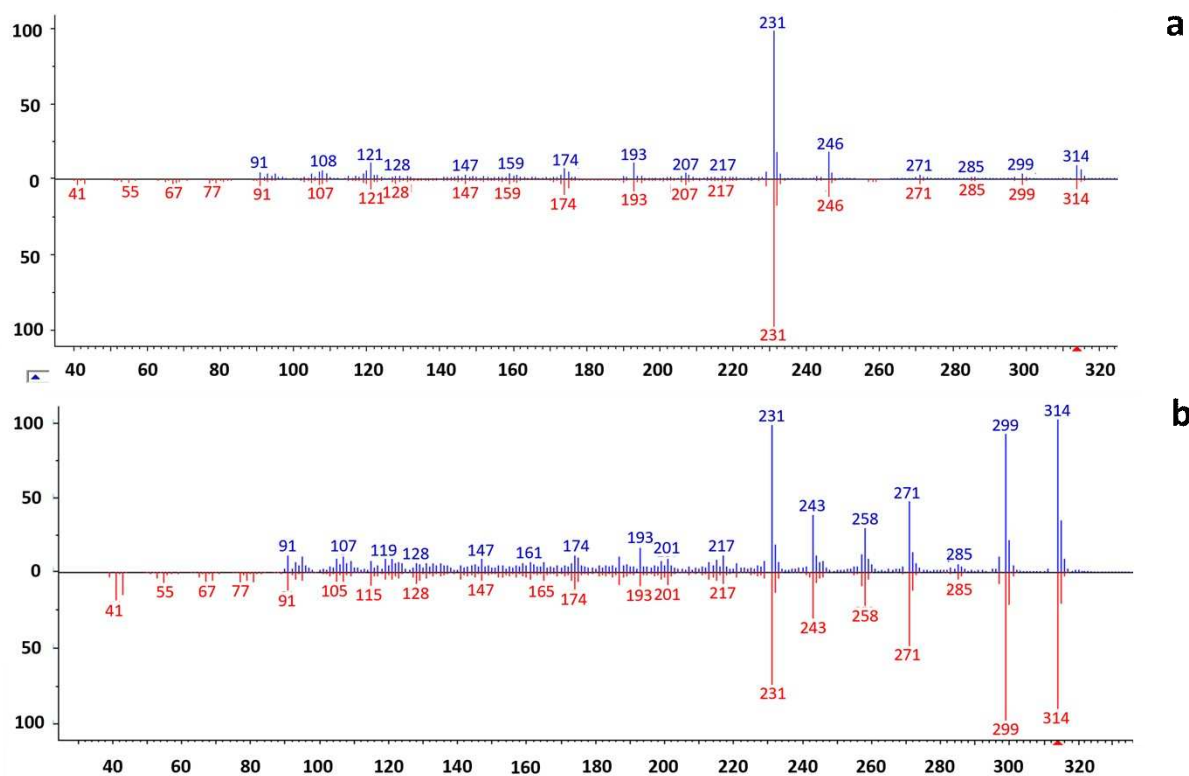


Figure S1 a-b. Low-resolution experiments. Comparison between the recorded and the NIST library reference spectra of a) CBD and b) THC. Top: experimental spectrum; Bottom: NIST reference spectrum. THC was identified with a matching factor of 790, and reverse match of 850; CBD with a matching factor of 820, and reverse match of 910.

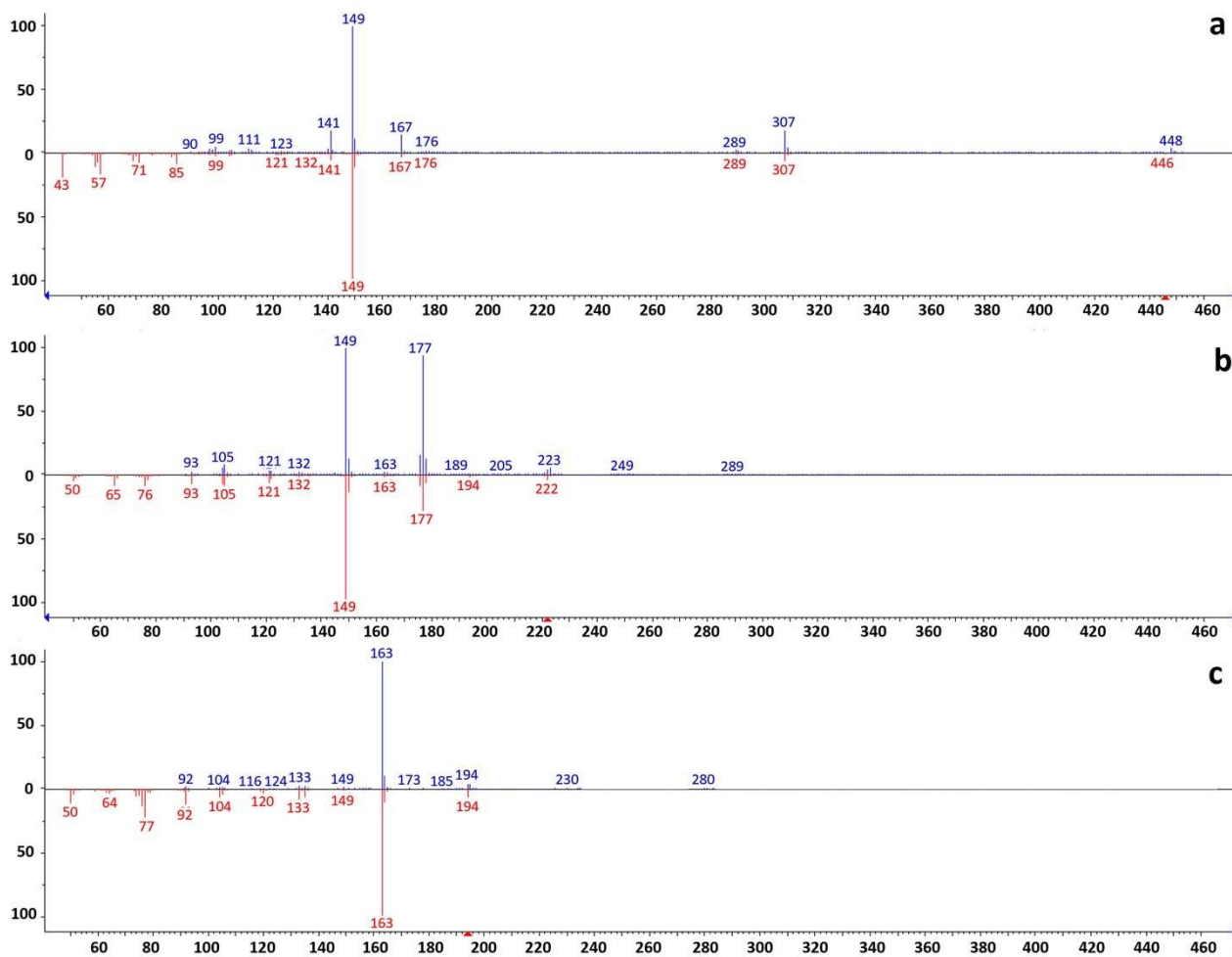


Figure S2 a-c. Low-resolution experiments. Comparison between the recorded and the NIST library reference spectra of a) diisodecyl phthalate; b) diethyl phthalate; c) dimethyl phthalate. Top: experimental spectrum; Bottom: NIST reference spectrum. Diisodecyl phthalate was identified with a matching factor of 771, and reverse match of 856; diethyl phthalate with a matching factor of 881, and reverse match of 891; dimethyl phthalate with a matching factor of 721, and reverse match of 840.

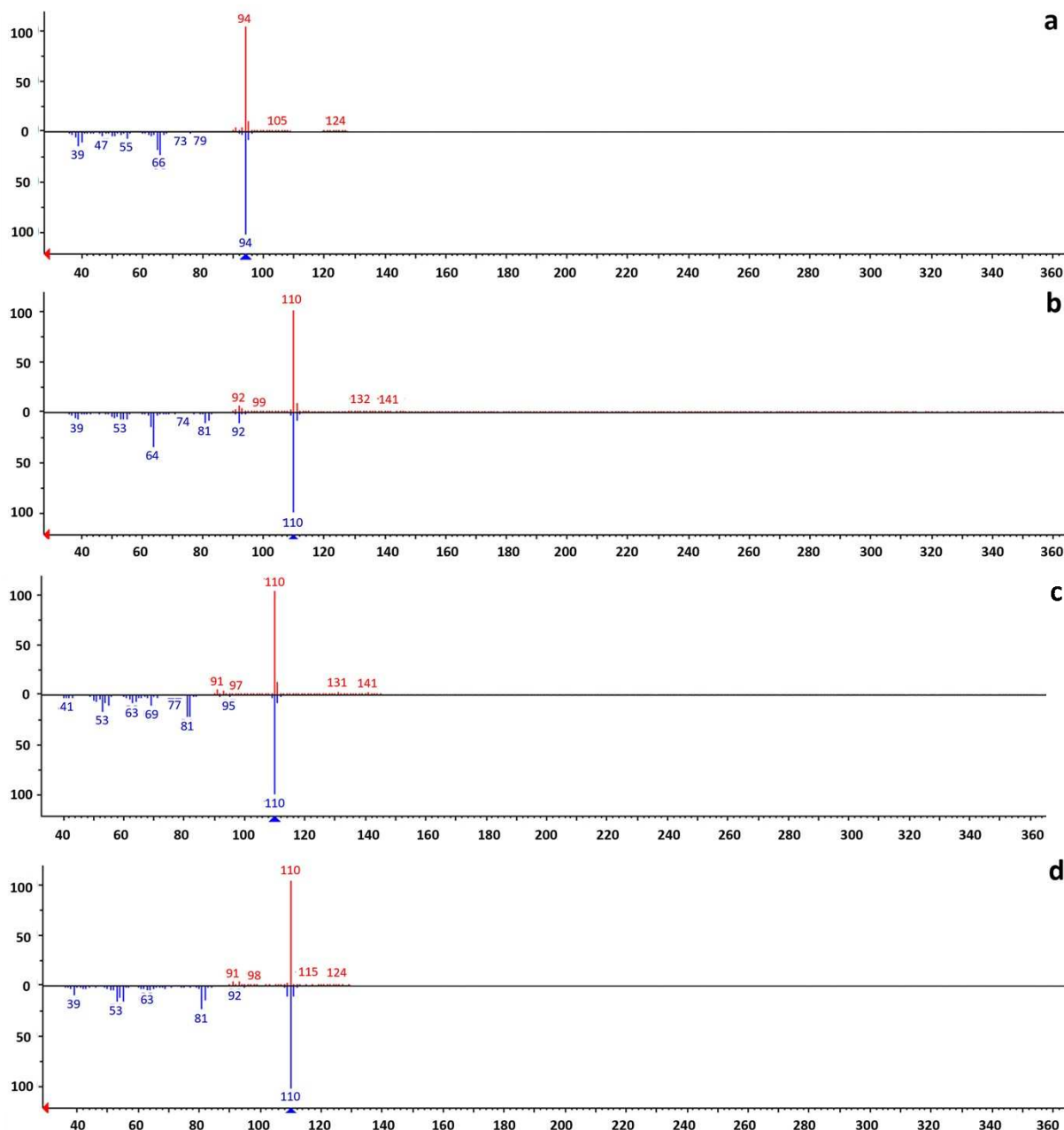


Figure S3 a-d. Low-resolution experiments. Comparison between the recorded and the NIST library reference spectra of a) phenol; b) catechol; c) resorcinol; d) hydroquinone. Top: experimental spectrum; Bottom: NIST reference spectrum. Phenol was identified with a matching factor of 700, and reverse match of 980; catechol with a matching factor of 850, and reverse match of 648; resorcinol with a matching factor of 636, and reverse match of 936; hydroquinone with a matching factor of 532, and reverse match of 888.

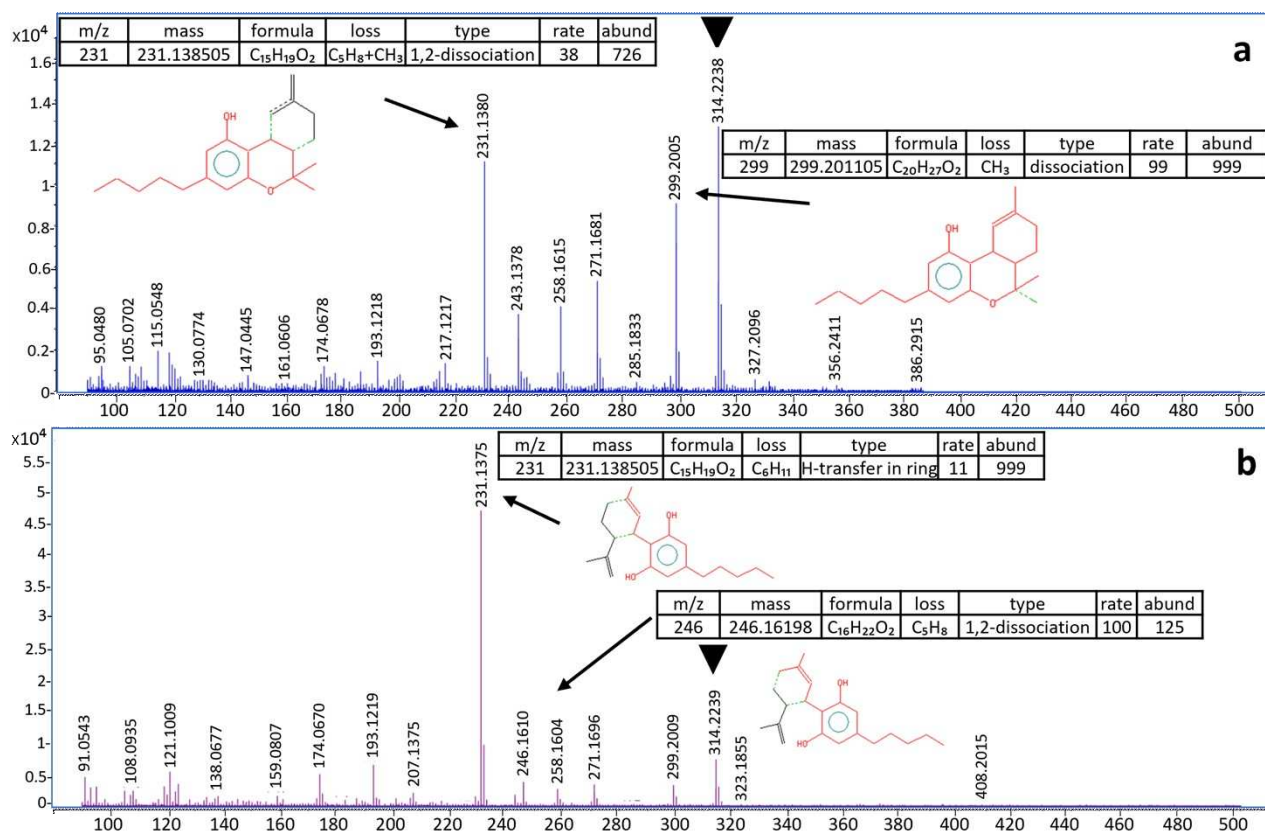


Figure S4 a-b. Recorded EI high-resolution full scan spectra of a) THC, b) CBD and assignment of the main fragments according to the MS Interpreter utility developed by NIST. Molecular ions are indicated by the black triangle.

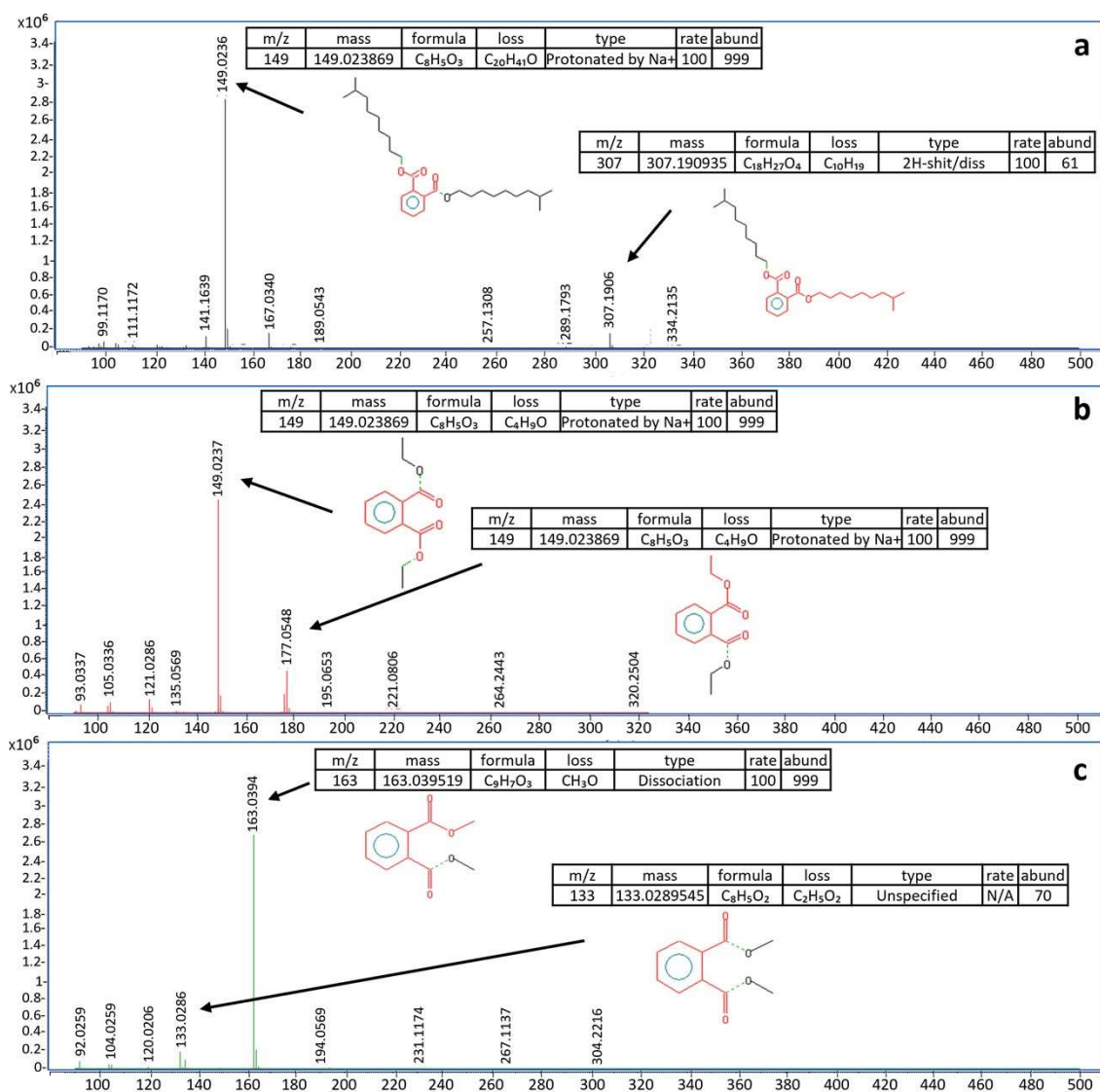


Figure S5 a-c. Recorded EI high-resolution full scan spectra of a) diisodecyl phthalate; b) diethyl phthalate; c) dimethyl phthalate and assignment of the main fragments according to the MS Interpreter utility developed by NIST.

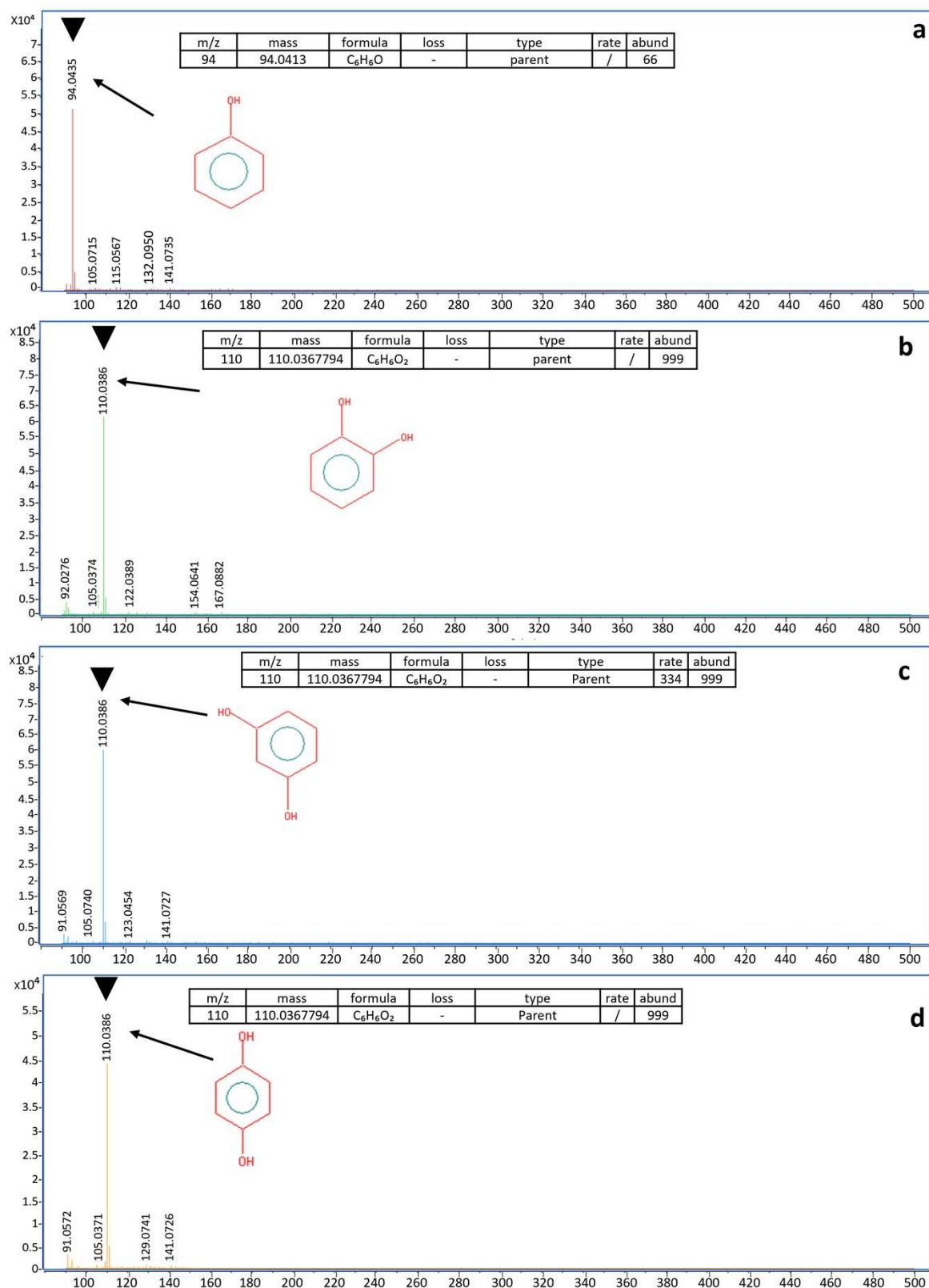


Figure S6 a-d. Recorded EI high-resolution full scan spectra of a) phenol; b) catechol; c) resorcinol; d) hydroquinone and assignment of the main fragments according to the MS Interpreter utility developed by NIST. Molecular ions are indicated by the black triangle.



Latest Developments in Direct and Non-Direct LC-MS Methods Based on Liquid Electron Ionization (LEI)

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Latest Developments in Direct and Non-Direct LC-MS Methods Based on Liquid Electron Ionization (LEI)

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ABSTRACT

Mass spectrometry (MS) enables precise identification and quantification of molecules, particularly when combined with chromatography. The advent of atmospheric pressure ionization (API) techniques allowed the efficient coupling of liquid chromatography with MS (LC-MS), extending analyses to nonvolatile and thermolabile compounds. API techniques present limitations such as low informative capacity and reproducibility of mass spectra, increasing instrument complexity and costs. Other challenges include analyzing poorly polar molecules and matrix effects (ME), which negatively impact quantitative analyses, necessitating extensive sample purification or using expensive labeled standards. These limitations prompted the exploration of alternative solutions, leading to the development of the Liquid Electron Ionization (LEI) interface. The system has demonstrated excellent robustness and reproducibility. LEI has been employed to analyze various compounds, including pesticides, drugs of abuse, phenols, polycyclic aromatic hydrocarbons (PAHs), phthalates, and many others. Its versatility has been validated with single quadrupole, triple quadrupole, and QToF detectors, operating in electron ionization (EI) or chemical ionization (CI) modes and with both reverse phase liquid chromatography (RPLC) and normal phase liquid chromatography (NPLC). LEI has also been successfully integrated with the Microfluidic Open Interface (MOI), Membrane Introduction Mass Spectrometry (MIMS), and Microfluidic Water-Assisted Trap Focusing (M-WATF), broadening its application scope and consistently demonstrating promising results in terms of sensitivity and identification power. The most recent advancement is the development of Extractive-Liquid Sampling Electron Ionization-Mass Spectrometry (E-LEI-MS), a surface sampling and real-time analysis technique based on the LEI concept. This review article offers a comprehensive and up-to-date picture of the potential of LEI.

KEYWORDS

Liquid chromatography; electron ionization; mass spectrometry; LEI; E-LEI

Introduction

Analytical techniques based on mass spectrometry (MS) are powerful tools that provide accurate compound identification and quantification.^[1–4] The mass spectrum generated from compound ionization and fragmentation can give much structural information with a minimum sample.

Initially, only volatile molecules could be analyzed using MS; indeed, the first ionization techniques developed, electron ionization (EI) and chemical ionization (CI), coupled with gas chromatography (GC), require only gas-phase molecules, excluding the possibility of analyzing nonvolatile and thermolabile substances.^[1,5] EI became the most used ionization technique due to the possibility of obtaining highly informative and reproducible spectra; working in gas-phase and high vacuum conditions, it provides library searchable spectra from different instruments, making molecule identifications easy.^[6] Library-based identification also provides sample names at the isomer level, which cannot be achieved *via* the elemental formula alone as provided by high-resolution LC-API-MS. Although EI produces unambiguous spectra, the hard ionization and the high molecule

fragmentation often do not make it possible to obtain the molecular ions of the compounds. For that reason, CI was developed by Munson and Field^[7] as a softer ionization technique coupled with GC. In CI, the electron beam impacts only the reagent gas, and then the gas ions react with the analytes, generating ions while reducing fragmentation.^[8,9] Later, to minimize fragmentation and implement molecular ion production, Amirav's group introduced Cold-EI.^[10] This ionization technique keeps the EI mass spectrum unchanged, increasing the abundance of the molecular ion and, consequently, facilitating the identification of unknown analytes.

However, GC amenable molecules were estimated to represent just 20% of the organic molecules. Hence, the possibility of coupling liquid chromatography (LC) to MS became the primary goal of extending these analytical tools to a wider variety of molecules.^[4,11] The problems of coupling LC with high vacuum ionization techniques rely on the high quantity of gas produced by mobile phase vaporization.^[1] With API, unlike EI and CI, compound ionizations occur at atmospheric pressure outside the high-vacuum

region of the MS.^[12] The API group includes atmospheric pressure chemical ionization (APCI), atmospheric pressure photoionization (APPI), and electrospray ionization (ESI). Despite APCI being one of the first ionization techniques developed for LC-MS, ESI is the most widely used. Unlike APCI, ESI does not require volatile and thermostable molecules due to its ability to form ions in solution and create multiple charged ions, allowing the analysis of compounds with higher mass concerning the m/z limit of the mass spectrometer.^[6,12,13]

Nevertheless, the excellent capabilities of API techniques can be compromised by matrix effects (ME), leading to signal suppression phenomena. This can occur when an analyte is ionized in the condensed phase during APCI or when the analyte reacts with another compound in the condensed phase to form an ion pair during ESI. Relatedly, ME complicates quantitative analyses in complex samples using API techniques. Hence, reducing the ME using these interfaces requires more accurate sample purification steps, efficient chromatographic separations, or internal standards (IS), leading to more time-consuming and higher costs.^[14–19] The lower limit of detection (LOD) and limit of quantification (LOQ) achieved with API techniques make them the preferred choice for quantitative analyses compared to the EI technique.^[20–22] Regarding the identification power of API techniques, different factors, such as mobile phase composition, ambient conditions, type of instruments, kind of ionization techniques, and polarity modes, can compromise the reproducibility of mass spectra, making identification more uncertain.^[5,22] Besides being soft ionization techniques, the production of fragments is very limited compared to EI, resulting in less information obtainable from the mass spectra. Different strategies involving tandem mass spectrometry (MS/MS) and high-resolution mass spectrometry (HRMS)^[23] were developed to enhance the identification power, and the number of MS/MS libraries for spectra comparison increased a lot.^[24] The spectra are still obtained in various conditions, unlike EI, where the conditions are universal.^[25,26] For these reasons, the possibility of coupling the LC with EI is arousing great interest.^[6,22,27] Different non-direct and direct LC-EI strategies have been developed to extend EI and CI techniques to low-volatile and thermolabile molecules, developing a universal interface for analyzing a wider variety of compounds.^[28–35] The history of EI in LC-MS was carefully described by Famiglini et al.^[6] The main problem with this approach relies on the low compatibility of the EI ion source with the liquid stream, which can break the high vacuum, inducing CI. Tal'roze and coworkers first attempted to develop the LC-EI-MS interface in the 1960s;^[36] afterward, Arpino and coworkers produced the direct liquid introduction (DLI) in 1974.^[37] As a consequence of the various problems experienced in achieving this coupling, in the same period, other LC-MS interfaces were developed trying offline approaches, where the mobile phase was separated from the analytes before entering the EI source: the moving belt (MB),^[38] the particle beam (PB),^[39] the micro flow rate particle beam (Micro-PB),^[32,40] and the Capillary-EI (Cap-EI).^[41] Despite the liquid flow elimination,

all these interfaces demonstrated a loss of sensitivity and reproducibility.^[38,40] Hence, with the introduction of the micro-LC instruments, the next attempt was focused on decreasing the solvent involved in the analyses.^[32,38,40] Direct-EI (DEI) was explored for several years, demonstrating remarkable versatility.^[29–31,35,42,43] The system involved a nano-LC (nLC) that worked at a low flow rate ($<1\ \mu\text{L}/\text{min}$), and the vaporization of the analytes and solvents occurred directly in the EI ion source. However, some limitations were still unsolved, such as the early solvent vaporization, causing capillary clogging with the consequent need to vent the instrument for replacing it, and thermal degradation of thermolabile molecules due to the contact with the hot metal of the ion source surface.^[6] Similar systems were also proposed by Rigano et al. in 2016,^[34] by Moini and coworkers,^[44] and by Lancas et al.^[28] Differently from the interface described previously, the LC-EI-MS interface developed by Amirav and coworkers^[33] was coupled with a supersonic molecular beam (SBM), and it is based on a stable pneumatic spray formation as in APCI or APPI. The SBM interface and fly-through ion source are the same as those used in GC-MS, demonstrating remarkable versatility, robustness, and high identification power.^[10,45–47]

Although different groups proposed several solutions for coupling LC with EI, the last configuration proposed by Termopoli and coworkers,^[48] called liquid electron ionization (LEI) interface, is the most employed. Different modifications have been made since its first configuration was presented in 2017.^[49,50] In addition, the same group proposed a novel prototype, coupling the ambient sampling with LEI for the first time, called Extractive-liquid sampling electron ionization-mass spectrometry (E-LEI-MS).^[51] This review carefully describes both devices and illustrates the latest innovations and applications. LEI and E-LEI-MS systems demonstrated robustness, reproducibility, and high identification power of various analytes in several matrices, enlarging the number of molecules amenable to the EI source.

Interfaces description

In 2017, the LEI interface was presented for the first time.^[48] The peculiarity of this new interface is in the vaporization of solvents and analytes at atmospheric pressure into the core of the LEI, the Vaporization Microchannel (VMC). The small diameter of VMC divides the high vacuum zone inside the MS from the atmospheric pressure zone where the vaporization occurs, helping to keep the pressure gradient. Furthermore, the VMC introduction allows for a quick replacement of the inlet capillary in case of clogging, avoiding venting the instrument as with the DEI interface. A tee junction is connected to three different capillaries: (1) VMC, (2) inlet capillary, and (3) He carrier capillary. The VMC consists of a fused silica capillary ($800\ \mu\text{m}$ O.D. and $400\ \mu\text{m}$ I.D.) positioned inside the standard GC metal transfer line that can be heated up to $400\ ^\circ\text{C}$. The VMC can be easily replaced in case of contamination. In addition, it avoids contact of the analytes with the transfer line walls, preventing the thermal degradation of the

analytes. The liquid flow reaches the VMC through the inlet capillary (150 μm O.D. and 25 μm I.D.). In the VMC, the inlet capillary releases the liquid flow and the analytes, which are vaporized due to the high temperature (Figure 1). The vaporization inside the bigger capillary solves the problem of inlet clogging caused by the precipitation of analytes

when the solvent evaporates too early. In this regard, in the first prototype, a Peltier unit was added before the entrance of the VMC to prevent early solvent vaporization. A 1.2 mL/min helium flow flows from the He carrier capillary to the VMC. Herein is mixed with vaporized solvent and analytes, helping to speed up their transfer to the ion source. An

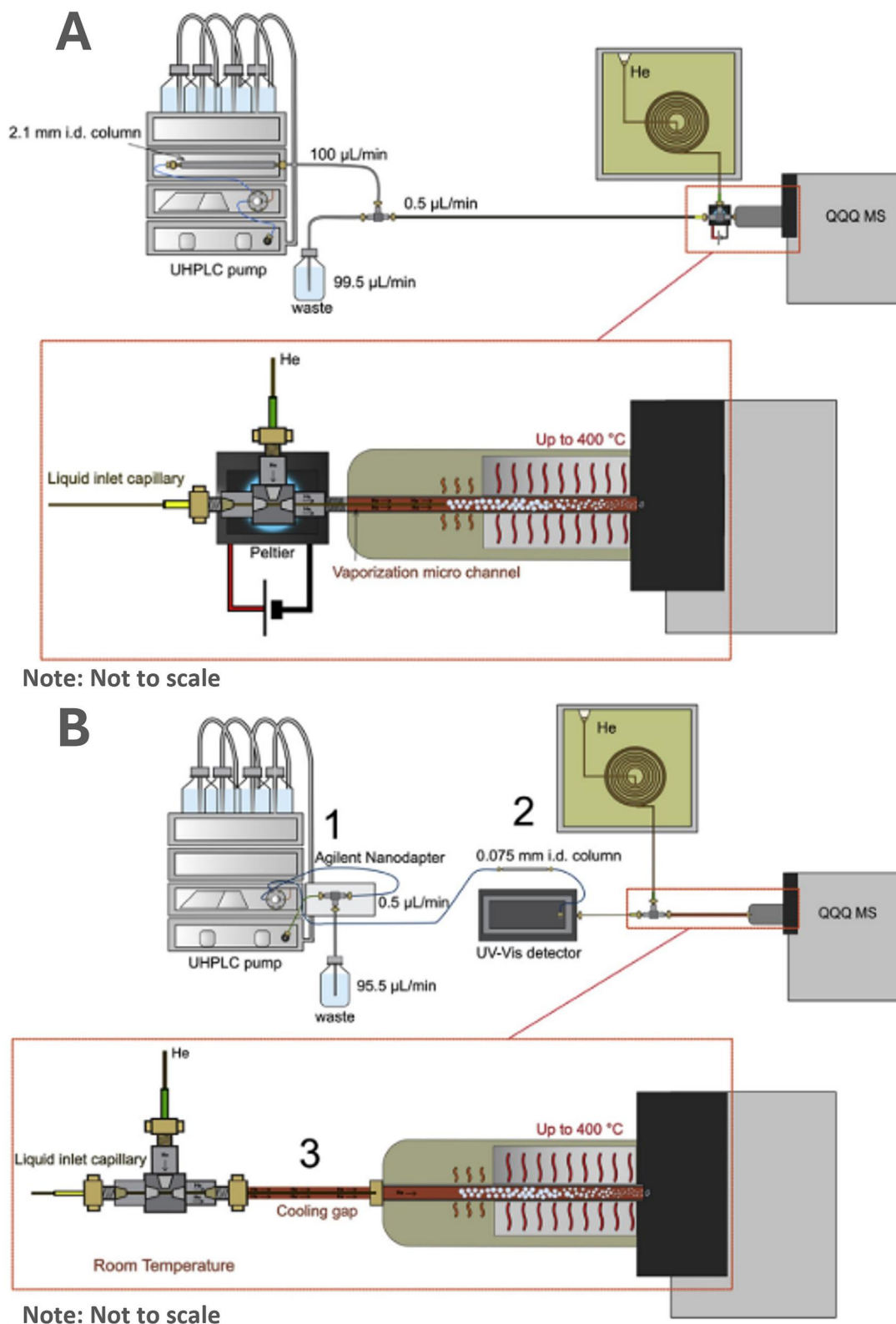


Figure 1. Schematic comparison of the LC–MS system equipped with the previous (a), and the new (b) LEI interface. 1: Nanodapter; 2: nano-LC column and online UV–vis detector; 3: cooling gap. Adapted from Ref.^[49] with permission from the copyright holder.

accurate liquid flow study was performed to fully understand the flow rate compatible with the EI ion source, suggesting that the ideal flow rate is between 300 nl/min and 800 nl/min.

A new version of the LEI interface was presented in 2019 from the same group;^[49] the main difference relies on the replacement of the Peltier unit by the so-called cooling gap. The cooling gap is realized by a longer VMC that protrudes out of the heated zone of ≈ 5 cm, avoiding the early vaporization of the solvent inside the inlet capillary. Other variables studied were the position of the inlet capillary and its dimensions. The results showed a reduction of capillary clogging and an improvement in vaporization efficiency using 25–30 μm I.D. as an inlet capillary positioned 2 mm inside the VMC (2).

More recently, a passive flow splitter (PFS) was introduced to allow higher flow rates and the use of more performing 1.0 or 2.1 i.d. LC columns (Figure 2). PFS consists of a tee junction connecting the capillary, which transports the eluate, the splitter capillary, and the inlet capillary of the LEI interface. The splitter capillary conveys a part of the liquid flow in the waste, and the length and diameter can be changed based on the flow rate used for the analyses. LEI inlet capillary and the capillary which transports eluate have fixed dimensions. The possibility of coupling LC with EI using a higher flow rate allows the expansion of the LEI interface to more applications.^[50]

Recently, a new prototype was proposed to combine EI with ambient sampling for the first time. Arigò and coworkers^[51] developed a new system called E-LEI-MS, which can perform targeted and untargeted screening in two-dimensional (2D) and three-dimensional (3D) analyses. In brief, the system's main components are the sampling tip,

the on/off valve, the tee connector, and the micromanipulator. As reported in Figure 3, two coaxial tubes form the sampling tip while the micromanipulator places it onto the sample surface. A syringe pump pushes a liquid solvent into the outside capillary; a solvent drop is deposited on the target surface, starting to extract the analytes; the internal capillary, connected to the under vacuum ion source through an on-off valve, sucks and transfers the solution inside the ion source. The on-off valve is necessary to isolate the source from the outside when sampling is not carried out. The vacuum effect enables the delivery of the extracted analytes from the sample into the ion source, and the MS signal is obtained in approximately 3 minutes. The versatility of this new device is demonstrated by the possibility of using any solvent depending on the type of surface and analytes sought.

Applications

Non-direct LC-MS systems coupled with EI

LEI interface

The possibility to couple LC with EI shows several advantages, such as the possibility to extend this ionization technique to low volatile and thermolabile molecules that usually require derivatization,^[52] reducing the time needed for the sample preparation step, and the possibility to analyze simultaneously molecules with different chemical/physical properties, which generally requires different MS-based techniques.^[53] Indeed, the fast vaporization and the short-time molecules are exposed to high temperatures using the LEI interface, prevent compound degradation and precipitation.^[48] In the first work, Termopoli and coworkers

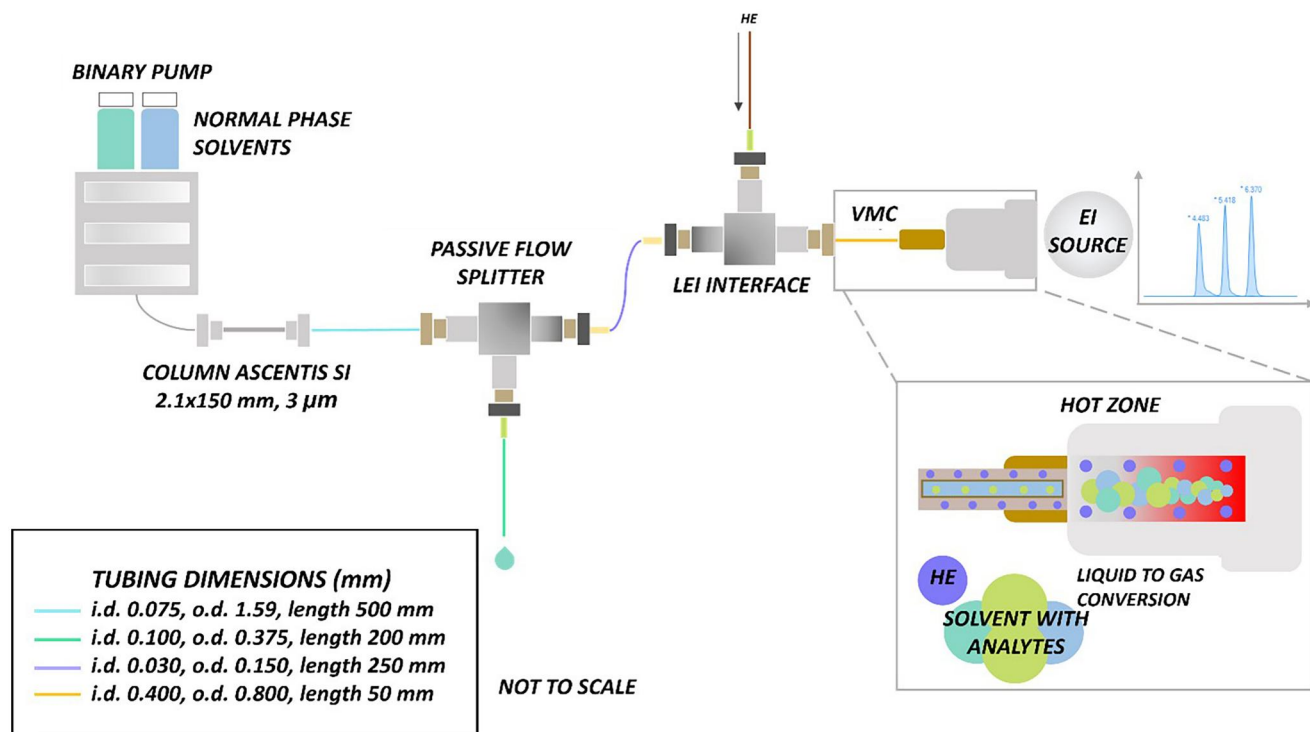
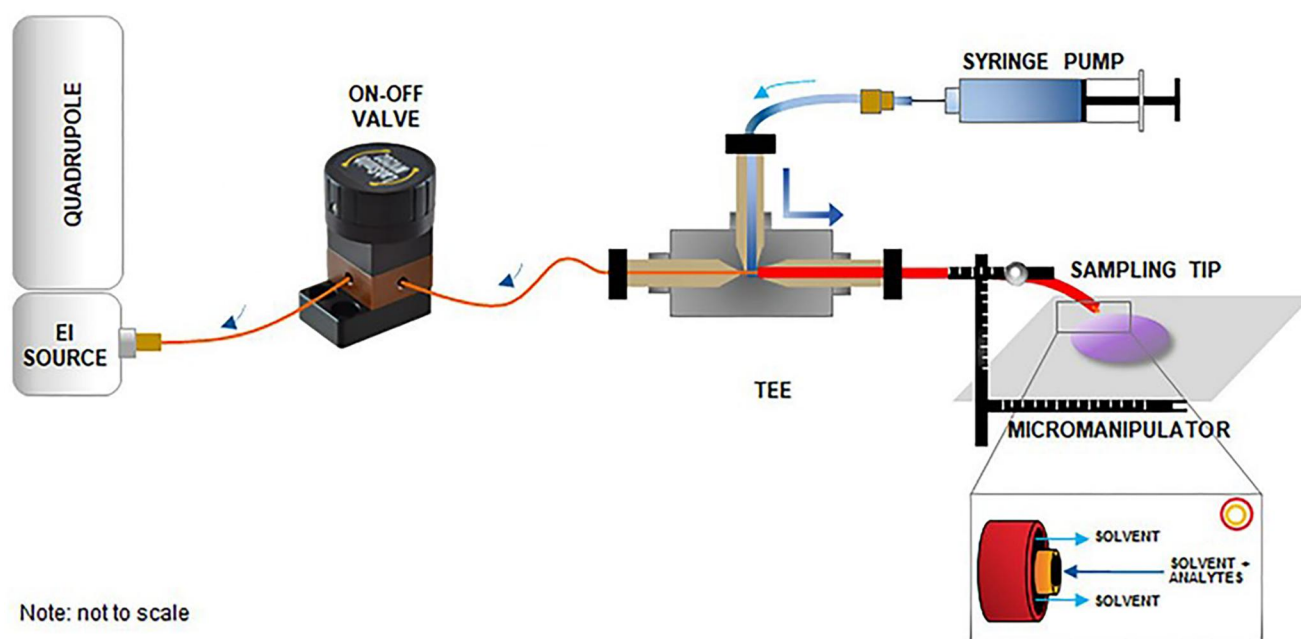


Figure 2. Scheme of the LC-LEI-MS/MS and LC-LEI-QTOF-HRMS systems. Adapted from Ref.^[77] with permission from the copyright holder.



Note: not to scale

Figure 3. Configuration of E-LEI-MS system. Adapted from Ref.^[51] with permission from the copyright holder.

performed the simultaneous analysis of 20 environmental priority pollutants with different polarities; the MS trace was compared with the UV trace, demonstrating the maintenance of a good S/N ratio, peak shape, and chromatographic resolution. In the same work, two compounds with higher molecular weight, deoxycholic acid, a bile acid, and imatinib, a chemotherapy agent not present in NIST (respectively 392.57 g/mol and 493.61 g/mol), were analyzed, demonstrating that LEI is helpful in structural elucidation and identification, of untargeted compounds.

A significant advantage of EI, already widely demonstrated,^[18] is the almost total absence of ME, which allows for a more straightforward and quicker analysis of complex samples. In the work of Cappiello and coworkers,^[54] tefluthrin, a pyrethroid pesticide commonly analyzed in GC-MS due to its non-polar nature, and dicamba, a highly polar pesticide usually analyzed with ESI-LC-MS or in GC-MS after derivatization,^[55,56] were analyzed simultaneously in a water-based commercial formulation, using LEI interface coupled with Negative Chemical Ionization (NCI). This matrix was very complex due to the presence of several other compounds and interfering additives. In addition, the analysis of dicamba in aqueous matrices using ESI requires accurate extraction procedures, mainly based on SPE,^[57,58] to the ME, which can affect ionization.^[59–61] The use of the LEI interface allowed the detection and quantification of the two pesticides only after filtration of the sample, reducing the analysis time and possible loss of sensitivity. Flow Injection Analysis (FIA)-LEI-MS/MS was successfully employed to analyze another very complex sample, olive oil, to detect and quantify Tyrosol (Tyr) and Hydroxytyrosol (Htyr).^[62] The work was complex and only possible thanks to the unique characteristics of the LEI since neither the use of chromatography nor complex sample preparation was envisaged, only the dilution of the sample. Usually, this type of analysis is based on liquid-liquid extraction before

instrumental detection, increasing time and costs.^[63–65] The ME measured was close to zero for both compounds in each sample, confirming the excellent qualitative and quantitative LEI capabilities.

One of the main limitations of the LC-EI-MS interfaces is the limited flow rate that can be employed, between 300 and 800 nL/min, to obtain the best performance. The cap-EI and DEI versions solved the problem using capillary and nanocolumns. Unfortunately, especially in the case of nanocolumns, the small injection volume (<100 nL) leads to a significant loss of sensitivity^[66] and chromatographic efficiency concerning larger columns. Different strategies were developed to overcome these limitations, such as sample enrichment using solid phase extraction (SPE)^[67] and solid phase microextraction (SPME),^[68] the use of a more sensitive detector, or large volume injection (LVI) strategies.^[69] However, offline sample preparation strategies involve a more extended analysis time and the risk of losing analytes. In the field of LVI strategies, in 2020, Termopoli et al. developed a new device called Microfluidic water-assisted trap focusing (M-WATF), which allows an increment of 400-fold of the sample injection volume in a nano column connected to the LEI interface.^[70] This device operates a water dilution of a sample dissolved in a large volume of organic solvent (up to 20 μ L) to focalize it onto a reverse phase (RP) trap column before sending it to the analytical one. M-WATF has two configurations: trap loading and elution (Figure 4). An HPLC pumps water at 20 μ L/min, and the flow is split using a tee junction (T1) in 18 μ L/min and 2 μ L/min. A part of the flow (2 μ L/min) is directed to a 6-port valve (V1), where the sample, dissolved in RP organic solvent, is loaded in a 20 μ L sample loop and pushed by the water flow in a second tee junction (T2). Herein, the sample and the split water flow are mixed with the 18 μ L/min of water to reestablish the 20 μ L/min flow rate, and they are pushed to a second 6-port valve (V2) connected to the

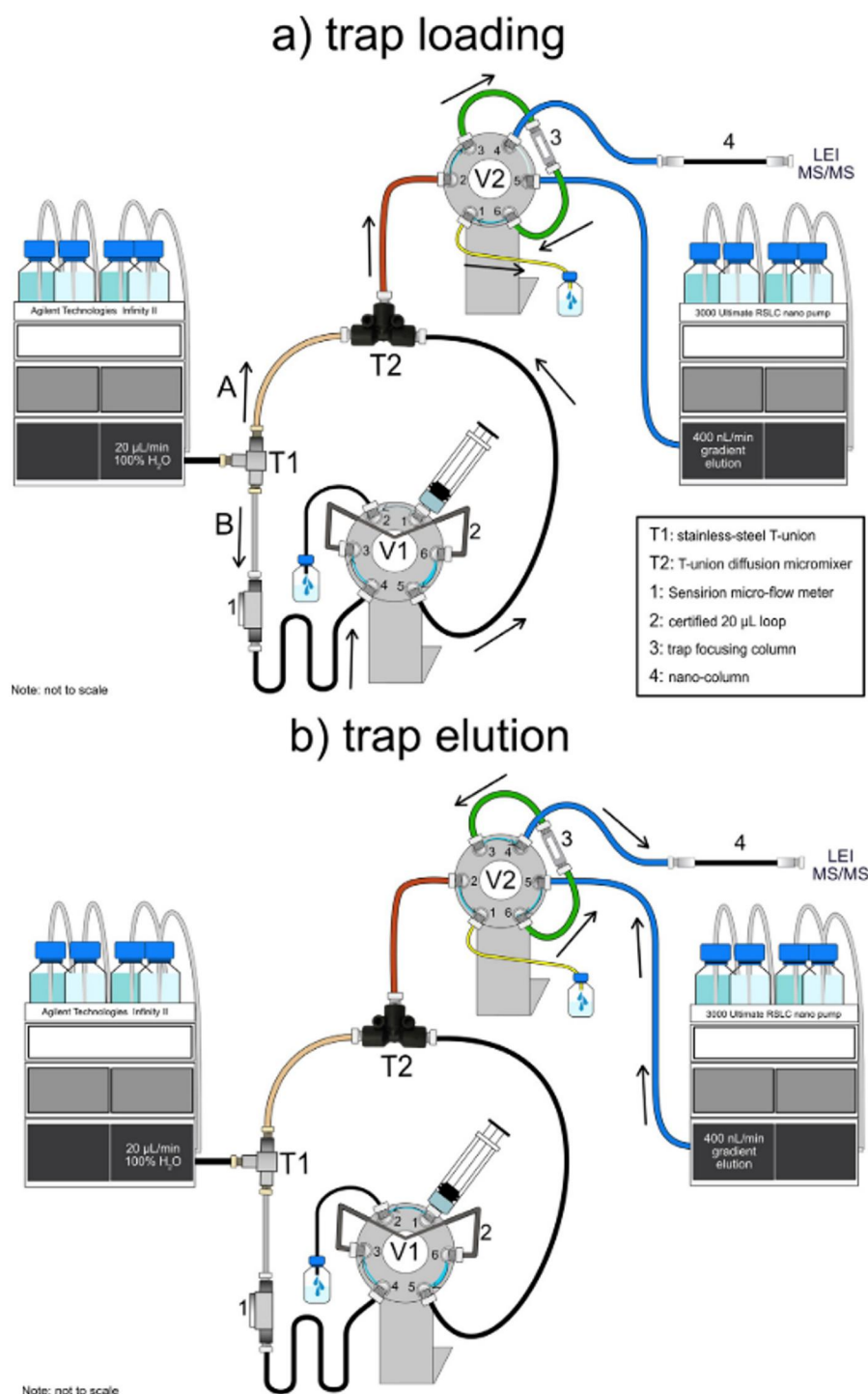


Figure 4. M-WATF operation steps in sequence: (a) trap loading; (b) trap elution. A: 18 µL/min of 100% H₂O; B: 2 µL/min of 100% H₂O. Adapted from Ref.^[70] with permission from the copyright holder.

nano-trap column. In the meantime, a second HPLC pumps the two mobile phases required to elute the trap column in back-flushing mode and perform the chromatographic separation.

Five pesticides (Clomazone, paclobutrazol, fludioxonil, metolachlor, and pirimiphos-methyl) were analyzed using the M-WATF system (Figure 5); the LOD and LOQ reached were in the range of ng/L, demonstrating that M-WATF increases significantly the sensitivity of the system using a nano-LC without affect the chromatographic resolution.^[70]

More recently, the LEI interface was coupled for the first time with a Microfluidic Open Interface (MOI) (Figure 6), allowing the direct coupling of solid phase micro-extraction (SPME) fibers with the LEI interface and the triple quadrupole MS (QQQ). The MOI-LEI-QQQ allowed direct analysis of extracted analytes without chromatographic separation due to SPME selectivity and MRM detection. This configuration for direct analysis has been tested with and without a passive flow splitter (PFS), which undoubtedly increases the speed of the process and reduces the peak width, allowing it

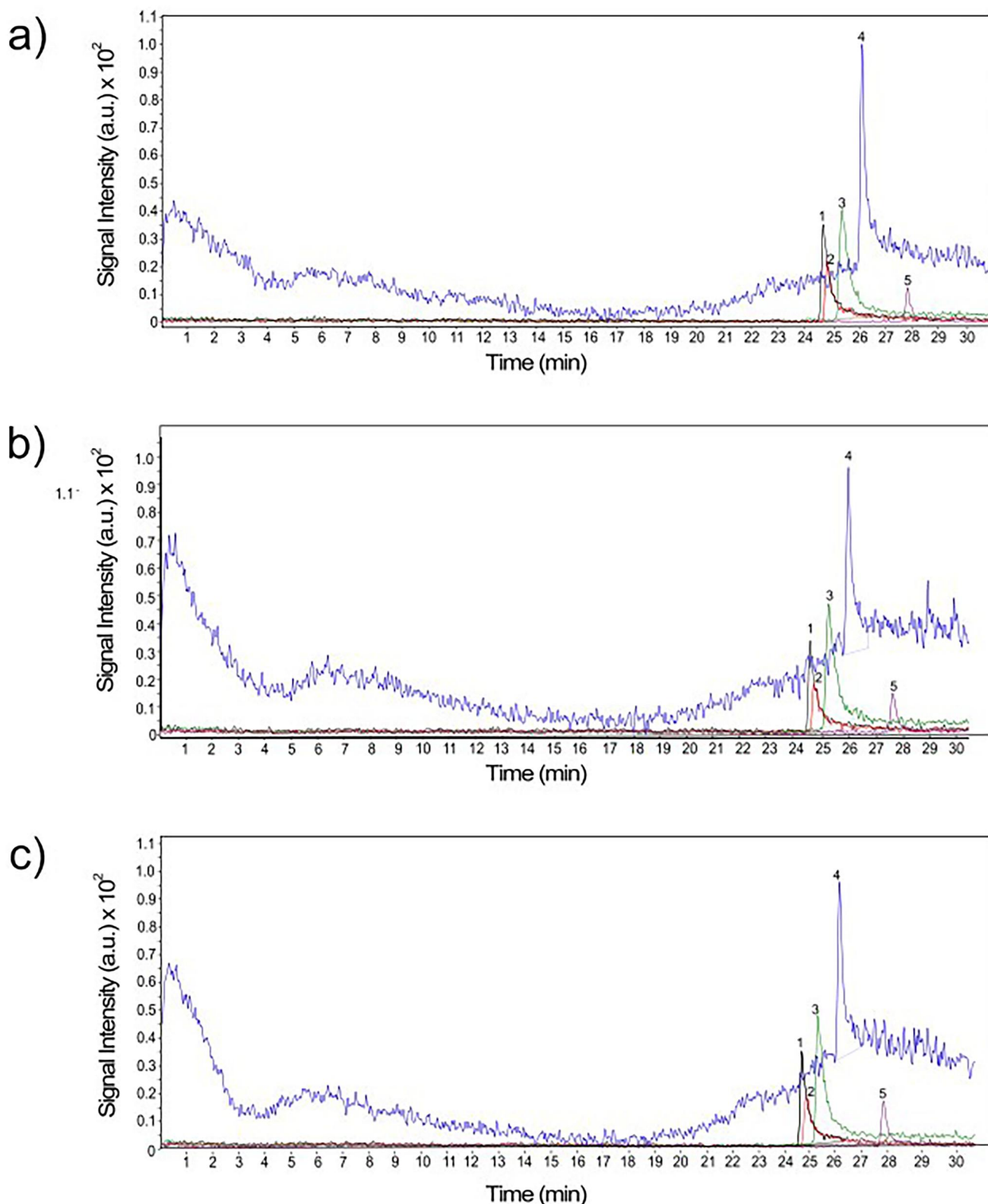


Figure 5. MRM profiles of a five-compound mixture at 10 ng/mL dissolved in a) 100% H₂O; b) 50:50 H₂O:ACN (v:v); c) 100% ACN, respectively. 1) clomazone; 2) paclobutrazol; 3) fludioxonil; 4) s-metolachlor; 5) pirimiphos-methyl. Adapted from Ref.^[70] with permission from the copyright holder.

to reach lower LOD and LOQ.^[50] As indicated in Figure 6, two steps are involved: A) stand-by – the HPLC pump, through a 6 ports-2 positions valve, pushes a solvent first into the desorption chamber of the MOI, filling it and second to the LEI interface and the MS; B) desorption – the

valve is actuated in the second position where the solvent coming from the HPLC goes directly to the waste, and MOI remains full of static solvent. The desorption chamber of the MOI is opened, and SPME fiber is introduced into it to be desorbed for one minute. After desorption, the fiber is taken

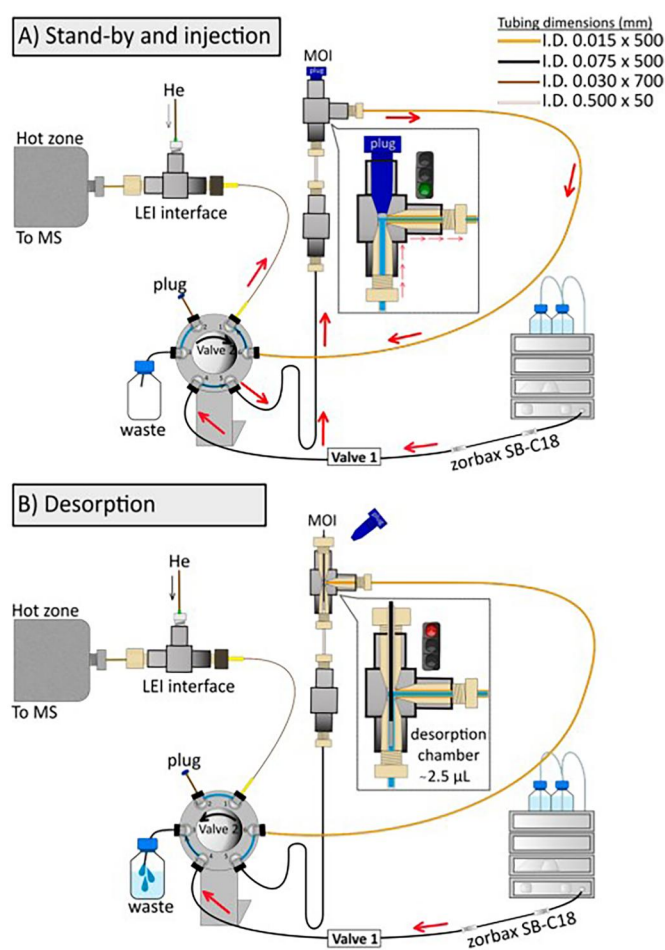


Figure 6. Schematics of the MOI-LEI-MS system. Adapted from Ref.^[71] with permission from the copyright holder.

away, and the chamber closed again; A) injection – the valve is actuated in the original position, and the solvent, coming from HPLC, transfers the content of the MOI to the LEI and the MS. The entire process takes less than 6 minutes.^[50] With this new technology, a well-known analgesic drug, fentanyl, was extracted from plasma and urine samples by the direct immersion (DI) of an SPME fiber and analyzed.^[71] Nevertheless, no sample clean-up procedure or chromatographic separation was performed, and a negligible ME was observed without using an internal standard (IS), compared with other *in situ* drug analyses based on ESI.^[72] MOI-LEI-MS/MS was successfully used to simultaneously analyze two common pesticides, dicamba and tefluthrin, into a complex commercial formulation (CF). Specificity and sensitivity were guaranteed by SPME, CI detection, and multiple reaction monitoring (MRM) acquisition mode even without chromatographic separation; in this case, the analyses showed negligible ME, as well.^[73] A comparison of the green evaluation was made between the previous work, where dicamba and tefluthrin were analyzed in CF using LC separation,^[56] and the new one based on MOI; the system MOI-LEI-QQQ showed a higher score due to the reduction of toxic solvents and energy consumption involved in the analysis, together with a higher sample throughput.^[71,74]

The versatility of the interface was again demonstrated, coupling normal phase liquid chromatography (NPLC) and

MS with the use of the LEI interface. NPLC-MS is still challenging due to the incompatibility of conventional LC-MS interfaces with non-polar mobile phases.^[75] At the same time, NPLC shows significant advantages in isomers and highly hydrophobic compound separation.^[75,76] Marittimo and coworkers^[77] realized the LC-MS separation of different compounds that benefited from NPLC due to their chemical-physical characteristics, such as tocopherols, THC and CBD, phthalates, and phenols (Figure 7). All the chromatographic separations were conducted in isocratic mode, eliminating the conditioning step and reducing the total time needed for the analyses. In addition, the mass spectra of the analytes were not modified by apolar solvents, demonstrating perfect compatibility with the LEI interface.

NPLC can be very useful when hydrophobic compounds have a too-long retention time using RPLC, and separations are time-consuming and lost in separation power. A comparison between RPLC and NPLC for hydrophobic impurities separation in a complex CF is reported (Figure 8); NPLC reduced the analysis time from 50 minutes to 13 minutes, maintaining the compound separations and EI mass spectrometry detection power.

Direct LC-MS systems coupled with EI

CP-MIMS-LEI-MS

LEI interface has also been used for direct analysis, providing quick results from real samples without chromatographic separation and sample extraction steps. An interesting coupling of the LEI interface was proposed by Vandergreef and coworkers^[78] with condensed phase membrane introduction mass spectrometry (CP-MIMS) (Figure 9). CP-MIMS involves a hollow fiber poly-dimethyl siloxane (PDMS) membrane assembled on a direct sampling probe immersed in the stirred sample; inside the membrane, the acceptor phase is pumped by an HPLC pump. The PDMS membrane allows the analytes to transfer from the sample to the acceptor phase, which is then split and vaporized in the VMC of the LEI interface.^[78–85]

Molecules from different complex matrices were analyzed using the CP-MIMS-LEI system without any sample pretreatment: 1) different polycyclic aromatic hydrocarbons (PAHs) were quantified in soil and water without any sample preparation step (Figure 10). The LOD and LOQ were in the range of ng/L for all compounds, and the R^2 was between 0.999 and 0.996.^[84] 2) phthalates were analyzed in house dust using a CI source; the authors demonstrated that the acceptor phase (acetonitrile, ACN, in this application), vaporized in the VMC, could act as reagent gas in the CI process, making the addition of a reagent gas unnecessary. Using EI and CI in the same instrument increases the potential to identify and quantify the analytes of interest.^[85] 3) benzophenone-3 (BP-3) was detected and quantified in water samples.^[78] This compound is present in many sunscreens, and being a confirmed endocrine disruptor, a sensitive method for its quantification/detection is necessary. Different analytical methods were developed to analyze BP-3 and related compounds in water; however, these methods required different purification and

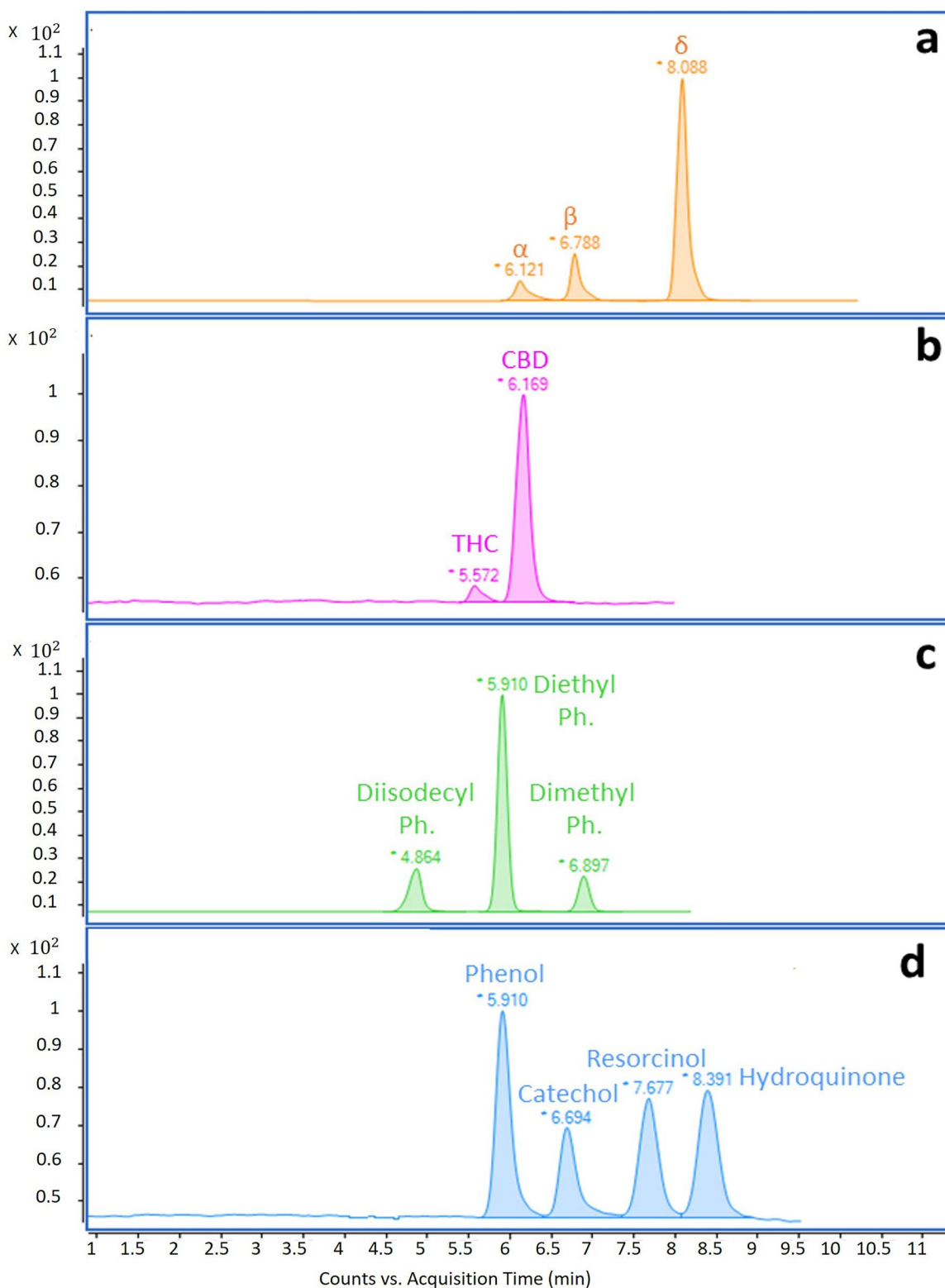


Figure 7. (a–d). NPLC MRM and SIM reconstructed ion chromatograms of the selected model compounds: (a) tocopherols; (b) cannabinoids; (c) phthalates; (d) phenols. Adapted from Ref.^[77] with permission from the copyright holder.

extraction techniques, extending the time and cost of analyses. CP-MIMS-LEI/CI requires 6 minutes of PDMS membrane immersion in the sample to have the result.^[78] In addition, due to the possibility of registering an online signal, the technique allows to obtain a geospatial assessment to correlate the number of swimmers and the quantity of BP-3 released in the water in real-time. CP-MIMS-LEI-MS was also employed

to monitor organic reactions in real-time^[80]. The catalytic oxidation of phenylacetylene to acetophenone (Figure 11) and the synthesis of alkyl glycinate (Figure 12) were observed efficiently and quantitatively following the formation of the products and the consumption of the reagents. In the second example, the simultaneous monitoring of the formation of various alkylation products is of significant synthetic interest.

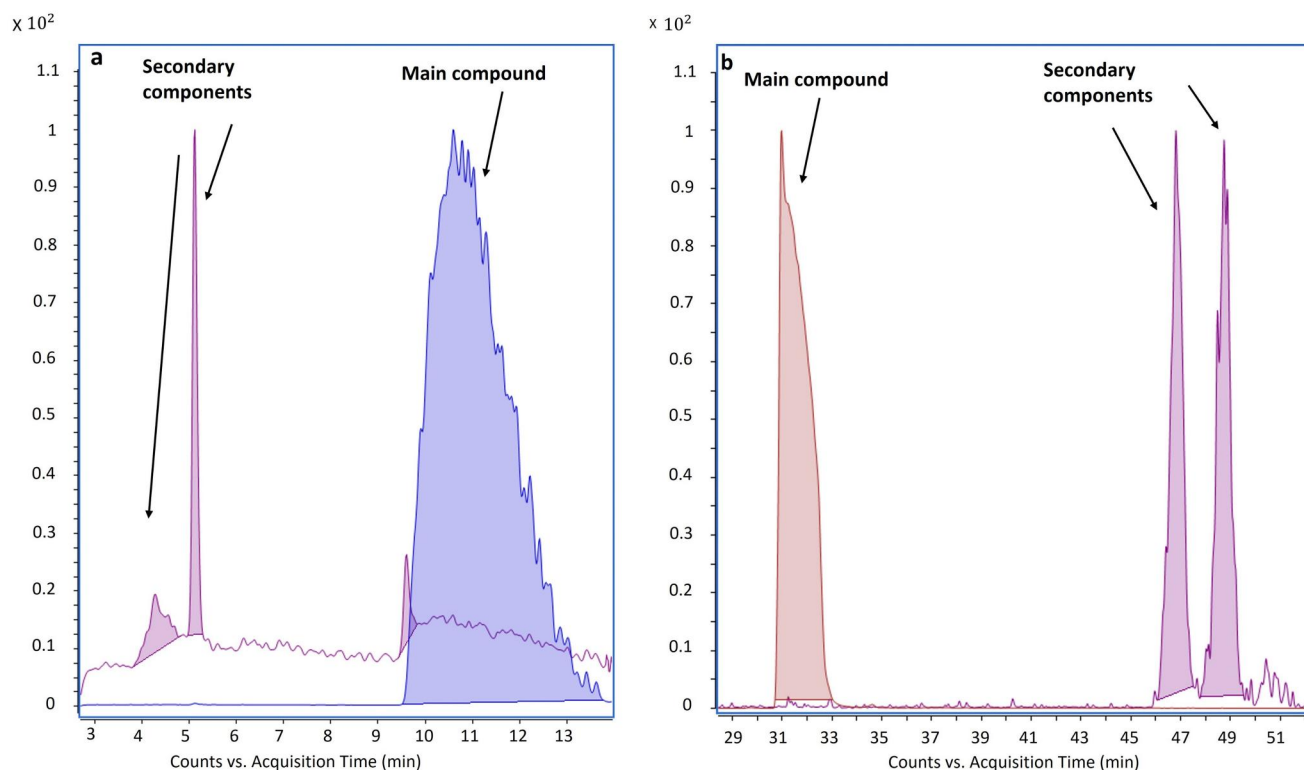


Figure 8. Extracted ion chromatograms of secondary components and main compound present in a commercial mixture obtained using the following separation conditions: (a) NPLC. A: HEX, B: ethyl acetate; gradient: 1–40% B in 25 minutes, 40–90% in 1 minute, 90% held for 10 minutes; column: Ascentis Si (2.1×150 mm, $3 \mu\text{m}$); flow rate: 0.1 mL/min; (b) RPLC. A: water + 0.3% formic acid; B: ACN + 0.3% formic acid; gradient: 0–100% B in 30 minutes, 100% B held for 20 minutes; column: Waters Acquity UHPLC C18 (1×10 mm, $1.7 \mu\text{m}$); flow rate: 0.1 mL/min. Adapted from Ref.^[77] with permission from the copyright holder.

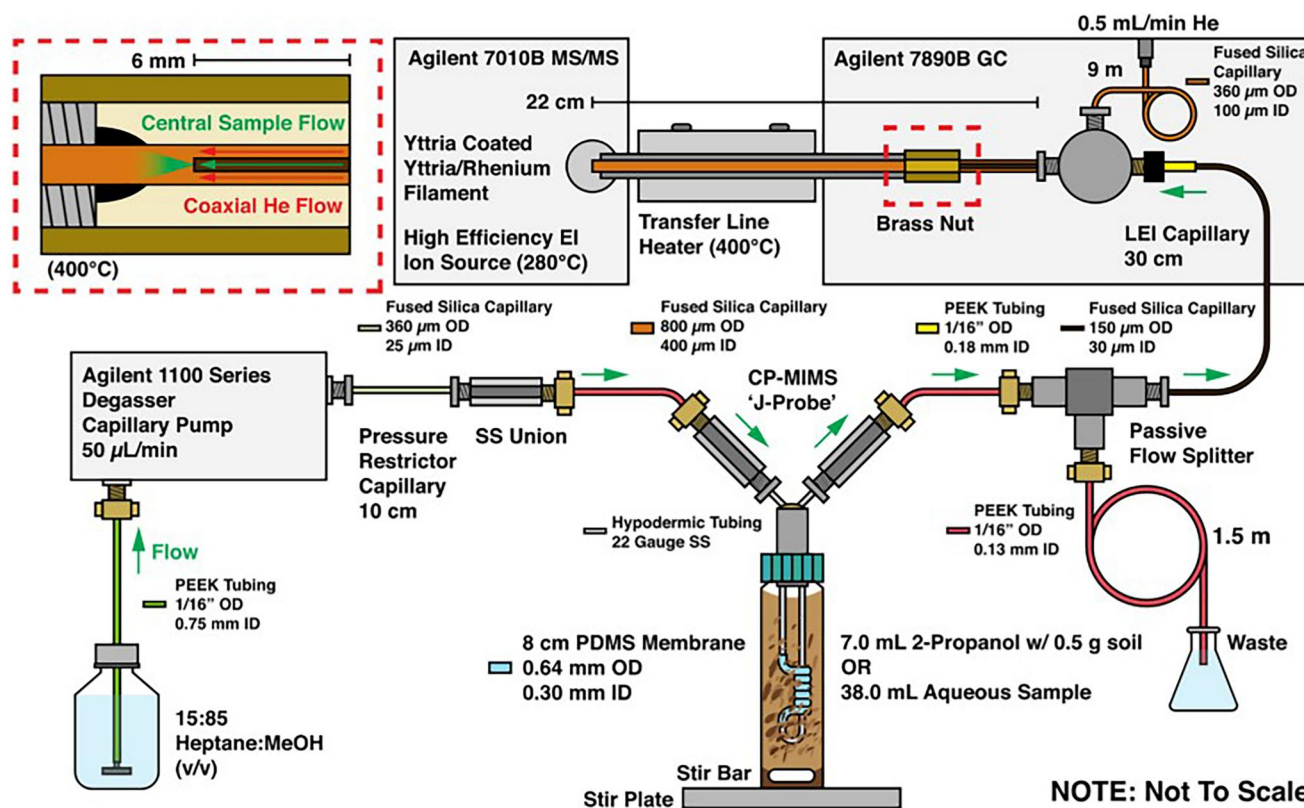


Figure 9. Schematic diagram of the CP-MIMS – LEI experimental apparatus. Adapted from Ref.^[83] with permission from the copyright holder.

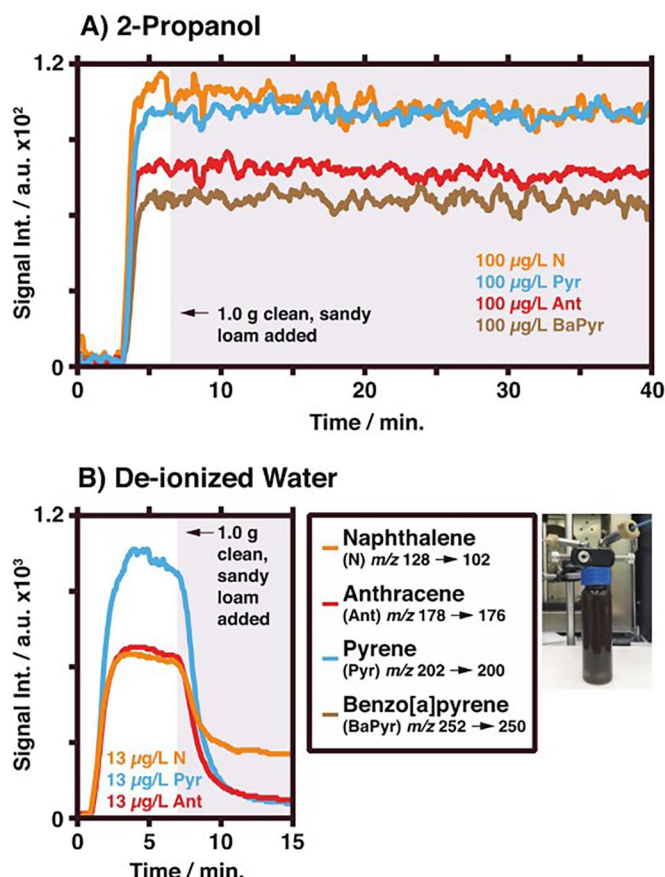


Figure 10. Comparison of signal chronograms for target PAHs in (A) 2-propanol and (B) de-ionized water (both 38.0 mL) with the addition of clean, sandy loam using CP-MIMS – LEI. Inset picture is of the aqueous sample after the addition of the clean, sandy loam. Adapted from Ref.^[83] with permission from the copyright holder.

Although the usefulness and potential of using CI with LEI interfacing have already been established, both with the use of reagent gases and with the use of the mobile phase alone, no study was performed to evaluate the role of the reagent gas in NCI together with a liquid mobile phase. Recently, a new study involving LEI coupled with NCI was presented to analyze per- and poly-fluoroalkyl substances (PFASs). Because they are persistent compounds in several matrices, PFAS analyses are acquiring much interest.^[86–90] The study aimed to find the optimal combination of some parameters that can affect the NCI mechanisms for the future development of a method for PFAS analyses. The evaluated parameters include different reagent gases, water in the mobile phase, and formic acid (FA) as a mobile phase modifier. The study confirmed a better performance of ACN alone, which can act as a reagent gas for CI, without adding other gases: the intensity of the base peak for all PFASs was magnified. At the same time, the addition of water and FA negatively affects the ionization efficiency.^[91]

E-LEI-MS

As described in the previous paragraph, the E-LEI-MS is a prototype of a surface analysis instrument that can be used for fast screening and real-time analysis.^[51] This technique represents an add-on to a common MS but can evolve into

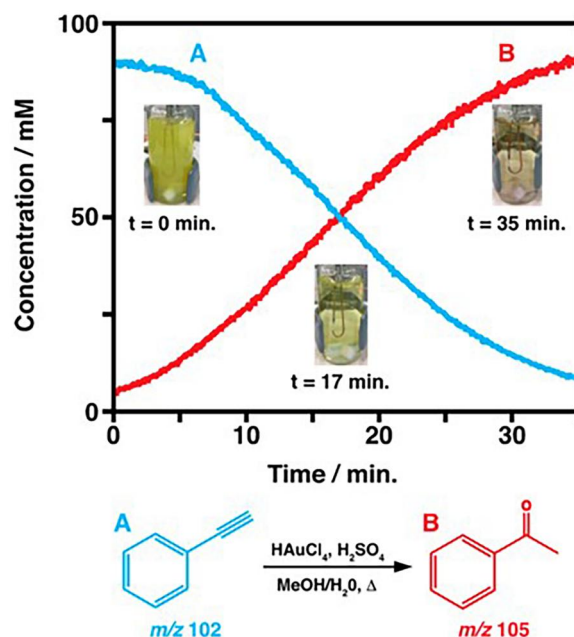


Figure 11. CP-MIMS-LEI online monitoring of the catalytic oxidation of phenylacetylene (A) to acetophenone (B) under highly acidic conditions. Adapted from Ref.^[80] with permission from the copyright holder.

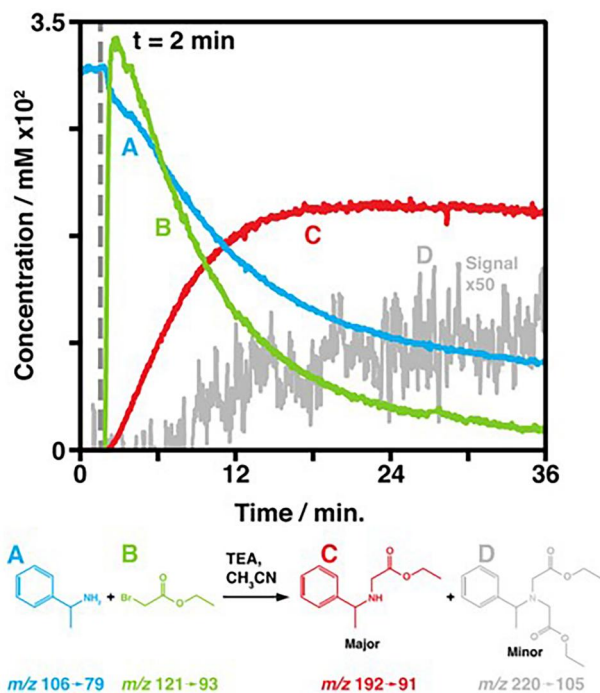


Figure 12. CP-MIMS-LEI demonstration of the quantitative online monitoring of an alkyl glycinate synthesis in dry acetonitrile with triethylamine (TEA) catalyst: (A) (R)-α-methyl benzylamine; (B) ethyl bromoacetate; (C) ethyl (R)-(1-phenylethyl)glycinate; (D) diethyl (R)-2,2'-((1-phenylethyl)-azanediyloxy)diacetate. Adapted from Ref.^[80] with permission from the copyright holder.

a small and portable instrument for in-site sampling. The present prototype has been coupled with single and triple quadrupoles in different pharmaceutical, food, forensic, and artwork applications with promising results. For targeted and untargeted analyses, full scan, SIM, and MRM acquisition modes were applied to several tablets. Despite excipients or multiple active ingredients simultaneously, the mass spectra allowed the identification of the contained

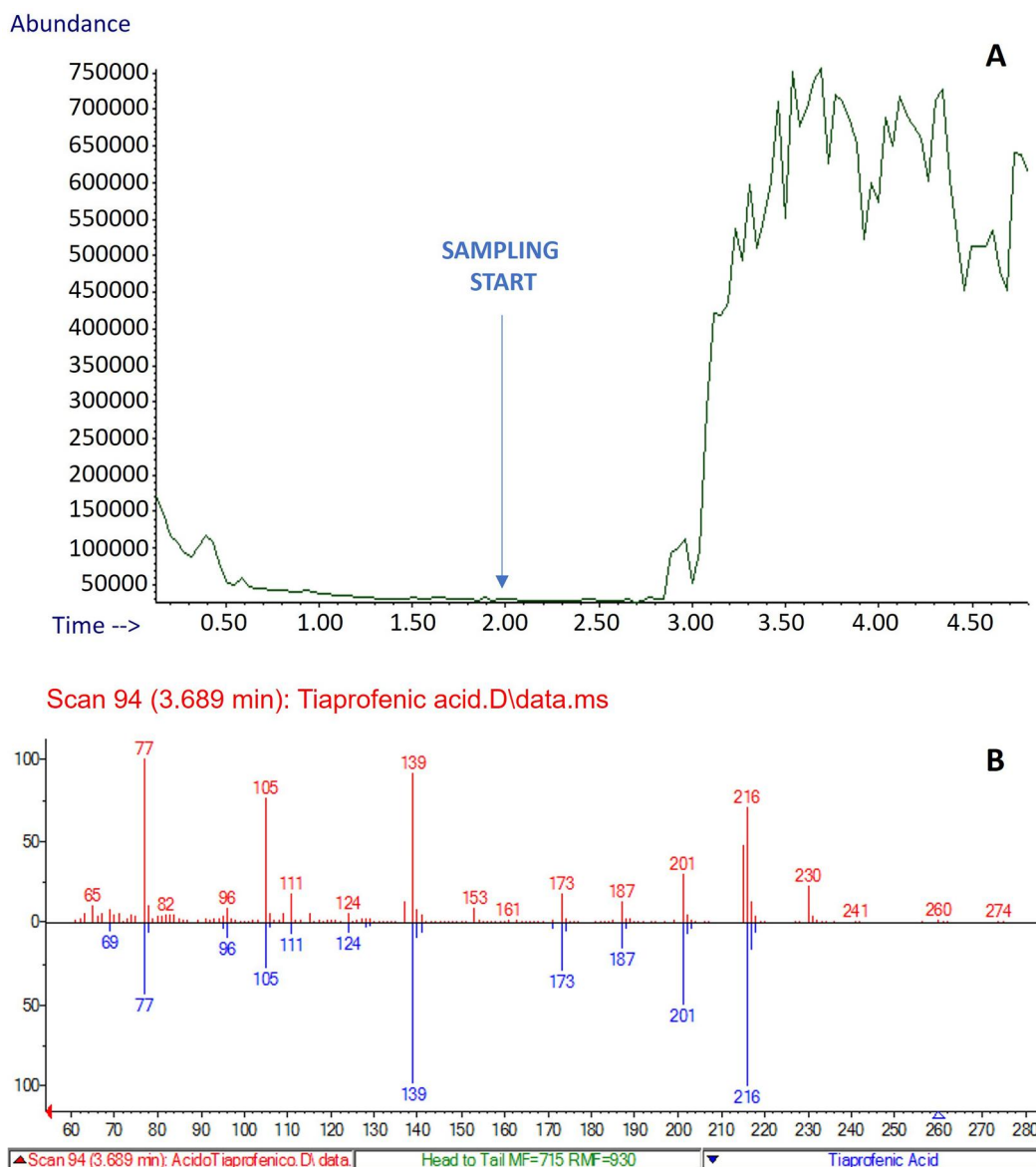


Figure 13. (A) Analysis of a Surgamyl tablet: TIC; (B) NIST library spectrum matching: red, experimental spectrum; blue, library spectrum. The arrow indicates the sampling start. Adapted from Ref.^[51] with permission from the copyright holder.

substances, showing satisfactory quality levels for untargeted analyses. In Figure 13, the detection of tiaprofenic acid on the surface of a Surgamyl tablet is reported. The mass spectrum obtained was compared with the NIST library, providing satisfactory probability values. The same good results were obtained for identifying ibuprofen in a Brufen tablet. Single ion monitoring (SIM) mode was employed to detect and separate the signals of three active principles in NeoNisidina tablets: acetylsalicylic acid, acetaminophen, and caffeine.

E-LEI-MS was involved in pesticide analysis on fruit peels, which is helpful in food quality control applications. In particular, orange peels were fortified with chlorpyrifos and imazalil at compatible concentrations and analyzed. Both compounds were detected and identified by mass spectra similar to those in the NIST library: probabilities were 90.3% and 97.0%, respectively. The same method was applied to analyze benomyl in banana peels, and good results were

obtained. Although the E-LEI, like most superficial analysis techniques, is a predominantly qualitative analysis tool, a semi-quantitative approach has also been tested. Orange peels were spiked at five different concentration levels (50, 100, 500, 1000, and 2000 mg/L) and analyzed. A calibration curve was produced with an R^2 of 0.9950. The analysis of cocaine residues on banknotes also demonstrated how the E-LEI-MS can be very useful in the forensic field. The surface of the banknote was then analyzed in both the contaminated and clean areas: the signal increased rapidly in the cocaine-spotted areas, and the spectrum of the analyte gave a high match score (>90%) with the NIST library. An interesting application where E-LEI-MS has been successfully employed was on a painting surface. The superficial analysis of a painting is fundamental to understanding how to approach a restoration process, which techniques the author used to confirm the authenticity and the attribution to a historical period. However, these analyses must respect the artwork with zero

or almost zero impact. The minimal volume of solvent used by the E-LEI-MS system allows a low impact on the paintings. If the sampling is prolonged over time, results from deeper layers can also be obtained, allowing 3D analysis. In this application, different solvents were tested due to the unknown characteristics of the surface. Acetonitrile was chosen due to its extractive and viscous properties. Two compounds, isopropyl myristate and isopropyl palmitate (Figure 14), were detected and recognized using scan mode analysis in different painting layers.^[50]

Both compounds are based components of binders utilized to fix the pigments on the layer; the first compound was found on the surface of the painting at the beginning of the sampling step; after other 3 minutes of sampling, the signal detected changed, and the spectrum recorded was that of the second compound. The solvent continued to dissolve the surface of the painting, going deeper to where the composition was different from that on the surface. Therefore, by moving the sampling tip in the two dimensions of space, it is possible to get a 2D surface mapping. Still, by staying longer at the same point, it is possible to obtain information on the layers a few millimeters deep, obtaining a 3D mapping. Despite the encouraging results, E-LEI-MS is still a prototype that can be improved.

Novel applications

LEI for non-targeted analyses (NTA) and a case study

The LEI interface represents the point of arrival of a study aimed at making LC compatible with the EI-MS, which has passed through previous projects and configurations. Having been conceived as an add-on to a standard instrument, the LEI has been successfully tested with single and triple quadrupoles using both EI and CI modes. The interface was recently coupled with a high-resolution spectrometer (QToF) to further explore the untargeted analysis capabilities of EI spectra with accurate masses. A tandem instrument, such as QToF, can be helpful for identification purposes and structural elucidation, especially with the EI fragmentation pattern. Usually, the absence of molecular ions in EI spectra can represent the principal limit for identifying unknown compounds.^[92] In this case, a low energy-capable EI source, able to operate with low energy electrons (15 eV; 9 eV), combines the benefit of high informative standard spectra with those obtained with a softer fragmentation, often providing molecular ions information.^[93] The analysis at classic 70 eV and low energies can be performed using the same ion source, giving the possibility to switch from the two modes very quickly without turning off the

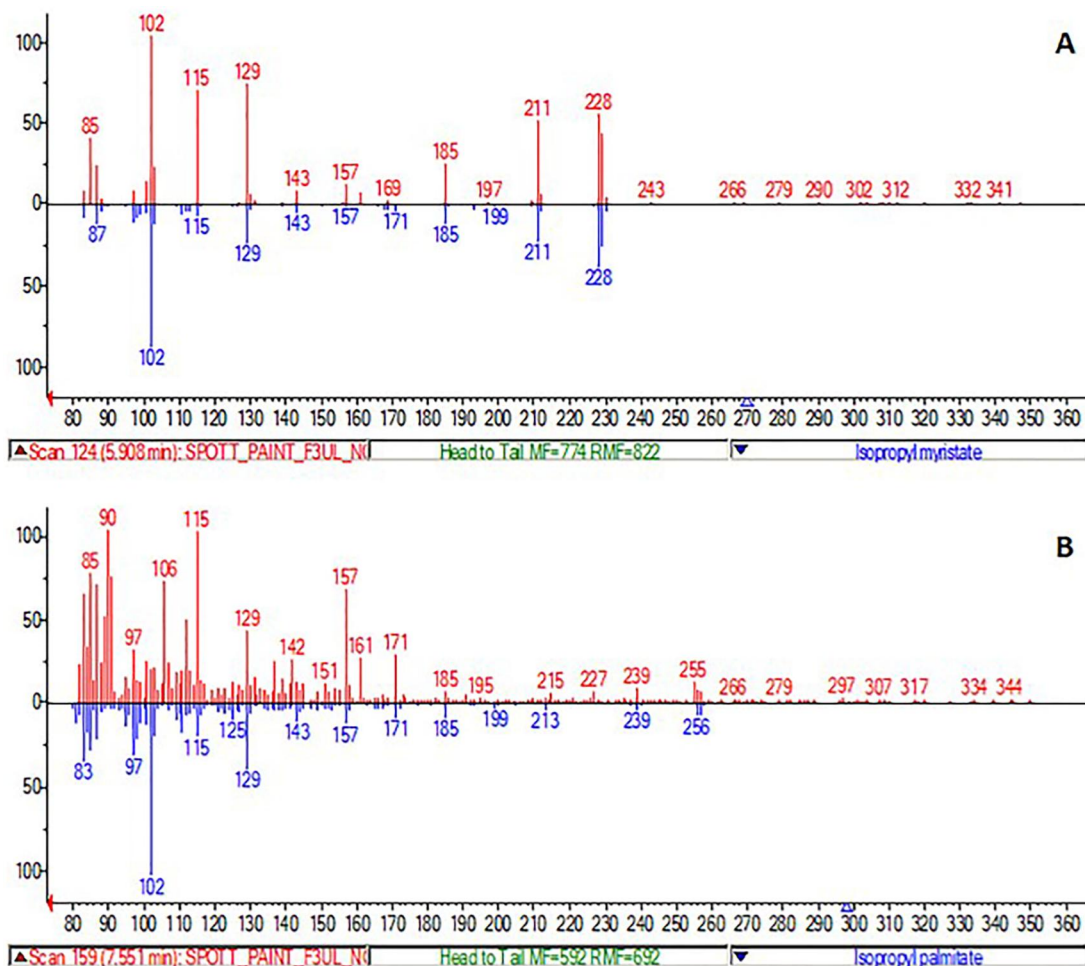


Figure 14. (A) Spectrum obtained from the analysis of the painting surface; (B) spectrum obtained from the analysis of the painting deeper layer. NIST library spectra matching: red, experimental spectra for isopropyl myristate and isopropyl palmitate; blue, library spectra. Adapted from Ref.^[51] with permission from the copyright holder.

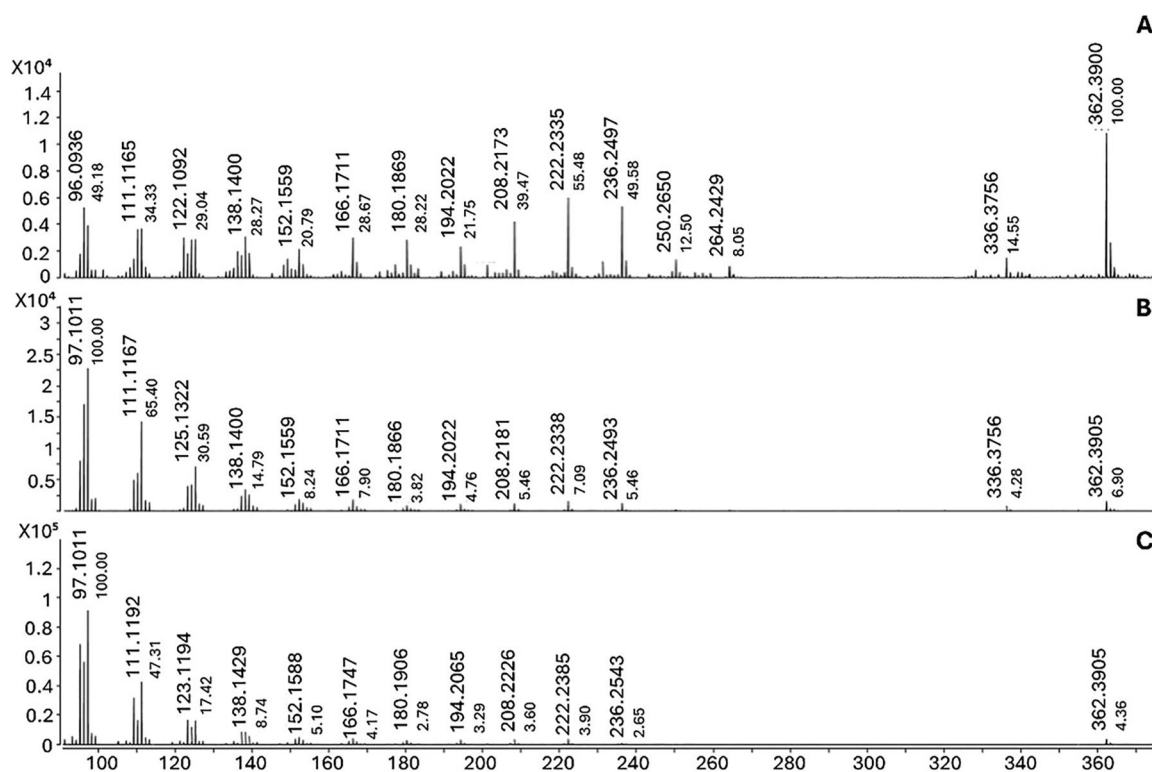


Figure 15. Impurity Y spectra were acquired at (A) 9 eV, (B) 15 eV, and (C) 70 eV.

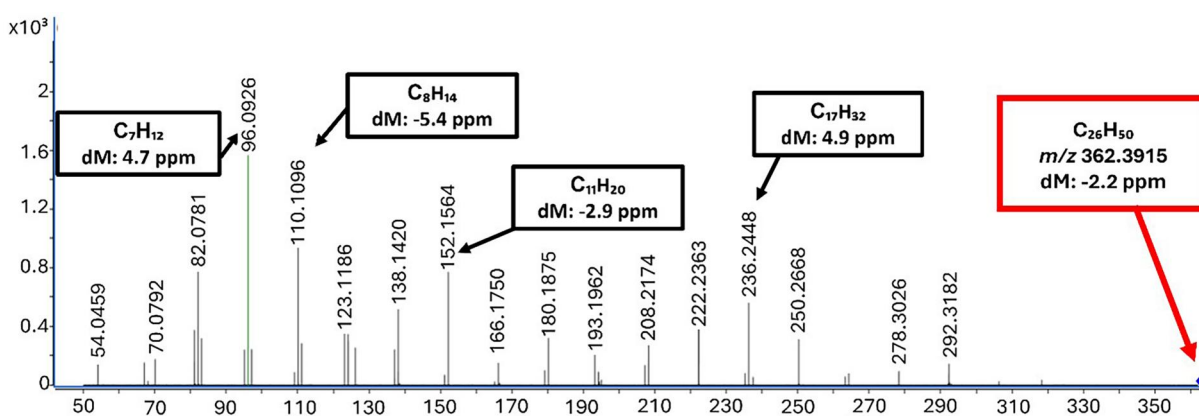


Figure 16. HR MS/MS spectrum of Impurity Y with the molecular formula and the accuracy of each fragment.

instrument. For this peculiarity, the LEI-QToF was used to develop a workflow for NTA to detect and identify an impurity (called impurity Y) present in a complex CF. Due to the high non-polar nature of impurity Y, its detection with the conventional atmospheric pressure ionization techniques was a challenge. Not knowing the nature of the substances present in the sample and considering the low concentration of impurity Y, compared to the main components of the sample, the CF itself was injected as is, without any preparation step. The LC separation was performed in NPLC using an Ascentis Si (2.1×150 mm, $3 \mu\text{m}$) column, the pump flow rate was set at $100 \mu\text{L}/\text{min}$, and the split ratio was set at 1:200 to reach the flow rate of $500 \text{ nL}/\text{min}$, which is suitable for an optimal LEI functioning. The separation was optimized in gradient mode using mobile phase A: hexane and phase B: ethyl acetate. The gradient was optimized

as follows: 1–40% B in 25 min, 40–90% in 1 minutes, and 90% held for 10 minutes. The injection volume was $1 \mu\text{L}$, and the analyses were conducted in triplicate. The analyses were performed in standard conditions at 70 eV and lower electron energies (15 eV and 9 eV) to determine the molecular ion (Figure 15). Molecular ions were necessary to reconstruct the molecular formula of the unknown compound. The suspected molecular ion (m/z 362.3940) reached 36.33% at 15 eV and 100% of relative abundance at 9 eV. The progressive increase in the presumed molecular ion's intensity as the electrons' energy decreases confirmed the choice. This ion was then fragmented in the collision cell to produce daughter ions. The high-resolution MS/MS fragmentation was used for determining the unknown compound structure using a molecular structure correlation software, which enables the assignment of the formula and structure starting

from the molecular ion fragmentation and the exact mass of each fragment, using an approach described by Alastair W. Hill and Russell J. Mortishire.^[94] MS/MS spectrum was compared with the structures in ChemSpider and PubChem databases, which assigned possible formulas and structures based on each compound's molecular ion fragmentation and exact masses. The assigned formula for the impurity Y was C₂₆H₅₀ (Figure 16).

Conclusions

The introduction of new materials and molecules permeates various fields complicating their identification and quantification. Consequently, it is essential to have tools for accurate and precise chemical analysis that can detect analytes with diverse chemical-physical properties, even at very low concentrations. The LEI interface addresses this analytical challenge by offering solutions for analytes that other instrumental techniques fail to adequately detect, positioning itself as a viable, non-competing alternative. This review aimed to collect what has been done in recent years to improve the performance of the LEI interface, the coupling with new and different instruments, and the use of CI. EI can still contribute significantly to chemical analyses due to the possibility of simultaneously detecting polar and non-polar compounds and the unparalleled tool of recognizing unknown substances represented by the mass spectrum. In addition, using low-energy-capable EI ion sources, exact masses, and CI extends the possibility of finding molecular ions, increasing the identification power. The low impact of ME has given a great impulse to use the LEI with direct analysis techniques without chromatographic separation. Therefore, E-LEI-MS and the coupling of LEI with MOI and CP-MIMS were born, opening the way for rapid analysis of superficial and complex samples. They represent the most significant innovations in the field with unpredictable margins for improvement. One of the points that certainly needs improvement is sensitivity. The low ionization efficiency of the EI is known, and it is not easy to act on it, but many technical aspects of the LEI and E-LEI still have a chance to be upgraded. One thing that is undoubtedly interesting about both systems is that they do not require particular modifications of the standard instrumentation, allowing to be installed in any of them and to switch from GC to LC very easily. However, the LEI and all instrumental couplings involving its use operate at low mobile phase flow rates or utilize splitting systems, which can limit its handling and sensitivity, respectively. Numerous substances of interest for various fields of application were already analyzed: pesticides, drugs, PFAS, PAHs, binders, and others, but many are ready to be tested with the hope of broadening the spectrum of use of a technique that is certainly useful and comparable to the others available in LC-MS.

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Disclosure statement

No potential conflict of interest was reported by the authors.

References

- [1] Watson, J. T.; Sparkman, O. D. *Introduction to Mass Spectrometry: Instrumentation, Applications, and Strategies for Data Interpretation*, 4th ed.; John Wiley & Sons, Ltd. England, 2007.
- [2] Gohlke, R. S.; McLafferty, F. W. Early Gas Chromatography/Mass Spectrometry. *J. Am. Soc. Mass Spectrom.* **1993**, *4*, 367–371. DOI: [10.1016/1044-0305\(93\)85001-E](https://doi.org/10.1016/1044-0305(93)85001-E).
- [3] Gohlke, R. S. Time-of-Flight Mass Spectrometry and Gas-Liquid Partition Chromatography. *Anal. Chem.* **1959**, *31*, 535–541. DOI: [10.1021/ac50164a024](https://doi.org/10.1021/ac50164a024).
- [4] Snyder, L. R.; Kirkland, J. J.; Dolan, J. W. *Introduction to Modern Liquid Chromatography*, 3rd ed.; John Wiley & Sons, Ltd. England, 2009.
- [5] Gross, J. H. *Mass Spectrometry. A Text Book*, 3rd ed.; Springer: Cham, Switzerland, 2017.
- [6] Famigliini, G.; Palma, P.; Termopoli, V.; Cappiello, A. The History of Electron Ionization in LC-MS, from the Early Days to Modern Technologies: A Review. *Anal. Chim. Acta.* **2021**, *1167*, 338350. DOI: [10.1016/j.aca.2021.338350](https://doi.org/10.1016/j.aca.2021.338350).
- [7] Field, F. H.; Munson, M. S. B. Chemical Ionization Mass Spectrometry. *J. Am. Chem. Soc.* **1966**, *88*, 2621–2630. DOI: [10.1021/ja00964a001](https://doi.org/10.1021/ja00964a001).
- [8] Bräkling, S.; Hinterleitner, C.; Cappellin, L.; Vetter, M.; Mayer, I.; Benter, T.; Klee, S.; Kersten, H. Gas Chromatography Coupled to Time-of-Flight Mass Spectrometry Using Parallel Electron and Chemical Ionization with Permeation Tube Facilitated Reagent Ion Control for Material Emission Analysis. *Rapid Commun. Mass Spectrom.* **2023**, *37*, e9461. DOI: [10.1002/rcm.9461](https://doi.org/10.1002/rcm.9461).
- [9] Munson, B. Chemical Ionization Mass Spectrometry. *Anal. Chem.* **1971**, *43*, 28A–43A. DOI: [10.1021/ac60307a042](https://doi.org/10.1021/ac60307a042).
- [10] Amirav, A.; Fialkov, A. B.; Gordin, A.; Elkabets, O.; Margolin Eren, K. J. Cold Electron Ionization (EI) Is Not a Supplementary Ion Source to Standard EI. It is a Highly Superior Replacement Ion Source. *J. Am. Soc. Mass Spectrom.* **2021**, *32*, 2631–2635. DOI: [10.1021/jasms.1c00241](https://doi.org/10.1021/jasms.1c00241).
- [11] Horvath, C. G.; Lipsky, S. R. Use of Liquid Ion Exchange Chromatography for the Separation of Organic Compounds. *Nature* **1966**, *211*, 748–749. DOI: [10.1038/211748a0](https://doi.org/10.1038/211748a0).
- [12] Horning, E. C.; Horning, M. G.; Carroll, D. I.; Dzidic, I.; Stillwell, R. N. New Picogram Detection System Base on Mass Spectrometer with an External Ionization Source at Atmospheric Pressure. *Anal. Chem.* **1973**, *45*, 936–943. DOI: [10.1021/ac60328a035](https://doi.org/10.1021/ac60328a035).
- [13] Sun, W.; Wu, S.; Wang, X.; Zheng, D.; Gao, Y. An Analysis of Protein Abundance Suppression in Data Dependent Liquid Chromatography and Tandem Mass Spectrometry with Tryptic Peptide Mixtures of Five Known Proteins. *Eur. J. Mass Spectrom. (Chichester)* **2005**, *11*, 575–580. DOI: [10.1255/ejms.776](https://doi.org/10.1255/ejms.776).
- [14] Nelson, M. D.; Dolan, J. W. Ion Suppression in LC-MS-MS- A Case Study. *LCGC. North Am.* **2002**, *20*, 24–32. DOI: [10.1021/acs.est.3c00663](https://doi.org/10.1021/acs.est.3c00663).
- [15] Matuszewski, B. K.; Constanzer, M. L.; Chavez-Eng, C.; M. Strategies for the Assessment of Matrix Effect in Quantitative Bioanalytical Methods Based on HPLC-MS/MS. *Anal. Chem.* **2003**, *75*, 3019–3030. DOI: [10.1021/ac020361s](https://doi.org/10.1021/ac020361s).
- [16] Cappiello, A.; Famigliini, G.; Palma, P.; Pierini, E.; Termopoli, V.; Trufelli, H. Overcoming Matrix Effects in Liquid Chromatography-Mass Spectrometry. *Anal. Chem.* **2008**, *80*, 9343–9348. DOI: [10.1021/ac8018312](https://doi.org/10.1021/ac8018312).
- [17] Taylor, P. J. Matrix Effects: The Achilles Heel of Quantitative High-Performance Liquid Chromatography-Electrospray-Tandem Mass Spectrometry. *Clin. Biochem.* **2005**, *38*, 328–334. DOI: [10.1016/j.clinbiochem.2004.11.007](https://doi.org/10.1016/j.clinbiochem.2004.11.007).

- [18] Trufelli, H.; Palma, P.; Famiglini, G.; Cappiello, A. An Overview of Matrix Effects in Liquid Chromatography-Mass Spectrometry. *Mass Spectrom. Rev.* **2011**, *30*, 491–509. DOI: [10.1002/mas.20298](https://doi.org/10.1002/mas.20298).
- [19] Robb, D. B.; Covey, T. R.; Bruins, A. P. Atmospheric Pressure Photoionization: An Ionization Method for Liquid Chromatography-Mass Spectrometry. *Anal. Chem.* **2000**, *72*, 3653–3659. DOI: [10.1021/ac0001636](https://doi.org/10.1021/ac0001636).
- [20] Cole, R. B. *Electrospray and MALDI Mass Spectrometry: Fundamentals, Instrumentation, Practicalities, and Biological Applications*, 2nd ed.; John Wiley & Sons, Inc., Hoboken, NJ, **2010**.
- [21] Yamashita, M.; Fenn, J. B. Electrospray Ion Source-Another Variation on the Free-Jet. *J. Phys. Chem.* **1984**, *88*, 4451–4459. DOI: [10.1021/j150664a002](https://doi.org/10.1021/j150664a002).
- [22] Maciel, E. V. S.; Pereira Dos Santos, N. G.; Vargas Medina, D. A.; Lanças, F. M. Electron Ionization Mass Spectrometry: Quo Vadis? *Electrophoresis* **2022**, *43*, 1587–1600. DOI: [10.1002/elps.202100392](https://doi.org/10.1002/elps.202100392).
- [23] Díaz, R.; Ibáñez, M.; Sancho, J. V.; Hernández, F. Target and Non-Target Screening Strategies for Organic Contaminants, Residues and Illicit Substances in Food, Environmental and Human Biological Samples by UHPLC-QTOF-MS. *Anal. Methods* **2012**, *4*, 196–209. DOI: [10.1039/C1AY05385J](https://doi.org/10.1039/C1AY05385J).
- [24] Kyle, J. E.; Crowell, K. L.; Casey, C. P.; Fujimoto, G. M.; Kim, S.; Dautel, S. E.; Smith, R. D.; Payne, S. H.; Metz, T. O. Liquid: An Open-Source Software for Identifying Lipids in LC-MS/MS-Based Lipidomics Data. *Bioinformatics* **2017**, *33*, 1744–1746. DOI: [10.1093/bioinformatics/btx046](https://doi.org/10.1093/bioinformatics/btx046).
- [25] Lee, S.; Hwang, S.; Seo, M.; Shin, K. B.; Kim, K. H.; Park, G. W.; Kim, J. Y.; Yoo, S. J.; No, K. T. BMDMS-NP: A Comprehensive ESI-MS/MS Spectral Library of Natural Compounds. *Phytochemistry* **2020**, *177*, 112427. DOI: [10.1016/j.phytochem.2020.112427](https://doi.org/10.1016/j.phytochem.2020.112427).
- [26] Volná, K.; Holcapek, M.; Kolárová, L.; Lemr, K.; Čáslavský, J.; Kacer, P.; Poustka, J.; Hubálek, M. Comparison of Negative Ion Electrospray Mass Spectra Measured by Seven Tandem Mass Analyzers towards Library Formation. *Rapid Commun. Mass Spectrom.* **2008**, *22*, 101–108. DOI: [10.1002/rcm.3334](https://doi.org/10.1002/rcm.3334).
- [27] Pereira dos Santos, N. G.; Maciel, E. V. S.; Vargas Medina, D. A.; Lanças, F. M. NanoLC-EI-MS: Perspectives in Biochemical Analysis. *Int. J. Mol. Sci.* **2023**, *24*, 11746. DOI: [10.3390/ijms241411746](https://doi.org/10.3390/ijms241411746).
- [28] Vargas Medina, D. A.; Burato, J. S. D. S.; Borsatto, J. V. B.; Lanças, F. M. Porous Layer Open Tubular Nano Liquid Chromatography Directly Coupled to Electron Ionization Mass Spectrometry. *J. Chromatogr. A* **2022**, *1674*, 463143. DOI: [10.1016/j.chroma.2022.463143](https://doi.org/10.1016/j.chroma.2022.463143).
- [29] Cappiello, A.; Famiglini, G.; Mangani, F.; Palma, P. A Simple Approach for Coupling Liquid Chromatography and Electron Ionization Mass Spectrometry. *J. Am. Soc. Mass Spectrom.* **2002**, *13*, 265–273. DOI: [10.1016/S1044-0305\(01\)00363-4](https://doi.org/10.1016/S1044-0305(01)00363-4).
- [30] Cappiello, A.; Famiglini, G.; Pierini, E.; Palma, P.; Trufelli, H. Advanced Liquid Chromatography-Mass Spectrometry Interface Based on Electron Ionization. *Anal. Chem.* **2007**, *79*, 5364–5372. DOI: [10.1021/ac070468l](https://doi.org/10.1021/ac070468l).
- [31] Flender, C.; Wolf, C.; Leonhard, P.; Karas, M. Nano-Liquid Chromatography—Direct Electron Ionization Mass Spectrometry: Improving Performance by a New Ion Source Adapter. *J. Mass Spectrom.* **2011**, *46*, 1004–1010. DOI: [10.1002/jms.1981](https://doi.org/10.1002/jms.1981).
- [32] Cappiello, A.; Bruner, F. Micro Flow Rate Particle Beam Interface for Capillary Liquid Chromatography/Mass Spectrometry. *Anal. Chem.* **1993**, *65*, 1281–1287. DOI: [10.1021/ac00057a028](https://doi.org/10.1021/ac00057a028).
- [33] Seemann, B.; Alon, T.; Tsizin, S.; Fialkov, A. B.; Amirav, A. Electron Ionization LC-MS with Supersonic Molecular Beams—The New Concept, Benefits and Applications. *J. Mass Spectrom.* **2015**, *50*, 1252–1263. DOI: [10.1002/jms.3695](https://doi.org/10.1002/jms.3695).
- [34] Rigano, F.; Albergamo, A.; Sciarro, D.; Beccaria, M.; Purcaro, G.; Mondello, L. Nano Liquid Chromatography Directly Coupled to Electron Ionization Mass Spectrometry for Free Fatty Acid Elucidation in Mussel. *Anal. Chem.* **2016**, *88*, 4021–4028. DOI: [10.1021/acs.analchem.6b00328](https://doi.org/10.1021/acs.analchem.6b00328).
- [35] Cappiello, A.; Famiglini, G.; Palma, P.; Siviero, A. Liquid Chromatography-Electron Ionization Mass Spectrometry: Fields of Application and Evaluation of the Performance of a Direct-EI Interface. *Mass Spectrom. Rev.* **2005**, *24*, 978–989. DOI: [10.1002/mas.20054](https://doi.org/10.1002/mas.20054).
- [36] Tal'roze, V. L.; Karpov, G. V.; Gordetskii, I. G.; Skurat, V. E. Capillary Systems for the Introduction of Liquid Mixtures into an Analytical Mass Spectrometer. *Russ. J. Phys. Chem.* **1968**, *42*, 1658–1664.
- [37] Arpino, P.; Baldwin, M. A.; McLafferty, F. W. Liquid Chromatography/Mass Spectrometry. II: Continuous Monitoring. *Biomed. Mass Spectrom.* **1974**, *1*, 80–82. DOI: [10.1002/bms.1200010117](https://doi.org/10.1002/bms.1200010117).
- [38] Hayes, M. J.; Lankmayer, E. P.; Vouros, P.; Karger, B. L.; McGuire, J. M. Moving Belt Interface with Spray Deposition for Liquid Chromatography/Mass Spectrometry. *Anal. Chem.* **1983**, *55*, 1745–1752. DOI: [10.1021/ac00261a024](https://doi.org/10.1021/ac00261a024).
- [39] Willoughby, R. C.; Browner, R. F. Monodisperse Aerosol Generation Interface for Combining Liquid Chromatography with Mass Spectroscopy. *Anal. Chem.* **1984**, *56*, 2626–2631. DOI: [10.1021/ac00278a003](https://doi.org/10.1021/ac00278a003).
- [40] Cappiello, A.; Famiglini, G. Evaluation of the Performance of a Microflow Rate LC/MS Particle. *Anal. Chem.* **1994**, *66*, 3970–3976. DOI: [10.1021/ac00094a019](https://doi.org/10.1021/ac00094a019).
- [41] Cappiello, A.; Balogh, M.; Famiglini, G.; Mangani, F.; Palma, P. An Efficient Liquid chromatography - Mass Spectrometry Interface for the Generation of Electron Ionization Spectra. *Anal. Chem.* **2000**, *72*, 3841–3846. DOI: [10.1021/ac991493x](https://doi.org/10.1021/ac991493x).
- [42] Palma, P.; Famiglini, G.; Trufelli, H.; Pierini, E.; Termopoli, V.; Cappiello, A. Electron Ionization in LC-MS: Recent Developments and Applications of the direct-EI LC-MS Interface. *Anal. Bioanal. Chem.* **2011**, *399*, 2683–2693. DOI: [10.1007/s00216-010-4637-0](https://doi.org/10.1007/s00216-010-4637-0).
- [43] Cappiello, A.; Famiglini, G.; Mangani, F.; Palma, P.; Siviero, A. Nano-High-Performance Liquid Chromatography-Electron Ionization Mass Spectrometry Approach for Environmental Analysis. *Anal. Chim. Acta* **2003**, *493*, 125–136. DOI: [10.1016/S0003-2670\(03\)00868-7](https://doi.org/10.1016/S0003-2670(03)00868-7).
- [44] Abonamah, J. V.; Eckenrode, B. A.; Moini, M. On-Site Detection of Fentanyl and Its Derivatives by Field Portable Nano-Liquid Chromatography-Electron Ionization-Mass Spectrometry (nLC-EI-MS). *Forensic Chem.* **2019**, *16*, 100180. DOI: [10.1016/j.forc.2019.100180](https://doi.org/10.1016/j.forc.2019.100180).
- [45] Amirav, A.; Gordin, A.; Poliak, M.; Fialkov, A. B. Gas Chromatography-Mass Spectrometry with Supersonic Molecular Beams. *J. Mass Spectrom.* **2008**, *43*, 141–163. DOI: [10.1002/jms.1380](https://doi.org/10.1002/jms.1380).
- [46] Fialkov, A. B.; Steiner, U.; Jones, L.; Amirav, A. A New Type of GC-MS with Advanced Capabilities. *Int. J. Mass Spectrom.* **2006**, *251*, 47–58. DOI: [10.1016/j.ijms.2005.12.045](https://doi.org/10.1016/j.ijms.2005.12.045).
- [47] Tsizin, S.; Fialkov, A. B.; Amirav, A. Electron Ionization Mass Spectrometry for Both Liquid and Gas Chromatography in One System without the Need for Hardware Adjustments. *J. Am. Soc. Mass Spectrom.* **2020**, *31*, 1713–1721. DOI: [10.1021/jasms.0c00136](https://doi.org/10.1021/jasms.0c00136).
- [48] Termopoli, V.; Famiglini, G.; Palma, P.; Piergiovanni, M.; Cappiello, A. Atmospheric Pressure Vaporization Mechanism for Coupling a Liquid Phase with Electron Ionization Mass Spectrometry. *Anal. Chem.* **2017**, *89*, 2049–2056. DOI: [10.1021/acs.analchem.6b04646](https://doi.org/10.1021/acs.analchem.6b04646).
- [49] Termopoli, V.; Famiglini, G.; Palma, P.; Piergiovanni, M.; Rocio-Bautista, P.; Ottaviani, M. F.; Cappiello, A.; Saeed, M.; Perry, S. Evaluation of a Liquid Electron Ionization Liquid Chromatography-Mass Spectrometry Interface. *J. Chromatogr. A* **2019**, *1591*, 120–130. DOI: [10.1016/j.chroma.2019.01.034](https://doi.org/10.1016/j.chroma.2019.01.034).
- [50] Marittimo, N.; Famiglini, G.; Palma, P.; Arigò, A.; Cappiello, A. Enhanced Microfluidic Open Interface for the Direct Coupling of Solid Phase Microextraction with Liquid Electron Ionization-

- Tandem Mass Spectrometry. *J. Chromatogr. A* **2022**, 1681, 463479. DOI: [10.1016/j.chroma.2022.463479](https://doi.org/10.1016/j.chroma.2022.463479).
- [51] Arigò, A.; Famiglini, G.; Marittimo, N.; Agostini, M.; Renzoni, C.; Palma, P.; Cappiello, A. Extractive-Liquid Sampling Electron Ionization-Mass Spectrometry (E-LEI-MS): A New Powerful Combination for Direct Analysis. *Sci. Rep.* **2023**, 13, 6429. DOI: [10.1038/s41598-023-33647-5](https://doi.org/10.1038/s41598-023-33647-5).
- [52] Imam, M. S.; Batubara, A. S.; Gamal, M.; Abdelazim, A. H.; Almrasy, A. A.; Ramzy, S. Adjusted Green HPLC Determination of Nirmatrelvir and Ritonavir in the New FDA Approved Co-Packaged Pharmaceutical Dosage Using Supported Computational Calculations. *Sci. Rep.* **2023**, 13, 137. DOI: [10.1038/s41598-022-26944-y](https://doi.org/10.1038/s41598-022-26944-y).
- [53] Famiglini, G.; Palma, P.; Termopoli, V.; Trufelli, H.; Cappiello, A. Single-Step LC/MS Method for the Simultaneous Determination of GC-Amenable Organochlorine and LC-Amenable Phenoxy Acidic Pesticides. *Anal. Chem.* **2009**, 81, 7373–7378. DOI: [10.1021/ac9008995](https://doi.org/10.1021/ac9008995).
- [54] Cappiello, A.; Termopoli, V.; Palma, P.; Famiglini, G.; Saeed, M.; Perry, S.; Navarro, P. Liquid Chromatography–Electron Capture Negative Ionization–Tandem Mass Spectrometry Detection of Pesticides in a Commercial Formulation. *J. Am. Soc. Mass Spectrom.* **2022**, 33, 141–148. DOI: [10.1021/jasms.1c00307](https://doi.org/10.1021/jasms.1c00307).
- [55] Catalina, M. I.; Dallüge, J.; Vreuls, R. J. J.; Brinkman, U. A. T. Determination of Chlorophenoxy Acid Herbicides in Water by in Situ Esterification Followed by in-Vial Liquid-Liquid Extraction Combined with Large-Volume on-Column Injection and Gas Chromatography-Mass Spectrometry. *J. Chromatogr. A* **2000**, 877, 153–166. DOI: [10.1016/S0021-9673\(00\)00242-9](https://doi.org/10.1016/S0021-9673(00)00242-9).
- [56] Rodríguez, I.; Rubí, E.; González, R.; Quintana, J. B.; Cela, R. On-Fibre Silylation following Solid-Phase Microextraction for the Determination of Acidic Herbicides in Water Samples by Gas Chromatography. *Anal. Chim. Acta* **2005**, 537, 259–266. DOI: [10.1016/j.aca.2004.12.083](https://doi.org/10.1016/j.aca.2004.12.083).
- [57] McManus, S. L.; Moloney, M.; Richards, K. G.; Coxon, C. E.; Danaher, M. Determination and Occurrence of Phenoxyacetic Acid Herbicides and Their Transformation Products in Groundwater Using Ultra High-Performance Liquid Chromatography Coupled to Tandem Mass Spectrometry. *Molecules* **2014**, 19, 20627–20649. DOI: [10.3390/molecules191220627](https://doi.org/10.3390/molecules191220627).
- [58] Caldas, S. S.; Arias, J. L. O.; Rombaldi, C.; Mello, L. L.; Cerqueira, M. B. R.; Martins, A. F.; Primel, E. G. Occurrence of Pesticides and PPCPs in Surface and Drinking Water in Southern Brazil: Data on 4-Year Monitoring. *J. Braz. Chem. Soc.* **2019**, 30, 71–80. DOI: [10.21577/0103-5053.20180154](https://doi.org/10.21577/0103-5053.20180154).
- [59] Guo, H.; Riter, L. S.; Wujcik, C. E.; Armstrong, D. W. Quantitative Analysis of Dicamba Residues in Raw Agricultural Commodities with the Use of Ion-Pairing Reagents in LC-ESI-MS/MS. *Talanta* **2016**, 149, 103–109. DOI: [10.1016/j.talanta.2015.11.043](https://doi.org/10.1016/j.talanta.2015.11.043).
- [60] Ghaste, M.; Hayden, N. C.; Osterholt, M. J.; Young, J.; Young, B.; Widhalm, J. R. Evaluation of a Stable Isotope-Based Direct Quantification Method for Dicamba Analysis from Air and Water Using Single-Quadrupole LC-MS. *Molecules* **2020**, 25, 3649. DOI: [10.3390/molecules25163649](https://doi.org/10.3390/molecules25163649).
- [61] Marić, S.; Jocić, A.; Tekić, D.; Mušović, J.; Milićević, J.; Dimitrijević, A. Customizable Cholinium-Based Aqueous Biphasic Systems as Ecofriendly Extraction Platform for Removal of Pesticide from Wastewaters. *Sep. Pur. Technol.* **2024**, 340, 126609. DOI: [10.1016/j.seppur.2024.126609](https://doi.org/10.1016/j.seppur.2024.126609).
- [62] Termopoli, V.; Piergiorganni, M.; Cappiello, A.; Palma, P.; Famiglini, G. Tyrosol and Hydroxytyrosol Determination in Extra Virgin Olive Oil with Direct Liquid Electron Ionization–Tandem Mass Spectrometry. *Separations* **2021**, 8, 173. DOI: [10.3390/separations8100173](https://doi.org/10.3390/separations8100173).
- [63] Khan, A.; Nadeem, M.; Ullah, R.; Gulzar, N.; Al-Asmari, F.; Imran, M.; Rahim, M. A.; Zongo, E.; Hussain, I.; Tayyab, M.; Almalki, R. S. Fatty Acid Composition, Phenolic Compounds, Phytosterols, and Lipid Oxidation of Single- and Double-Fractionated Olein of Safflower Oil Produced by Low-Temperature Crystallization. *ACS Omega*. **2023**, 9, 6787–6796. DOI: [10.1021/acsomega.3c08099](https://doi.org/10.1021/acsomega.3c08099).
- [64] Senizza, B.; Ganugi, P.; Trevisan, M.; Lucini, L. Combining Untargeted Profiling of Phenolics and Sterols, Supervised Multivariate Class Modelling and Artificial Neural Networks for the Origin and Authenticity of Extra-Virgin Olive Oil: A Case Study on Taggiasca Ligure. *Food Chem.* **2023**, 404, 134543. DOI: [10.1016/j.foodchem.2022.134543](https://doi.org/10.1016/j.foodchem.2022.134543).
- [65] Mohamed, M. B.; Rocchetti, G.; Montesano, D.; Ali, S. B.; Guasmi, F.; Grati-Kamoun, N.; Lucini, L. Discrimination of Tunisian and Italian Extra-Virgin Olive Oils according to Their Phenolic and Sterolic Fingerprints. *Food Res. Int.* **2018**, 106, 920–927. DOI: [10.1016/j.foodres.2018.02.010](https://doi.org/10.1016/j.foodres.2018.02.010).
- [66] Zotou, A. An Overview of Recent Advances in HPLC Instrumentations. *Cent. Eur. J. Chem* **2012**, 10, 554–569. DOI: [10.2478/s11532-011-0161-0](https://doi.org/10.2478/s11532-011-0161-0).
- [67] Cappiello, A.; Famiglini, G.; Palma, P.; Mangani, F. Trace Level Determination of Organophosphorus Pesticides in Water with the New Direct-Electron Ionization in LC/MS Interface. *Anal. Chem.* **2002**, 74, 3547–3554. DOI: [10.1021/ac015685f](https://doi.org/10.1021/ac015685f).
- [68] González-Fuenzalida, R. A.; Moliner-Martínez, Y.; Molins-Legua, C.; Campíns-Falcó, P. Miniaturized Liquid Chromatography Coupled on-Line to in-Tube Solid-Phase Microextraction for Characterization of Metallic Nanoparticles Using Plasmonic Measurements. A Tutorial. *Anal. Chim. Acta*. **2019**, 1045, 23–41. DOI: [10.1016/j.aca.2018.07.073](https://doi.org/10.1016/j.aca.2018.07.073).
- [69] Buonasera, K.; D’Orazio, G.; Fanali, S.; Dugo, P.; Mondello, L. Separation of Organophosphorus Pesticides by Using Nano-Liquid Chromatography. *J. Chromatogr. A* **2009**, 1216, 3970–3976. DOI: [10.1016/j.chroma.2009.03.005](https://doi.org/10.1016/j.chroma.2009.03.005).
- [70] Termopoli, V.; Famiglini, G.; Vocale, P.; Morini, G. L.; Palma, P.; Rocío-Bautista, P.; Saeed, M.; Perry, S.; Cappiello, A. Microfluidic Water-Assisted Trap Focusing Method for Ultra-Large Volume Injection in Reversed-Phase Nano-Liquid Chromatography Coupled to Electron Ionization Tandem-Mass Spectrometry. *J. Chromatogr. A* **2020**, 1627, 461421. DOI: [10.1016/j.CHROMA.2020.461421](https://doi.org/10.1016/j.CHROMA.2020.461421).
- [71] Rocío-Bautista, P.; Famiglini, G.; Termopoli, V.; Palma, P.; Nazdrajić, E.; Pawliszyn, J.; Cappiello, A. Direct Coupling of Bio-SPME to Liquid Electron Ionization-MS/MS via a Modified Microfluidic Open Interface. *J. Am. Soc. Mass Spectrom.* **2021**, 32, 262–269. DOI: [10.1021/jasms.0c00303](https://doi.org/10.1021/jasms.0c00303).
- [72] Liu, S.; Xu, W.; Zhai, Y. Swab-in-Capillary Electrospray Ionization and a Miniature Mass Spectrometer for In Situ Drug Analysis. *Anal. Chem.* **2023**, 95, 16987–16995. DOI: [10.1021/acs.analchem.3c03279](https://doi.org/10.1021/acs.analchem.3c03279).
- [73] Marittimo, N.; Grasselli, G.; Arigò, A.; Famiglini, G.; Palma, P.; Saeed, M.; Perry, S.; Navarro, P.; Clarke, P.; Brittin, M.; Cappiello, A. Sustainable and Rapid Determination of Two Halogenated Pesticides in a Commercial Formulation by Solid Phase Microextraction and Liquid Phase Chemical Ionization Mass Spectrometry. *Separations* **2023**, 10, 325. DOI: [10.3390/separations10060325](https://doi.org/10.3390/separations10060325).
- [74] Sajid, M.; Plotka-Wasyłka, J. Green Analytical Chemistry Metrics: A Review. *Talanta* **2022**, 238, 123046. DOI: [10.1016/j.talanta.2021.123046](https://doi.org/10.1016/j.talanta.2021.123046).
- [75] Jiang, P.; Lucy, C. A. Coupling Normal Phase Liquid Chromatography with Electrospray Ionization Mass Spectrometry: Strategies and Applications. *Anal. Methods* **2016**, 8, 6478–6488. DOI: [10.1039/C6AY01419D](https://doi.org/10.1039/C6AY01419D).
- [76] Cooper, W. T. Normal-Phase Liquid Chromatography. In *Encyclopedia of Analytical Chemistry*; Meyers, R. A.; Dorsey, J. G., Eds.; John Wiley & Sons: UK, **2006**. DOI: [10.1002/9780470027318.a5913](https://doi.org/10.1002/9780470027318.a5913).
- [77] Marittimo, N.; Grasselli, G.; Arigò, A.; Famiglini, G.; Agostini, M.; Renzoni, C.; Palma, P.; Cappiello, A. Liquid Electron Ionization-Mass Spectrometry as a Novel Strategy for Integrating Normal-Phase Liquid Chromatography with Low

- and High-Resolution Mass Spectrometry. *Analyst* **2024**, *149*, 2664–2670. DOI: [10.1039/D3AN02109B](https://doi.org/10.1039/D3AN02109B).
- [78] Vandergrift, G. W.; Lattanzio-Battle, W.; Rodgers, T. R.; Atkinson, J. B.; Krogh, E. T.; Gill, C. G. Geospatial Assessment of Trace-Level Benzophenone-3 in a Fish-Bearing River Using Direct Mass Spectrometry. *ACS Est. Water* **2022**, *2*, 262–267. DOI: [10.1021/acsestwater.1c00205](https://doi.org/10.1021/acsestwater.1c00205).
- [79] Monaghan, J.; Steenis, D.; Vander Meulen, I. J.; Peru, K. M.; Headley, J. V.; Gill, C. G.; Krogh, E. T. Online Membrane Sampling for the Mass Spectrometric Analysis of Oil Sands Process Affected Water-Derived Naphthenic Acids in Real-World Samples. *Separations* **2023**, *10*, 228. DOI: [10.3390/separations10040228](https://doi.org/10.3390/separations10040228).
- [80] Termopoli, V.; Torrisi, E.; Famigliani, G.; Palma, P.; Zappia, G.; Cappiello, A.; Vandergrift, G. W.; Zvekic, M.; Krogh, E. T.; Gill, C. G. Mass Spectrometry Based Approach for Organic Synthesis Monitoring. *Anal. Chem.* **2019**, *91*, 11916–11922. DOI: [10.1021/acs.analchem.9b02681](https://doi.org/10.1021/acs.analchem.9b02681).
- [81] Termopoli, V.; Piergiovanni, M.; Ballabio, D.; Consonni, V.; Cruz Muñoz, E.; Gosetti, F. Condensed Phase Membrane Introduction Mass Spectrometry: A Direct Alternative to Fully Exploit the Mass Spectrometry Potential in Environmental Sample Analysis. *Separations* **2023**, *10*, 139. DOI: [10.3390/separations10020139](https://doi.org/10.3390/separations10020139).
- [82] Monaghan, J.; Xin, Q.; Aplin, R.; Jaeger, A.; Heshka, N. E.; Hounjet, L. J.; Gill, C. G.; Krogh, E. T. Aqueous Naphthenic Acids and Polycyclic Aromatic Hydrocarbons in a Meso-Scale Spill Tank Affected by Diluted Bitumen Analyzed Directly by Membrane Introduction Mass Spectrometry. *J. Hazard. Mater.* **2022**, *440*, 129798. DOI: [10.1016/j.jhazmat.2022.129798](https://doi.org/10.1016/j.jhazmat.2022.129798).
- [83] Vandergrift, G. W.; Monaghan, J.; Krogh, E. T.; Gill, C. G. Direct Analysis of Polyaromatic Hydrocarbons in Soil and Aqueous Samples Using Condensed Phase Membrane Introduction Tandem Mass Spectrometry with Low-Energy Liquid Electron Ionization. *Anal. Chem.* **2019**, *91*, 1587–1594. DOI: [10.1021/acs.analchem.8b04949](https://doi.org/10.1021/acs.analchem.8b04949).
- [84] Vandergrift, G. W.; Krogh, E. T.; Gill, C. G. Direct, Isomer-Specific Quantitation of Polycyclic Aromatic Hydrocarbons in Soils Using Membrane Introduction Mass Spectrometry and Chemical Ionization. *Anal. Chem.* **2020**, *92*, 15480–15488. DOI: [10.1021/acs.analchem.0c03259](https://doi.org/10.1021/acs.analchem.0c03259).
- [85] Vandergrift, G. W.; Lattanzio-Battle, W.; Krogh, E. T.; Gill, C. G. Condensed Phase Membrane Introduction Mass Spectrometry with in Situ Liquid Reagent Chemical Ionization in a Liquid Electron Ionization Source (CP-MIMS-LEI/CI). *J. Am. Soc. Mass Spectrom.* **2020**, *31*, 908–916. DOI: [10.1021/acs.analchem.0c03259](https://doi.org/10.1021/acs.analchem.0c03259).
- [86] D'Agostino, L. A.; Mabury, S. A. Certain Perfluoroalkyl and Polyfluoroalkyl Substances Associated with Aqueous Film Forming Foam Are Widespread in Canadian Surface Waters. *Environ. Sci. Technol.* **2017**, *51*, 13603–13613. DOI: [10.1021/acs.est.7b03994](https://doi.org/10.1021/acs.est.7b03994).
- [87] Newton, S.; McMahan, R.; Stoeckel, J. A.; Chislock, M.; Lindstrom, A.; Strynar, M. Novel Polyfluorinated Compounds Identified Using High Resolution Mass Spectrometry Downstream of Manufacturing Facilities near Decatur, Alabama. *Environ. Sci. Technol.* **2017**, *51*, 1544–1552. DOI: [10.1021/acs.est.6b05330](https://doi.org/10.1021/acs.est.6b05330).
- [88] Liu, Y.; Pereira, A. D. S.; Martin, J. W. Discovery of C5-C17 Poly- and Perfluoroalkyl Substances in Water by in-Line SPE-HPLC-Orbitrap with in-Source Fragmentation Flagging. *Anal. Chem.* **2015**, *87*, 4260–4268. DOI: [10.1021/acs.analchem.5b00039](https://doi.org/10.1021/acs.analchem.5b00039).
- [89] Boone, J. S.; Vigo, C.; Boone, T.; Byrne, C.; Ferrario, J.; Benson, R.; Donohue, J.; Simmons, J. E.; Kolpin, D. W.; Furlong, E. T.; Glassmeyer, S. T. Per- and Polyfluoroalkyl Substances in Source and Treated Drinking Waters of the United States. *Sci. Total Environ.* **2019**, *653*, 359–369. DOI: [10.1016/j.scitotenv.2018.10.245](https://doi.org/10.1016/j.scitotenv.2018.10.245).
- [90] Kaboré, H. A.; Vo Duy, S.; Munoz, G.; Méité, L.; Desrosiers, M.; Liu, J.; Sory, T. K.; Sauvé, S. Worldwide Drinking Water Occurrence and Levels of Newly-Identified Perfluoroalkyl and Polyfluoroalkyl Substances. *Sci. Total Environ.* **2018**, *616–617*, 1089–1100. DOI: [10.1016/j.scitotenv.2017.10.210](https://doi.org/10.1016/j.scitotenv.2017.10.210).
- [91] Dutt, M.; Arigò, A.; Famigliani, G.; Zappia, G.; Palma, P.; Cappiello, A. Exploring Negative Chemical Ionization of Per- and Polyfluoroalkyl Substances via a Liquid Electron Ionization LC-MS Interface. *J. Am. Soc. Mass Spectrom.* **2023**, *35*, 890–901. DOI: [10.1021/jasms.3c00432](https://doi.org/10.1021/jasms.3c00432).
- [92] Hollender, J.; Schymanski, E. L.; Singer, H. P.; Ferguson, P. L. Nontarget Screening with High Resolution Mass Spectrometry in the Environment: Ready to Go? *Environ. Sci. Technol.* **2017**, *51*, 11505–11512. DOI: [10.1021/acs.est.7b02184](https://doi.org/10.1021/acs.est.7b02184).
- [93] Lau, H.; Liu, S. Q.; Tan, L. P.; Lassabliere, B.; Sun, J.; Yu, B. A Systematic Study of Molecular Ion Intensity and Mass Accuracy in Low Energy Electron Ionisation Using Gas Chromatography-Quadrupole Time-of-Flight Mass Spectrometry. *Talanta* **2019**, *199*, 431–441. DOI: [10.1016/j.talanta.2019.02.089](https://doi.org/10.1016/j.talanta.2019.02.089).
- [94] Hill, A. W.; Mortishire-Smith, R. J. Automated Assignment of High-Resolution Collisionally Activated Dissociation Mass Spectra Using a Systematic Bond Disconnection Approach. *Rapid Comm. Mass Spectrometry* **2005**, *19*, 3111–3118. DOI: [10.1002/rcm.2177](https://doi.org/10.1002/rcm.2177).