1506
UNIVERSITÀ
DEGLI STUDI
DI URBINO
CARLO BO

Università degli Studi di Urbino Carlo Bo

Department of Pure and Applied Sciences

Ph.D. PROGRAMME in: **Research Methods in Science and Technology**

CYCLE: **XXXV**

INNOVATIVE APPROACHES TO LC-MS: THE ROLE OF ELECTRON AND CHEMICAL IONIZATION

ACADEMIC DISCIPLINE: **CHIM/01**

Coordinator: Chiar.mo Prof. Alessandro Bogliolo

Supervisor: Chiar.mo Prof. Achille Cappiello

Ph.D. Student: Nicole Marittimo

ACADEMIC YEAR: **2021/2022**

ABSTRACT

The liquid electron ionization (LEI) interface is a device that converts a liquid phase to a gas phase in a mass spectrometer (MS) equipped with an electron ionization (EI) source. Analytes in the liquid phase vaporize at atmospheric pressure inside a specific vaporization microchannel (VMC) before entering the ion source where they are ionized under the typical EI conditions, generating library searchable spectra with strong matrix effects mitigation, even in complex samples. Because LEI is coupled with a conventional EI-based MS, it can operate both in EI and chemical ionization (CI) modes. LEI can be applied in the identification of unknown compounds (non-targeted analysis) or for the detection, with and without chromatographic separation, of targeted analytes in complex matrices.

In this study, the first goal was to explore the versatility of LEI, from one side, in direct analysis (without chromatography) using EI and CI with low and high-resolution MS and, from the other side, to assess the possibility of coupling normal phase liquid chromatography (NPLC) with EI in low and high-resolution MS using LEI.

LEI performances in direct analysis were evaluated by developing a sustainable and rapid method for the analysis of complex biological and environmental samples. The system is composed of a microfluidic open interface (MOI) for solid phase microextraction (SPME) liquid desorption, connected to LEI. MOI operates under the concept of flow isolated desorption and consists of an open to ambient desorption chamber (volume ~2.5 µL). A triple quadrupole tandem MS (QQQ, low-resolution) and a quadrupole time of flight MS (QTOF, high-resolution), operating in EI and negative CI (NCI) conditions were used. NCI is indicated for electrophilic compounds, increasing specificity, and reducing background noise. Between MOI and LEI, a passive flow splitter (PFS) reduces the flow rate (100 µL/min) needed for rapidly emptying the MOI chamber to the flow rate (500 nL/min) required for the correct LEI functioning. The MOI-PFS configuration was designed to speed up the sample transfer to MS, improving the signal to noise ratio (S/N) and peak shape, leading to fast and sensitive results. Fentanyl and 2 halogenated pesticides (dicamba, tefluthrin) were chosen as model compounds and extracted respectively from human blood serum and from a commercial formulation (CF) using a C18 fiber by direct immersion. Analytes desorption occurs in static conditions inside MOI filled with acetonitrile (ACN). Extraction and desorption steps were optimized to

increase efficiency and accelerate the process. Because chromatography is not involved, the system fully exploits MS/MS selectivity and HRMS accuracy demonstrating good linearity, repeatability, and LODs and LOQs in the µg/L range for fentanyl in serum and in the pg/mL range for the 2 pesticides in CF. Although complex matrices were used without chromatography, low-resolution experiments show that matrix effects (ME) do not affect the results and LEI identification potential is enhanced through the coupling with HRMS. The fast workflow makes the system suitable for high throughput analysis observing the principles of green analytical chemistry (GAC).

NPLC coupled to MS is challenging due to the incompatibility of the nonpolar solvents with the main ionization techniques. In this study, a new analytical approach based on the coupling of NPLC with EI in low and high-resolution MS using LEI is presented. Compounds with relevance in food, forensic, and environmental areas (tocopherols, cannabinoids, phenols, phthalates) were used as model analytes. A silica column with NPLC typical solvents was used to perform the separations in isocratic mode and the VMC temperature together with the mobile phase composition have been optimized. Low and high-resolution EI spectra of all targets were acquired and compared with the National Institute of Standards and Technology (NIST) library obtaining satisfactory match values, demonstrating the applicability of NPLC coupled to EI using the LEI interface. With LEI, the advantages of NPLC, such as the separation of nonpolar compounds and isomers, can be successfully combined with the generation of EI spectra and the HRMS identification power.

Another part of this work concerned the development of a prototypal device called extractive liquid sampling electron ionization mass spectrometry (E-LEI-MS). E-LEI-MS allows the coupling of ambient sampling with EI, avoiding sample preparation and providing spectral identification based on the comparison with the NIST library. It consists of a dispositive for solvent release and sampling at ambient conditions coupled with an EI source of a single quadrupole MS. The system was tested for targeted/non-targeted analysis and semi quantitative detection. E-LEI-MS was applied with satisfactory results for the analysis of active ingredients in pharmaceutical tablets, pesticides on fruit peel, a drug of abuse determination in banknotes, and analysis of unknown components on painting surfaces. Both forensic and artwork applications allowed the determination of the spatial distribution of the analytes. E-LEI-MS is also an environmentally friendly approach, as demonstrated by AGREE evaluation.

ACKNOWLEDGEMENTS

During my PhD I have worked under the supervision of Prof. Achille Cappiello and his group at the LC-MS Laboratory of the University of Urbino. I express my deepest gratitude to my supervisor, Prof. Achille Cappiello, who gave me the opportunity to work at the LC-MS Laboratory. I am also grateful to Prof. Giorgio Famiglini, Pierangela Palma, Adriana Arigò and Elisabetta Pierini for their support and precious help. Very special thanks go to my fellow PhD students Genny Grasselli and Malvika Dutt for sharing part of this journey with me. Finally, I would like to thank my family and my forever friends for always being by my side.

CONTENTS

LIST OF ORIGINAL PUBLICATIONS AND CONGRESSES

This thesis is based on the following original publications and congress presentations:

Scientific publications		
N.	**Type**	**Title**
1	Journal article	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. *Journal of Chromatography A* **2022**, 1681, 463479; https://dx.doi.org/10.1016/j.chroma.2022.463479.
2	Journal article	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 micro-extraction and direct liquid phase negative chemical ionization mass spectrometry. *Separations* **2023**, 10(6), 325; https://doi.org/10.3390/separations10060325.
3	Journal article	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. *Scientific Report* **2023**, 13, 6429; https://doi.org/10.1038/s41598-023-33647-5.

Congress presentations				
N.	**Type**	**Title of the presentation**	**Title of congress**	**Institution**
4	Oral	Advancements in direct-MS using SPME coupled to liquid-EI and CI	XXVII Congresso Nazionale della Società Chimica Italiana	Società Chimica Italiana (SCI)
5	Poster	Direct mass spectrometry using SPME coupled to a liquid-electron ionization and chemical ionization interface	ASMS 69th Conference on Mass Spectrometry and Allied Topics	American Society for Mass Spectrometry (ASMS)
6	Poster	Direct mass spectrometry using SPME coupled to liquid electron ionization and chemical ionization: a new green approach in direct analysis	1st Green and Sustainable Analytical Chemistry e-Conference (GSAC2022)	European Chemical Society (EuChemS)
7	Oral	Advantages of high-resolution mass spectrometry coupled to a liquid-electron Ionization Interface for pesticides analysis	ASMS 70th Conference on Mass Spectrometry and Allied Topics	American Society for Mass Spectrometry (ASMS)
8	Oral	A new approach for coupling normal phase chromatography and mass spectrometry	Incontri di Scienza delle Separazioni 2022	Società Chimica Italiana (SCI)

ABBREVIATIONS

ACN	Acetonitrile
AGREE	Analytical GREEnness Metric Approach
CapEI	capillary-EI
CBD	cannabidiol
CF	commercial formulation
CI	chemical ionization
CP	condense phase
DEI	direct electron ionization
DI-SPME	direct-solid phase micro extraction
DLI	direct liquid introduction
EI	electron ionization
EIC	extracted ion chromatogram
ESI	electrospray ionization
EtOH	ethanol
eV	electron volt
GAPI	Green Analytical Procedure Index
GC-MS	gas chromatography-mass spectrometry
HEX	hexane
ISO	isopropanol
K	distribution constant
KFS	partition coefficient between fiber coating and sample matrix
LC	liquid chromatography
LC-MS	liquid chromatography-mass spectrometry
LEI	liquid electron ionization
LOD	limit of detection
LOQ	limit of quantification

MAGIC	monodisperse aerosol generation interface for chromatography
MALDI	matrix-assisted laser desorption/ionization
MB	moving belt
ME	matrix effect
micro-PB	micro flow rate particle beam interface
MIMS	membrane introduction mass spectrometry
MOI	microfluidic open interface
MP	mobile phase
MRM	multiple reaction monitoring
MS	mass spectrometry
MW	molecular weight
NCI	negative chemical ionization
NIST	National Institute of Standards and Technology
NP	normal phase
NPLC	normal phase liquid chromatography
PA	phosphoric acid
PAHs	polycyclic aromatic hydrocarbons
PAN	polyacrylonitrile
PB	particle beam
PFS	passive flow splitter
POPs	priority organic pollutants
QQQ	triple quadruple
QTOF	quadruple time od flight
R2	determination coefficient
RSD	relative standard deviation
S/N	signal to noise ratio
SIDS	sudden infant death syndrome

SIM	selected ion monitoring
SPME	solid phase microextraction
THC	tetrahydrocannabinol
UV	ultraviolet
VMC	vaporization microchannel

1. AIMS OF THE STUDY

In this study, the first goal was to explore the versatility of LEI in direct analysis (without chromatography) using EI and CI with low and high-resolution mass spectrometry and to assess the possibility of coupling NPLC with EI in low and high-resolution mass spectrometry using LEI. In this context, the more detailed aims are the following:

- Developing a new method for the analysis of complex biological and environmental samples (fentanyl in human blood serum and pesticides dicamba and tefluthrin in a CF) based on a modified MOI connected to a PFS device for the direct coupling of a C18 SPME fiber to a LEI interface in a QQQ-MS (low-resolution) and in a QTOFMS (high-resolution), operating in EI and NCI mode.
- Developing a new approach for the coupling of NPLC with low and high-resolution MS using the LEI interface for the separation and analysis of model compounds with relevance in food, forensic, and environmental areas (tocopherols, cannabinoids, phenols, phthalates).

The second goal of this study was to develop a new dispositive called E-LEI-MS that allows the coupling of ambient sampling with EI providing identification based on the comparison with NIST library for the analysis in pharmaceuticals, forensic and artwork fields and in pesticide analysis and food quality control.

2. INTRODUCTION

2.1. BREAF HISTORY AND PRINCIPLES OF MASS SPECTROMETRY

According to the 2013 IUPAC recommendations MS is defined as an analytical technique that measures matter through the creation of ions in the gas phase that are sought and determined based on their m/z ratio [1]. MS has been used for many years in numerous scientific areas, including chemistry, medicine, pharmacology, forensic sciences, and food sciences, due to its adaptability. MS began in 1912 when Sir Joseph J. Thomson invented the spectrographic dish, the precursor of today's mass spectrometer. Thomson's apparatus used gas discharge tubes to produce ions, which were then transported toward electric and magnetic fields. When subjected to this setting, the ions followed parabolic paths based on their m/z ratio before being detected, by impact, on a photographic plate. The quadrupole analyzer and the ion trap analyzer, both described by Paul Wolfgang in 1953 and 1958, are two examples of developments that interested MS during that period. During the 1960s, the diffusion of GC coupled to MS broadened the field of application of this analytical technology to more complex mixtures. The development of MS/MS and CI belong to the same period. The necessity to widen the range of applicability to biologically relevant substances with large molecular masses led to the development of novel ionization modes such as ESI and MALDI in the 1980s. With the invention of the ESI interface, the coupling of HPLC and MS as a tool for evaluating larger and less volatile substances was solidified. Ambient ionization techniques have recently been established in the field of MS. Ambient MS allows mass spectra to be recorded from untreated samples that are examined in their natural environment [2]. DESI was the first ambient ionization approach to be introduced in 2004 [3]. DART was introduced in 2005, after DESI [4]. The potential of ambient ionization has paved the way for the development of innovative techniques that can be used to meet a variety of analytical needs.

MS is an analytical technique that uses a molecule's weight to identify a sample to be studied. It is a destructive analysis approach since the analytes are subjected to an ionization process that results in the development of a molecular ion and fragments derived from it, each with its own m/z ratio. Given that the charge is frequently equal to one, the m/z ratio is traditionally equal to the ion's mass. The processes involved in MS analysis are illustrated in **Figure 1**.

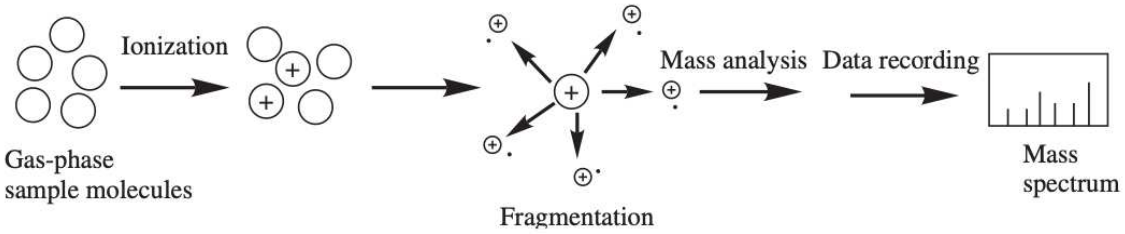


Figure 1. Processes involved in MS analysis. [5].

The mass spectrum, which graphically displays the relative abundance of each m/z ratio, is the result of the analysis. The "base peak" is the peak that corresponds to the ion with the greatest abundance, which can be the molecular ion or any other ion generated during molecule fragmentation. The mass spectrum is unique to each substance. As a result, the analyte is identified by comparing the experimental spectrum to spectra stored in electronic libraries or by performing a specialized interpretation of the spectrum itself. In fact, by observing the fragmentation profile, even unknown compound formulas can be deduced.

The MS apparatus is generally composed of:

- A sampling system that collects samples from the outside environment (atmospheric pressure) and transfers them inside the ionization source (vacuum condition).
- The ionization source where the molecules are converted from neutral species to ions in the gas phase.
- The mass analyzer that separates and analyzes ions as they move through a magnetic or electric field.
- The detector that measures and amplifies the ionic current.
- A computer system that reprocesses the data in the form of a mass spectrum.
- A vacuum system that maintains the optimal pressure inside the instrument.

2.2. IONIZATION SOURCES AND TECHNIQUES

The ion source is where neutral analytes are converted into gas phase ions [1]. In some techniques, ionization is performed by ejection or capture of an electron by an analyte to produce a radical cation $[M^{+\bullet}]$ or anion $[M^{-\bullet}]$, respectively. In others, a proton is added or subtracted to yield $[M + H]^+$ or $[M - H]^-$

ions. In some methods, a molecule can also be ionized by creating adducts with Na^+ and K^+ ions, forming cationic species, or with Cl^- ions, forming anionic species. The choice of the ionization technique used is determined mostly by the nature of the sample to be studied and the type of information required. Ionization techniques can be distinguished based on the approach used to convert neutral analytes into ionic species in the gas phase.

The first distinction is based on the energy used to ionize the molecule, which classifies ionization processes as hard or soft. Hard ionization techniques are those in which the ionization energy that strikes the molecule inside the ionization chamber is substantially greater than the energy required to lose an electron. The surplus energy is distributed throughout the molecule's bonds, resulting in extensive fragmentation. EI is the most popular of hard ionization techniques.

Soft techniques use less energy and are hence less destructive. CI, ESI, APCI, and MALDI are examples of this sort of method. It is also possible to distinguish between techniques that operate under vacuum conditions and those in which ionization occurs at ambient temperature and pressure. Among the techniques that operate under vacuum conditions, EI and CI will be discussed.

2.2.1. ELECTRON IONIZATION

EI, introduced by Dempster in 1918, is the oldest ionization mechanism used in MS. EI is widely employed in the analysis of thermally stable and relatively volatile organic compounds [5]. These properties make this type of ionization suitable for coupling with GC. GC-MS has become one of the most popular methods for evaluating the presence of target compounds and in non-targeted analysis due to the separation capabilities of GC and the identification potential of MS [6]. GC-MS capability to identify a substance is also due to the development of electronic libraries containing spectra of already known substances which act as a comparison with the spectra obtained experimentally. The applicability of EI to HPLC has also been demonstrated via LEI interface which will be discussed more in depth in the next paragraph [7]. In the EI process, the vaporized sample molecules are bombarded with a beam of energetic electrons (usually generated by a tungsten filament held at an energy of 70 eV). An electron from the target molecule is expelled during this collision process to convert the molecule to a positive ion with an odd number of electrons. This positive ion, called a molecular ion or radical cation, is represented by the

symbol [$M^{+\bullet}$]. This process occurs in a high vacuum condition and the ionization source is maintained at high temperatures to avoid condensation of the sample. In **Figure 2**, the scheme of an EI ion source is presented. The kinetic energy of the bombarding electrons must exceed the ionization energy of the sample molecule. The excess energy gained by the ionized molecule causes it to dissociate into structurally diagnostic smaller-mass-fragment ions, some of which may still have sufficient energy to fragment further into second-generation product ions. The fragmentation pattern thus obtained is diagnostic of the structure of the sample molecule.

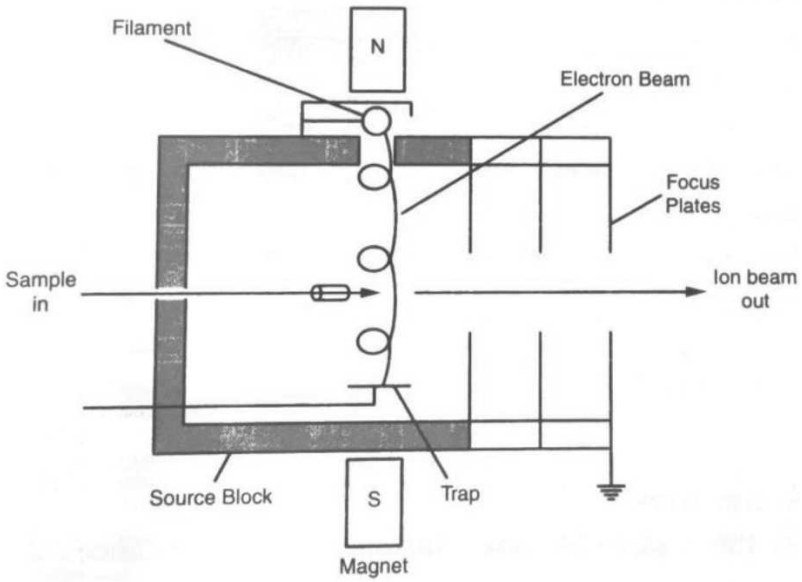


Figure 2. Scheme of an EI ion source [Herbert, C.G.; Johnstone, R. A. W. Mass Spectrometry Basics. **2002**, CRC Press.]

2.2.2. CHEMICAL IONIZATION

CI is a soft technique, with lower ionization energy compared to EI, suitable for the analysis of those substances that fail to yield molecular ion signal in EI mode. Basically, CI shares a common ion-source assembly with EI, but the CI process is quite distinct from EI. In fact, CI is accomplished through gas-phase acid-base reactions between the sample molecules and the reagent gas ions. Ionization of the sample occurs via a proton transfer mechanism in which the molecules of the reagent gas behave like Bronsted acids, which are chemical species capable of donating H^+ ions. The first step of the CI process is based on the same mechanism that characterizes EI and involves the ionization of the reagent gas molecules which

are bombarded inside the source by a beam of electrons. This interaction determines the loss of an electron from a reagent molecule, resulting in the formation of a radical that will interact with another neutral reagent molecule. The product of this reaction reacts with the sample molecules through a protonation process which favors the molecule with higher proton affinity. When the proton affinity of an analyte molecule exceeds that of the reagent gas, ions $[M + H]^+$ are produced (positive CI). When the proton affinity of the reagent gas molecule is greater than that of the analyte molecule, $[M + H]^-$ ions are produced (negative CI). For this reason, reagent gases with different proton affinity can be used. Methane, isobutane, and ammonia are the most common reagent gases.

The mass spectrum acquired with CI is simpler than that obtained via EI analysis. It usually contains the signal of the molecular ion and the signal of a few fragment ions in case they are formed. The extent of fragmentation can be controlled by a correct choice of reagent gases.

2.3. HISTORY OF THE LC-EI-MS INTERFACES

Over the years, several attempts have been made to ionize analytes from the liquid phase using EI (**Figure 3**). Initially, EI was considered incompatible with LC-MS systems due to the presence of a liquid mobile phase and the limitations imposed by the high vacuum and high-temperature conditions required for EI operation. The main obstacle was the apparent flow rate incompatibility, in fact, the presence of a liquid mobile phase at high flow rates could break the vacuum needed in the EI ion source, and the mobile phase transition from the liquid state to the gaseous state inside the ion source mainly induced chemical ionization, as a result, clean EI spectra cannot be obtained [8,9]. According to McLafferty [10], LC-MS interfaces must overcome the problem of the tremendous disparity in operating pressure between the LC exit ($\geq$ atmospheric pressure) and the MS ion source ($< 10^{-4}$ torr for EI-MS).

The first LC-EI-MS coupling was proposed in 1968 by Tal'roze and coworkers [11]. Through a capillary leak system, they succeeded in continuously introducing volatile organic liquids inside a conventional EI ion source, however, due to the $< 10^{-4}$ torr pressure requirements of the ion source, only flow rates of $\sim 10^{-6}$ ml/min were possible. Furthermore, other problems of the capillary interface system were absorption of polar components, cross-contamination of samples, flow rates irreproducibility, and occlusions [12].

In the following years, three general types of LC-MS interfaces have been proposed: interfaces that introduce part of the effluent directly into the MS ion source, those which employ solvent separation, and those which vaporize the total effluent at atmospheric pressure.

In 1974, Arpino et al. [13] developed an LC-MS interface based on CI called direct liquid introduction (DLI). The liquid mobile phase was directly conveyed into the ion source and converted into the gas phase, acting as a CI reagent gas. The flow rate was adjusted to the spectrometer operating conditions by introducing a splitter and differential pumping systems. DLI encountered various problems related to the non-formation of EI spectra, low sensitivity due to the high splitting of mobile phase and sample, and frequent occlusion of the capillaries.

One of the most successful systems for solvent separation, called moving belt (MB), was proposed in the same period by Scott and coworkers [14]. The LC effluent was sprayed over a moving wire or tape and evaporated at atmospheric pressure. Through a series of vacuum chambers, the analytes on the belt were carried into the mass spectrometer's ion source where the sample was heated, vaporized, and ionized. Either EI or CI spectra can be obtained. Although quite successful for some years, this interface also had several limitations due to the complexity and mechanical fragility and the high memory effect, especially with low volatile substances.

Another interface called particle beam (PB), also known as the monodisperse aerosol generator interface for chromatography (MAGIC), proposed by Willoughby and Browner in 1984 [15], exploited the same principle as MB of separating the mobile phase before the analytes enter the ion source. With PB, the column eluate is first passed to a nebulizer, where the mobile phase is dispersed into a fine mist of droplets. The resulting aerosol then passes through a desolvation chamber, kept slightly above ambient temperature, where the volatile solvent evaporates, and dissolved analyte condenses to form solid particles. The resulting mixture of particles, solvent molecules, and helium atoms is drawn through a small nozzle into a pumped chamber causing a rapid expansion to occur. The relatively massive solute particles will gain high momentum from the expansion and therefore continue in a linear beam, while the low mass, low momentum solvent molecules and helium atoms will undergo rapid radial expansion. Separation is achieved by sampling the particle beam through a small opening, or skimmer, leaving the other components to be pumped away. The resulting dry, high-velocity particle beam passes through a

short transfer line to the mass spectrometer. The heated walls of the ion source provide thermal energy for flash volatilization of the particles and ionization of the gas phase analyte can then proceed by EI or CI. Since the first introduction, the development of the PB interface has proceeded rapidly and several modifications to the basic design have been made in terms of methods for aerosol formation and gas-solid separation. However, problems with the PB interface were drawbacks related to the transfer and loss of analytes while crossing the momentum separators, sensitivity issues, and limitations in quantification due to high volumes of solvent in the ion source [16].

As previously stated, the main reason why LC-MS was difficult to realize was the presence of large amounts of liquid solvent that had to be removed by the vacuum system. Typical LC flow rates around that time were at least 1 mL/min, which was highly challenging for the MS pumping system [17]. In that period, due to the emergence of green chemistry, there was a drive toward the miniaturization of the last environmentally friendly instruments. The advent of capillary and nanoscale liquid chromatography has made the flow of the mobile phase compatible with the EI vacuum, facilitating LC-MS coupling. A consequence of this evolution was the micro flow rate particle beam (micro-PB) developed in 1993 by Cappiello and Bruner [18]. It was a modified version of the PB designed to work at a lower flow rate of mobile phase (1-5 μL/min). By using a lower flow, the performance of the interface was increased. Micro-PB was widely studied to try to optimize the parameters involved in the interface and to explore its real potential [19,20]. The different experiments carried out using this new interface showed that the impact of the mobile phase on the ion source had to be reduced and many applications published from 1994 to 2001 demonstrated the merits of micro-PB [21-29].

The Capillary-EI interface (CapEI) represented the evolution of the micro-PB: it guaranteed a lower loss of the analytes, thus increasing the sensitivity [30].

Despite the good results obtained, the mechanical complexity of all those interfaces exceeded their popularity, resulting in these approaches rapidly disappearing from the LC-MS portfolio. Nevertheless, these milestones indicated the way for more advanced solutions for the application of LC to EI-MS [8].

In 2001, Professor Cappiello's research group proposed a new LC-EI-MS interface prototype [31], taking into consideration the advantages deriving from LC nano columns. In fact, considering these advantages, the nebulization process, solvent evaporation, particle formation and conversion into the gaseous phase

all took place within the ion source. The temperature of the ion source (150-350°C) was high enough to favor the passage of the analytes from the liquid to the gaseous phase, according to their physical and chemical characteristics. This interface was called Direct-EI (DEI) due to the direct introduction of the LC eluate into the ion source. The mobile phase flow rate (<1 μL/min) and some modifications of the ion source positively contributed to the DEI performance, such as a more efficient pumping system and a new vent window in the ionization chamber to facilitate the dispersion of solvent vapors. This configuration minimized sample loss and efficiently removed solvent vapors, resulting in more efficient analyte ionization. DEI was used for 15 years in various applications, both in association with LC and in direct analyses. Indeed, DEI has been used in many research fields, demonstrating the lack of the matrix effect. It is unaffected by coelution of analytes or matrix components, making it suitable for direct introduction of diluted samples [31-63]. However, DEI also had several disadvantages, which have not yet been resolved, such as premature evaporation of the solvent causing subsequent solute precipitation and the occasional occlusion of the nebulizer capillary. Another obstacle is associated with the surface of the ion source: inside the stainless-steel surface of the ion source, polar, low volatile and thermally unstable molecules can undergo either adsorption or thermal degradation, resulting in peak tailing phenomena, drastic reduction of sensitivity, poor reproducibility, and anomalies in the EI spectra.

In 2017, the same research group of Professor Cappiello evaluated the possibility of making the conversion from liquid to gas take place outside the ion source. A new interface was developed: the liquid electron ionization interface (LEI) [64,65], which will be discussed in the next paragraph.

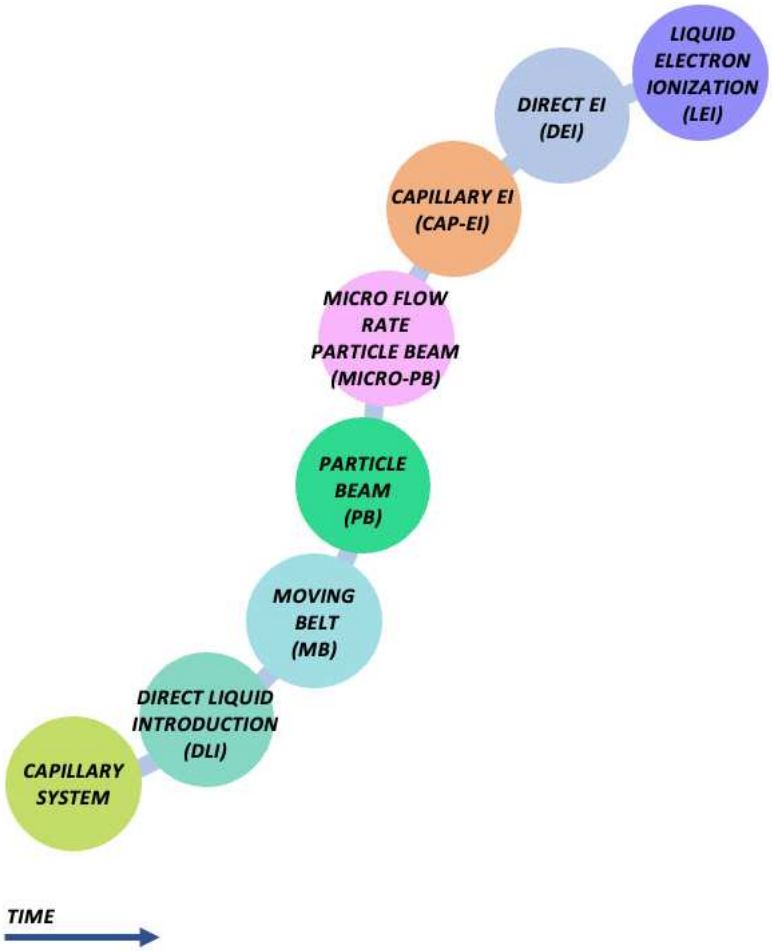


Figure 3. Summary of the development of the major LC-EI-MS interfaces.

2. 4. LIQUID ELECTRON IONIZATION INTERFACE

LEI was recently developed by Prof. Achille Cappiello and his research group with the aim of solving the problems arising in the conversion of the liquid phase into the gaseous phase, preserving the integrity of the compounds and the chromatographic information, also allowing to obtain EI spectra that can be searched in the libraries [7]. LEI is a novel device that converts a liquid phase to the gas phase in a mass spectrometer equipped with an EI source. LEI is completely independent from the rest of the instrumentation and can be adapted to any GC-MS system, as an add-on for a rapid LC-MS conversion [65]. In the LEI interface, the vaporization of the eluate from the column takes place at atmospheric pressure, inside a specific VMC right before reaching the ion source. A stream of inert gas carries molecules in the gaseous state in the high vacuum ion source. In this way, solute vaporization takes place

just outside the ion source in a space that is most suitable considering the size, pressure, temperature, and surface materials and is free of sensitive components and electrical potentials. Once in the ion source, the analytes are ionized under the typical EI conditions (70 eV) generating library searchable EI spectra with strong matrix effects mitigation and small solvent consumption (LEI works at nano-flow rates). By extending the electronic spectra libraries access to LC, LEI can provide a powerful tool in the identification of unknown polar and nonpolar compounds (non-targeted analysis), which is of increasing importance in food safety, environmental, forensic, and in many other investigation areas [65]. Due to the negligible matrix effects, this technique can be used in many different applications, not only in combination with chromatography but also in direct analysis for the detection of targeted analytes in complex samples without sample preparation steps [66]. LEI offers a reliable and simple solution for small molecule applications and, by operating in the nano-field, it accomplishes guidelines and principles of "green analytical chemistry" to combine performance and sustainability, limiting reagent consumption and waste generation. The processes involved in the functioning of the LEI interface are shown in **Figure 4**.

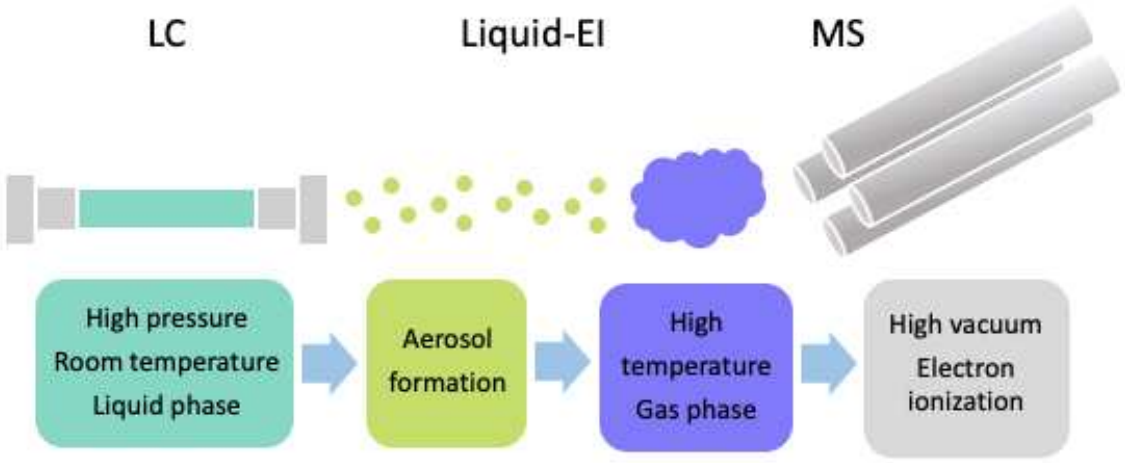


Figure 4. Processes involved in the functioning of the LEI interface.

As illustrated in **Figure 5** and **Figure 6**, the most updated version of the interface is characterized by three fundamental parts [7]:

- liquid-gas connector for adding the helium flow: the liquid flow rate exiting from the LC column is conveyed inside the inlet capillary and meets the liquid-gas connector. This component ensures proper mixing between helium and mobile phase once both merge as gas phase in the vaporization microchannel. Helium has multiple functions in this interface: to accelerate the transport of the sample into the ion source, to avoid vapor backflow, to promote sample vaporization, and to assist the correct ionization and ion ejection from the ion source. The inlet capillary is very thin and carries the eluate deriving from the LC column into the vaporization microchannel. It is made of fused silica with an external diameter of 150 µm, while the internal diameter can vary from 25-50 µm. The vaporization microchannel is considered the heart of the interface: here the conversion from the liquid to the gaseous phase takes place. Several attempts have been made to minimize the adverse effects of the metal surface by using other materials, such as Teflon or ceramics [67, 68]. Those materials allowed to obtain several advantages, allowing new applications, and improving the shape of the peaks, especially in the analysis of thermolabile and high molecular weight compounds. However, they also had several disadvantages, due to their low thermal resistance, fragility, and cost. Then, a fused silica capillary is used as VMC inside the metal transfer line. The fused silica vaporization microchannel has an external diameter of 800 µm and an internal diameter of 400 µm, wide enough to accommodate the inlet capillary, leaving a ring-shaped space between the two elements of 125 µm. This space enables the passage of the helium flow. The liquid-gas connector is kept at room temperature to avoid premature vaporization of the mobile phase. At the end of the inlet capillary, helium combines with liquid vapors in the liquid-to-gas conversion zone and the sample is vaporized. Typically, the helium flow is 1 mL/min.

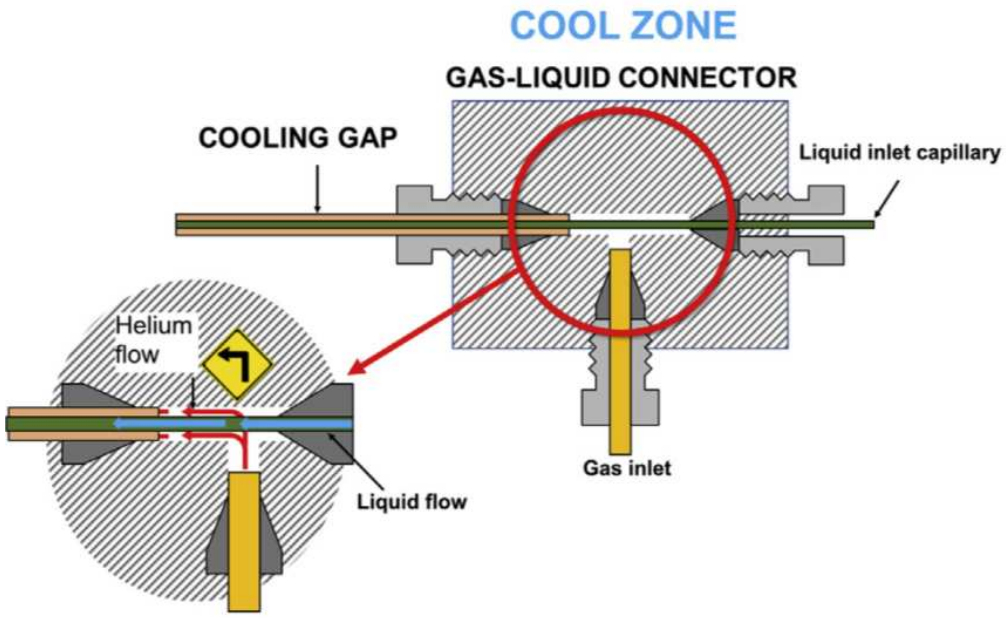


Figure 5. Liquid-gas connector [7].

- Cooling gap to separate the hot vaporization area from the connector: it is an extension of the vaporization micro channel consisting in a fused silica capillary that protrudes 5 centimeters from the heated transfer block to prevent heat from diffusing back from the hot region of the interface.

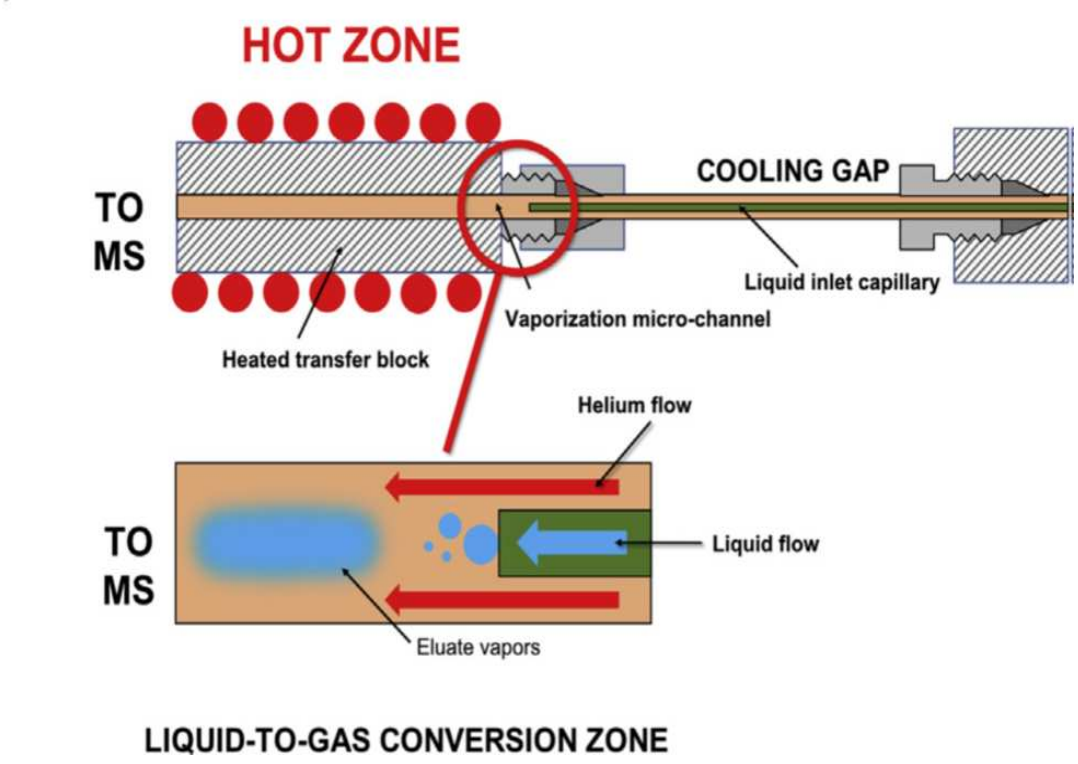


Figure 6. Cooling gap and vaporization microchannel. (7).

In a previous version of LEI, a Peltier cell kept the liquid-gas connector temperature cool. The Peltier cell, shown in **Figure 7**, subtracted some of the back-diffusing heat from the heated region of the interface. When the Peltier was turned off, the mobile phase reached a temperature of 55°C with a microchannel temperature set at 350°C. This value was high enough to promote early vaporization of most of the mobile phase resulting in solute precipitation and capillary clogging. After the Peltier cell was turned on, the liquid temperature was 40°C, 15°C below the boiling point of methanol, which is the most volatile solvent used in reverse-phase chromatography [65]. The cooling gap, on the other hand, is more efficient and keeps these values limited to ambient temperature, regardless of the vaporization temperature used, preventing sample precipitation.

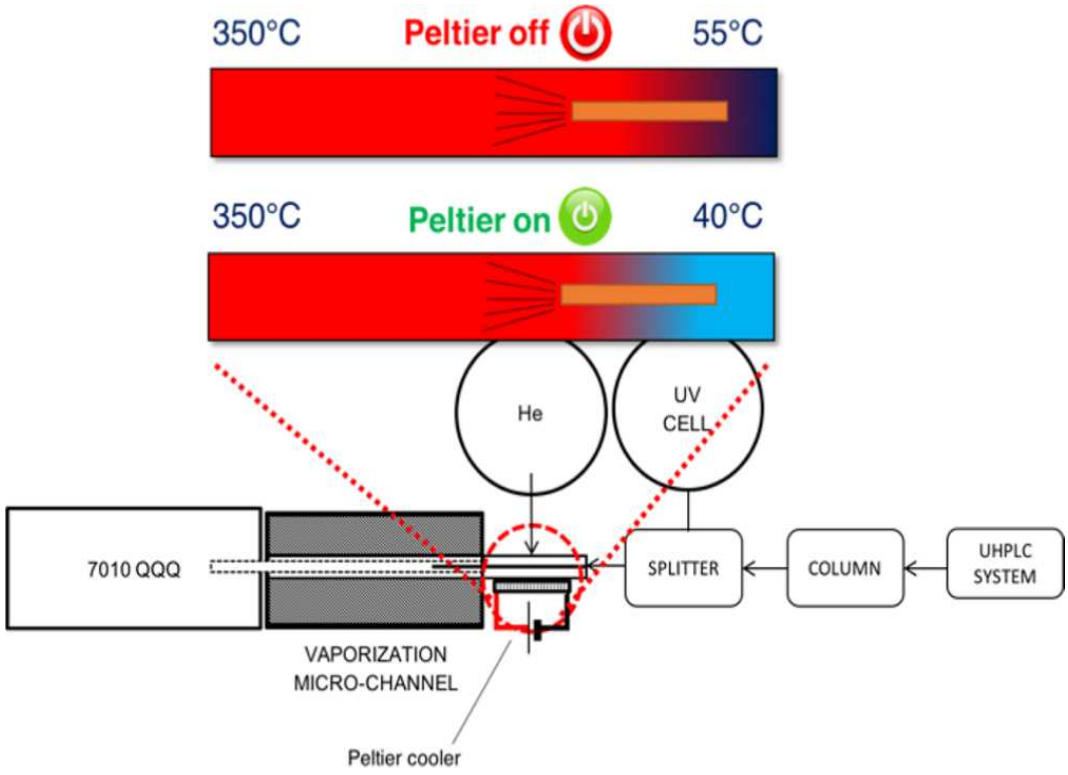


Figure 7. Temperature distribution between the vaporization microchannel set at 350 °C and the liquid-gas connector with the Peltier cell in both ON and OFF positions [65].

- liquid to gas conversion zone where the liquid is vaporized and mixed with the helium flow: here the liquid sample is finally converted to the gas-phase. This process takes place inside the vaporization microchannel, at the end of the cooling gap and where the inlet capillary ends, approximately 2 mm inside the heated transfer block. At this exact point, the inlet capillary releases the liquid effluent at atmospheric pressure inside the tight space of the hot vaporization microchannel, and flash vaporization takes place upon liquid contact with the hot internal surface of the microchannel. The other end of this narrow channel terminates in the high vacuum region of the EI source. The analytes in the gas phase are conveyed by the vacuum gradient and helium flow inside the EI ion source for the ionization under typical conditions (70 eV).

2.4.1. ROLE OF LEI IN COMBINATION WITH CHROMATOGRAPHIC TECHNIQUES

The effectiveness of LEI in combination with chromatographic techniques has been demonstrated by the excellent results obtained from the analysis of pesticides, PAHs, hormones, and phenols, as demonstrated by the experiment, shown in **Figure 8**, using a mixture of 20 priority organic pollutants (POPs) (acetamiprid, bisphenol A, thiram, fludioxonil, phosmet, fenhexamid, imazalil, boscalid, naphthalene, chlorfenvinfos, lindane, zoxamide, anthracene, pyriproxyfen, 4-n-octyl phenol, endosulfan II, chlorpyrifos, 4-n-nonyl phenol, benzo[k]fluoranthene, benzo[a]pyrene), and 4 internal standards (bisphenol A D16, Phosmet D6, Chlorpyrifos-methyl D6, Pyrene D10). They were analyzed both in full scan and MRM and a UV profile was detected at the column outlet before the LEI. The compounds belong to different classes with different polarities, and they all gave an excellent response both for the S/N and for the shape of the peak. The results obtained demonstrated the ability of the mass spectrometer, equipped with an LEI interface, to generate high-quality EI mass spectra even if deriving from a liquid phase. Through the comparison between the results obtained in MRM with UV, a perfect match is observed in terms of chromatographic resolution and peak width [65].

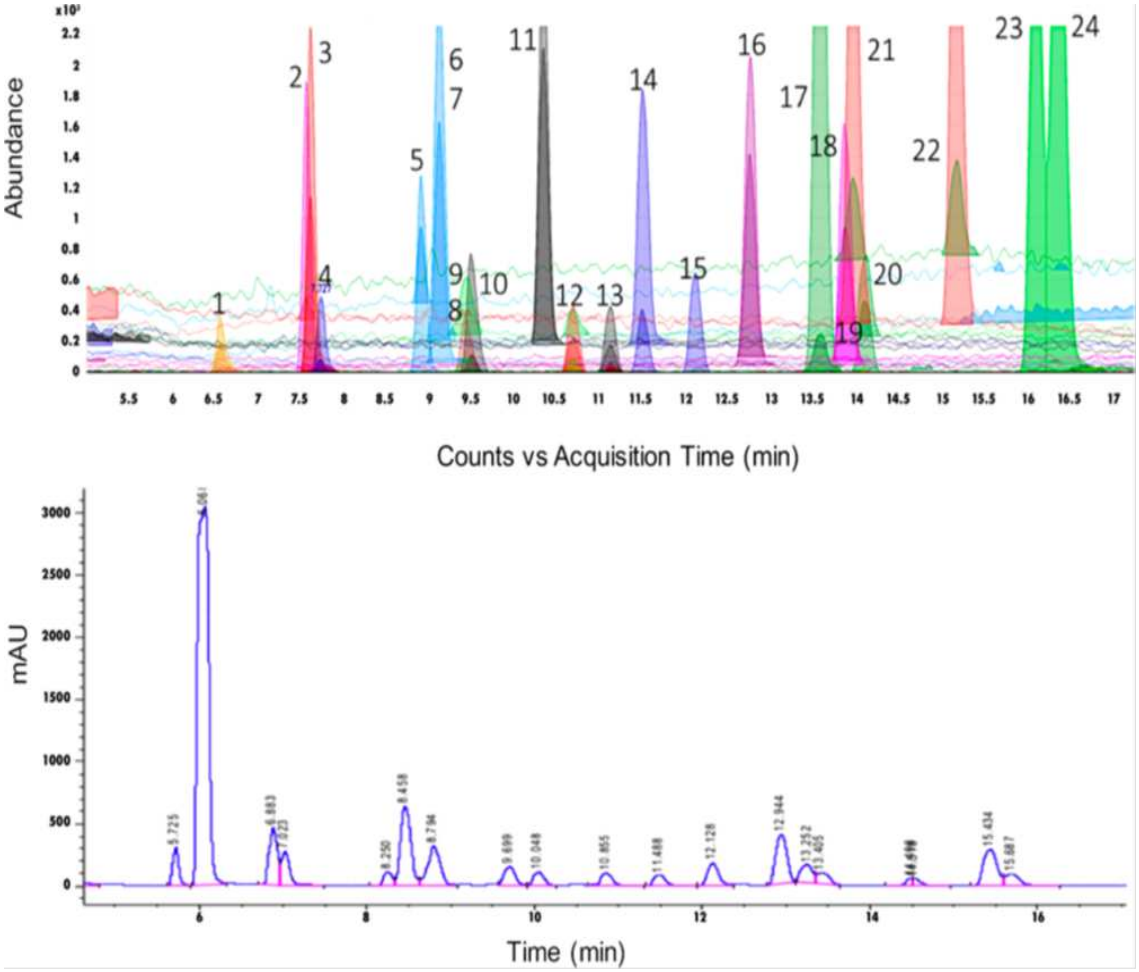


Figure 8. Results obtained from the separation and analysis of 20 pollutants, including four internal standards.1, acetamiprid; 2, BPA-d16; 3, BPA; 4, thiram; 5, fludioxonil; 6, phosmet D6; 7, phosmet; 8, fenhexamid; 9, imazalil; 10, boscalid; 11, naphthalene; 12, chlorfenvinfos; 13, lindane; 14, zoxamide; 15, chlorpyrifos-methyl D6; 16, anthracene; 17, pyrene D10; 18, pyriproxyfen; 19, 4-n-octyl phenol; 20, endosulfan II; 21, chlorpyrifos; 22, 4-n-nonyl phenol; 23, benzo[k]fluoranthene; 24, benzo[a]pyrene. [65].

Another recent application concerning the development of an LC-LEI-QQQ method using a reversed-phase column to detect dicamba and tefluthrin in a commercial formulation (CF), demonstrated that a further advantage of LEI is the ability to work in CI mode [69]. CF is a complex matrix due to active ingredients and additives, reaching LODs and LOQs of 0.08 and 0.3 ng/mL for dicamba and 0.05 and 0.2 ng/mL for tefluthrin. The simultaneous analysis of dicamba and tefluthrin, two halogenated pesticides, is challenging due to their opposite chemical-physical 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 [69, 70]. NCI is typically used in GC-MS for ionizing compounds containing electronegative atoms, increasing 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 [71, 72]. In ref. [69], the analyses were performed using chromatographic separations (**Figure 9**), and the CF, fortified with dicamba and tefluthrin standards, was injected after dilution, filtration, and pH adjustment.

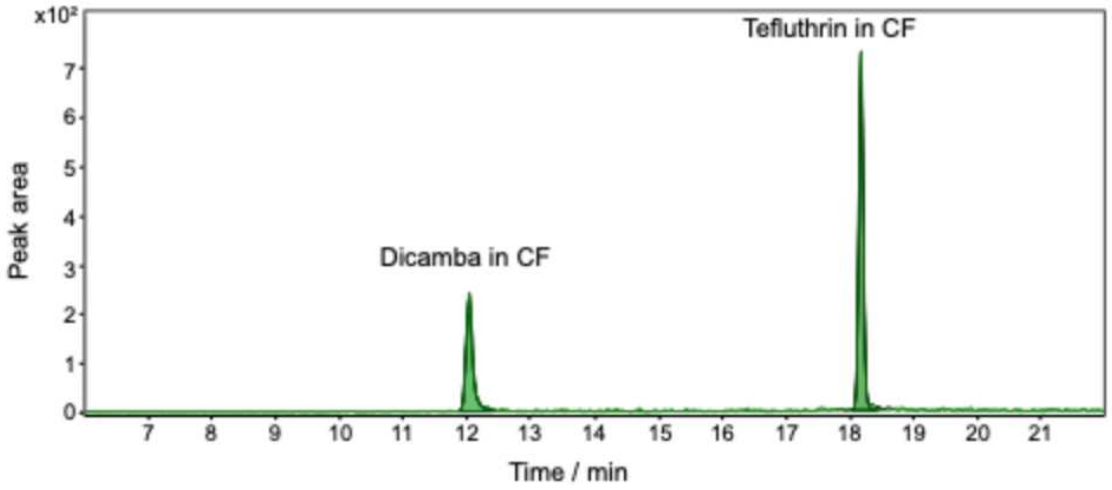


Figure 9. MRM profiles of dicamba and tefluthrin two-compound mixture in diluted CF [69].

These experiments demonstrated that LEI interface efficiently converts liquid eluate into a gas stream without loss of chromatographic information and preserving the chemical integrity of the analytes.

When dealing with new mass spectrometric instruments, one of the most important characteristics is the mass spectrum quality. With LEI interface, mass spectra comparable to those found in libraries (such as NIST) can be obtained, allowing the precise identification of unknown analytes. During the identification, the comparison between the experimental spectrum obtained and those present in the libraries can be facilitated using deconvolution programs, these programs can separate ions of coeluted compounds,

aiding identification. Indeed, a point of strength of LEI is the ability to identify non-targeted compounds. This ability was highlighted through an experiment, shown in **Figure 10**, conducted on the brain tissue of a SIDS victim (sudden infant death syndrome), to search for a possible toxic environmental contaminant [65]. Several peaks in full scan mode were obtained due to the complexity of the matrix. Considering that the LEI can benefit from modern deconvolution programs that help to minimize the background noise and to separate each single mass spectrum generated during the sample analysis, it was possible to identify the presence of benzo(a)pyrene, under a large peak arising from the matrix. The identification was confirmed by MRM analysis and by comparison with a standard solution of benzo(a)pyrene.

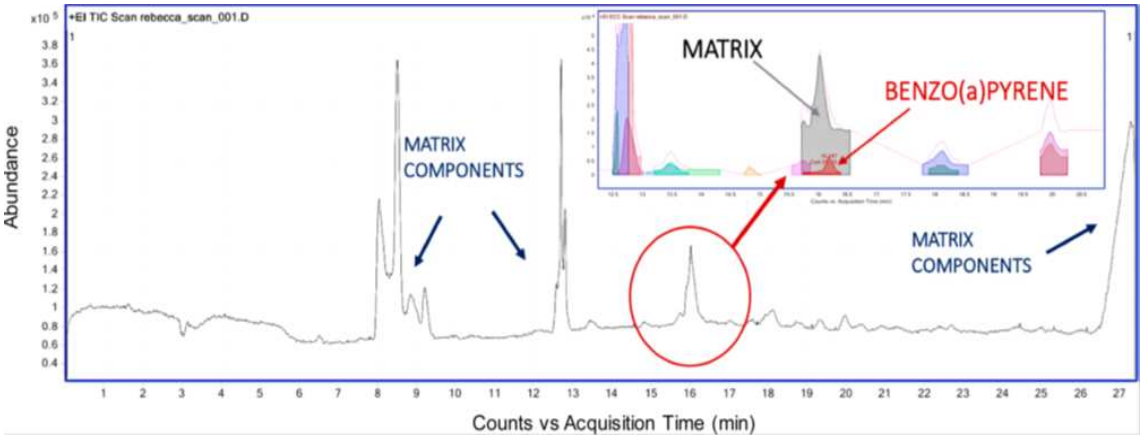


Figure 10. Results obtained from the analysis of the brain tissue of a SIDS victim [65].

2.4.2. ROLE OF LEI IN DIRECT ANALYSIS

Direct MS can benefit from coupling LEI with specific extraction and preconcentration techniques such as membrane introduction mass spectrometry (MIMS). With MIMS, molecules permeate the membrane according to their individual permselectivity and are transferred to the mass spectrometer as a mixture by a continuous flowing acceptor phase which can be either gaseous or a condensed phase such as a liquid suitable for semi to nonvolatile compounds [73]. Co-permeating analytes in the mixture can be resolved by the mass spectrometer according to their unique m/z or by using tandem MS/MS techniques. The membrane also rejects bulk matrix components, as a result, potential problems from interfering matrix components can be mitigated or significantly reduced [74]. An interesting application of CP-MIMS-LEI-MS with encouraging results concerned the possibility of monitoring synthetic organic reactions. CP-MIMS-

LEI-MS was employed to analyze polar and non-polar compounds, using a semipermeable membrane immersed directly in the reaction chamber. Changes in the concentrations of reagents and products were monitored, while, at the same time, interfering molecules and particles formed during the synthesis process were eliminated. As reported **in Figure 11**, in-situ quantitative continuous monitoring of catalytic oxidation and alkylation reactions were investigated [73].

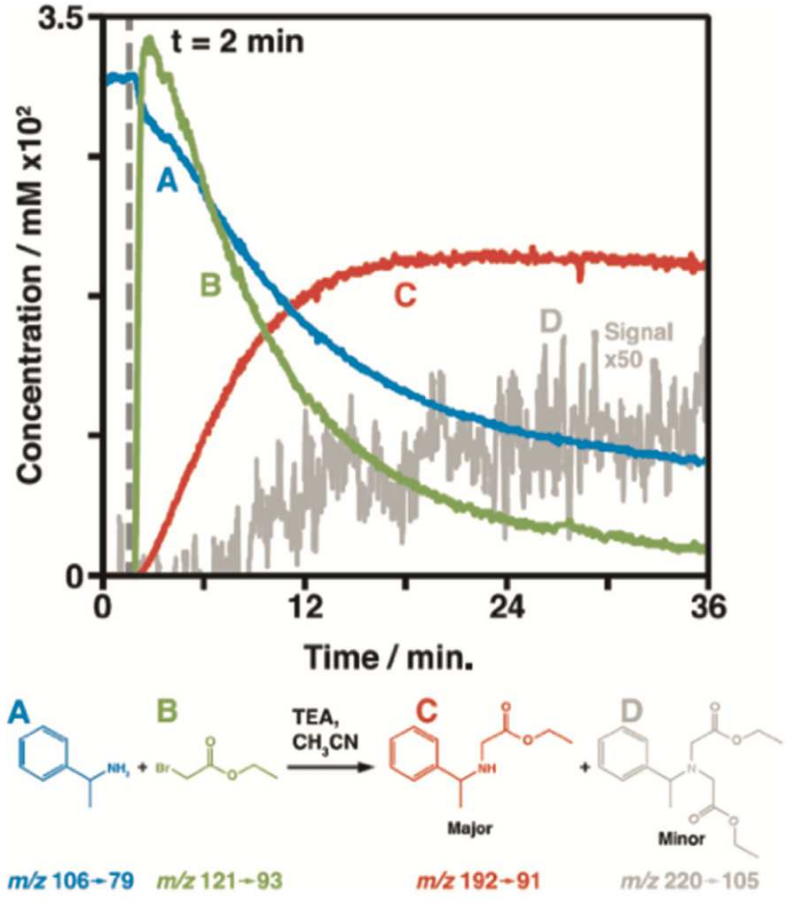


Figure 11. Quantitative online monitoring of an alkyl glycinate synthesis in dry acetonitrile with triethylamine catalyst with CP-MIMS-LEI: (A) (R)-a-methyl benzylamine; (B) ethyl bromoacetate; (C) ethyl (R)-(1-phenylethyl)glycinate; (D) diethyl (R)-2,20-((1- phenylethyl)-azanediyl)-diacetate. [73].

Another recent approach involving direct MS was the coupling of LEI with MOI [75]. MOI, developed by Pawliszyn et al. [76], is a rapid elution system for solid phase microextraction (SPME) fibers. MOI is based on the concept of a flow-isolated desorption chamber of 7 µL connected to an ionization source Analytes,

selectively extracted from various matrices, are rapidly eluted into the MOI and introduced directly to the MS ion source without chromatographic separation. Recently, a modified MOI configuration in which the desorption chamber was reduced at ~2.5 µL was proposed and coupled with an LEI-MS system. LEI-MOI-MS system was tested on fentanyl in urine and plasma, used as a model compound showing good results.

3. MATERIALS AND METHODS

3.1. SOLVENTS AND REAGENTS, STANDARDS, AND SAMPLES/MATRICES

LC-MS grade acetonitrile (ACN), dichloromethane (CH_2Cl_2), ethanol (EtOH), hexane (HEX), isopropanol (IPA), and octanol, were 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). Phosphoric acid (PA, 85%) was purchased from Merck (Milan, Italy). Standards of fentanyl (1000 mg/L in MeOH), (+)-alpha-tocopherol, (+)-delta-tocopherol (purity ≥ 98%) and caffeine (purity > 99.0%) were purchased from Sigma-Aldrich-Cerilliant (Milan, Italy). Standards of chlorpyriphos (purity 99.9%), imazalil (purity 99.8%), and benomyl (purity ≥ 98.0%) were supplied by Sigma Aldrich-Pestanal (Milan, Italy). Dicamba and tefluthrin (purity >99%) were provided by Syngenta Ltd (Bracknell, UK). Standard of (+)-beta-tocopherol (purity ≥ 98%) was supplied by Extrasynthese (Genay, France). Solutions of cannabinoids ((-)-delta9-trans-tetrahydrocannabinol 100 mg/L in MeOH, cannabidiol 1000 mg/L in MeOH) and cocaine (1000 mg/L in ACN) were provided by the Toxicology Lab. A.S.T. AV1 (Pesaro, Italy). Phthalates (diisodecyl phthalate, diethyl phthalate and dimethyl phthalate, purity ≥ 99%), and phenols (phenol, catechol, resorcinol and hydroquinone, purity ≥ 99%), were provided by the Department of Biomolecular Sciences (DISB) of Urbino University (Urbino, Italy). All the standards were stored at -20°C. Human blood serum was purchased from Sigma Aldrich-Cerilliant (Milan, Italy) and stored at -20°C. Commercial formulation (CF) samples were provided by Syngenta Ltd (Bracknell, UK) and stored at -5°C. The anti-inflammatory drugs used for the analysis of active ingredients in pharmaceutical tablets with E-LEI-MS were purchased at a local pharmacy and were the following: Surgamyl (produced by Scharper S.p.a., Milan, Italy) containing tiaprofenic acid (300 mg), Brufen (produced by Mylan S.p.a., Milan, Italy) containing ibuprofen lysine salt acid (200 mg), NeoNisidina (produced by Pharmaidea S.r.l., Travagliato, BS, Italy), containing acetylsalicylic acid (250 mg), acetaminophen (200 mg), and caffeine (25 mg). Oranges and bananas, for the determination of pesticides on fruit peel with E-LEI-MS, were bought at a local market. A 5-euro banknote was used for the analysis of drugs of abuse (cocaine) with E-LEI-MS. Paintings used for the analysis of unknown components on painting surfaces with E-LEI-MS, were provided by the “Scuola di Conservazione e Restauro” laboratories, of the University of Urbino.

The solvents and reagents, standards, and samples/matrices used in this study are listed in **Table 1**–**3**.

Table 1. List of chemicals: solvents and reagents used in the study.

Solvent/Reagent	Manufacturer/Supplier	Publication/Congress
Acetonitrile (ACN)	VWR International-Avantor. Milan, Italy	1, 2, 3, 4, 5, 6, 7
Dichloromethane (CH_2Cl_2)	VWR International-Avantor. Milan, Italy	3
Ethanol (EtOH)	VWR International-Avantor. Milan, Italy	8
Hexane (HEX)	VWR International-Avantor. Milan, Italy	3, 8
Isopropanol (IPA)	VWR International-Avantor. Milan, Italy	8
Phosphoric acid (PA)	Merck. Milan, Italy	2, 4, 5, 6, 7
Octanol	VWR International-Avantor. Milan, Italy	3
Water (milli-Q, H_2O)	Merck Millipore Co. Milan, Italy	1, 2, 3, 4, 5, 6, 7

Table 2. List of chemicals: standards used in the study.

Standard	Molecular weight	Molecular formula	Manufacturer/Supplier	Publication/ Congress
Benomyl	290.32	$C_{14}H_{18}N_4O_3$	Sigma Aldrich-Pestanal. Milan, Italy	3
Caffeine	194.19	$C_8H_{10}N_4O_2$	Sigma-Aldrich-Cerilliant. Milan, Italy	3
Cannabidiol (CBD)	314.5	$C_{21}H_{30}O_2$	Toxicology Lab. A.S.T. AV1. Pesaro, Italy	8
Catechol	110.11	$C_6H_6O_2$	DISB University of Urbino. Italy	8
Chlorpyriphos	350.59	$C_9H_{11}Cl_3NO_3PS$	Sigma Aldrich-Pestanal. Milan, Italy	3
Cocaine	303.35	$C_{17}H_{21}NO_4$	Toxicology Lab. A.S.T. AV1. Pesaro, Italy	3
Dicamba	221.03	$C_8H_6Cl_2O_3$	Syngenta Ltd. Bracknell, UK	2, 4, 5, 6, 7
Diethyl phthalate	222.24	$C_{12}H_{14}O_4$	DISB University of Urbino. Italy	8
Diisodecyl phthalate	446.7	$C_{28}H_{46}O_4$	DISB University of Urbino. Italy	8
Dimethyl phthalate	194.18	$C_{10}H_{10}O_4$	DISB University of Urbino. Italy	8
Fentanyl	336.5	$C_{22}H_{28}N_2O$	Sigma-Aldrich-Cerilliant. Milan, Italy	1, 4, 5, 6
Hydroquinone	110.11	$C_6H_6O_2$	DISB University of Urbino. Italy	8
Imazalil	297.18	$C_{14}H_{14}Cl_2N_2O$	Sigma Aldrich-Pestanal. Milan, Italy	3
Phenol	94.11	C_6H_6O	DISB University of Urbino. Italy	8
Resorcinol	110.11	$C_6H_6O_2$	DISB University of Urbino. Italy	6
Tefluthrin	418.7	$C_{17}H_{14}ClF_7O_2$	Syngenta Ltd. Bracknell, UK	2, 4, 5, 6, 7
(+)-alpha-tocopherol	430.7	$C_{29}H_{50}O_2$	Sigma-Aldrich-Cerilliant. Milan, Italy	8
(+)-beta-tocopherol	416.7	$C_{28}H_{48}O_2$	Extrasynthese. Genay, France	8
(+)-delta-tocopherol	402.7	$C_{27}H_{46}O_2$	Sigma-Aldrich-Cerilliant, Milan, Italy	8
(-)-delta9-trans-tetrahydrocannabinol (THC)	314.5	$C_{21}H_{30}O_2$	Toxicology Lab. A.S.T. AV1. Pesaro, Italy	8

Table 3. List of chemicals: samples/matrices used in the study.

Sample/matrix	Manufacturer/Supplier	Publication/ Congress
Human blood serum	Sigma-Aldrich-Cerilliant. Milan, Italy	1, 4, 5, 6
Commercial formulation (CF)	Syngenta Ltd. Bracknell, UK	2, 4, 5, 6, 7
Surgamyl	Scharper S.p.a. Milan, Italy	3
Brufen	Mylan S.p.a. Milan, Italy	3
NeoNisidina	Pharmaidea S.r.l. Travagliato, BS, Italy	3
Oranges and bananas	Local market	3
5-banknote		3
Paintings	"Scuola di Conservazione e Restauro" laboratories, University of Urbino. Italy	3

3.2. STANDARD SOLUTION AND SAMPLE PREPARATION

Diluted solutions of fentanyl were prepared volumetrically in ACN from the reference standard (1000 mg/L) at the following concentrations: 0.2, 1, 2, 4, 10, 20, 40, 80, 200, 400 mg/L, and stored in dark vials (Agilent Technologies, Santa Clara, CA, USA) at -20°C. Diluted solutions were used to volumetrically prepare working standard solutions of fentanyl in ultrapure water and serum, containing ACN 0.25% (v/v) and 5.0% (v/v), respectively. These concentrations were: 1000 µg/L for DI-SPME studies, PFS experiments, and repeatability tests, and 5, 10, 50, 100, 200, 500, and 1000 µg/L for calibration curves. Each solution was injected in triplicate. Serum was not treated nor diluted before use. No filtration of serum samples was needed because polyacrylonitrile (PAN) particles, used as precursors for coating the fiber, does not bind hydrophobic groups, thus preventing the matrix from sticking onto the coating surface. Stock solutions of dicamba and tefluthrin 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 were prepared volumetrically at the concentrations of 0.5, 2.5, 5, 25, 50, and 100 µg/mL in ACN. 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 solution of 5 mg/mL. The diluted CF solution was vortexed for 5 minutes and divided into 1 mL aliquots used 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. 1 mL aliquots of water acidified with 0.2 % PA, without CF, were fortified with 1 µL of working standard solutions 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. Stock solutions of tocopherols and phthalates were prepared volumetrically at a concentration of 1000 mg/L in HEX and stored in dark vials (VWR International, part of Avantor Milan, Italy) at 4°C. Stock solutions of phenols were prepared gravimetrically at a concentration of 1000 mg/L in EtOH and stored in dark vials at 4°C. Working standard solutions of tocopherols were prepared volumetrically as combined suits (100 µL of α at 1000 mg/L+100 µL of β at 1000 mg/L+100 µL of δ at 1000 mg/L) at a concentration of 333 mg/L in HEX. Working standard solutions of cannabinoids were prepared volumetrically as combined suits (200 µL of THC at 100 mg/L+100 µL of CBD at 1000 mg/L) at a concentration of 66 mg/L for THC and 333 mg/L for CBD in MeOH. Working standard solutions of phthalates were prepared volumetrically as combined suits (100 µL of diisodecyl at 1000 mg/L+100 µL of diethyl at 1000 mg/L+100 µL of dimethyl at 1000 mg/L) at a concentration of 333 mg/L in HEX. Working standard solutions of phenols were prepared volumetrically as combined suits (100 µL of phenol at 1000 mg/L+100 µL of catechol at 1000 mg/L+100 µL of resorcinol at 1000 mg/L+100 µL of hydroquinone at 1000 mg/L) at a concentration of 250 mg/L in EtOH. For E-LEI-MS experiments, chlorpyrifos, imazalil, benomyl, cocaine and caffeine stock solutions were prepared gravimetrically at a concentration of 1000 mg/L in ACN. Solutions of chlorpyrifos at 50, 100, and 500 mg/L were prepared by diluting the stock solution with ACN. The banana peel was fortified with benomyl (20 µL of a 1000 mg/L standard solution). In two different experiments, the orange peel was fortified with 20 µL of chlorpyrifos and 20 µL of imazalil, both at a concentration of 1000 mg/L. Chlorpyrifos was added to the orange peel at increasing concentrations spanning from 20 to 1000 mg/L. The 5-euro banknote (62 × 120 mm) was signed with circles to perform 2D and 3D experiments. Some circles were wetted with ACN and represented the negative control, whereas others were spotted with 20 µL of a cocaine solution at 100 mg/L as positive controls. Water, ACN, MeOH, CH_2Cl_2, hexane, and octanol were tested to select the most suitable solvent for dissolving the painting sample surface. All measurements were performed in triplicate and the relative standard deviation was calculated. LODs and LOQs were calculated as the minimum concentration with a S/N ratio equal to or higher than 3 and 10, respectively.

3.3. INSTRUMENTATION

3.3.1. LEI AND DIRECT ANALYSIS: MICROFLUIDIC OPEN INTERFACE AND PASSIVE FLOW SPLITTER

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 ~2.5 µL (**Figure 12**). The MOI chamber dimensions must be as small as possible but sufficiently large to allow fiber insertion promoting the desorption of the correct analyte. During the desorption step, which occurs in static conditions, the chamber filled with organic solvent is isolated and closed with a plug and the analytes partition from the fiber to the solvent.

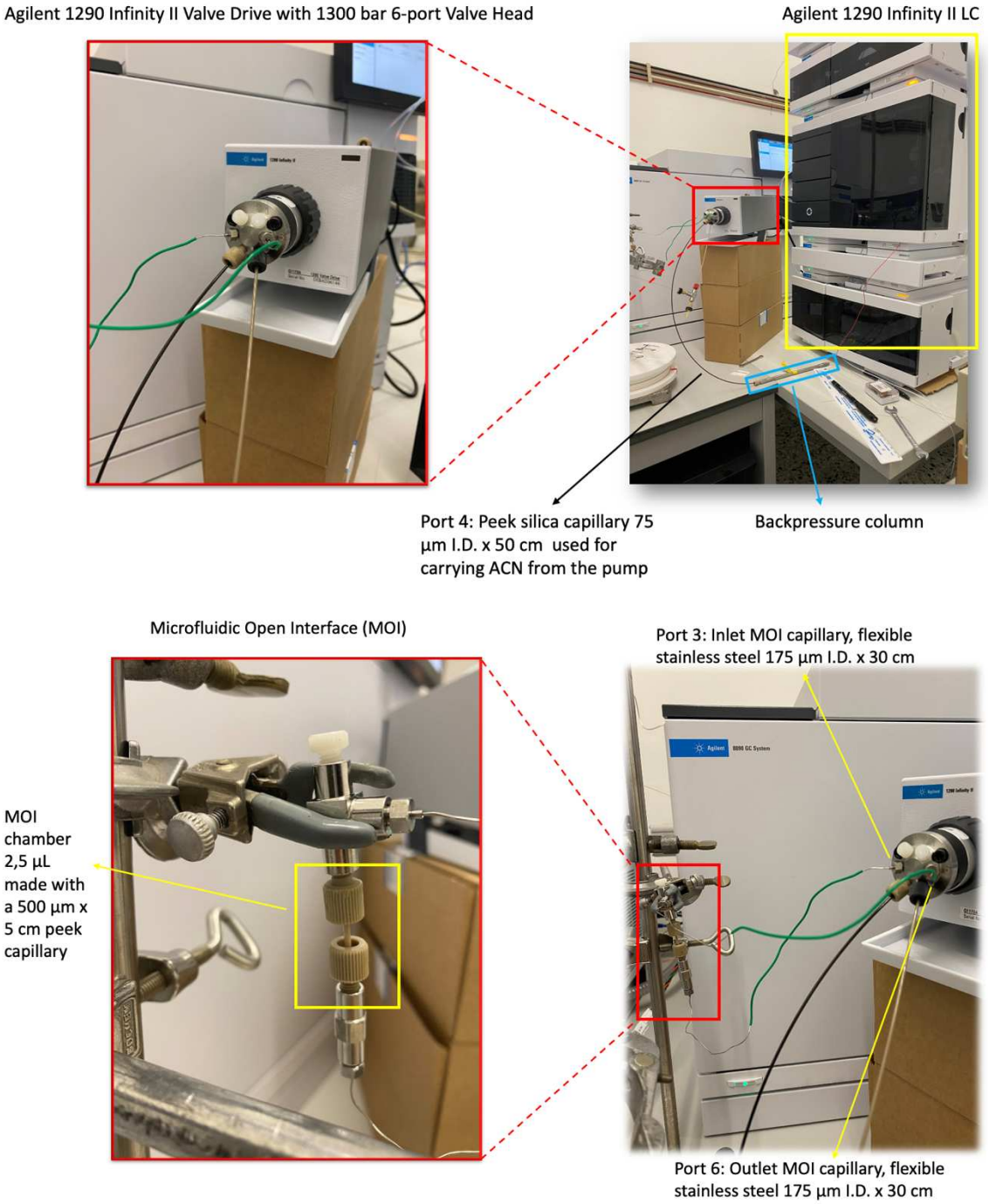


Figure 12. Microfluidic open interface.

PFS reduces the flow rate from 10 µL/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. 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 (**Figure 13**). The length and diameter of the splitter fused silica capillary can be adjusted according to the split ratio needed.

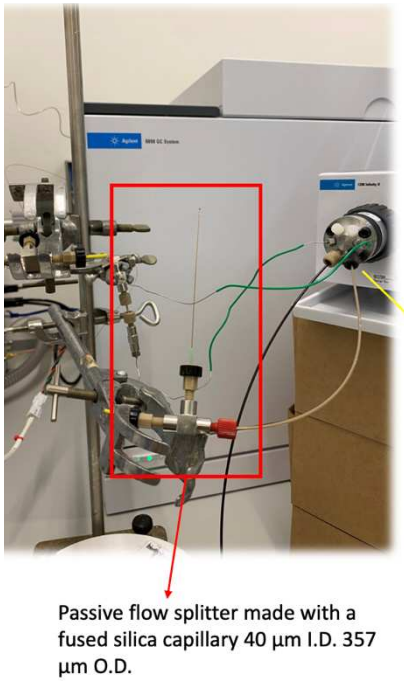


Figure 13. Passive flow splitter**.**

3.3.2. LEI AND DIRECT ANALYSIS: 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. An Agilent 1290 Infinity II binary pump (Agilent Technologies Inc., Santa Clara, CA, USA) was used to deliver 100% ACN (acidified with 0.2% PA for dicamba and tefluthrin analysis) through the system, and an Agilent Zorbax Eclipse XDB C18 backpressure column (4.6x150 mm, 5 µm particle size) was employed for stabilizing the flow rate. 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.). The scheme of the MOI-PFS-LEI-QQQ and MOI-PFS-LEI-QTOF systems is reported **in Figure 14**.

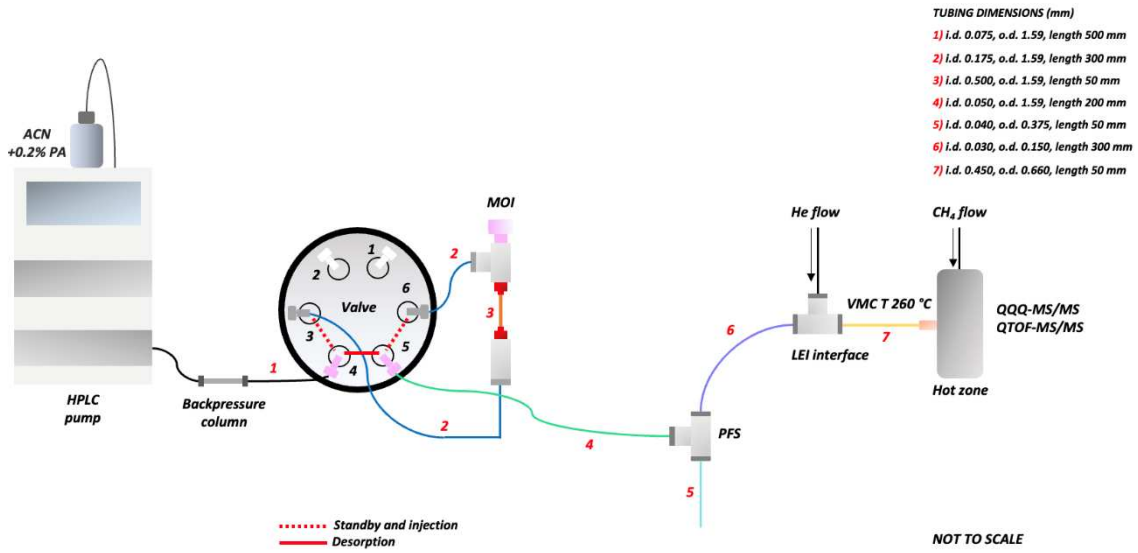


Figure 14. Scheme and operation of the MOI-PFS-LEI-QQQ and MOI-PFS-LEI-QTOF systems. Step 1: MOI is filled in the standby position. The solvent flows from the pump to port 4 of the valve at 10 µL/min, which is in line with port 3 (MOI inlet). Once the MOI chamber is filled, the liquid flows to port 6 (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.

As indicated in **Table 4**, for fentanyl analysis in EI mode, the VMC temperature was set at 350 °C, quadrupoles temperature was 150 °C and the EI ion source was kept at 280 °C. Fentanyl data acquisition was carried out in MRM using the following transitions and collision energies: Q = 245→189 (10 eV) and q = 245→146 (5 eV) (**Table 4**). For dicamba and tefluthrin analysis in NCI mode, VMC and quadrupole temperatures were 260 °C and 150 °C, respectively (**Table 5**). The NCI ion source temperature was 150 °C for providing low-kinetic energy electrons [69]. 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 [69]. Dicamba and tefluthrin data acquisitions were carried out in MRM using the following transitions and collision energies: Q = 149→105 (10 eV) and q = 184→104 (5 eV) for dicamba, Q = 241→205 (10 eV) and q = 243→205 (10 eV) for tefluthrin (**Table 5**).

Table 4. Operational parameters for MOI-PFS-LEI-QQQ (low-resolution) analysis of fentanyl in water and human blood serum.

Compound name	Mobile phase	VMC T °C	Quadrupoles T °C	Ion source T °C	MRM transitions and CE
Fentanyl	100% ACN	350	150	280	245→189 (10 eV) 245→146 (5 eV)
Flow rate 10 µL/min split at 500 nL/min (1:20 split ratio)					
Backpressure column Eclipse XDB C18 4.6x150 mm, 5 µm particle size					
Ionization mode EI					

Table 5. Operational parameters for MOI-PFS-LEI-QQQ (low-resolution) simultaneous analysis of dicamba and tefluthrin in water and commercial formulation.

Compound name	Mobile phase	VMC T °C	Quadrupoles T °C	Ion source T °C	MRM transitions and CE
Dicamba	100% ACN with 0.2% PA	260	150	150	149→105 (10 eV) 184→104 (5 eV)
Tefluthrin					241→205 (10 eV) 243→205 (10 eV)
Flow rate 10 µL/min split at 500 nL/min (1:20 split ratio)					
Backpressure column Eclipse XDB C18 4.6x150 mm, 5 µm particle size					
Ionization mode NCI					
Reagent gas Methane ~2 mL/min					

The setup described above was also employed for high-resolution analysis of dicamba and tefluthrin in NCI mode 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 (**Table 6**). Q-TOF mass calibration was performed after each analysis. During calibration, no mobile phase was admitted into the ion source.

Table 6. Operational parameters for MOI-PFS-LEI-QTOF-MS (high-resolution) simultaneous analysis of dicamba and tefluthrin in water and commercial formulation. LEI-QTOF-MS.

Compound name	Mobile phase	VMC T °C	Ion source T °C
Dicamba	100% ACN with 0.2% PA	260	150
Tefluthrin			
Flow rate 10 µL/min split at 500 nL/min (1:20 split ratio)			
Backpressure column Eclipse XDB C18 4.6x150 mm, 5 µm particle size			
Ionization mode NCI			
Reagent gas Methane ~2 mL/min			
Acquisition mode full scan from m/z 80 to m/z 500			

3.3.3. DIRECT IMMERSION-SPME METHOD OPTIMIZATION

A C18 SPME fiber was used in direct immersion mode (DI-SPME) for fentanyl sampling in human blood serum and ultrapure water and for dicamba and tefluthrin sampling in CF and ultrapure 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 300 µL aliquots of serum and water spiked with fentanyl at 1000 µg/L, and 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 analysis of dicamba and tefluthrin. Before use, the SPME fiber was preconditioned in H_2O/ACN (50/50, v/v) for 10 minutes using a magnetic stir bar. Sampling was conducted by completely immersing the fiber in 1 mL of the sample. Before desorption, in the case of dicamba and tefluthrin analysis in CF, a 5-second 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 for dicamba and tefluthrin analysis).

3.3.4. LEI AND NORMAL PHASE CHROMATOGRAPHY: LC-LEI-MS/MS AND LC-LEI-QTOF-MS APPARATUS

LC separations in low and high resolution were performed with an Agilent 1290 Infinity II UHPLC (Agilent Technologies, Santa Clara, CA, USA) using an Ascentis silica column (2.1 x 150 mm, 3 µm particle size) purchased from Sigma-Aldrich-Supelco (Milan, Italy). The pump flow rate was set at 100 µL/min with a passive postcolumn splitter to decrease the flow rate to 500 nL/min (1:200 split ratio). 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 and the composition of the mobile phases was optimized for each class of compounds. For tocopherols, HEX (solvent A) and IPA (solvent B) were tested in different percentages as follows: A 100%, A:B 99:1 (v:v), A:B 98:2 (v:v), and A:B 97:3 (v:v). For cannabinoids, HEX (solvent A) and EtOH (solvent B) were tested as follows: A 100%, A:B 99:1 (v:v), A:B 97:3 (v:v), A:B 95:5 (v:v), and A:B 93:7 (v:v). For phthalates, HEX (solvent A) and IPA (solvent B) were tested as follows: A 100%, A:B 99:1 (v:v), A:B 97:3 (v:v), A:B 95:5 (v:v), A:B 93:7 (v:v), and A:B 90:10 (v:v). For phenols HEX (solvent A) and EtOH (solvent B) were tested as follows: A 100%, A:B 90:10 (v:v), and A:B 80:20 (v:v). As indicated in **Table 7**, the most efficient separations were achieved using HEX:IPA 98:2 (v:v) as mobile phases for tocopherols, HEX:EtOH 95:5 (v:v) for cannabinoids, HEX:ISO 90:10 (v:v) for phthalates, and HEX:EtOH 80:20 (v:v) for phenols. The Agilent OpenLab CDS ChemStation software was used for HPLC instrument control. For the detection of the analytes in low-resolution experiments, an Agilent triple quadrupole 7010 B MS (Agilent Technologies, Santa Clara, CA, USA) coupled with an LEI interface was used. The core of the LEI is the vaporization microchannel (VMC) where the liquid phase is mixed with the helium flow, heated, and converted into the gas phase. Different VMC temperatures, ranging from 250 to 400 °C, were tested to ensure rapid and efficient vaporization of the eluate from the LC, avoiding thermal degradation of the analytes. The optimal VMC temperature for tocopherols was set at 250 °C, for cannabinoids at 400 °C, and at 350 °C for phthalates and phenols (**Table 7**). The ion source and quadrupoles temperatures were kept at 280 °C and 150 °C, respectively, for all low-resolution experiments. For tocopherols, cannabinoids, and phthalates, data acquisition was carried out in multiple reaction monitoring (MRM) using the following transitions and collision energies (**Table 7**): Q = 430→165 (15 eV) and q = 430→205 (5 eV) for α tocopherol, Q = 416→151 (20 eV) and q = 191→135 (10 eV) for β tocopherol, Q = 402→137 (15 eV) and q = 402→177 (10 eV) for δ tocopherol, Q = 314→299 (5 eV) and q = 231→174 (20 eV) for THC, Q =

246→174 (25 eV) and q = 231→174 (20 eV) for CBD, Q = 149→93 (15 eV) and q = 149→121 (10 eV) for diisodecyl phthalate, Q = 177→149 (5 eV) and q = 222→149 (5eV) for diethyl phthalate, 163→133 (5 eV) for dimethyl phthalate. Phenols data acquisition was carried out in selected ion monitoring (SIM) using the following characteristic ions (**Table 7**): m/z 94 for phenol and m/z 110 for catechol, resorcinol, and hydroquinone. Data were acquired with the Agilent Mass Hunter GC/MS acquisition software (Version B.07.04.2260) and processed with the Agilent Mass Hunter Qualitative Analysis software (Version B.07.00).

Table 7. Operational parameters for NPLC-LEI-QQQ-MS (low-resolution) separations of tocopherols, cannabinoids, phthalates, and phenols.

Compound name	Mobile phase	Injection volume	Acquisition mode	MRM transitions and CE/Selected ions	VMC T °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 (5eV)	
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 split at 500 nL/min (1:200 split ratio)					
Column Ascentis Si 2.1x150 mm, 3 µm particle size					
Ion source temperature 280 °C					
Quadrupoles temperature 150 °C					

An Agilent quadrupole time of flight 7250 MS (Agilent Technologies Inc., Santa Clara, CA, USA) equipped with an LEI interface was used for the detection of the analytes in high-resolution experiments. To make a comparison with the low-resolution setup, the same conditions in terms of the column, flow rate, injection volume, mobile phase composition, and VMC temperature were applied. The ion source temperature was kept at 260 °C for all high-resolution experiments. For each class of compounds, data

acquisition was carried out in full scan. The acquisition range was set from m/z 90 to m/z 500 to avoid the detection of the ions deriving from the hexane (MW 86.18 g/mol) in the mobile phase. The data extraction window was set at 25 ppm. The operational parameters used for NPLC-LEI-QTOF-MS (high-resolution) experiments are presented in **Table 8**. After each analysis, Q-TOF mass calibration was performed, during this procedure, 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 8 Operational parameters for NPLC-LEI-QTOF-MS (high-resolution) separations of tocopherols, cannabinoids, phthalates, and phenols.

Compound name	**Mobile phase**	**Injection volume**	**VMC T °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 µL/min split at 500 nL/min (1:200 split ratio)			
Column Ascentis Si 2.1x150 mm, 3 µm particle size			
Ion source temperature 260 °C			
Acquisition mode full scan from m/z 90 to m/z 500			

The scheme of low and high-resolution systems and all tubing dimensions are illustrated in **Figure 15**.

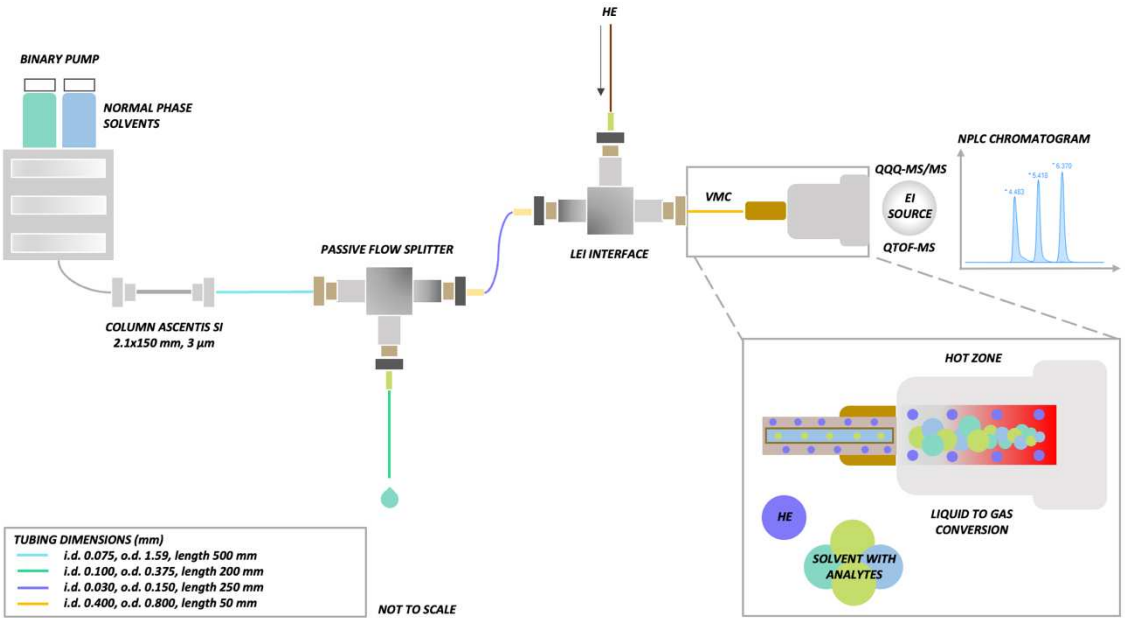


Figure 15. Scheme of the LC-LEI-MS/MS and LC-LEI-QTOF-MS apparatus.

3.3.5. E-LEI-MS SYSTEM

The E-LEI-MS apparatus is composed of a single quadrupole mass spectrometer Agilent Technologies 5975 inert Mass Selective Detector (Agilent Technologies, Santa Clara, CA, USA) equipped with an EI source, a sampling tip realized with a fused silica capillary (30 µm i.d., 375 µm o.d), an MV201 manual microfluidic 3-port valve (LabSmith, Livermore, CA, USA), a tee connector, a peek capillary for solvent delivery (450 µm i.d., 660 µm o.d., 10 cm length), and a micromanipulator (Standa, Vilnius, Lithuania). The solvent is delivered by a KD Scientific syringe pump (KD Scientific Inc., Holliston, MA, USA) equipped with a 1 mL syringe (Hamilton, Bonaduz, Switzerland), directly connected to the tee through a Teflon tubing. The flow of solvent (ACN) delivered by the syringe pump on the sample surfaces was set at 3 µL/min for all the experiments. **Figure 16** shows the system configuration.

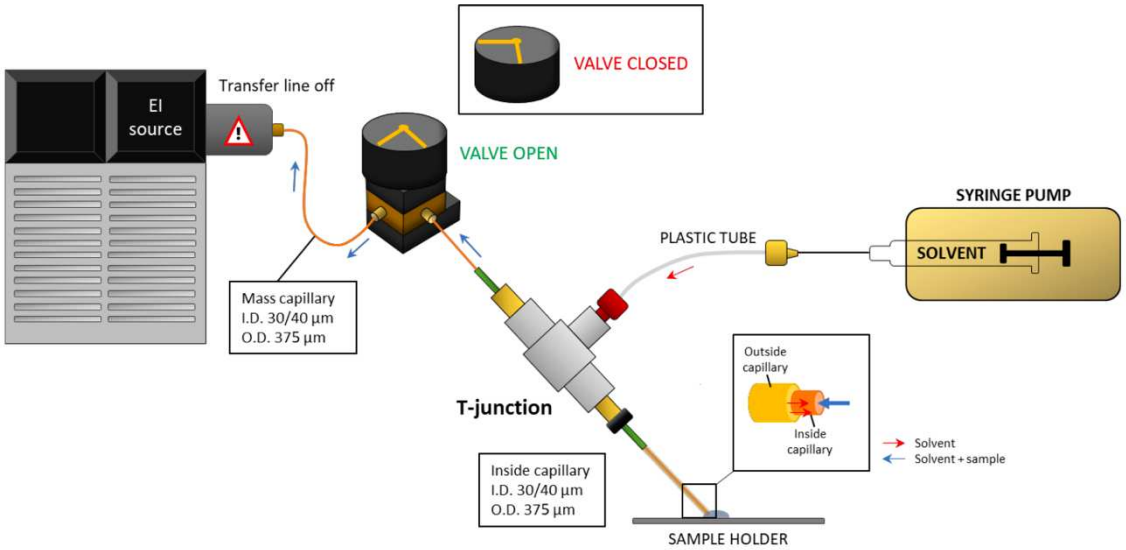


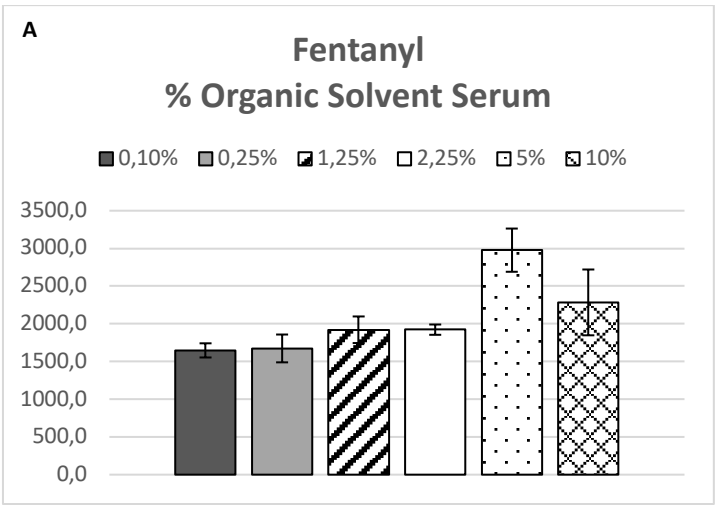
Figure 16. E-LEI-MS apparatus. The sampling tip consists of two coaxial capillaries. The inner capillary (orange) is connected to the EI source passing through an on/off valve and crossing a tee up to the sampling spot. The outer capillary (red) carries the solvent surrounding the inner tubing from the tee to the sampling spot. The syringe pump filled with the solvent is connected to the tee. When the syringe pump is turned on, the solvent flows between the two capillaries up to the sampling spot, where it mixes with the analytes. The solvent and analytes aspiration is ensured by the instrument's high vacuum pumps. Access to the ion source is controlled by an on/off valve. The sample signal appears approximately 1 minute after the valve opening. MS acquisition is turned on before valve actuation. The micromanipulator can operate on x-y-z axes and angle degrees allowing to adjust the sample and tip relative position with an accuracy of 0.1 mm.

MS tuning was performed daily at an ion source temperature of 280 °C, no mobile phase was admitted into the source during this procedure. The ion source can operate up to 350 °C depending on the nature, molecular weight, and boiling point of the selected compounds. The single quadrupole mass spectrometer was operated at 150 °C. MS data acquisition was carried out in scan and SIM modes. Scan analyses were conducted in an m/z range of 60–500, depending on the solvent used, with a sampling rate of 0.43 scan/s, and a threshold of 10. Depending on the targeted molecules, SIM analyses were conducted by selecting two or three specific ions. Blank analyses were performed after each acquisition. Enhanced ChemStation MSD E.02.00.493 (Agilent Technologies) software was used for data acquisition and processing.

4. RESULTS AND DISCUSSION

4.1. LEI AND DIRECT ANALYSIS: SAMPLING AND DESORPTION STEPS

Extraction and desorption efficiency can be affected by several factors. 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 [77], when the concentration of organic solvent in water exceeds 1% of the total sample volume, it can change water properties and distribution constant (K). The amount of organic solvent should be kept at the lowest possible because it may alter DI-SPME extraction efficiency due to the decrease of the analytes partition coefficient between fiber coating and sample matrix (KFS). However, standard solutions were prepared in ACN; hence, using an organic solvent is inevitable. In addition, K is also affected by the nature of the SPME fiber, and in some cases, a higher percentage of organic modifiers can positively affect the extraction yield [77-80]. 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, in the case of fentanyl, it was observed that extraction efficiency was higher at high percentages (5%) of ACN in serum and at low percentages (0.25%) of ACN in water, as shown in the graphics in **Figure 17 A, B.** For dicamba and tefluthrin, it was observed that extraction efficiencies were higher at low percentages (0.1%) of ACN for both compounds and matrices, as shown in the graphics in **Figure 18 A–D**.



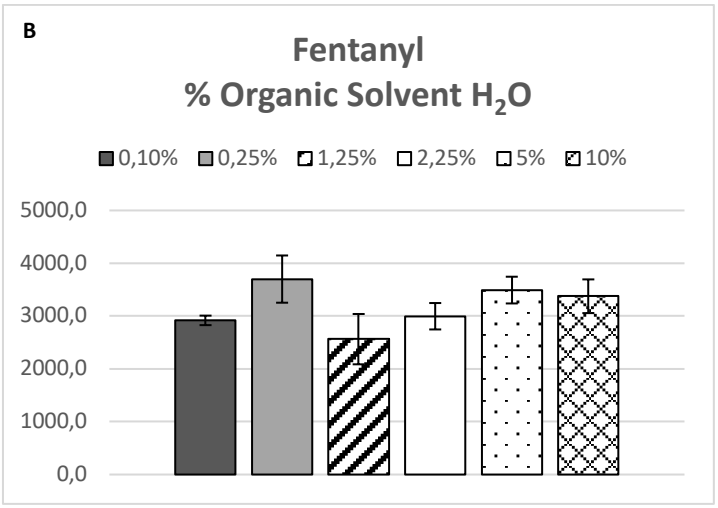


Figure 17 A, B. Peak area values at different percentages of ACN in (A) fentanyl in serum, (B) fentanyl in H_2O. Analyte concentration: 1000 µg/L.

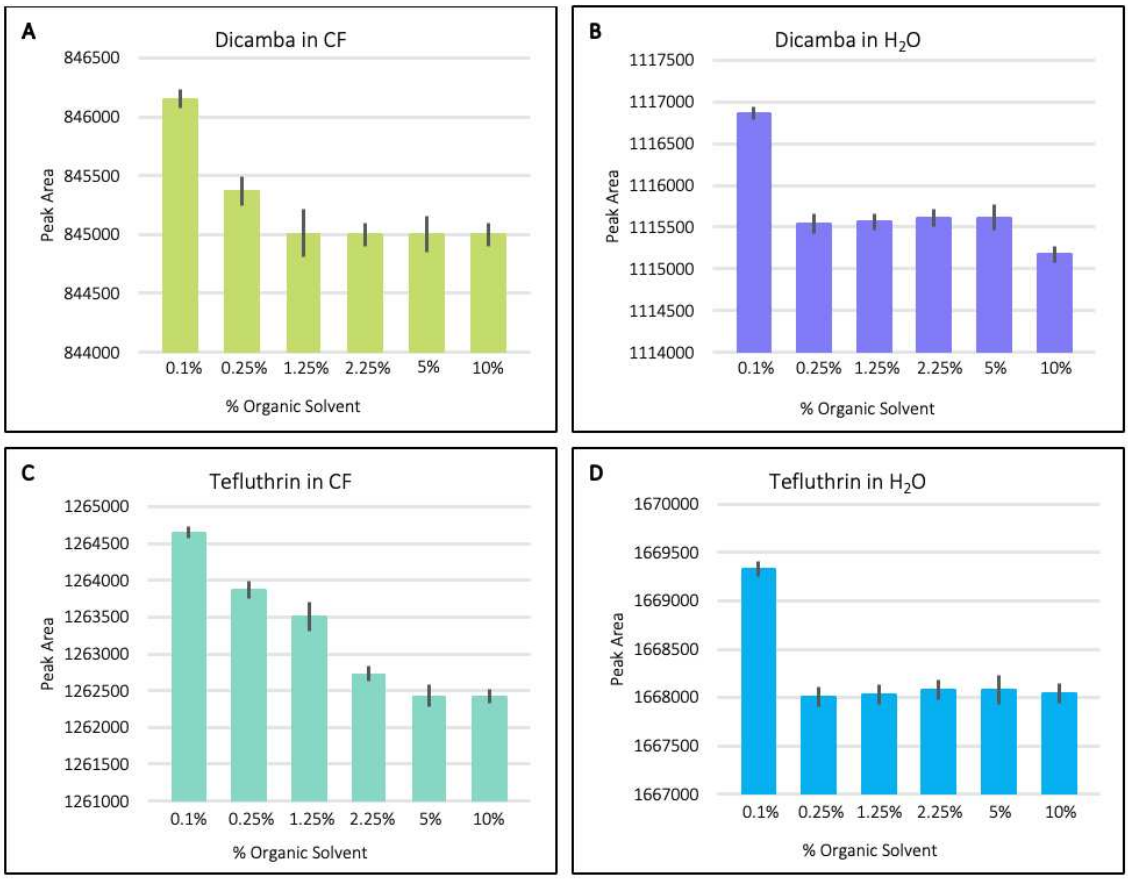


Figure 18 A–D. Peak area values at different percentages of ACN in (A) dicamba in CF, (B) dicamba in H_2O, (C) tefluthrin in CF, (D) tefluthrin in H_2O. Analytes concentration: 100 ng/mL.

Concerning extraction time, several factors, such as molecular size, coating thickness, distribution constant (K), and boundary layer thickness can affect the time required to reach equilibrium [81]. Different extraction times (15, 20, 25, and 30 minutes) using a magnetic stir bar as an agitation method were initially considered. The differences in area values for fentanyl in water, dicamba and tefluthrin in CF and water are reported in **Figure 19** and **Figure 20 A, B** which shows that the highest response was obtained at 25 and 30 minutes.

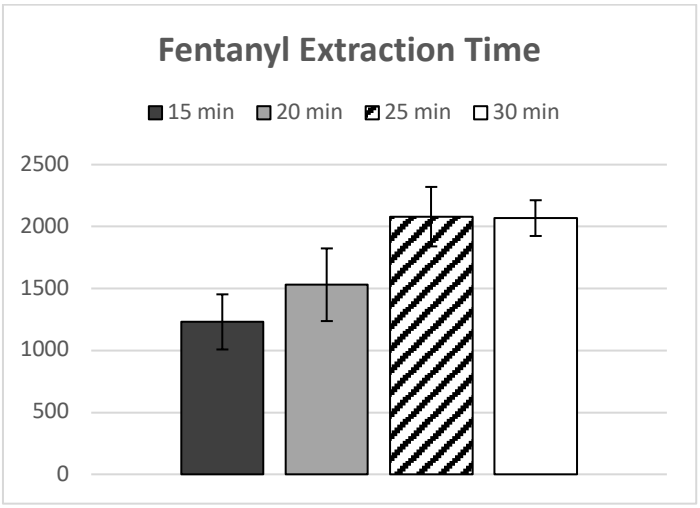


Figure 19. Effects of different extraction times on the integrated peaks area values of fentanyl in water at 1000 µg/L using magnetic stir bar as agitation method.

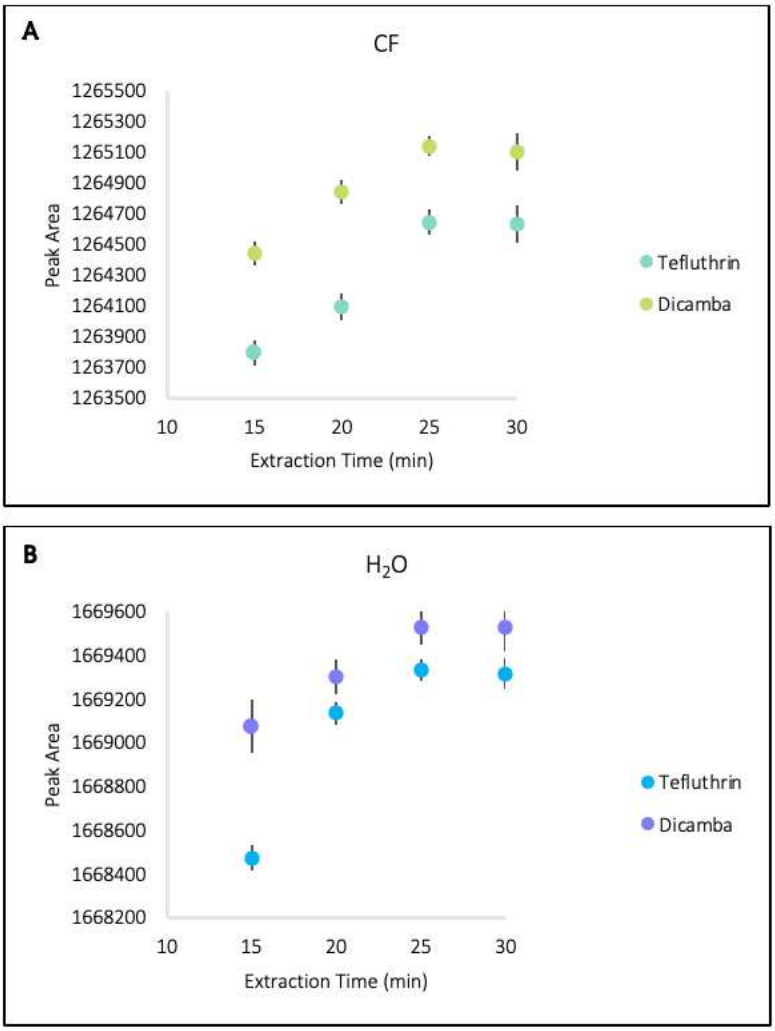


Figure 20 A,B. Effects of different extraction times on the integrated peaks area values of dicamba and tefluthrin at 100 ng/mL using magnetic stir bar as agitation method in (A) CF and (B) H_2O.

The use of vortex was also tested as an alternative to magnetic stir bar agitation to reduce the extraction time, maintaining the same efficiency. According to Pawliszyn and co-workers [82], vibration reduces sampling time, ensuring the same extraction efficiency and good repeatability. Different extraction times were evaluated: 1, 2, 3, and 4 minutes. As demonstrated in **Figure 21** and **Figure 22 A–D**, the equilibrium for tefluthrin in water and dicamba and tefluthrin in CF and water was achieved after 3 minutes, after which the signal remained constant with area values comparable to the ones obtained with the magnetic stir bar. Hence, 3 minutes of vortex agitation was chosen as the extraction time step.

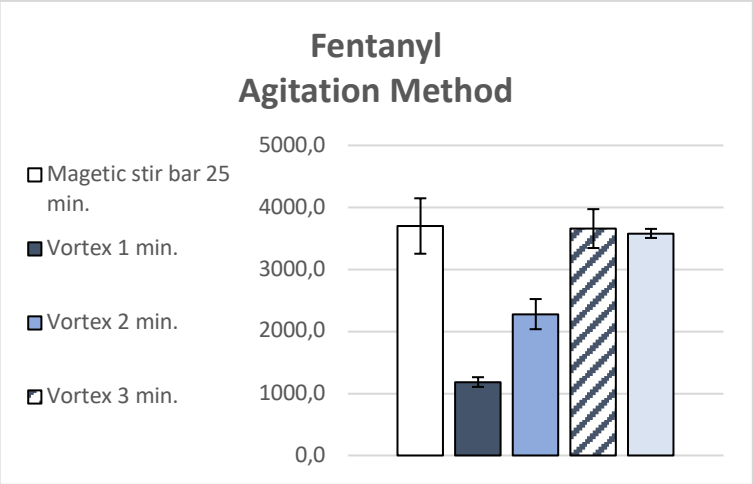


Figure 21. Effects of different extraction times on the integrated peaks area values of fentanyl in water using vortex as agitation method compared with magnetic stir bar. Analyte concentration: 1000 µg/L.

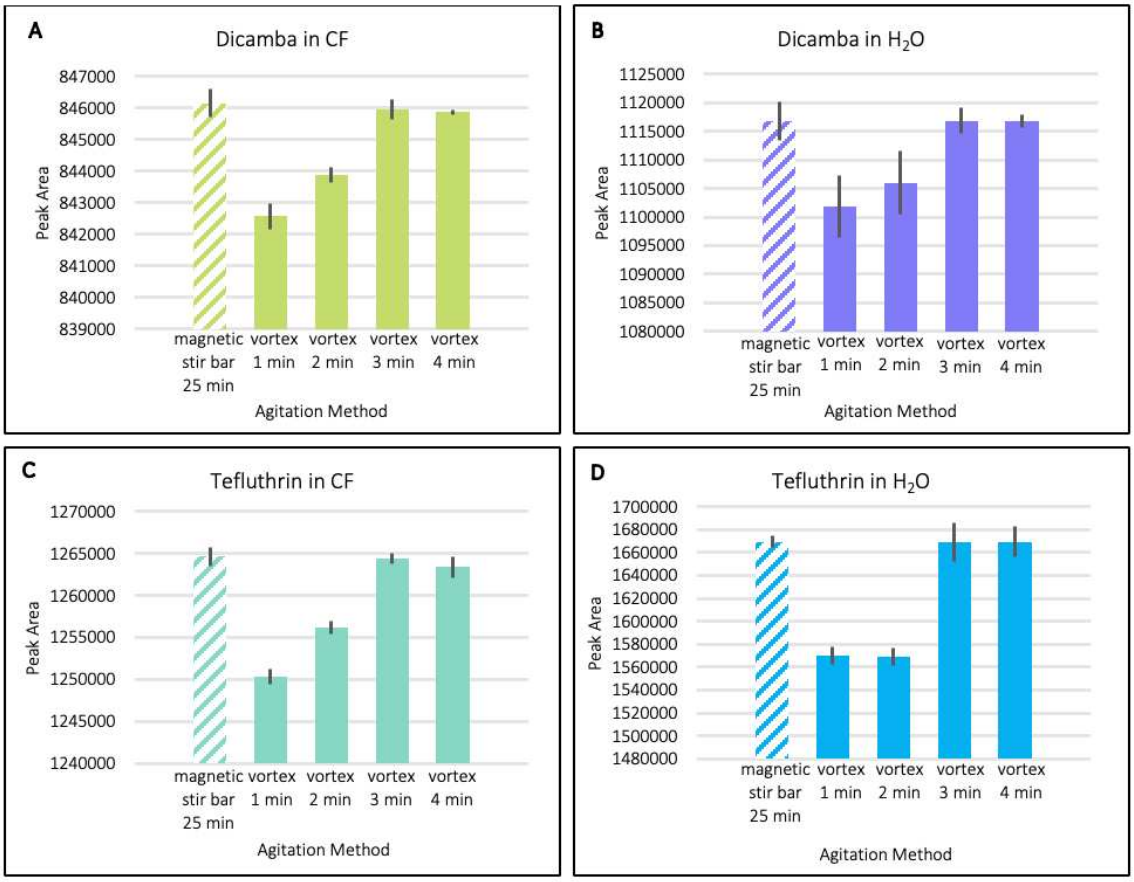


Figure 22 A–D. Effects of different extraction times on the integrated peaks area values of dicamba in (A) CF and (B) H_2O and tefluthrin in (C) CF and (D) H_2O using vortex as agitation method compared with magnetic stir bar. Analytes concentration: 100 ng/mL.

Desorption occurs in static conditions by inserting the SPME fiber inside the MOI chamber filled with ACN (acidified with 0.2% PA for dicamba and tefluthrin analysis). The static desorption efficiency is determined by desorption time and desorption solvent. 30 seconds, 1, 2, and 3 minutes were considered as the desorption times. **Figure 23 and Figure 24 A,B** show the effects of different desorption times on peak areas for fentanyl in water and dicamba and tefluthrin in both matrices; 1 minute was selected as the proper desorption time.

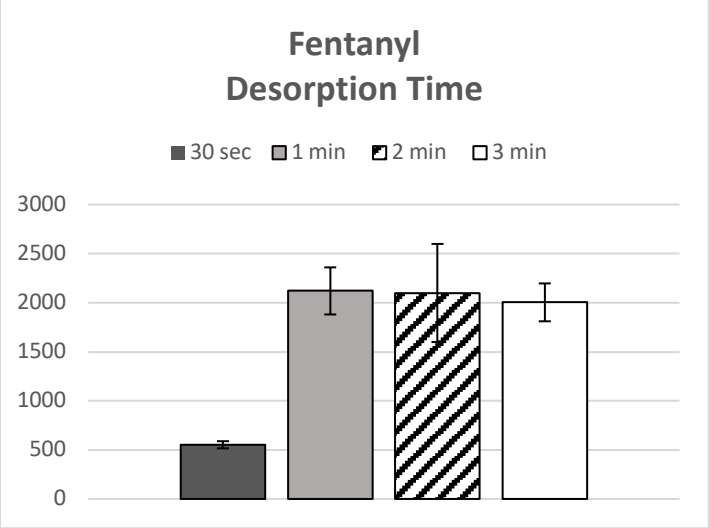


Figure 23. Effects of different desorption times on the integrated peaks area values of fentanyl and tefluthrin at 1000 1000 µg/L using magnetic stir bar as agitation method in water H_2O.

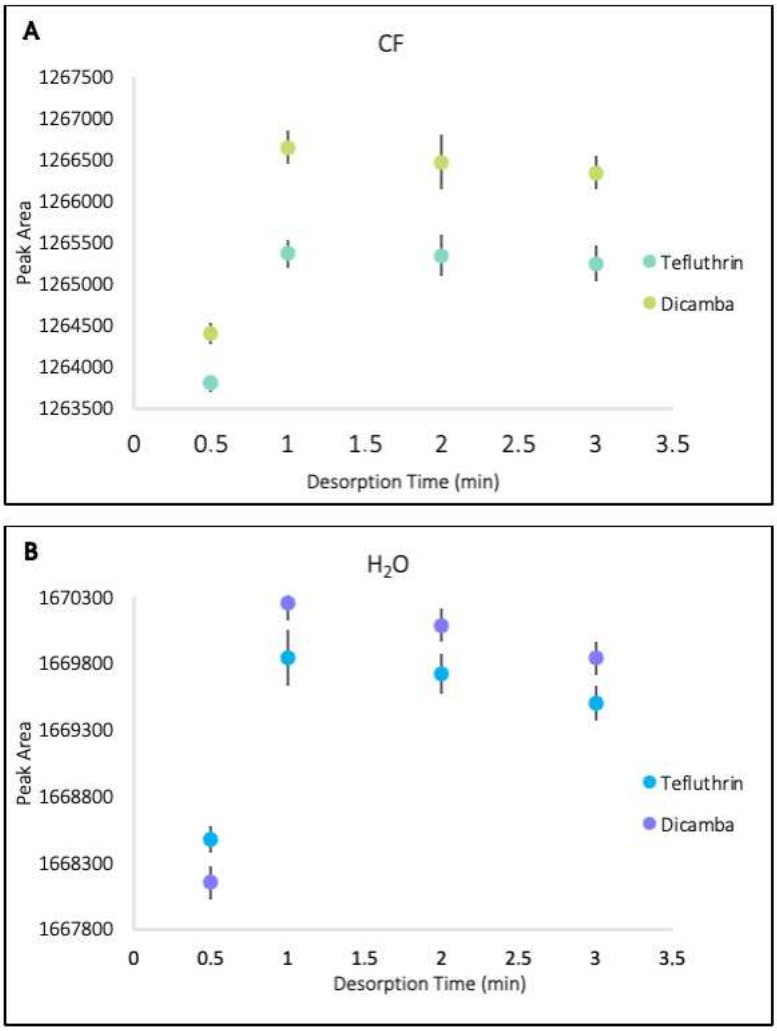


Figure 24 A, B. Effects of different desorption times on the integrated peaks area values of dicamba and tefluthrin at 100 ng/mL using magnetic stir bar as agitation method in (A) CF and (B) H_2O.

To summarize, the optimized DI-SPME method consists of stirring the fiber for 3 minutes with vortex, using a sample solution with 5% and 0.25% ACN for fentanyl in serum and water respectively, and 0.1% ACN for dicamba and tefluthrin in CF and water, and desorbing the fiber for 1 minute in the MOI chamber filled with ACN (acidified with 0.2% PA for dicamba and tefluthrin analysis). As an example, the DI-SPME workflow for dicamba and tefluthrin analysis is shown in **Figure 25**.

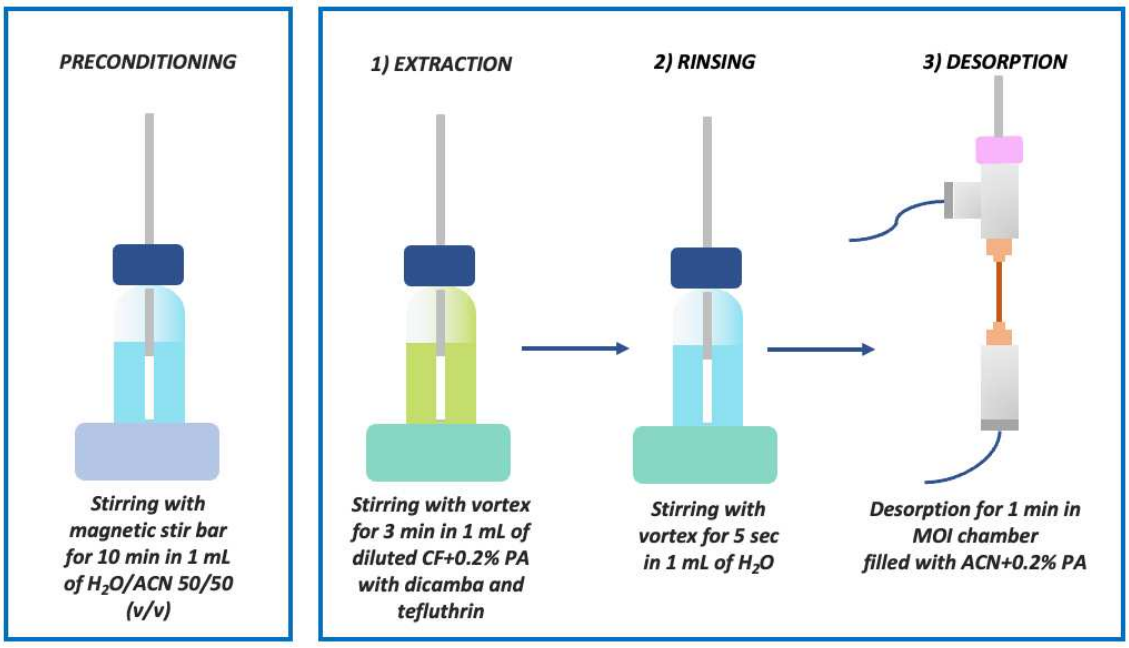


Figure 25. DI-SPME optimized workflow for dicamba and tefluthrin analysis. The procedure consists of (1) extraction, (2) rinsing, and (3) desorption. The C18 fiber was preconditioned before use.

4.2. LEI AND DIRECT ANALYSIS: METHOD VALIDATION

4.2.1. 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 fentanyl in serum and water and dicamba and tefluthrin in CF, as reported in **Table 9 and 10**. 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 LODs and LOQs calculations. Ten consecutive analyses of 300 µL of serum and water fortified with fentanyl at 1000 µg/L and 10 consecutive analysis 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 5 analyses for 5 consecutive days. Regarding intraday and interday measurements, without internal standard correction, good repeatability was obtained in all the experiments with RSD values lower than 25%. For fentanyl, LODs were 1.5 µg/L1 in water and 2.5 µg/L in serum, and LOQs were 5 µg/L in water and 10 µg/L in serum. For dicamba and tefluthrin, 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.

For fentanyl, linearity was satisfactory in both matrices, with a determination coefficient (R^2) of 0.9995 in water and 0.9981 in serum. Dicamba and tefluthrin calibration curves showed good linearity in CF with an R^2 of 0.9925 and 0.9958, respectively.

Table 9. Calibration data, LODs, and LOQs for fentanyl in water and serum obtained with the MOI-PFS-LEI-QQQ system.

Matrix	Linearity range (µg/L)	R^2	LOQ (µg/L)	LOD (µg/L)	Intraday RSD% at 1000 µg/L	Interday RSD% at 1000 µg/L
Water	5-1000	0.9995	5	1.5	15	16
Serum	10-1000	0.9981	10	2.5	18	24

Table 10. Method validation data for dicamba and tefluthrin in CF obtained with the MOI-PFS-LEI-QQQ system.

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

Ion suppression and signal enhancement are the major drawbacks affecting the analytical performance when matrix components compete with the analytes of interest. Different methods can be exploited to calculate MEs [83-85]. MEs evaluation was performed by comparing the slopes of calibration curves for fentanyl analyzed in serum and water and for dicamba and tefluthrin analyzed in CF and water (**Figure 26 A-B and Figure 27 A-B**) using the following formula:

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

A result of 100% indicates no MEs. The results were 84.0% dicamba and tefluthrin were 76.80% and 79.09%, for fentanyl, dicamba and tefluthrin, respectively (with a variance of 16%, 23.2% and 20.91%), demonstrating the system's suitability for trace-level analysis in a complex matrix.

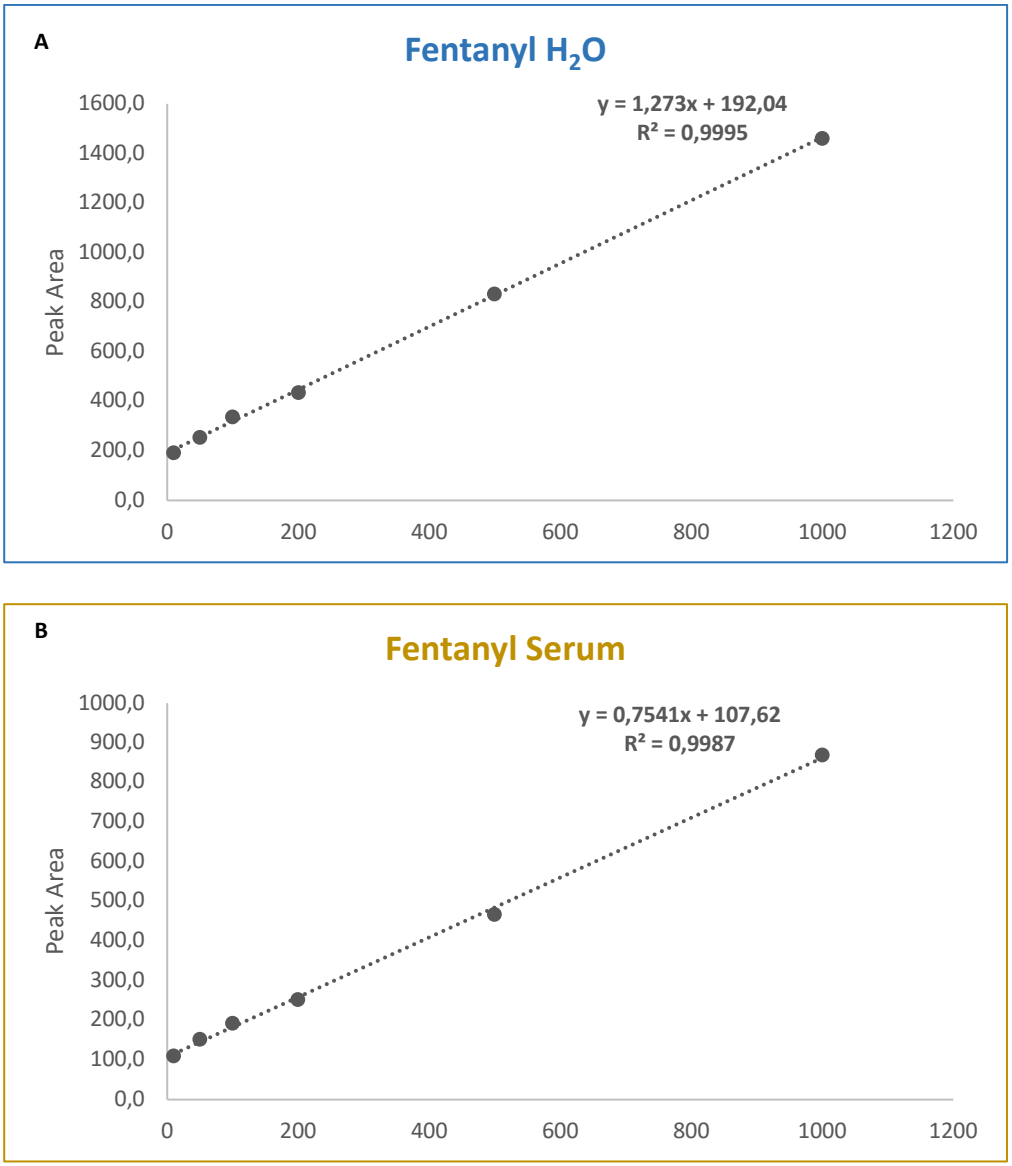


Figure 26 A-B. Calibration curves (5, 10, 50, 100, 200, 500, 1000 µg/L) of (A) fentanyl in water and (B) fentanyl in serum.

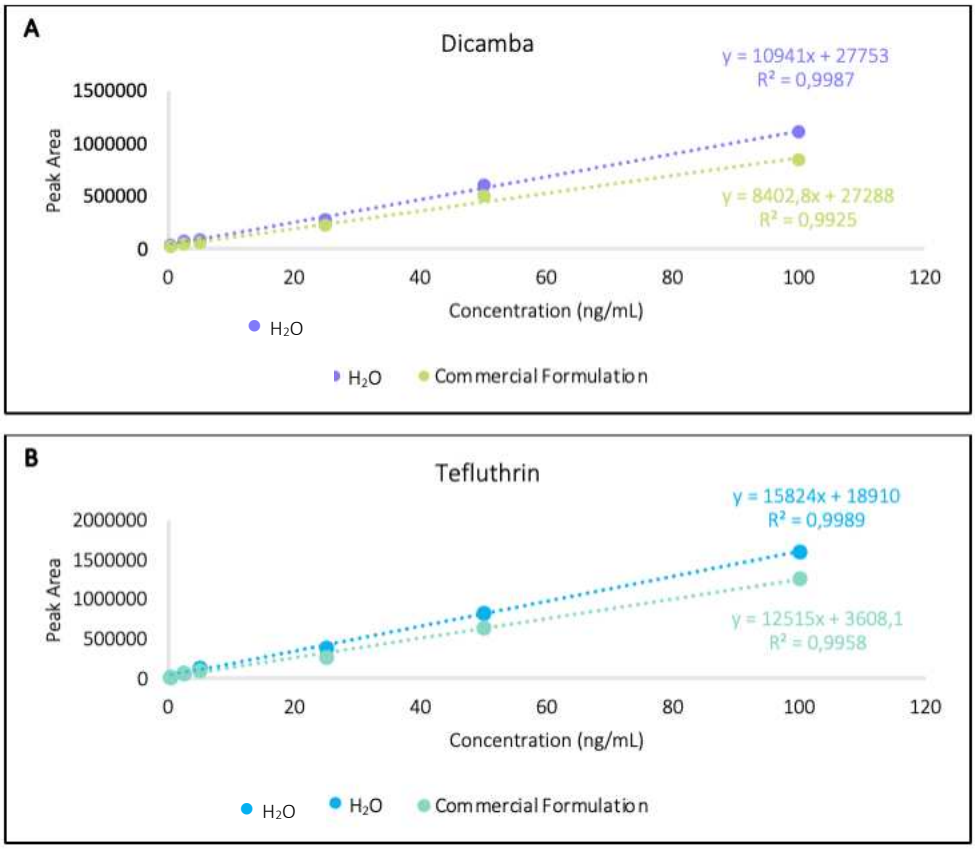


Figure 27 A-B. Calibration curves (0.5, 2.5, 5, 25, 50, and 100 ng/mL) of (A) dicamba and (B) tefluthrin in CF and H_2O.

4.2.2. 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 mass spectra and NCI fragmentation pathways are shown in **Figure 28 A,B.** The results follow those reported in the literature [69], demonstrating the applicability of the system and the potential advantages of using HRMS, which gives accurate masses in complex matrices combined with NCI, which ionize exclusively electrophilic compounds, reducing the background noise.

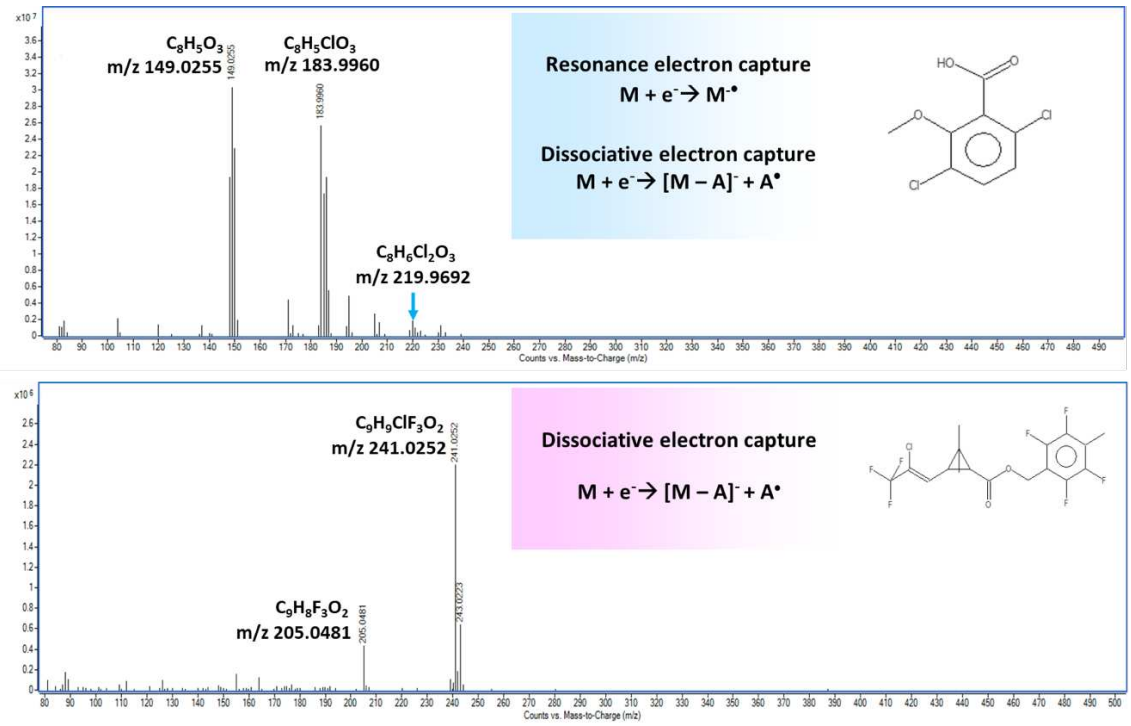


Figure 28 A,B. High-resolution mass spectra and NCI fragmentation pathways of (A) dicamba and (B) tefluthrin at 100 ng/mL in H_2O recorded with the MOI-PFS-LEI-QTOF-MS/MS system.

The performances of the system were evaluated considering intraday repeatability, performing ten consecutive analyses of 1 mL aliquots of CF fortified with dicamba and tefluthrin at 100 ng/mL, LODs, LOQs, and linearity of dicamba and tefluthrin in CF. 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 29 A,B)**.

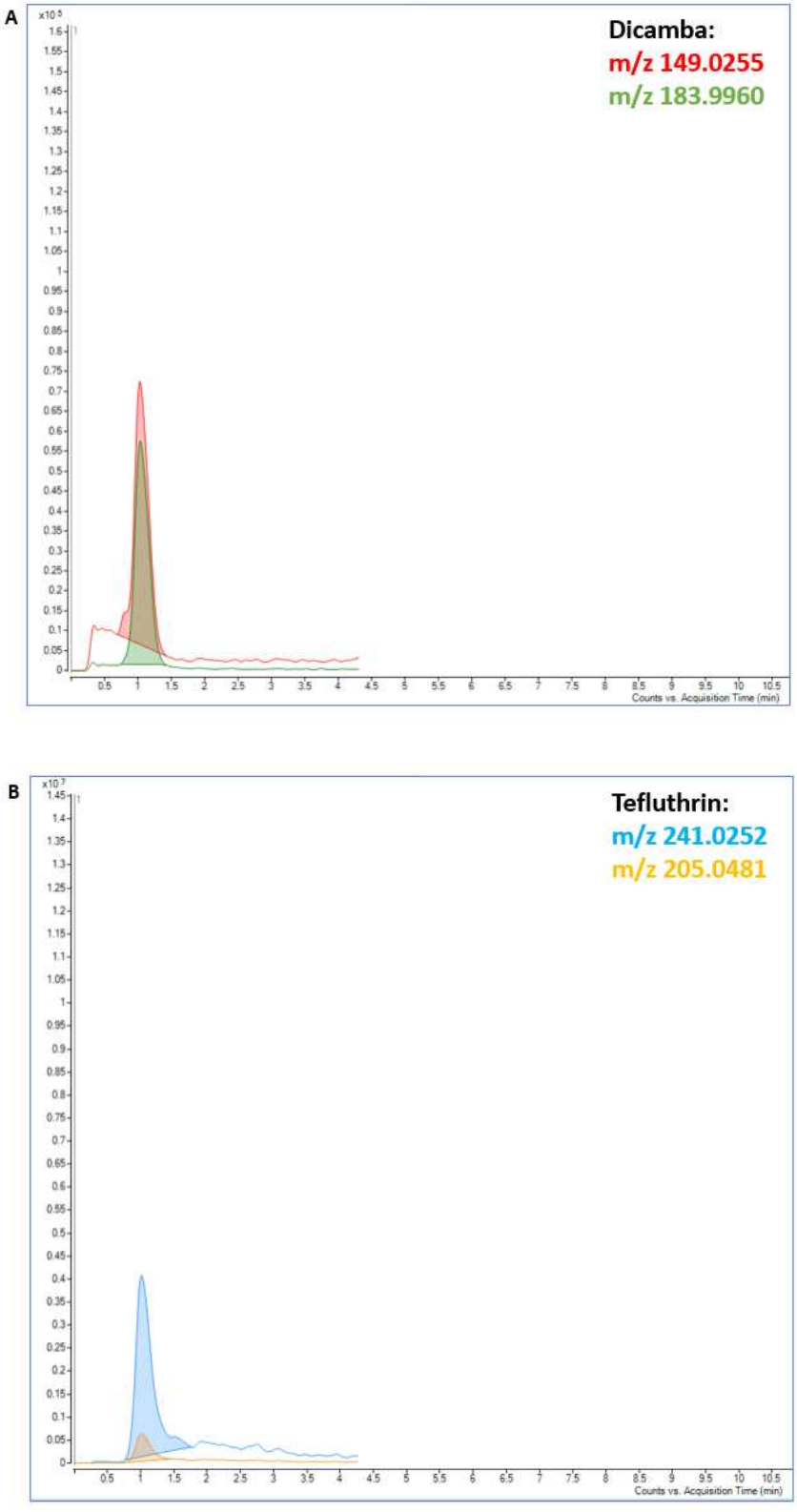


Figure 29 A, B. EIC chromatogram of dicamba (A) and tefluthrin (B) at 100 ng/mL in CF obtained with the MOI-PFS-LEI-QTOF system in NCI. Dicamba peaks: m/z 149.0255 (red), m/z 183.9960 (green). Tefluthrin

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.

As shown in **Table 11**, 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. R^2 values are slightly worse than those obtained in low-resolution. However, it must be considered that no chromatographic separation was involved, and, unlike from low-resolution, the analyses were performed in scan mode.

Table 11. Method validation data for dicamba and tefluthrin in CF obtained with the MOI-PFS-LEI-QTOF system.

Compound	Matrix	Linearity range (ng/mL)	R^2	LOD (ng/mL)	LOQ (ng/mL)	RSD% intraday at 100 ng/mL
Dicamba	CF	0.5-100	0.9752	0.05	0.5	26%
Tefluthrin	CF	0.5-100	0.9397	0.05	0.5	24%

4.3. GREENNESS EVALUATION

According to Sajid and Płotka-Wasylka [86], different methods such as Analytical GREEnness Metric Approach (AGREE) [87], Green Analytical Procedure Index (GAPI) [88], and Analytical Eco-Scale [89] can be used to evaluate the greenness 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 30 A, 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 [69] 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).

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. 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 minutes 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 minutes, two compounds are detected in a single analysis, and sample throughput is twelve analyses/hour. The LC-MS method is penalized by the total power consumption (kWh), which is high considering 20 minutes of analysis. 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 µL/min (2 mL of organic solvent/analysis), whereas in the proposed method, minimal solvent quantities are employed (the flow rate 10 µL/min and only 0.05 mL in 5 minutes were used).

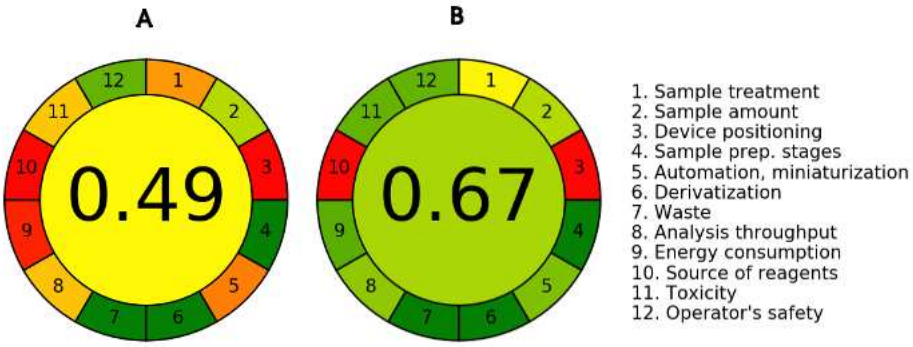


Figure 30 A, B. AGREE comparison between LC-LEI-QQQ (A) and MOI-PFS-LEI-QQQ (B) methods for detecting dicamba and tefluthrin in CF.

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.

4.4 LEI AND NORMAL PHASE CHROMATOGRAPHY

The initial experiments were dedicated to acquiring the EI full scan low and high-resolution mass spectra of all target analytes. The results were obtained in flow injection analysis (FIA) using an external manual injector with an internal loop of 0.1 µL (Vici, Switzerland). Each compound was injected and analyzed separately using the following solutions: α, β, and δ tocopherol at 1000 mg/L in HEX; THC and CBD in MeOH at 100 mg/L and 1000 mg/L, respectively; diisodecyl, diethyl, and dimethyl phthalate at 1000 mg/L in HEX; phenol, catechol, resorcinol, and hydroquinone at 1000 mg/L in EtOH. In both low and high-resolution experiments, the pump flow rate was set at 100 µL/min split at 500 nL/min (1:200 split ratio), 100% HEX was used as the mobile phase, the scan range was set from m/z 90 to m/z 500, and the VMC temperature was set at 250 °C for tocopherols, 400 °C for cannabinoids, and 350 °C for phthalates and phenols. The ion source temperature was kept at 280 °C in low-resolution mode and at 260 °C in high-resolution. As an example, in **Figure 31 A-C** and **Figure 32 A, B** are reported the recorded low-resolution full scan spectra of tocopherols and cannabinoids. These spectra were compared with the reference spectra present in the NIST library. Regarding tocopherols, the comparison showed that α tocopherol was recognized with a matching factor of 74%, a reverse match of 87%, and probability of 63%; β tocopherol was identified with a matching factor of 91%, a reverse match of 95%, and probability of 60%; δ tocopherol was recognized with a matching factor of 88%, a reverse match of 90%, and probability of 86% (**Figure 33 A-C**). For cannabinoids, THC was identified with a matching factor of 79%, a reverse match of 85%, and probability of 70%; CBD with a matching factor of 74%, a reverse match of 82%, and probability of 74% (**Figure 34 A, B**). These match values can be considered satisfactory demonstrating a proof of concept on

the applicability of the combination of NPLC and EI, made possible thanks to the LEI interface. Concerning high-resolution experiments, the coupling of NPLC with QTOF-MS permitted the acquisition of EI-HRMS spectra allowing the determination of the exact structure of each fragment by applying the utility Mass Spectrum Interpreter developed by NIST which finds possible structural origins of peaks in a mass spectrum providing the molecular formula. As an example, the recorded high-resolution mass spectra and the assignment of the main fragments, according to MS Interpreter, of tocopherols and cannabinoids are reported in **Figure 35 A-C** and **Figure 36 A, B**, respectively. These results demonstrate the potential advantages of using the system for structural elucidation purposes.

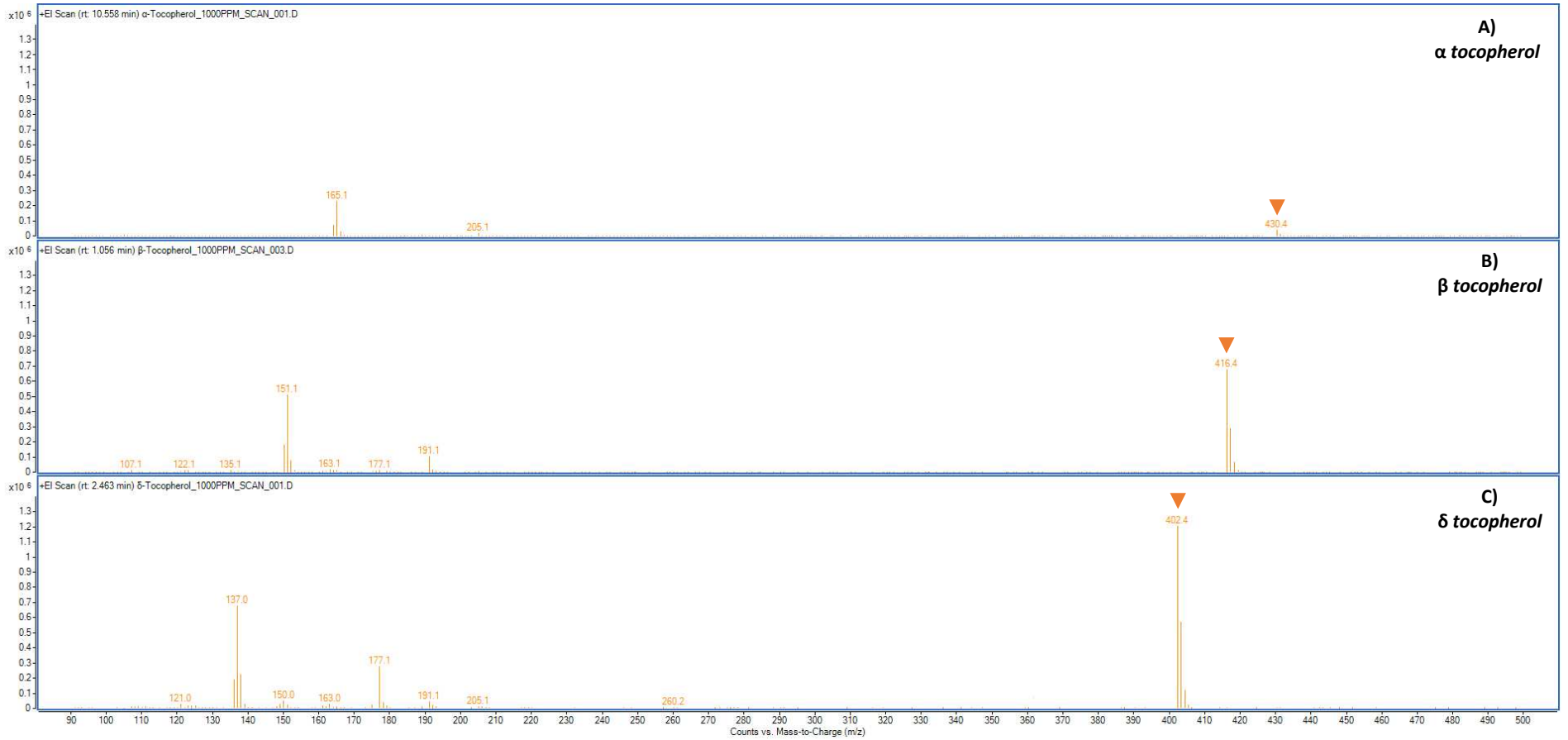


Figure 31 A-C. Recorded EI low-resolution full scan spectrum of A) α tocopherol, B) β tocopherol, and C) δ tocopherol. Each compound molecular ion is indicated by the orange triangle.

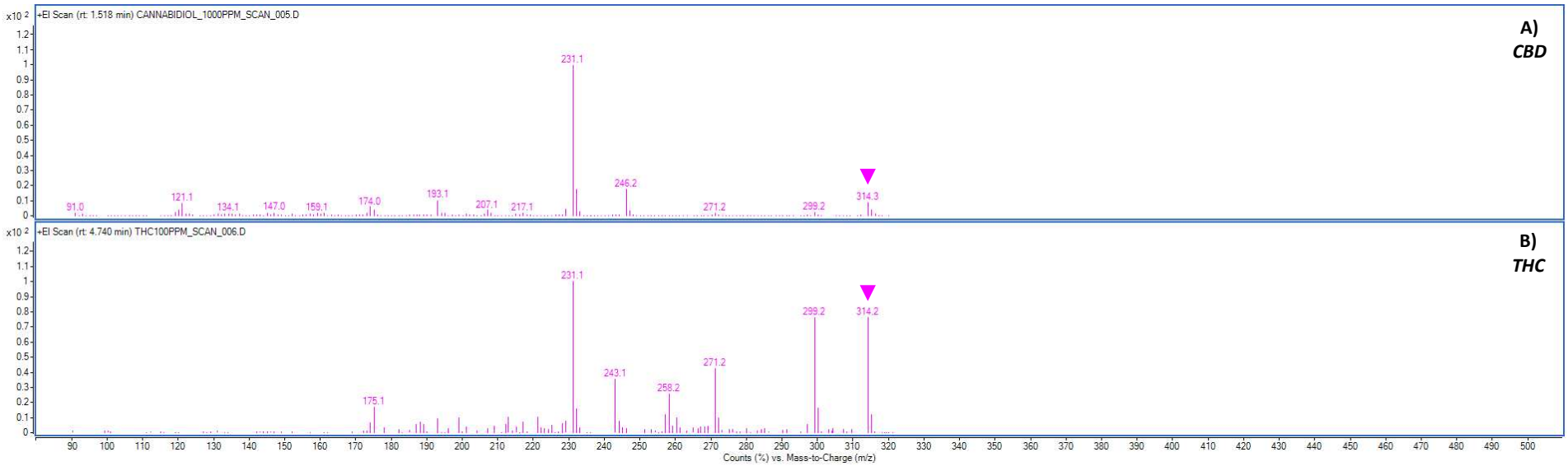


Figure 32 A,B. Recorded EI low-resolution full scan spectrum of A) CBD, and B) THC. Each compound molecular ion is indicated by the pink triangle.

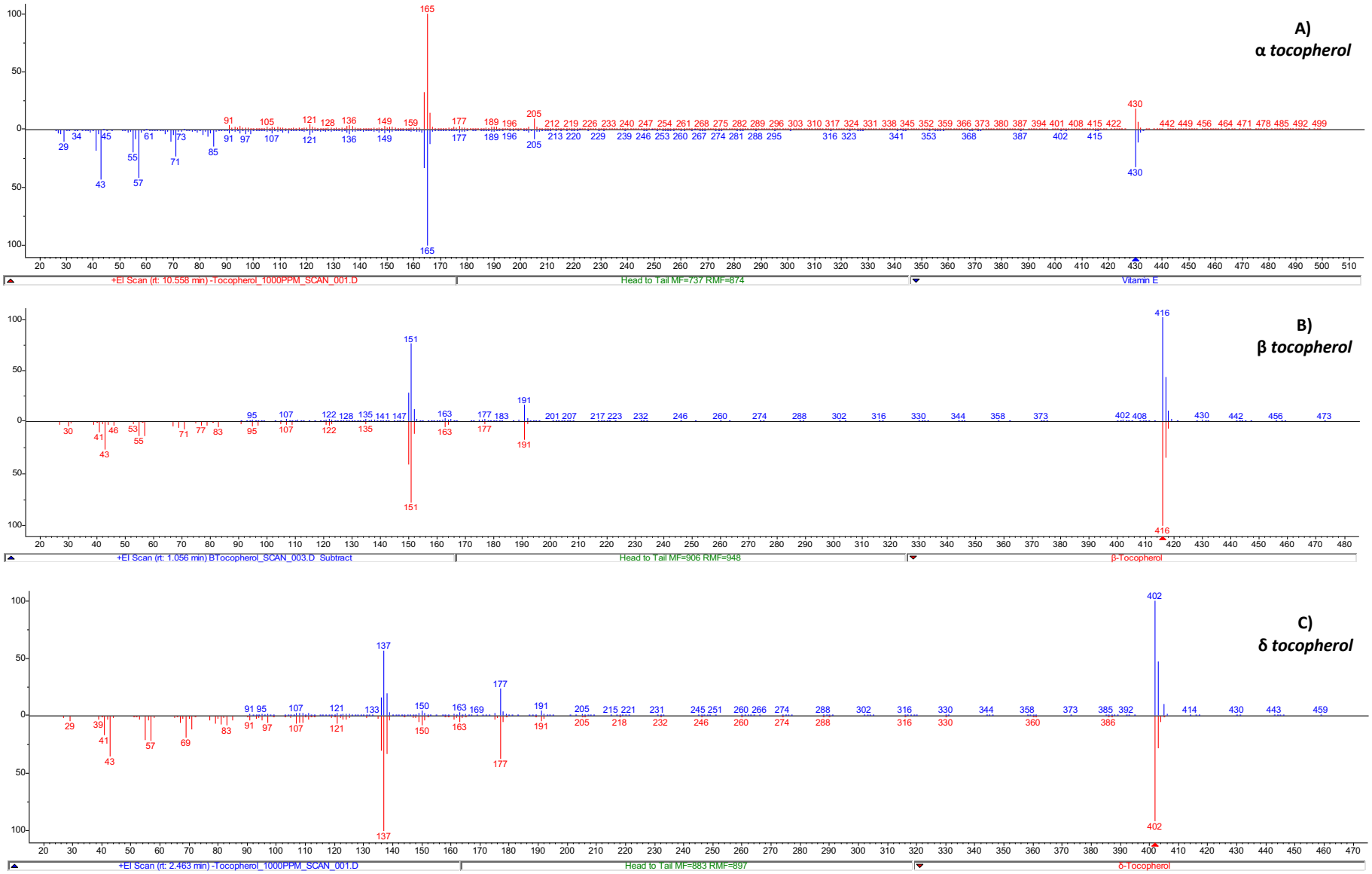


Figure 33 A-C. Comparison between the recorded EI low-resolution mass spectrum of A) α tocopherol, B) β tocopherol, and C) δ tocopherol and the reference spectrum of the NIST library. Blu trace recorded spectrum, red trace reference spectrum.

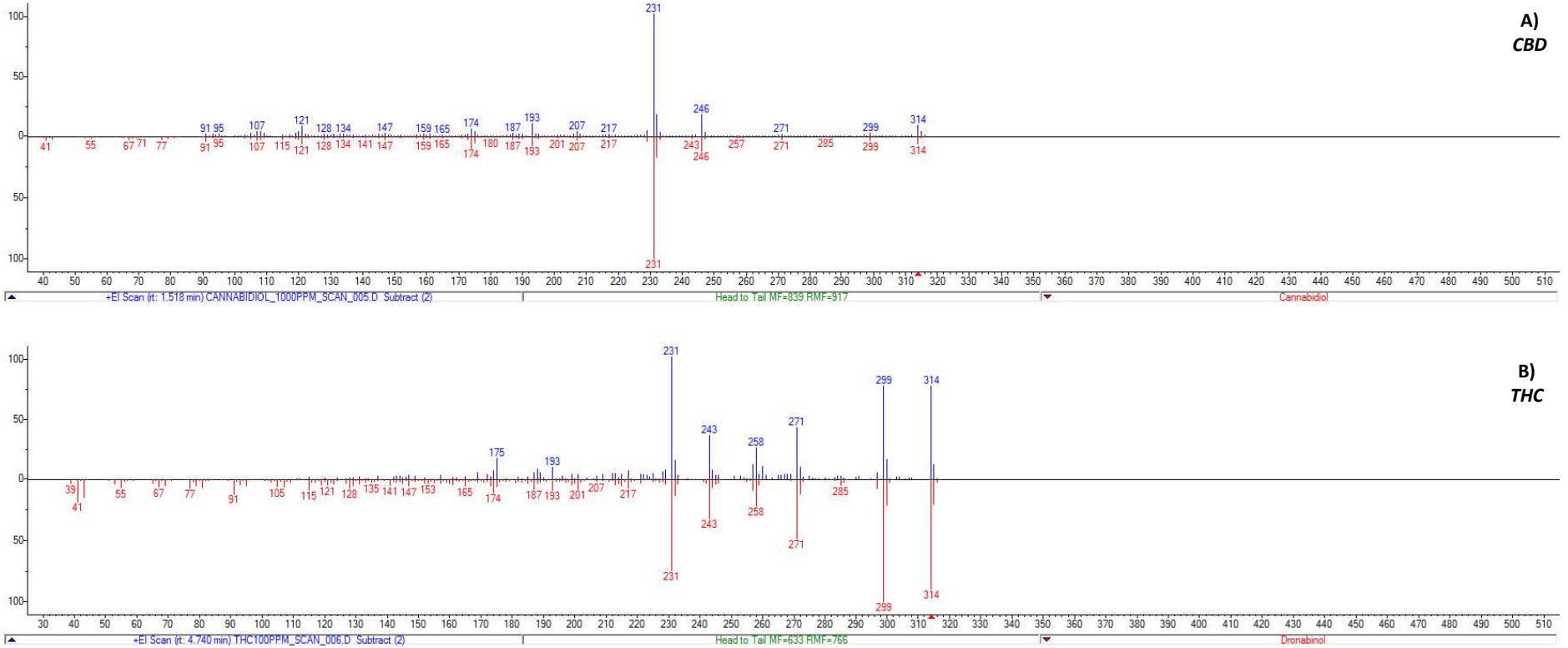


Figure 34 A, B. Comparison between the recorded EI low-resolution mass spectrum of of A) CBD, B) THC and the reference spectrum of the NIST library. Blu trace recorded spectrum, red trace reference spectrum.

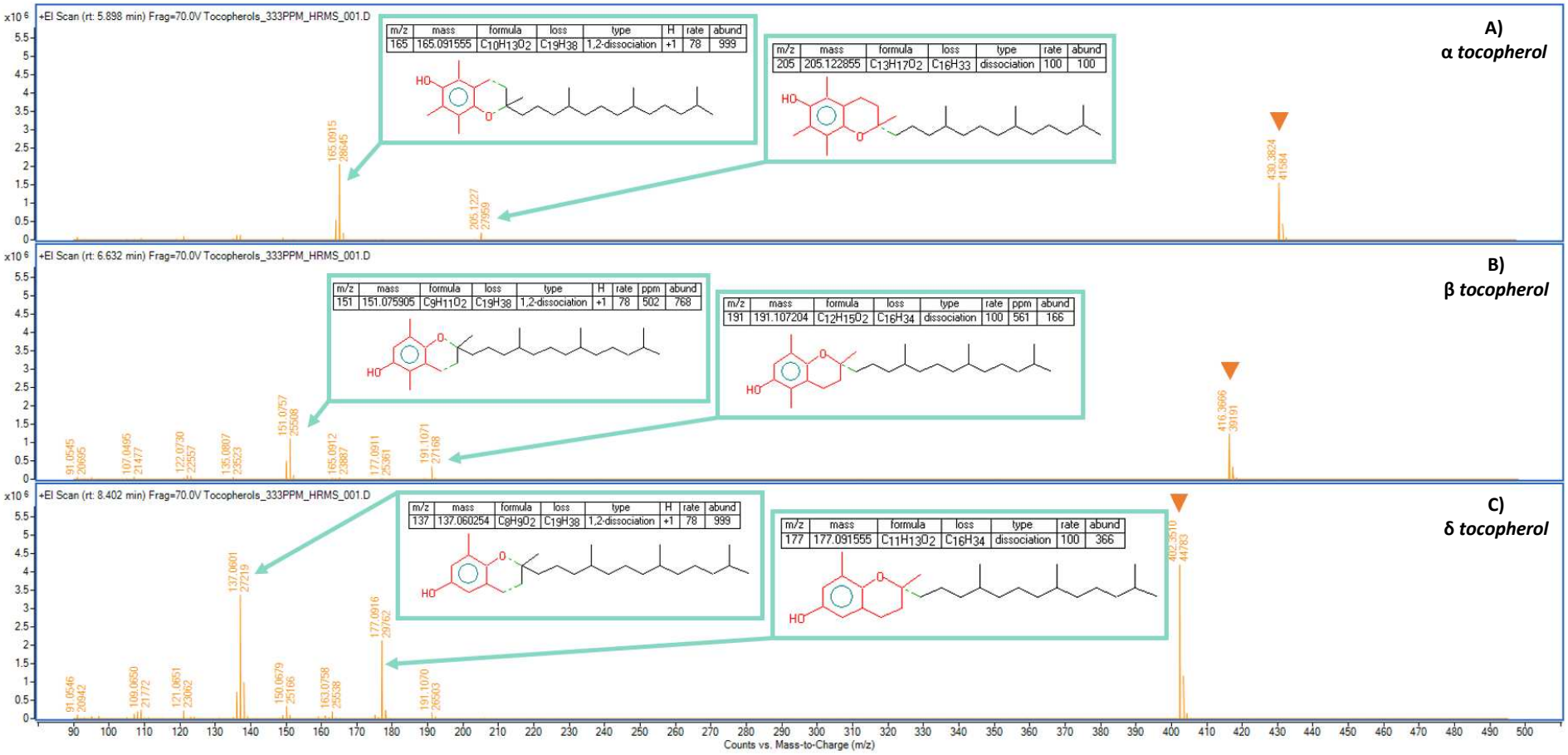


Figure 35 A–C. Recorded EI high-resolution full scan spectrum of A) α tocopherol, B) β tocopherol, and C) δ tocopherol and the assignment of the main fragments according to the MS Interpreter utility developed by NIST. Each compound molecular ion is indicated by the orange triangle.

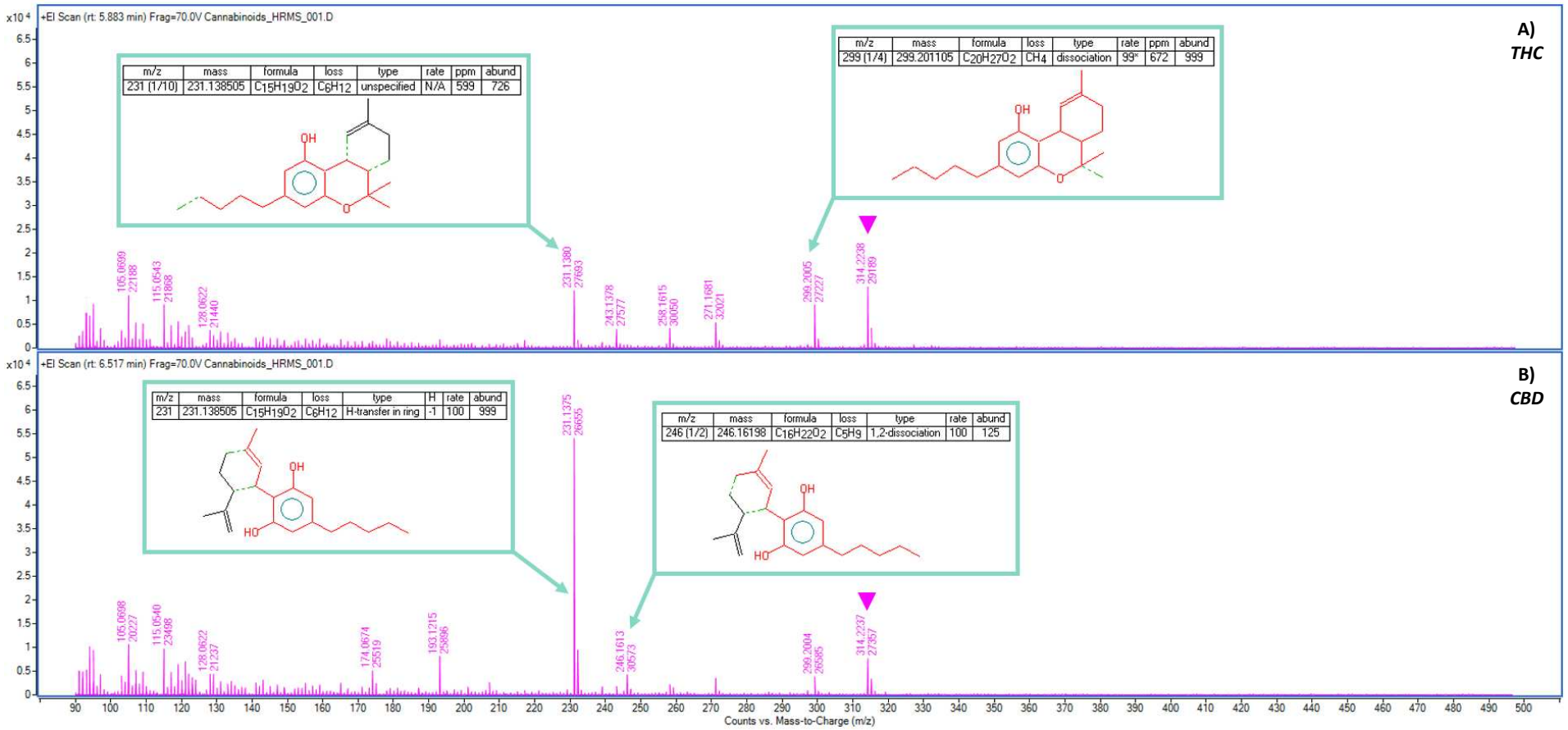


Figure 36 A,B. Recorded EI high-resolution full scan spectrum of A) THC, B) CBD and the assignment of the main fragments according to the MS Interpreter utility developed by NIST. Each compound molecular ion is indicated by the pink triangle.

4.4.1. MOBILE PHASE COMPOSITION AND VMC TEMPERATURE OPTIMIZATION

The mobile phase composition and VMC temperature optimization for tocopherols was carried out in MRM using a standard mixture of α, β, and δ tocopherol at a concentration of 333 mg/L in HEX. The pump flow rate was set at 100 µL/min split at 500 nL/min (1:200 split ratio). The injection volume was 4 µL. The ion source and quadrupoles temperatures were kept at 280 °C and 150 °C, respectively. HEX (solvent A) and IPA (solvent B) were tested in different percentages as follows: A 100%, A:B 99:1 (v:v), A:B 98:2 (v:v), and A:B 97:3 (v:v) (**Figure 37 A-D**). Tocopherols eluted in the following order: α, β, δ tocopherol. Their identification was possible due to the precursor ions and product ions characteristic of each compound selected. Using the last composition of MP, the overall duration of the analysis was reduced (about 7.5 minutes) but an overlap was also observed between the α and β tocopherol peaks. Instead, with 98% of hexane and 2% of isopropanol the analysis time was extended by a few minutes (duration of almost 9 minutes) but there was no overlap, hence, this MF composition was selected.

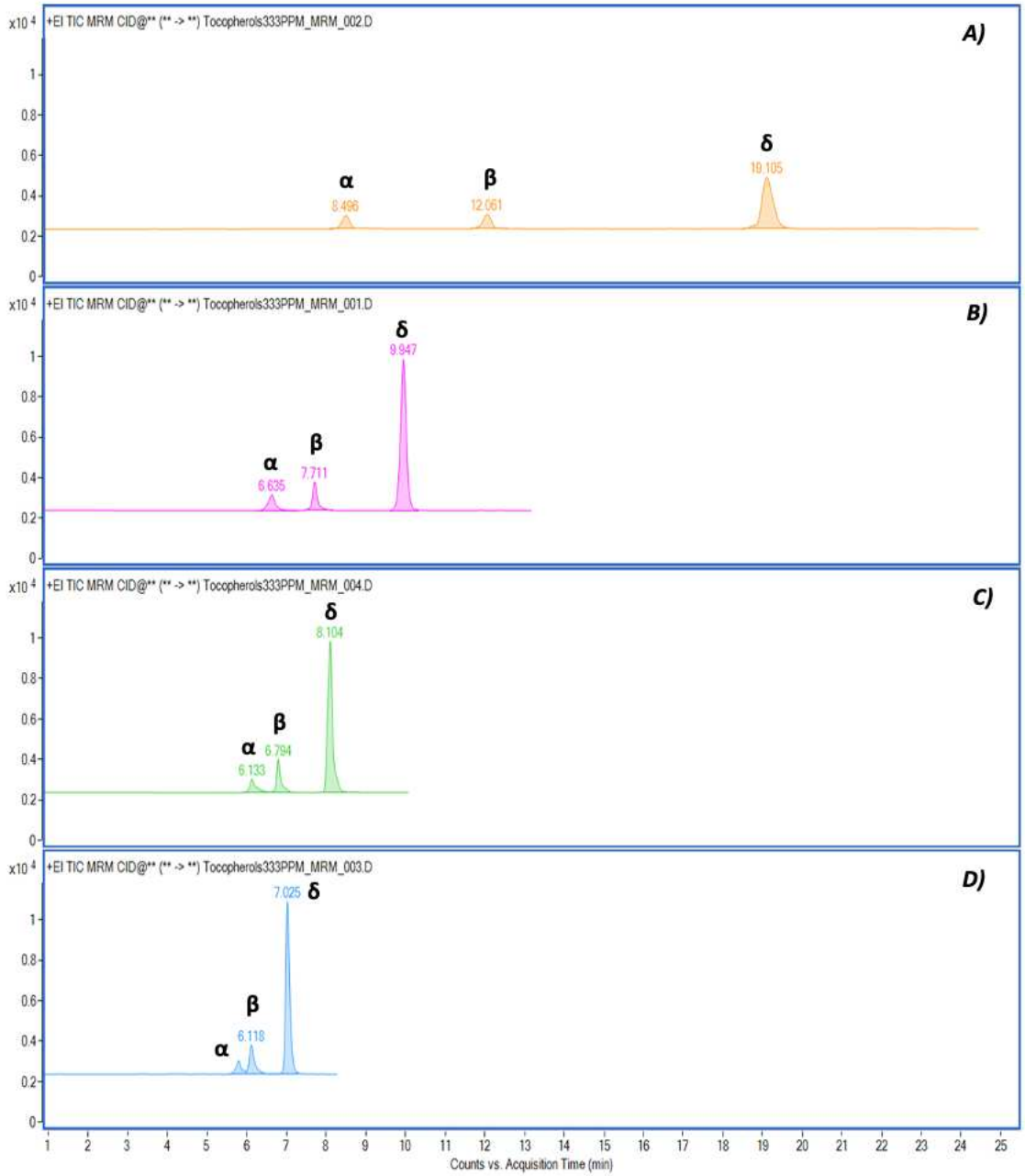


Figure 37 A-D. Results obtained by modifying the % of solvents used as MP for tocopherols. A) 100% HEX, B) HEX:ISO 99:1 (v:v), C) HEX:ISO 98:2 (v:v), and C) HEX:ISO 97:3 (v:v).

The results shown in **Figure 37 A-D** were obtained with a VMC temperature of 250 °C. Different VMC temperatures, ranging from 250 to 400 °C, were tested to ensure rapid and efficient vaporization of the eluate from the LC, avoiding thermal degradation of the analytes. The influence of the VMC temperature was evaluated in terms of the integrated peak area value of each tocopherol and the results (**Figure 38**) demonstrate that the optimal VMC temperature is 250 °C for all three compounds.

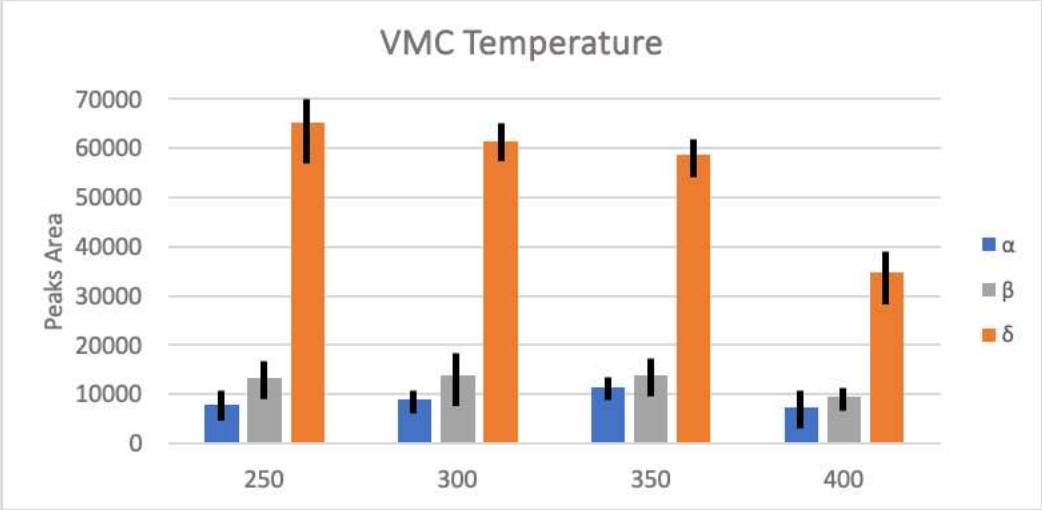


Figure 38. Effects on the integrated peak area values of different VMC temperatures for α, β, and δ tocopherol.

For cannabinoids the mobile phase composition and VMC temperature optimization was carried out in MRM using a standard mixture of THC and CBD at a concentration of 66 mg/L for THC and 333 mg/L for CBD in MeOH. The pump flow rate was set at 100 µL/min split at 500 nL/min (1:200 split ratio). The injection volume was 1 µL. The VMC temperature was set at 400°C. The ion source and quadrupoles temperatures were kept at 280 °C and 150 °C, respectively. HEX (solvent A) and EtOH (solvent B) were tested as follows: A 100%, A:B 99:1 (v:v), A:B 97:3 (v:v), A:B 95:5 (v:v), and A:B 93:7 (v:v) (**Figure 39 A-E**).

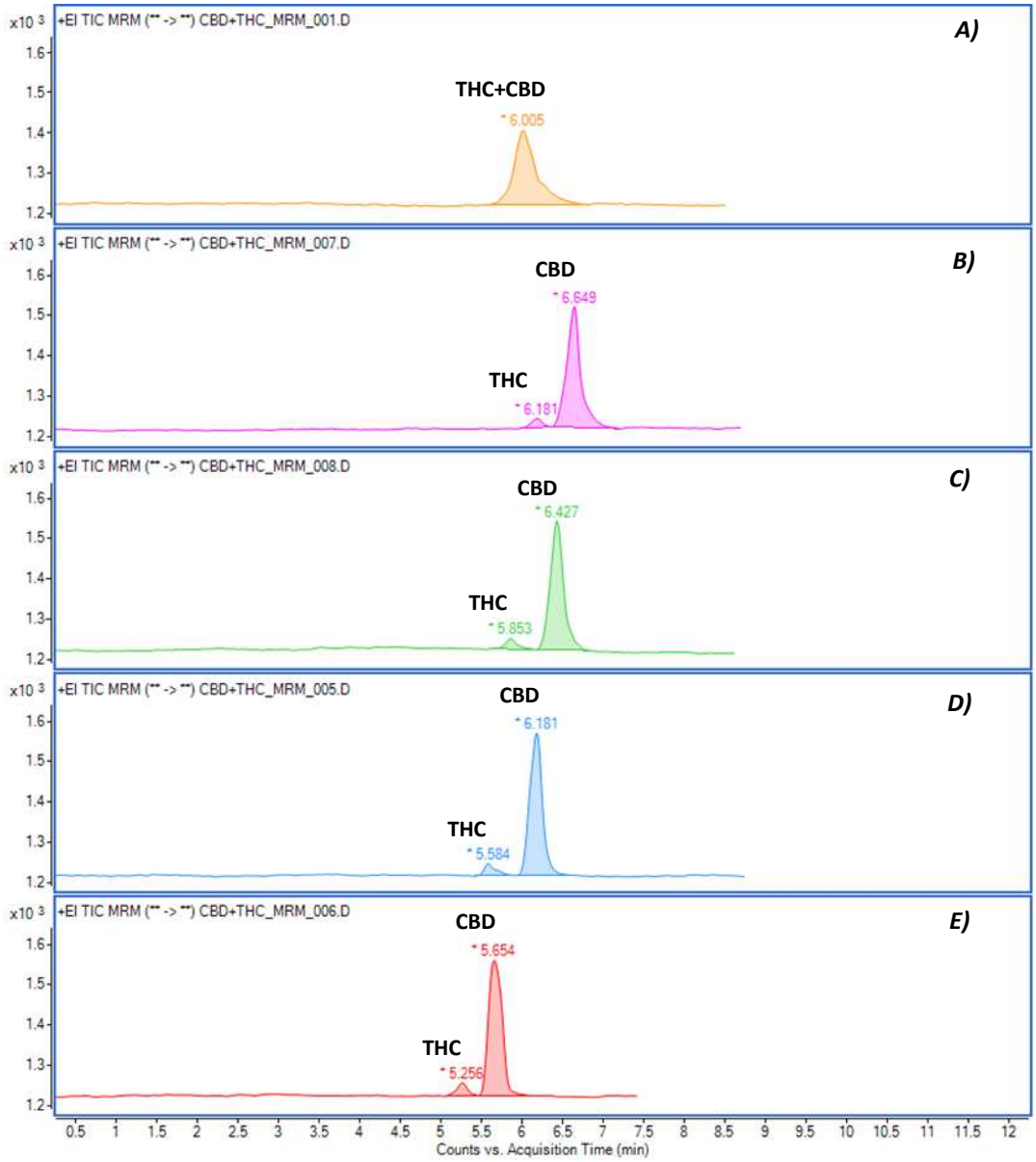


Figure 39 A-E. Results obtained by modifying the % of solvents used as MP for cannabinoids. A) 100% HEX, B) HEX:EtOH 99:1 (v:v), C) HEX:EtOH 97:3 (v:v), D) HEX:EtOH 95:5 (v:v), and D) HEX:EtOH 93:7 (v:v).

No separation of the two cannabinoids was obtained using 100% of hexane. the best separation was obtained using 95% of hexane and 5% of ethanol, in fact, using 93% hexane and 7% ethanol the peaks are slightly overlapping. As demonstrated in **Figure 40**, It was observed that, in the case of cannabinoids the VMC temperature is very significant in terms of integrated peak area values. Using a VMC temperature of 250°C, THC was detected and the optimal VMC temperature is 400 °C for the two compounds.

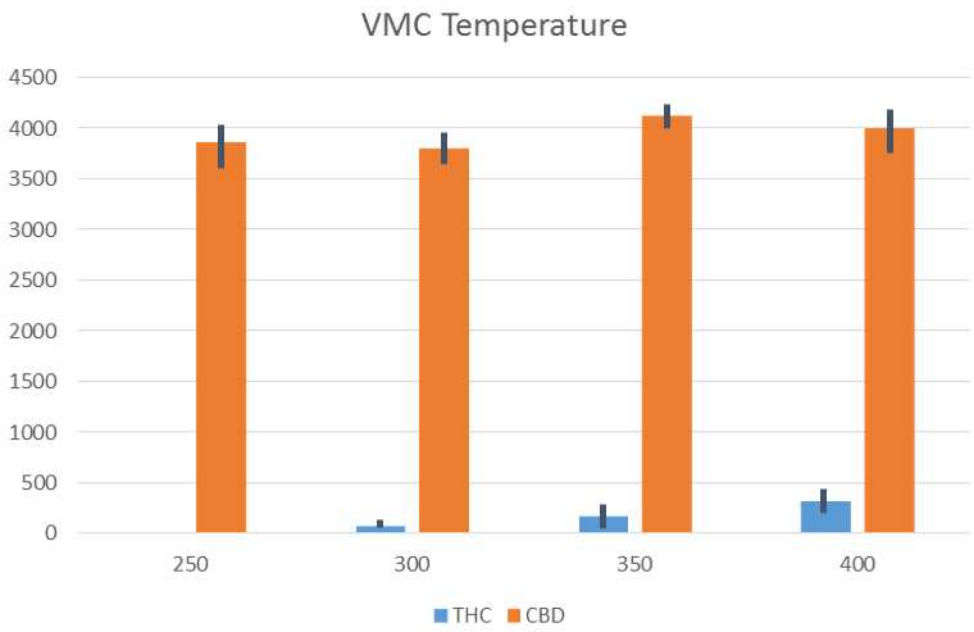


Figure 40. Effects on the integrated peak area values of different VMC temperatures for THC and CBD.

For phthalates, the mobile phase composition and VMC temperature optimization was carried out in MRM using a standard mixture of diisodecyl, diethyl and dimethyl phthalate at 333 mg/L in HEX. The pump flow rate was set at 100 µL/min split at 500 nL/min (1:200 split ratio). The injection volume was 4 µL. The VMC temperature was set at 350°C. HEX (solvent A) and IPA (solvent B) were tested as follows: A 100%, A:B 99:1 (v:v), A:B 97:3 (v:v), A:B 95:5 (v:v), A:B 93:7 (v:v), and A:B 90:10 (v:v) (**Figure 41 A-G**) and the best separation in terms of analysis time was obtained using 90% of hexane and 10% of isopropanol.

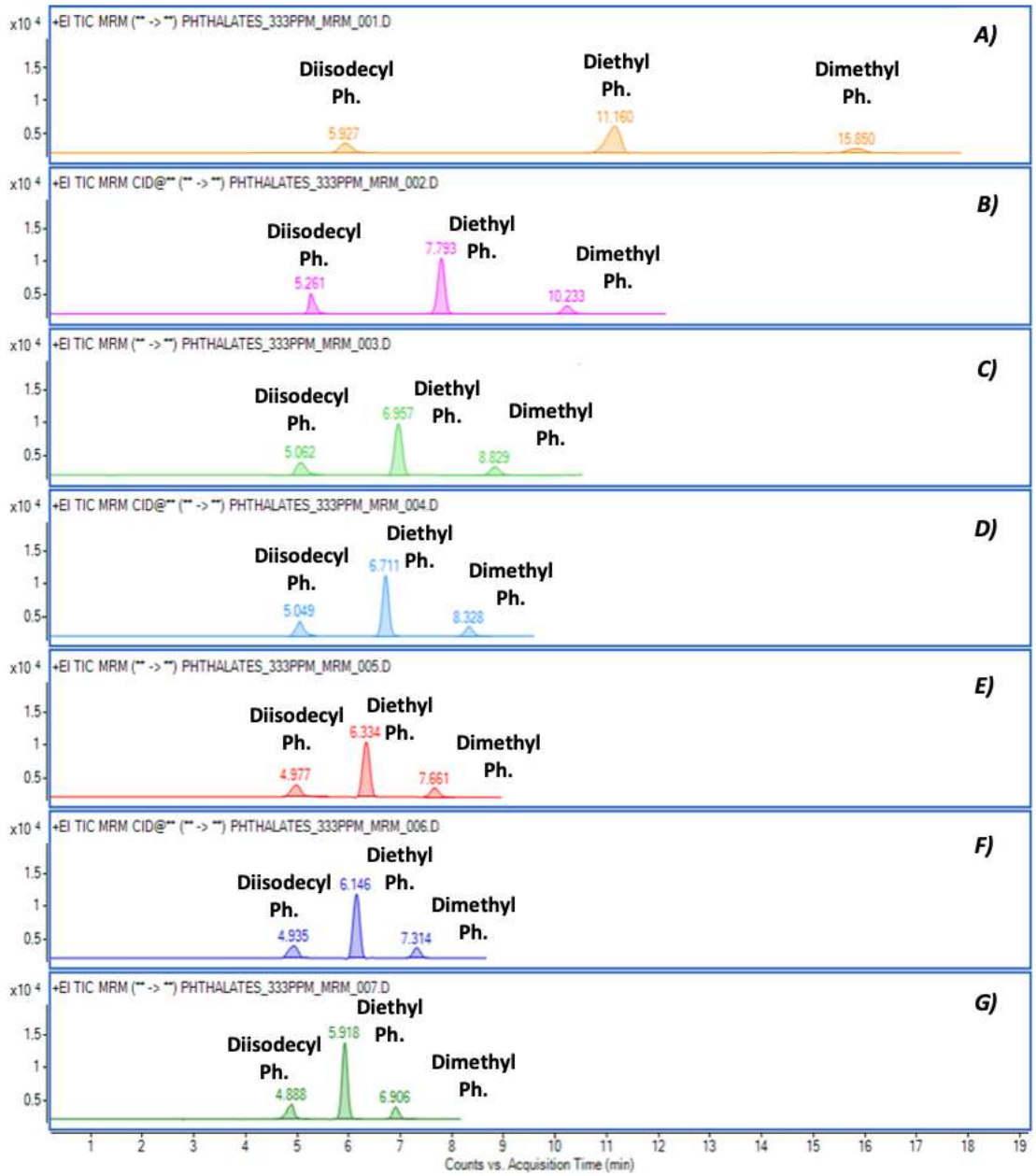


Figure 41 A-G. Results obtained by modifying the % of solvents used as MP for phthalates. A) 100% HEX, B) HEX:ISO 99:1 (v:v), C) HEX:ISO 98:2 (v:v), D) HEX:ISO 97:3 (v:v), E) HEX:ISO 95:5 (v:v), F) HEX:ISO 93:7, and G) HEX:ISO 95:5 (v:v).

As demonstrated in **Figure 42**, It was observed that, in the case of phthalates the VMC temperature is not very significant, however integrated peak area values slightly increased using a VMC temperature of 350 °C for the three compounds.

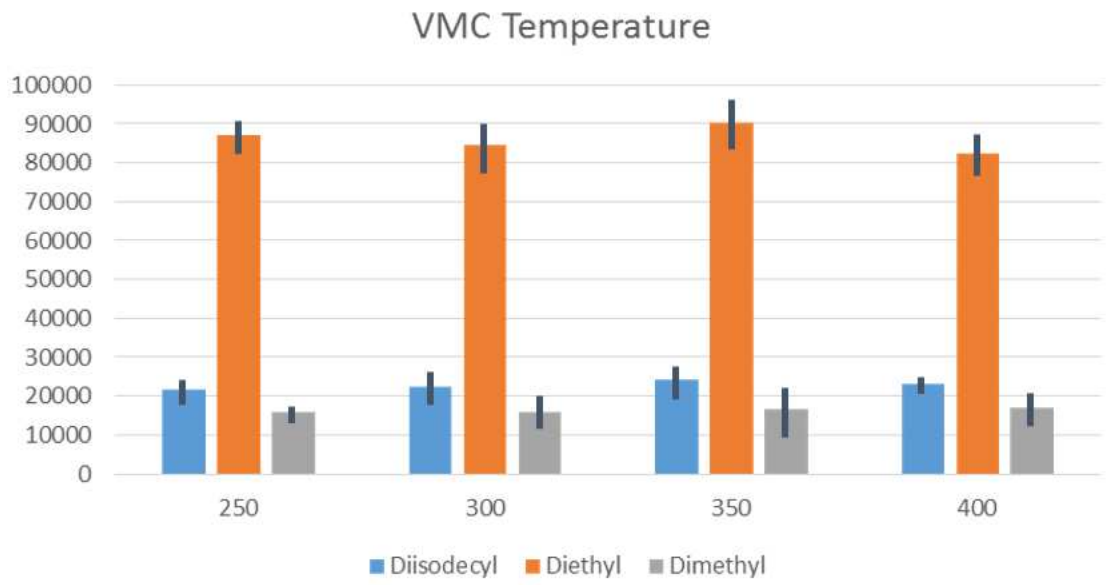


Figure 42. Effects on the integrated peak area values of different VMC temperatures for diisodecyl, diethyl and dimethyl phthalate.

For phenols, the mobile phase composition and VMC temperature optimization was carried out in SIM using a standard mixture of phenol, catechol, resorcinol and hydroquinone at a concentration of 250 mg/L in EtOH. The pump flow rate was set at 100 µL/min split at 500 nL/min (1:200 split ratio). The injection volume was 1 µL. The VMC temperature was set at 350°C. HEX (solvent A) and IPA (solvent B) were tested as follows: HEX (solvent A) and EtOH (solvent B) were tested as follows: A 100%, A:B 90:10 (v:v), and A:B 80:20 (v:v) (**Figure 43 A**,**B**) and the best separation in terms of analysis time was obtained using 80% of hexane and 20% of ethanol.

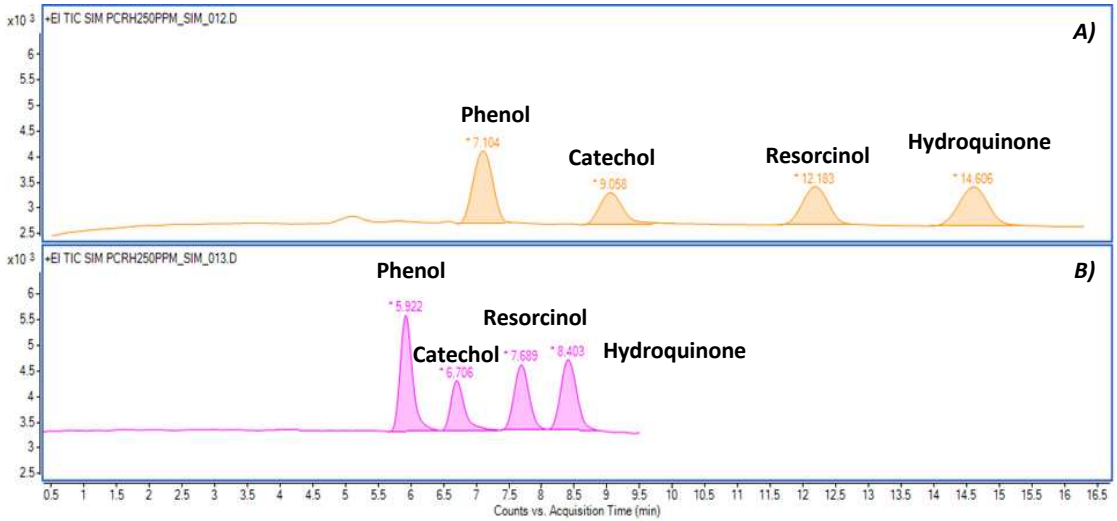


Figure 43. Results obtained by modifying the % of solvents used as MF for phenols. A) HEX:EtOH 90:10 (v:v), B) HEX:EtOH 80:20 (v:v).

As demonstrated in **Figure 44**, It was observed that, in the case of phenols the VMC temperature is not very significant, hence and intermediate VMC temperature of 350 °C for the three compounds was selected.

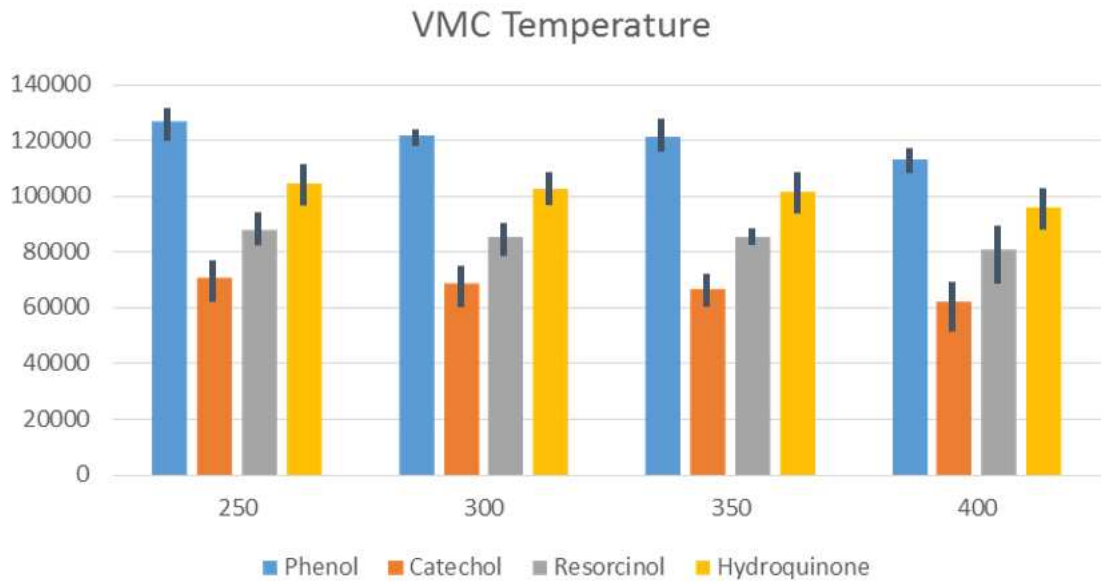


Figure 44. Effects on the integrated peak area values of different VMC temperatures for phenol, catechol, resorcinol and hydroquinone.

In **Figure 45 A-D** and **Figure 46 A-D** are illustrated all the optimized separations obtained in low and high-resolution experiments.

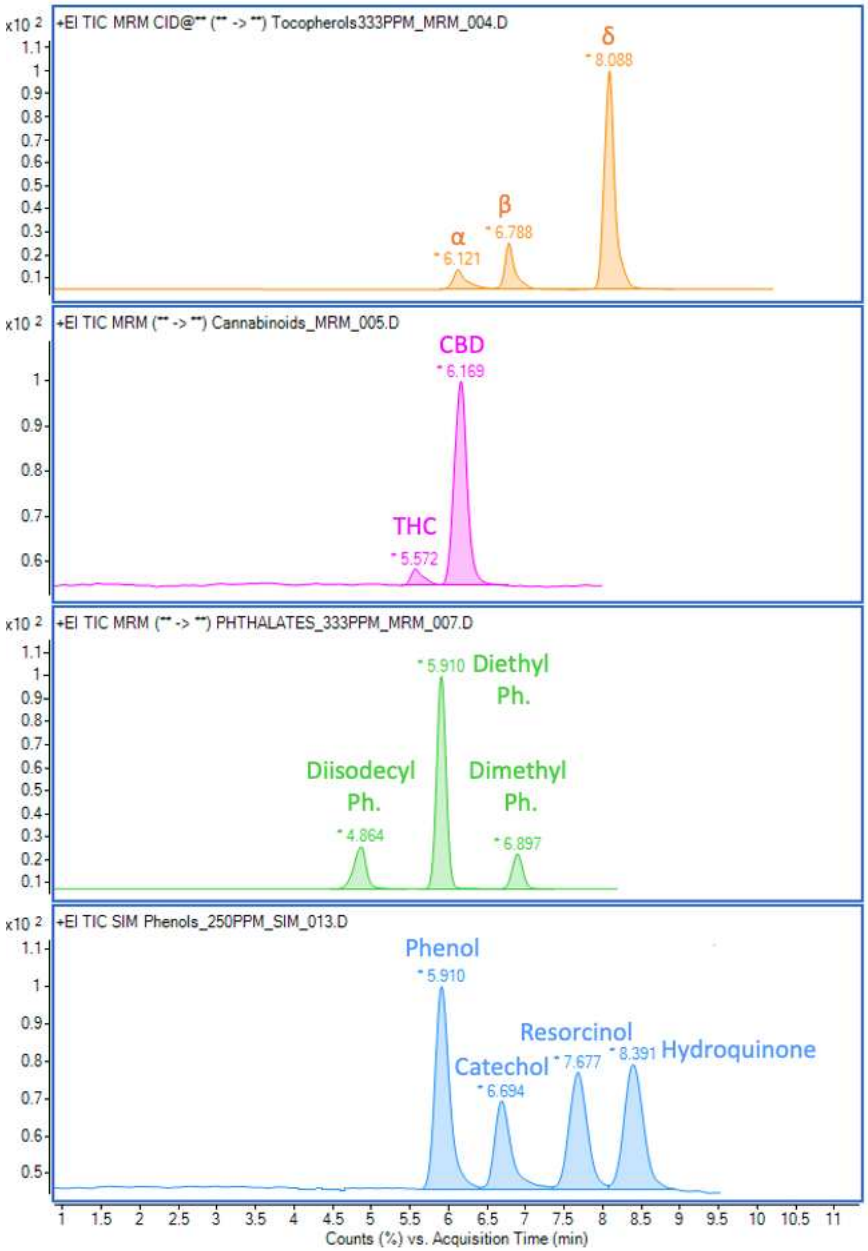


Figure 45 A-D. Results obtained from NPLC-LEI-QQQ-MS (low-resolution) separations of A) tocopherols (MP HEX:IPA 98:2 (v:v), VMC T 250 °C), B) cannabinoids (MP HEX:EtOH 95:5 (v:v), VMC T 400 °C), C) phthalates (MP HEX:IPA 90:10 (v:v), VMC T 350 °C), D) phenols (MP HEX:EtOH 80:20 (v:v), VMC T 350 °C).

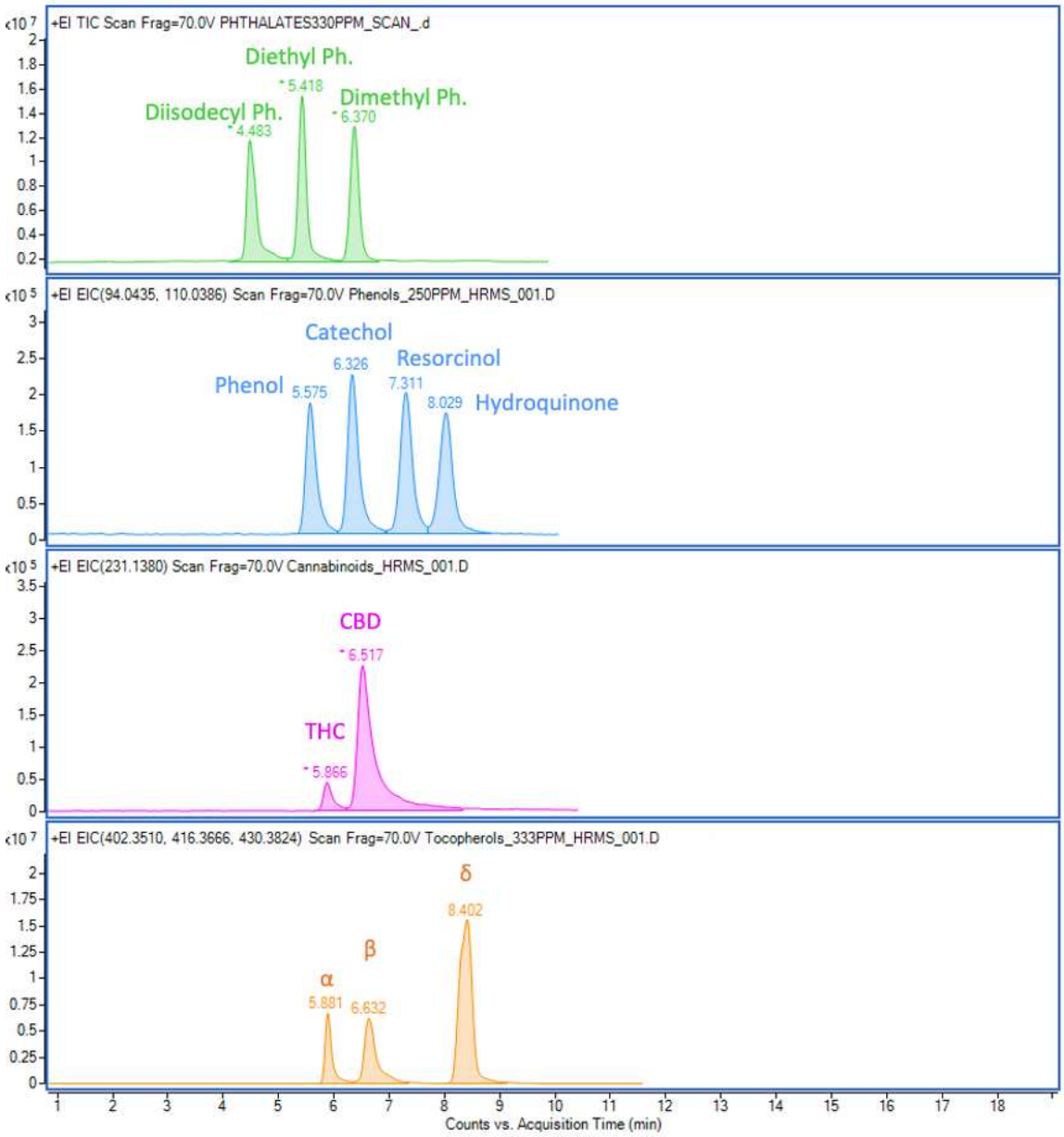


Figure 46 A-D. Results obtained from NPLC-LEI-QTOF-MS (high-resolution) separations of A) phthalates (MP HEX:IPA 90:10 (v:v), VMC T 350 °C), B) phenols (MP HEX:EtOH 80:20 (v:v), VMC T 350 °C), C) cannabinoids (MP HEX:EtOH 95:5 (v:v), VMC T 400 °C), D) tocopherols (MP HEX:IPA 98:2 (v:v), VMC T 250 °C).

4.5. E-LEI-MS EXPERIMENTS

E-LEI-MS was applied for the identification of the main active ingredients in selected medicinal tablets. Tiaprofenic acid contained in Surgamyl tablets was detected and correctly identified by the NIST library in scan acquisition mode. **Figure 47 A** shows the total ion current (TIC) signal acquired during the E-LEI-MS

analysis of Surgamyl, showing a signal increase at 3.00 min when the aspirated solution in ACN reached the EI source. Tiaprofenic acid identification by the NIST library search, with a spectral match of 93.6%, is shown in **Figure 47 B**.

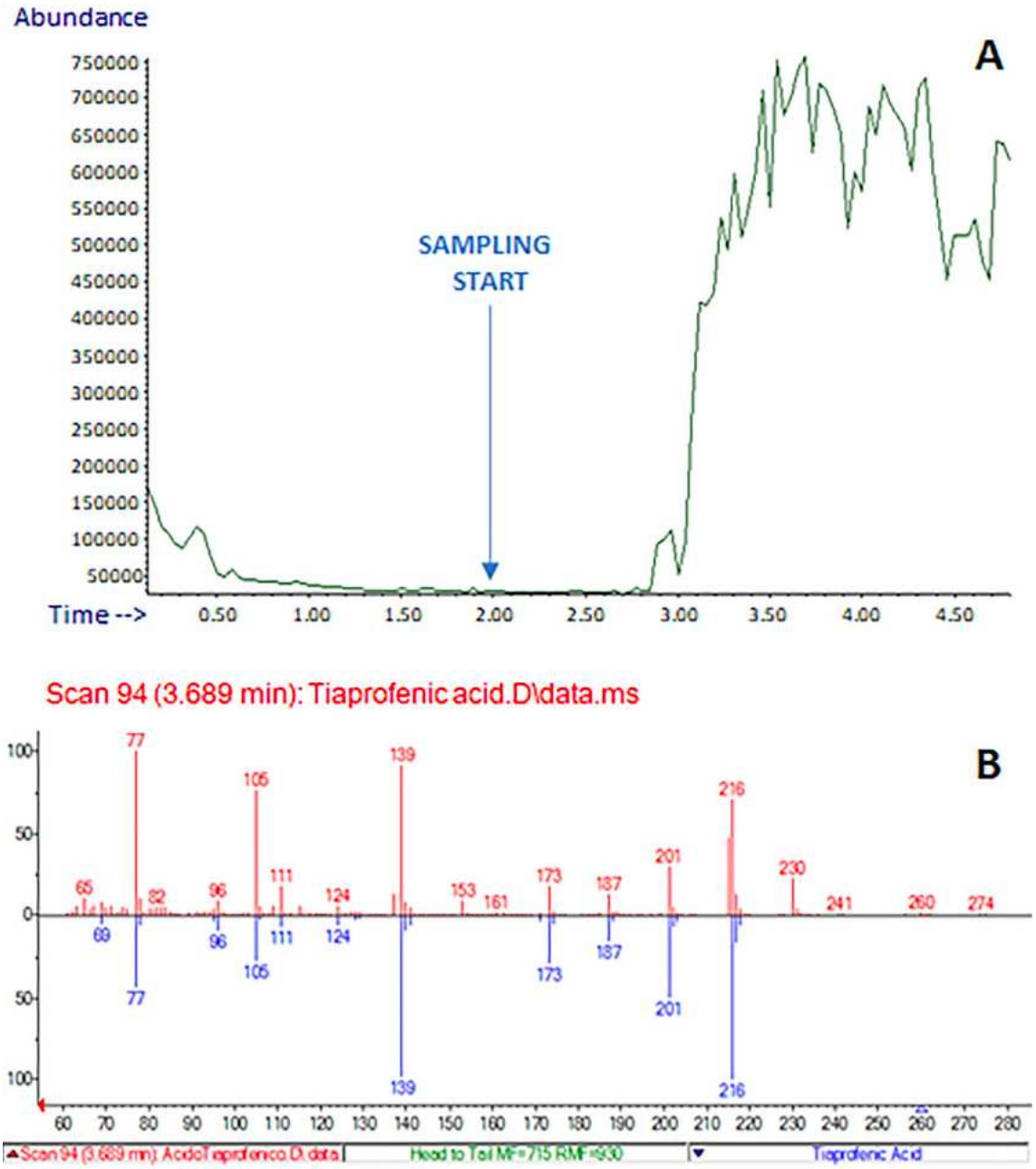


Figure 47 A, B. A) Analysis of a Surgamyl tablet: TIC, B) NIST library spectrum matching: red, experimental spectrum; blue, library spectrum. The arrow indicates the sampling start.

Figure 48 A,B shows a similar experiment carried out using Brufen tablets in which ibuprofen was identified using the NIST library.

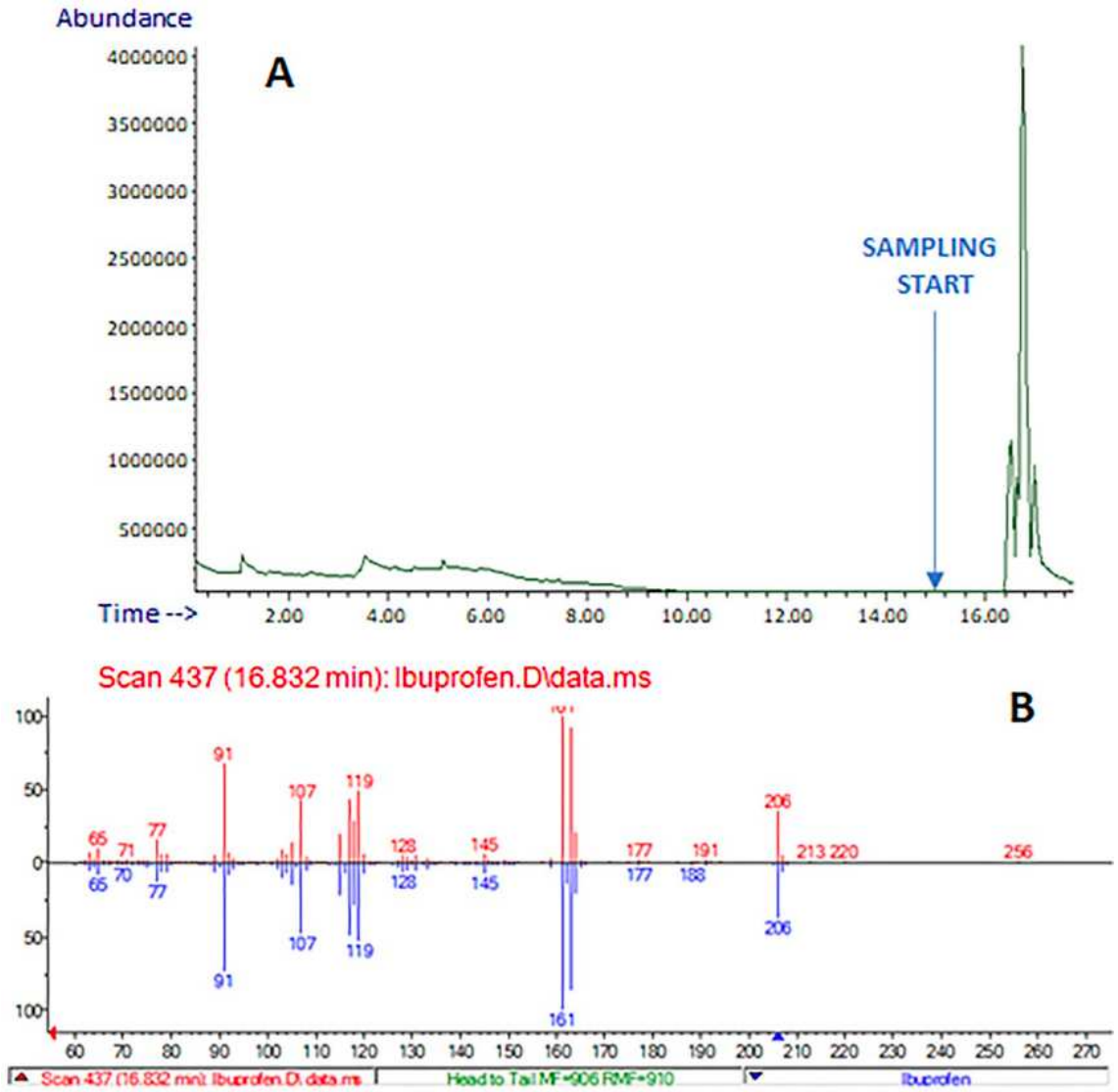


Figure 48 A, B. A) Analysis of a Brufen tablet: TIC, B) NIST library spectrum matching: red, experimental spectrum; blue, library spectrum. The arrow indicates the sampling start.

The analysis of NeoNisidina tablets (**Figure 49 A-E**), containing acetylsalicylic acid (250 mg), acetaminophen (200 mg), and caffeine (25 mg) was carried out in sim acquisition mode, using ACN as solvent. Due to the simultaneous presence of three different targeted compounds in the same formulation, selected ions were monitored to visualize each specific signal. The selected ions were m/z 92, 120, and 138 for acetylsalicylic acid; m/z 109 and 151 for acetaminophen; m/z 109 and 194 for

caffeine. All fragments were properly detected in SIM, demonstrating E-LEI-MS capability of identifying multiple target compounds in the same untreated matrix.

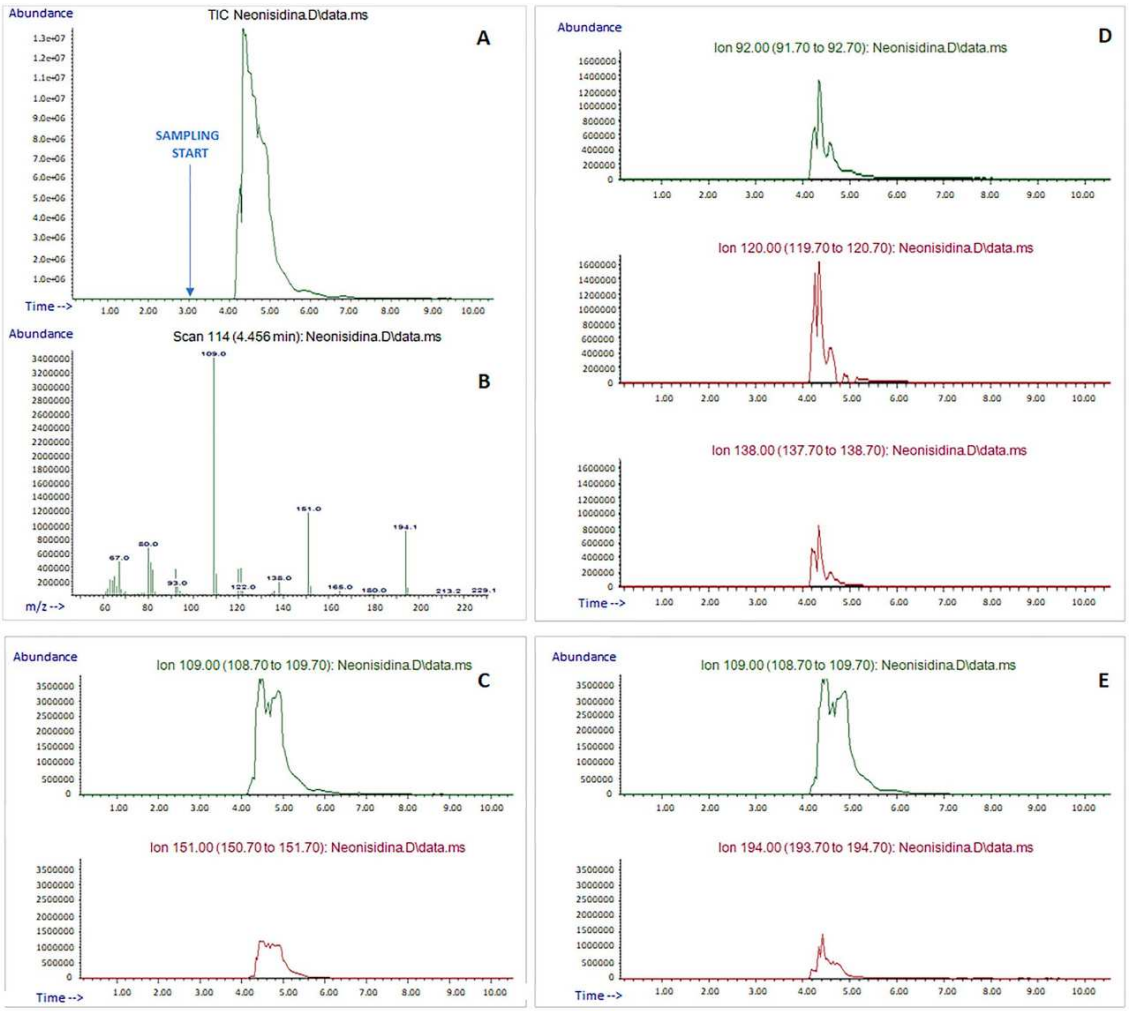


Figure 49 A-E. A) Analysis of a NeoNisidina tablet: TIC, B) EI experimental full spectrum, C) acetaminophen selected ions: SIM, D) acetylsalicylic acid selected ions: SIM, E) caffeine selected ions: SIM. The arrow indicates the sampling start.

These applications demonstrate E-LEI-MS capability to identify active ingredients in commercial drugs without sample preparation underlining its utility in quality control and forensic applications when a rapid identification of substances is needed.

A targeted determination of pesticides in fruit peels was carried out to demonstrate the applicability of E-LEI-MS in food and environmental analysis. Pesticides were selected based on their specific use on

orange and banana fruits; in particular, chlorpyrifos and imazalil were used to fortify orange peels, and benomyl was used on banana peels. An orange peel specimen (3 × 3 cm, 0.5 cm thickness) was fortified with 20 µL of chlorpyrifos solution at a concentration of 1000 mg/L. The orange specimen was stored for 24 h at room temperature to allow the complete standard adsorption on the surface and solvent evaporation before analysis. The same procedure was repeated using the same volume of an imazalil solution at a concentration of 1000 mg/L. The concentrations of the two pesticide solutions were calculated to match approximately those present in common commercial formulations used in agriculture. The experimental results are shown in **Figure 50 A-D**. TIC signals were clearly defined, and chlorpyrifos and imazalil EI spectra were correctly identified by the NIST library with a spectral similarity of 90.3 and 97.0%, respectively, despite the presence of the matrix. A banana peel specimen (3 × 3 cm, 0.5 cm thickness) was fortified with 20 µL of a benomyl solution at a concentration of 1000 mg/L. In this case, the identification resulted in a match equal to 75.2%

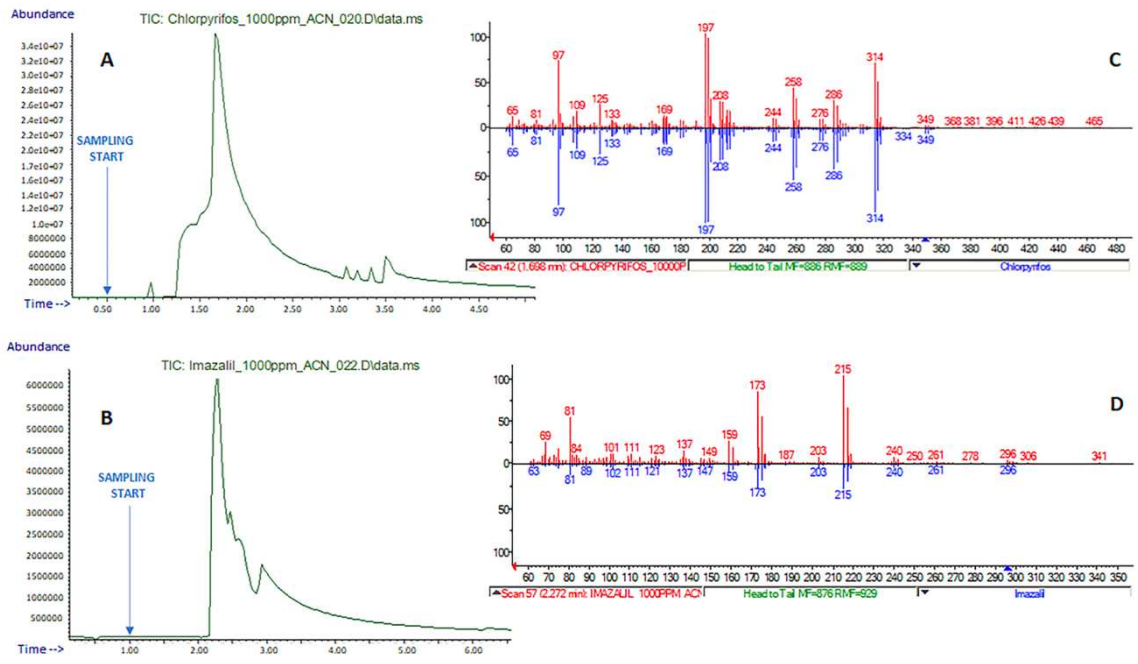


Figure 50 A-D. A) Analysis of an orange peel fortified with chlorpyrifos: TIC, B) analysis of an orange peel fortified with imazalil: TIC. NIST library spectra matching: in red experimental spectra for chlorpyrifos C) and imazalil D), in blue library spectra. The arrows indicate the sampling start.

A quantitative approach was attempted by calculating a calibration curve for chlorpyrifos. Solutions of chlorpyrifos at five concentration levels (50, 100, 500, 1000, and 2000 mg/L) were used to fortify orange peel specimens. The analyses were carried out by sampling each peel for 1 minute, and the curves were plotted based on the concentration levels (x-axis) and the recorded areas of the signals corresponding to m/z 314 ion (y-axis). The resulting equation was y = 48,302.6x − 2,371,974.6 with a coefficient of determination R2 of 0.9950 and completely satisfactory, considering the wide range of concentrations tested, the total absence of sample preparation, and, especially, the prototype stage of E-LEI-MS.

E-LEI-MS was applied to the analysis of a cocaine-spotted banknote. These experiments aimed to provide 2-D tests to verify if the system can fast recognize specific substances on selected surface spots, compared to controls, thus rendering analytes spatial distribution. The banknote was spotted with the target solution, then dried at ambient conditions before analysis. **Figure 51 A-C** shows the signals corresponding to the sampling of three negative control spots (1–3) and one (4) positive control spot corresponding to the stain of 20 µL of a cocaine solution at a concentration of 100 mg/L on a 1 cm^2 spot. The cocaine EI spectrum was unambiguously identified by matching the NIST library with a high spectral similarity score (> 90%). In these experiments, E-LEI-MS demonstrated an unparalleled identification power which is mandatory in forensic applications and represents the main point of strength of this approach.

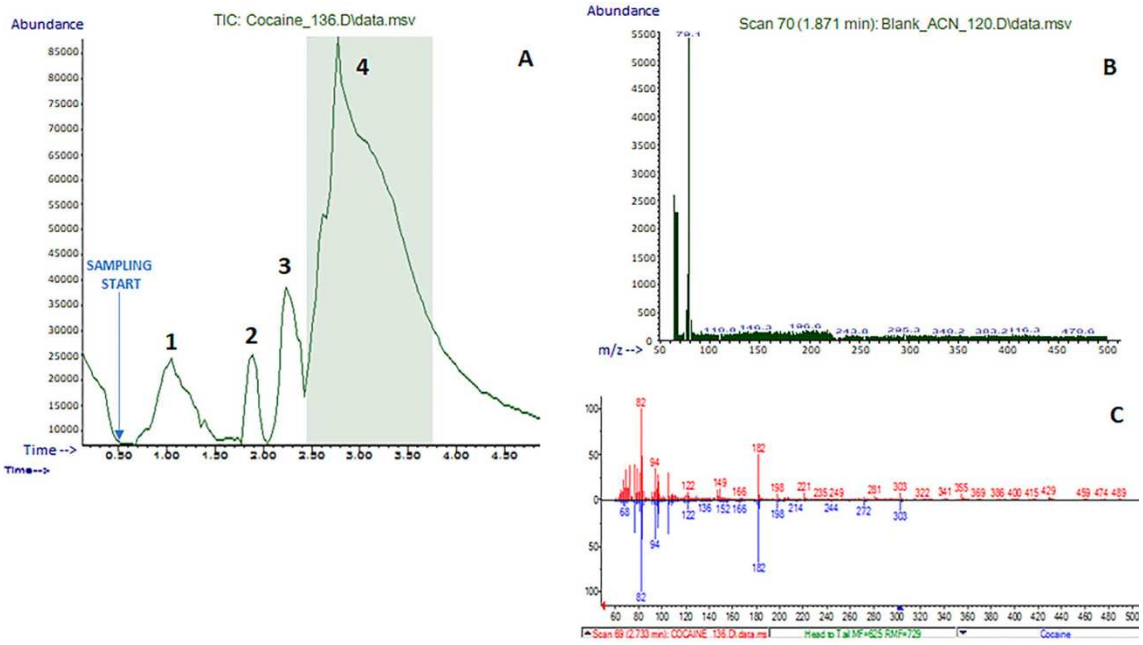


Figure 51 A-C. A) Analysis of spots 1–3 (intact) and 4 (cocaine) on a banknote: TIC, B) EI experimental full

spectrum of a negative spot (matrix), C) NIST library spectrum matching: red, experimental spectrum; blue, library spectrum. The arrow indicates the sampling start.

The potential of E-LEI-MS to identify completely unknown components in a complex matrix was tested in artwork analysis, particularly in detecting untargeted compounds in paintings for compounds attributable to the type of colors or fixative used, which helps select the proper materials in restoration interventions. An accurate understanding of the materials used in a particular painting is also fundamental for attributing the artwork to an artist or a historical period. several solvents were tested on small squares of painting samples: water, ACN, MeOH, dichloromethane (CH_2Cl_2), hexane, and octanol. ACN was selected for the combination of proper dissolving power and viscosity. This study aimed to also investigate E-LEI-MS's ability to perform unknown and multilayer analyses permitting the sample mapping in three dimensions, where the third dimension corresponds to the deeper layers. For this reason, the sample was exposed to ACN for a longer time allowing the in-depth extraction of the compounds. Sampling started after 2 min of solvent release from the tip. As shown in **Figure 52 A-B**, the first spectrum at 5.91 min corresponded to isopropyl myristate, with a NIST spectral similarity of 93.4%. This result is satisfactory, considering the matrix complexity, which could affect the spectrum quality by limiting unknown compounds' identification. The identified compound was concordant with the sample nature; isopropyl myristate is an esterified fatty acid, potentially a component of fixative varnish applied on paintings. After another three minutes of sampling in the same surface area, another spectrum was acquired corresponding to isopropyl palmitate (spectral similarity 43.2%), also used as a fixative component. Isopropyl palmitate was detected later than isopropyl myristate, probably because present in a deeper layer of the painting; therefore its extraction required a longer time. This application clearly shows the E-LEI-MS identification power of unknown compounds since it was conducted using a matrix whose surface components were totally unknown. As a micro-invasive technique, E-LEI-MS is particularly suitable for this kind of application when preserving prestigious matrices is mandatory.

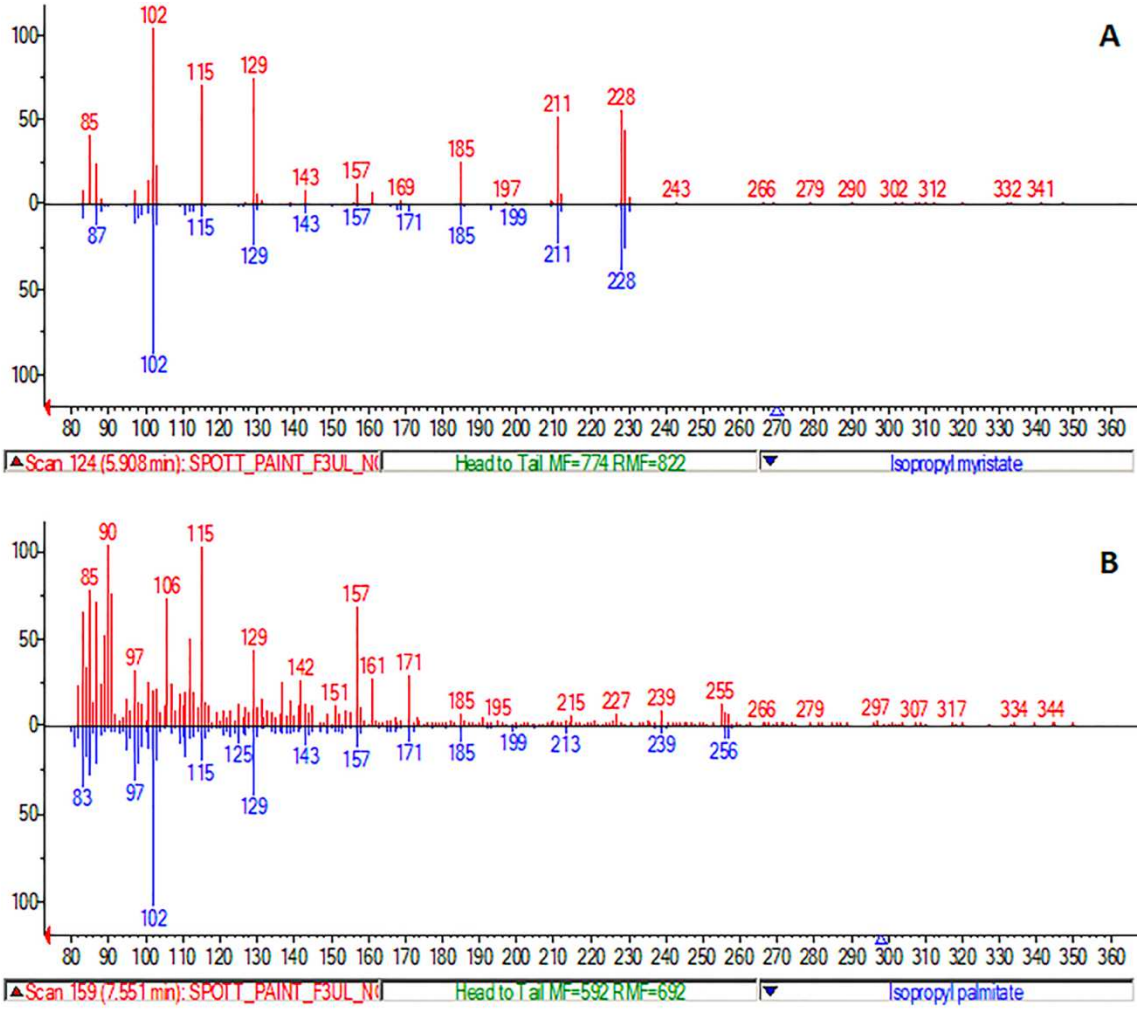


Figure 52 A-B. A) Spectrum for isopropyl myristate obtained from the analysis of the painting surface: TIC. B) spectrum isopropyl palmitate obtained from the analysis of the painting deeper layer: TIC. NIST library spectra matching: in red experimental spectra in blue library spectra.

5 CONCLUSIONS

The first aim of this research was to explore the versatility of LEI in direct analysis (without chromatographic separations) using EI and CI with low and high-resolution mass spectrometry and to assess the possibility of coupling normal phase liquid chromatography with electron ionization in low and high-resolution mass spectrometry using LEI.

A new method involving the use of MOI-PFS coupled to a triple quadrupole tandem MS (QQQ, low-resolution) and a quadrupole time of flight MS (QTOF, high-resolution), operating in EI and NCI was proposed for the analysis of complex biological and environmental samples without chromatographic separation. The proposed method presents the significant advantage of a fast sample transfer to MS and demonstrated to improve the signal-to-noise ratio and peak shape, leading to fast, sustainable and sensitive results. Using the LEI interface in NCI mode is a powerful tool to reduce the ME, allowing the selective ionization of electrophilic compounds and reaching very low LODs and LOQs.

MOI allows direct coupling of the SPME fiber with LEI-QQQ and LEI-QTOF systems for a more sustainable and reliable alternative for trace levels compound analyses in serum and in a commercial formulation. The overall procedure is fast: extraction from the actual sample requires only 3 minutes, and because no chromatography is involved, the analysis time is approximately 5 minutes. Although, in MRM mode was demonstrated to be a selective detector for this application, halogenated compounds with the same nominal mass cannot be distinct. MOI and LEI interface were coupled with Q-TOF for the first time for high-resolution experiments, and the results obtained are encouraging for future applications involving complex matrices and no chromatographic separation. Future studies will be focused on investigating sustainable and non-toxic solvents to enhance the green character of this approach.

In this work, the possibility of using the LEI interface in combination with NPLC was explored for the first time. Some model compounds have been chosen such as tocopherols, cannabinoids, phthalates and phenols which benefit from NPLC. Preliminary experiments were dedicated to the full scan acquisition of the spectra of the selected compounds and their MRM transitions. The separations of the compound mixtures were performed in MRM using a UHPLC pump and a silica column in isocratic mode. Parameters such as the type and percentage of solvents used and the VMC temperature have been optimized. The

spectra obtained showed good correspondence with the NIST library demonstrating that this technique can represent a valid alternative to reverse phase chromatography combined with EI. Coupling NPLC with QTOF-MS generated EI-HRMS spectra allowing the determination of the structure of each fragment by applying specific tools of the software as well as for unknown identification. LEI combined with HRMS demonstrated to be a powerful analytical tool for targeted and untargeted component analysis and to have great potential in different application fields, to succeed where other analytical strategies have failed. Future experiments will include the study of new applications and the evaluation of the quantitative aspect.

Finally, E-LEI-MS, a new system for direct analysis in EI mode, is introduced in this work. E-LEI-MS demonstrated to be a novel and competitive real-time technology that uses ambient sampling and EI-MS to identify analytes quickly and accurately. The method allowed to perform quick analysis of targeted and unknown compounds without the need for sample manipulation or preparation, which is required for separation techniques, demonstrating great potential and versatility in several application fields. In 2D and multi-layer studies, satisfactory results were achieved with various matrices and samples, emphasizing the utility of E-LEI-MS in surface/spatial analysis. E-LEI-MS proved to be also an environmentally friendly approach, as indicated by the AGREE evaluation, and future research will focus on improving E-LEI-MS performance and making it a portable device, with subsequent gains in environmental performance.

REFERENCES

[1] Murray, K. K.; Boyd, R. K.; Eberlin, M. N.; Langley, G. J.; Li, L.; Naito, Y. Definition of terms relating to mass spectrometry (IUPAC Recommendations). *Pure Appl. Chem.* **2013**, 85, 1515-1609. https://doi.org/10.1351/PAC-REC-06-04-06.

[2] Cooks, R. G.; Ouyang, Z.; Takats, Z.; Wiseman, J. M. Ambient Mass Spectrometry. *Science.* **2006**, 311, 5767, 1566-1570. https://doi.org/10.1126/science.1119426.

[3] Takats, Z.; Wiseman, J. M.; Gologan, B.; Cooks, R. G. Mass spectrometry sampling under ambient conditions with desorption electrospray ionization. *Science.* **2004**, 306, 5695, 471-3. https://doi.org/10.1126/science.1104404.

[4] Cody, R.B.; Laramée, J.A.; Durst, H. D. Versatile new ion source for the analysis of materials in open air under ambient conditions. *Anal Chem.* **2005**, 77, 2297-302. https://doi.org/10.1021/ac050162j.

[5] Dass, C. Fundamental of contemporary mass spectrometry. **2007**, John Wiley & Sons.

[6] Herbert, C.G.; Johnstone, R. A. W. Mass Spectrometry Basics. **2002**, CRC Press.

[7] Termopoli V.; Famiglini, G.; Palma, P.; Piergiovanni, M.; Rocio-Bautista, P.; Ottaviani, M.F.; Cappiello, A.; Saeed, M.; Perry, S.J. 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].

[8] 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. *Analytica Chimica Acta*. **2021**, 1167, 338350. https://doi.org/10.1016/j.aca.2021.338350.

[9] Niessen, W.M.A.; Tinke, A.P. Liquid Chromatography-Mass Spectrometry General Principles and Instrumentation. *J. Chromatogr. A*. **1995**, 703, 37-57.

[10] McLafferty, F.W. Current Topics in Organic Mass Spectrometry. *Pure & Appl. Chem.* **1979**, 50, 831–835.

[11] 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.

[12] Tal'roze, V.L.; Karpov, G.V.; Gordetskii, I.G.; Skurat, V.E. Analysis of Mixtures of Organic Substances on a Mass Spectrometer with a Capillary System for the Introduction of Liquid Specimens. *Russ. J. Phys. Chem.* **1969**, 43, 198–201.

[13] Arpino, P.J.; Baldwin, M.A.; McLafferty, F.W. A Liquid Chromatography/Mass Spectrometry System Providing Continuous Monitoring with Nanogram Sensitivity. *Biomed. Mass Spectrom.* **1974**, 1, 80-82.

[14] Scott, R. P. W.; Scott, C. G.; Munroe, M.; Hess Jr, J. (1974). Interface for Online Liquid Chromatography-Mass Spectroscopy Analysis. *J. Chromatogr. A.* **1974**, 99, 395-405. https://doi.org/10.1016/S0021-9673(00)90872-0.

[15] Willoughby, R.C.; Browner, R.F. Monodisperse Aerosol Generation Interface for Combining Liquid Chromatography with Mass Spectroscopy. *Anal. Chem.* **1984**, 56, 2626-2631. https://doi.org/10.1021/ac00278a003.

[16] Cappiello, A. Is Particle Beam an Up-to-date LC-MS Interface? State of the Art and Perspectives. *Mass Spectrom. Rev.* **1996**, 15, 283–296. https://doi.org/10.1002/(SICI)1098-2787(1996)15:5%3C283::AID-MAS1%3E3.0.CO;2-E.

[17] Koster, C.G.; Schoenmakers, P.J. History of liquid chromatography-mass spectrometry couplings, in: P.Q. Tranchida, L. Mondello (Eds.), Hyphenations of Capillary Chromatography with Mass Spectrometry, **2020**, ISBN 978-0-12- 809638-3, pp. 279-295.

[18] Cappiello, A.; Bruner, F.; Micro flow rate particle beam interface for capillary liquid chromatography/mass spectrometry. *Anal. Chem.* **1993**, 65, 9, 1281-1287. https://doi.org/10.1021/ac00057a028.

[19] Cappiello, A.; Famiglini G. Evaluation of the performance of a microflow rate LC/MS particle beam interface. *Anal. Chem.* **1994**, 66, 3970-3976. https:// doi.org/10.1021/ac00094a019.

[20] Cappiello, A.; Famiglini G. Capillary-scale particle-beam liquid chromatography/mass spectrometry interface: can electron ionization sustain the competition? *J. Am. Soc. Mass Spectrom.* **1998**, 9, 993-1001. https://doi.org/ 10.1016/S1044-0305(98)00084-1.

[21] Cappiello, A.; Famiglini G.; Bruner, F. Determination of acidic and basic/ neutral pesticides in water with a new microliter flow rate LC/MS particle beam interface. *Anal. Chem.* **1994**, 66, 1416-1423 https://doi.org/10.1021/ ac00081a009.

[22] Cappiello, A.; Famiglini G.; Palma, P.; Berloni, A.; Bruner F. New approach for the analysis of acidic pesticides in water by LC/MS with a particle beam interface. *Environ. Sci. Technol*. **1995**, 29, 2295-2300. https://doi.org/ 10.1021/es00009a021.

[23] Cappiello, A.; Famiglini G.; Mangani, F.; Angelino, S.; Gennaro, M.C. Simultaneous determination of acidic and basic-neutral pesticides in water at ppt concentration level by ion-interaction micro-HPLC/MS. *Environ. Sci. Technol.* **1999**, 33, 3905-3910. https://doi.org/10.1021/es990340o.

[24] Cappiello, A.; Berloni, A.; Famiglini, G.; Mangani, F.; Palma, P. Micro-SPE method for sample introduction in capillary HPLC/MS. *Anal. Chem.* **2001**, 73 298-302. https://doi.org/10.1021/ac000859z.

[25] Cappiello, A.; Berloni, A.; Famiglini, G.; Mangani, F.; Tirillini, B. Analysis of coumarins by micro high-performance liquid chromatography-mass spectrometry with a particle beam interface, *J. Am. Soc. Mass Spectrom.* **1995**, 6, 132-139. https://doi.org/10.1016/S1044-0305(94)00096-I.

[26] Cappiello, A.; Famiglini, G.; Tirillini, B. Determination of aflatoxins in peanut meal by lc/ms with a particle beam interface. *Chromatographia* **1995**, 40 411-416. https://doi.org/10.1007/BF02269904.

[27] Cappiello, A.; Famiglini, G.; Lombardozzi, A.; Massari, A.; Vadala, G.G. Electron capture ionization of explosives with a micro-flow rate particle beam interface, *J. Am. Soc. Mass Spectrom.* **1996**, 7, 753-758. https://doi.org/ 10.1016/1044-0305(96)00015-3.

[28] Cappiello, A.; Famiglini, G.; Rossi, L.; Magnani, M. Use of Non-Volatile Buffers in liquid chromatography-mass spectrometry: advantages of capillary-scale particle beam interfacing, *Anal. Chem.* **1997**, 69, 5136-5141. https:// doi.org/10.1021/ac970765y.

[29] 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. https://doi.org/ 10.1021/ac991493x.

[30] Cappiello, A.; Famiglini, G.; Mangani, F.; Careri, M.; Lombardi, P.; Mucchino, C. Liquid chromatographic-mass spectrometric determination of phenolic compounds using a capillary-scale particle beam interface. *J. Chromatogr. A.* **1999**, 855, 515-527. https://doi.org/10.1016/S0021-9673(99)00708-6.

[31] 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. https://doi.org/10.1002/mas.1004.

[32] Cappiello, A.; Palma, P.; Famiglini, G. LC-MS interfaces: state of the art and emerging techniques, in: D. Corradini (Ed.), Handbook of HPLC, Second Edition, Chromatographic Science Series, vol. 101, CRC Press, Taylor & Francis Group, (USA), Boca Raton, T.M. Phillips, 2010, ISBN 978-1-57444- 554-1, pp. 233-255.

[33] Cappiello, A.; Famiglini, G.; Palma, P. Electron ionization for LC/MS. *Anal. Chem.* **2003**, 75 497a-503a, https://doi.org/10.1021/ac0313116.

[34] Cappiello, A.; Famiglini, G.; Palma, P.; Siviero, A. Liquid chromatography- electron ionization mass spectrometry: field of application and evaluation of the performance of a Direct-EI interface, *Mass Spectrom. Rev.* **2005**, 24, 978-989. https://doi.org/10.1002/mas.20054.

[35] Cappiello, A.; Famiglini, G.; Palma, P., Perspectives of EI in LC-MS, particle-beam and other approaches, in: W. M. A. Niessen the Encyclopedia of Mass Spectrometry Hyphenated Methods, vol. 8, Elsevier, Amsterdam (The Netherlands), 2006, ISBN 978-0-08-043847-4, pp. 145-156.

[36] Cappiello, A.; Palma, P.; Palma, P.; Pierini, E.; Termopoli, V.; Trufelli, H. Direct- EI in LC-MS: towards a universal detector for small-molecule applications. *Mass Spectrom. Rev.* **2011**, 30, 1242-1255. https://doi.org/10.1002/ mas.20329.

[37] 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, 8, 2683-2693. https://doi.org/10.1007/s00216-010-4637-0.

[38] 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. https://doi.org/10.1016/ S1044-0305(01)00363-4.

[39] 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. https://doi.org/10.1016/S0003-2670(03)00868-7.

[40] 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. https://doi.org/10.1021/ac070468l.

[41] 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, 10, 1004-1010. https://doi.org/10.1002/jms.1981.

[42] Cappiello, A.; Famiglini, G.; Palma, P.; Mangani, F. Trace level determination of organophosphorus pesticides in water with the new direct-electron ionization LC/MS interface. *Anal. Chem.* **2002**, 74, 3547-3554. https://doi.org/ 10.1021/ac015685f.

[43] Famiglini, G.; Trufelli, H.; Pierini, E.; De Simoni, E.; Mangani, F.; Cappiello, A. Comparison of solid-phase extraction and micro-solid-phase extraction for liquid chromatography/mass spectrometry analysis of pesticides in water samples. *J. AOAC Int.* **2003**, 86, 5, 941-946. https://doi.org/10.1093/jaoac/ 86.5.941.

[44] Famiglini, G.; Palma, P.; Pierini, E.; Trufelli, H.; Cappiello, A. Organochlorine pesticides by LC-MS. *Anal. Chem.* **2008**, 80, 3445-3449. https://doi.org/ 10.1021/ac8000435.

[45] 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. https://doi.org/10.1021/ac9008995.

[46] Famiglini, G.; Palma, P.; Siviero, A.; Rezai, M.A.; Cappiello, A. Determination of endocrine disrupting compounds in marine water by nano liquid chromatography/direct-electron ionization mass spectrometry. *Anal. Chem.* **2005**, 77, 7654-7661. https://doi.org/10.1021/ac050842m.

[47] Cappiello, A.; Famiglini, G.; Palma, P.; Pierini, E.; Trufelli, H.; Maggi, C.; Manfra, L.; Mannozzi, M. Application of nano-FIA-Direct-EI-MS to determine diethylene glycol in produced formation water discharges and seawater samples. *Chemosphere* **2007**, 69, 554-560. https://doi.org/10.1016/ j.chemosphere.2007.03.026.

[48] Tomasini, D.; Cacciola, F.; Rigano, F.; Sciarrone, D.; Donato, P.; Beccaria, M.; Caramaeo, E.B.; Dugo, P.; Mondello, L. Complementary analytical liquid chromatography methods for the characterization of aqueous phase from pyrolysis of lignocellulosic biomasses. *Anal. Chem.* **2014**, 86, 11255-11262. https://doi.org/10.1021/ac5038957.

[49] Albergamo, A.; Rigano, F.; Purcaro, G.; Mauceri, A.; Fasulo, S.; Mondello, L. Free fatty acid profiling of marine sentinels by nano LC-EI-MS for the assessment of environmental pollution effects. *Sci. Total Environ.* **2016**, 571, 955-962. https://doi.org/10.1016/j.scitotenv.2016.07.082.

[50] Trufelli, H.; Famiglini, G.; Termopoli, V.; Cappiello, A. Profiling of non-esterified fatty acids in human plasma using liquid chromatography-electron ionization mass spectrometry. *Anal. Bioanal. Chem.* **2011**, 400, 2933-2941, https://doi.org/10.1007/s00216-011-4955-x.

[51] 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. https://doi.org/10.1021/acs.analchem.6b00328.

[52] Cappiello, A.; Famiglini, G.; Termopoli, V.; Trufelli, H.; Zazzeroni, R.; Jacquoilleot, S.; Radici, L.; Saib, O. Application of LC-Direct-EI-MS in vitro dermal absorption study: quantitative determination of trans-cinnamaldehyde. *Anal. Chem.* **2011**, 83, 8537-8542. https://doi.org/10.1021/ ac201839x.

[53] Cappiello, A.; Famiglini, G.; Palma, P.; Termopoli, V.; Trufelli, H. A new liquid chromatography-mass spectrometry approach for generic screening and quantitation of potential genotoxic alkylation compounds without derivatization. *J. Chromatogr. A* **2012**, 1255, 286-290. https://doi.org/10.1016/ j.chroma.2011.12.068.

[54] Cappiello, A.; Tirillini, B.; Famiglini, G.; Trufelli, H.; Termopoli, V.; Flender, C. Determination of natural pyrethrins by LC-EI-MS. *Phytochem. Anal*. **2012,** 23, 191-196. https://doi.org/10.1002/pca.1342.

[55] Russo, M.; Rigano, F.; Arigo, A.; Sciarrone, D.; Calabro, M.L.; Farnetti, S.; Dugo, P.; Mondello, L. Rapid isolation, reliable characterization, and water solubility improvement of polymethoxyflavones from cold-pressed mandarin essential oil. *J. Separ. Sci.* **2016**, 39, 2018-2027. https://doi.org/10.1002/jssc.201501366.

[56] Cappiello, A.; Famiglini, G.; Palma, P.; Termopoli, V.; Capriotti, F.; Cellar, N. Identification potential of direct-ei lc-ms interfacing in small-molecule applications. *Separ. Sci.* **2013**, 5, 7, 13-17.

[57] Famiglini, G.; Termopoli, V.; Palma, P.; Cappiello, A. Liquid chromatography electron ionization tandem mass spectrometry with the Direct-EI interface in the fast determination of diazepam and flunitrazepam in alcoholic beverages. *Electrophoresis* **2016**, 37, 1048-1054. https://doi.org/10.1002/ elps.201500517.

[58] Famiglini, G.; Termopoli, V.; Palma, P.; Capriotti, F.; Cappiello A. Rapid LC-MS method for the detection of common fragrances in personal care products without sample preparation. *Electrophoresis* **2014**, 35, 1339-1345. https:// doi.org/10.1002/elps.201300462.

[59] Flender, C.; Leonhard, P.; Wolf, C.; Fritzsche, M.; Karas, M. Analysis of boronic acids by nano liquid chromatography-direct electron ionization mass spectrometry. *Anal. Chem.* **2010**, 82, 10, 4194-4200. https://doi.org/10.1021/ ac1004585.

[60] Rigano, F.; Tranchida, P.Q.; Dugo, P.; Mondello, L. High-performance liquid chromatography combined with electron ionization mass spectrometry: a review. *Trends Anal. Chem.* **2019**, 118 112-122. https://doi.org/10.1016/ j.trac.2019.05.032.

[61] Schepler, C.; Sklorz, M.; Passig, J.; Famiglini, G.; Cappiello, A.; Zimmermann, R. Flow injection of liquid samples to a mass spectrometer with ionization under vacuum conditions: a combined ion source for single-photon and electron impact ionization. *Anal. Bioanal. Chem* **2013**, 405, 22, 6953-6957. https://doi.org/10.1007/s00216-013-7151-3.

[62] Kruth, C.; Czech, H.; Sklorz, M.; Passig, J.; Ehlert, S.; A. Cappiello, A.; Zimmermann, R. Direct infusion resonance-enhanced multiphoton ionization mass spectrometry of liquid samples under vacuum conditions*. Anal. Chem.* **2017**, 89, 20, 10917-10923. https://doi.org/10.1021/acs.analchem.7b02633.

[63] 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. https://doi.org/10.1016/j.forc.2019.100180.

[64] Cappiello, A.; Famiglini, G.; Piergiovanni, M.; Termopoli V. 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, p. 332.

[65] 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.

[66] Cappiello, A. The benefits of a liquid-electron ionization liquid chromatography-mass spectrometry interface, *The Column* **2019**, 32, 16-19.

[67] Cappiello, A.; Famiglini, G. Analysis of thermally unstable compounds by liquid chromatography/mass spectrometry particle beam interface with a modified ion source. *Anal. Chem.* **1995**, 67, 412-419. https://doi.org/ 10.1021/ac00098a029.

[68] Magrini, L.; Famiglini, G.; Palma, P.; Termopoli, V.; Cappiello, A. Boosting the detection potential of liquid chromatography-electron ionization mass spectrometry using a ceramic coated ion source. *J. Am. Soc. Mass Spectrom.* **2016**, 27, 1, 153-160. https://doi.org/10.1007/s13361-015-1257-0.

[69] 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.

[70] 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. doi:10.1002/bmc.1513.

[71] Dougherty, R.C. Negative Chemical Ionization Mass Spectrometry. *Anal. Chem.* **1981**, 53, 625–636, doi:10.1021/ac00227a813.

[72] 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. doi:10.1016/j.chroma.2022.463186.

[73] Termopoli, V.; Famiglini, G.; Palma, P.; Cappiello, A. Vandergrift, G.W.; Krogh, E.T.; Gill, C. G. Condensed phase membrane introduction mass spectrometry with direct electron ionization: on-line measurement of PAHs in complex aqueous samples. *J. Am. Soc. Mass Spectrom.* **2016**, 27, 301-308. doi: 10.1007/s13361-015-1285-9.

[74] Krogh, E.T.; Gill, C. G. Membrane introduction mass spectrometry (MIMS): a versatile tool for direct, real time chemical measurements. *J. Mass Spectrom.* **2014**, 49, 1205-1213. doi: 10.1002/jms.3447.

[75] 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.

[76] 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. doi:10.1021/acs.analchem.7b04295.

[77] 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. doi:10.1016/j.talanta.2020.120746.

[78] Zhang, Z.; Yang, M.J.; Pawliszyn, J. Solid-Phase Microextraction. A Solvent-Free Alternative for Sample Preparation. **1994**, 66, 844A-853A, doi:10.1021/ac00089a001.

[79] 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. doi:10.1016/j.aca.2009.02.049.

[80] Pawliszyn, J. *Handbook of Solid Phase Microextraction*; Elsevier: Amsterdam, The Nedertherlands, 2012. ISBN 9780124160170, doi:10.1016/C2011-0-04297-7.

[81] Górecki, T.; Yu, X.; Pawliszyn, J. Theory of Analyte Extraction by Selected Porous Polymer SPME Fibres.

Analyst **1999**, 124, 643-649. doi:10.1039/a808487d.

[82] 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. doi:10.1016/S0021-9673(97)00332-4.

[83] 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. doi:10.1016/j.chroma.2009.11.060.

[84] Zhou, W.; Yang, S.; Wang, P.G. Matrix Effects and Application of Matrix Effect Factor. *Bioanalysis* **2017**, 9, 1839–1844. doi:10.4155/bio-2017-0214.

[85] 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. doi:10.1371/journal.pone.0246108.

[86] Sajid, M.; Płotka-Wasylka, J. Green Analytical Chemistry Metrics: A Review. *Talanta* **2022**, 238. doi:10.1016/j.talanta.2021.123046.

[87] Gomes, J.V.D.; Tosta, C.L.; Cunha Neto, Á. da; 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. doi:10.1016/j.sajb.2021.09.034.

[88] Płotka-Wasylka, J. A New Tool for the Evaluation of the Analytical Procedure: Green Analytical Procedure Index. *Talanta* **2018**, *181*, 204–209, doi:10.1016/j.talanta.2018.01.013.

[89] 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. doi:10.1016/j.trac.2012.03.013.

APPENDIX: ORIGINAL PUBLICATIONS

Journal of Chromatography A 1681 (2022) 463479

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Short communication

Enhanced microfluidic open interface for the direct coupling of solid phase microextraction with liquid electron ionization-tandem mass spectrometry

Nicole Marittimo[a], Giorgio Famiglini[a], Pierangela Palma[a,b], Adriana Arigò[a], Achille Cappiello[a,b,*]

[a] University of Urbino Carlo Bo, Department of Pure and Applied Sciences, LC-MS Lab, Piazza Rinascimento 6 61029 Urbino, Italy
[b] Department of Chemistry, Vancouver Island University, B360-R306 – 900 Fifth St., Nanaimo, BC, Canada

ARTICLE INFO

Article history:
Received 10 June 2022
Revised 1 September 2022
Accepted 4 September 2022
Available online 6 September 2022

Keywords:
Direct mass spectrometry
Liquid-EI interface – LEI
Microfluidic open interface – MOI
SPME
Passive Flow Splitter (PFS)

ABSTRACT

Solid-phase microextraction (SPME) directly coupled to MS is a widespread technique for determining small molecules in different matrices in many application fields. Here we present a modified microfluidic open interface (MOI) connected to a passive-flow-splitter device (PFS) for the direct coupling of SPME to a liquid-electron ionization (LEI) interface in a tandem mass spectrometer for the analysis of complex biological samples. No chromatographic separation is involved. The new MOI-PFS configuration was designed to speed up the sample transfer to MS, improving the signal-to-noise ratio and peak shape and leading to fast and sensitive results. MOI-PFS-LEI-MS/MS experiments were conducted using fentanyl as a model compound in water and blood serum. The method uses a C18 Bio-SPME fiber by direct immersion (3 min) in 300 µL of the sample followed by rapid desorption (1 min) in a flow isolated volume (MOI chamber, 2.5 µL) filled with 100% acetonitrile. The PFS permits the rapid transfer of a fraction of the sample into the MS via the LEI interface. The optimal conditions were obtained at a flow rate of 10 µL·min^{-1} and a 1:20 split ratio. Altogether, extraction, desorption, and analysis require approximately 5 min. Good interday and intraday precision, excellent linearity and LOQs in the µg·L^{-1} range were obtained for fentanyl in water and serum. Greenness evaluation demonstrated a limited environmental impact of this technique.

1. Introduction

The conventional MS process is preceded by several steps (sampling, sample preparation, chromatographic separation) that, on the one hand, improve performance but, on the other hand, increase the overall analysis time. The integration of sampling and sample preparation steps, followed by the direct introduction to MS without chromatography, can provide an integrated workflow for high throughput analyses [1].

Different avenues can be exploited to obtain reliable and sensitive results in a short time. One avenue can use direct MS techniques for fast analytes desorption and ambient ionization (AMS). The development of the desorption electrospray ionization (DESI) by Cooks and co-workers [2,3] generated a flowering of different approaches sharing the same philosophy in which MS in the open air can analyze the surface of a sample with little or no pre-treatment [4–6]. However, the drawbacks of the direct introduction of complex samples into the MS could be matrix effects (ME), low sensitivity, poor quantitative data, and instrument contaminations. Nowadays, many miniaturized extraction techniques are available, meeting the principles of "green analytical chemistry," reducing the use of toxic solvents and reagents, energy consumption, waste generation, and providing good performance and environmental sustainability [7]. Among these "green" extraction techniques, SPME is a well-established, non-exhaustive technique, widely used in many applications for sampling, sample preparation, and enrichment in one step. A small amount of a suitable sorbent is exposed to the sample either by direct immersion (DI-SPME) or from the headspace (HS-SPME). The accurate control of the operative conditions (time of exposure, temperature, sample stirring, and others) is the basis of the proper quantification of the extracted analyte.

Since its first introduction in 1990, SPME underwent various developments in geometries, stationary and extraction phases, as described in detail in recent review articles. Different configurations and instrumental set-ups are compatible with SPME offering

* Corresponding author.
E-mail address: achille.cappiello@uniurb.it (A. Cappiello).

https://doi.org/10.1016/j.chroma.2022.463479
0021-9673/

a multifaceted approach that was proved to be robust and reliable, sensibly reducing the analysis time [8–12].

GC-MS and LC-MS are commonly used to separate and detect the extracted analytes, though, in recent years, SPME fibers have been coupled directly to MS, circumventing the chromatographic separation [13]. Thousands of papers on SPME and its application to various fields have been published over the years, demonstrating the worldwide success of the technique. An interesting recent review article summarizes all review articles on SPME, presenting theory, applications, new features, and future perspectives [14].

Recently, Pawliszyn and co-workers have developed a novel microfluidic technology called microfluidic open interface (MOI) to directly couple SPME fibers to MS. A flow isolated, small desorption chamber (V ≤ 7 mL) at atmospheric pressure is connected to the ion source of an MS driven by the vacuum pumps. MOI allows the direct hyphenation of SPME fibers to different ionization sources if constant liquid flow and aspiration toward the MS are provided. MOI was used in the simultaneous SPME-MS/MS detection of immunosuppressive drugs from whole human blood and in modified versions to determine drugs of abuse from blood, urine, and plasma [15–18].

In most cases, these MOI-MS couplings involve electrospray ionization (ESI) as the ionization mechanism, which is prone to matrix effects such as signal suppression or enhancement. High mass flow, mobile phase modifiers, or other compounds coming from the matrix can influence the ionization mechanism and should be addressed in the quantitative analysis (post-extraction addition or post-column infusion methods) [19–25].

Recently, MOI was successfully coupled to a liquid electron ionization-tandem mass spectrometry system (LEI-MS/MS) to directly analyze fentanyl in biological samples without sample pretreatment and measurable ME [26]. The ionization mechanism of LEI is electron ionization (EI), typically used for sample detection in GC-MS and occurs in the gas-phase in a high vacuum, high-temperature ion source, promoted by high energy electrons generated by a tungsten filament crossed by electric current. The collision between these electrons and the gas-phase molecules results in a positive radical cation, M·+, the molecular ion, and a series of characteristic fragments. Compounds identification is possible through comparison with mass spectra library databases and matching. This ionization mechanism is highly reproducible, often providing molecular ion information and a reproducible fragmentation. This fragmentation is a distinctive characteristic of the analyte, depending on its molecular structure and the energy involved in the ionization process [27]. The use of EI in the analysis of LC amenable compounds has been described in the literature exploiting different approaches, offering the advantage of a careful identification and quantification that with atmospheric pressure ionization (API) techniques is possible only with tandem mass spectrometry (MS/MS) or high-resolution mass spectrometry (HRMS) [28–34].

In the LEI interface, the vaporization of the analytes occurs at atmospheric pressure inside a microchannel called vaporization microchannel (VMC). Inside the VMC, the right combination of temperature, pressure, and surface materials can promote the optimal vaporization of the analytes, depending on their physical-chemical properties. No electric nor magnetic fields are applied to the VMC. The analytes are conveyed by a vacuum gradient and a helium flow inside an EI ion source for the ionization under the typical conditions (70 eV) [34–36].

Here we present a fast and straightforward sampling tool providing an updated MOI coupled to an LEI-MS/MS system via a PFS to analyze fentanyl, as a model compound, in water and blood serum using Bio-SPME fibers by direct immersion (DI). No sample preparation nor chromatographic separations are needed, and negligible matrix effects (ME) are observed. Compared to our previous attempt, the novel MOI-PFS configuration was designed to speed up the sample transfer to MS, improving the signal-to-noise ratio and peak shape and leading to fast and sensitive results .

In terms of intra- and interday RSD, limits of detection, and quantification, the validation method parameters were satisfactory.

When presenting new analytical methods, “greenness" evaluation is essential in reducing anthropogenic environmental impacts. The greenness of the presented method was evaluated, giving noticeable results.

2. Material and methods

2.1. Material 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). Fentanyl (1000 mg·L^{-1} in MeOH) and human blood serum were purchased from Sigma-Aldrich-Cerilliant (Milan, Italy) and stored at -20°C. For all experiments, 1.5 mL amber vials with 0.2 mL micro-inserts and slitted septa screw caps were used, supplied by VWR International. Bio-SPME fibers were assembled for the extraction procedure using 5 cm nitinol wires (200 µm diameter) coated with a mixture of polyacrylonitrile (PAN) and C18 particles. The fibers' coating thickness and length were 20 µm and 1 cm, respectively. A thorough description of the fibers manufacturing procedure is reported elsewhere . PEEKsil capillaries of all dimensions used in this study were purchased from IDEX (Oak Harbor, WA, USA), fused silica capillaries were from Molex Polymicro (Lisle, IL, USA), and flexible stainless-steel tubings were from Agilent Technologies Inc. (Santa Clara, CA, USA). Capillaries used to connect all parts were carefully selected according to internal diameter and length to avoid system overpressure and ensure the lowest possible volume for a rapid transfer of the analyte to the MS. Capillaries dimensions are reported in Fig. 1.

2.2. Standard solution preparation

Diluted solutions of fentanyl were prepared volumetrically in ACN from the reference standard (1000 mg·L^{-1}) at the following concentrations (0.2 mg·L^{-1}, 1 mg·L^{-1}, 2 mg·L^{-1}, 4 mg·L^{-1}, 10 mg·L^{-1}, 20 mg·L^{-1}, 40 mg·L^{-1}, 80 mg·L^{-1}, 200 mg·L^{-1}, 400 mg·L^{-1}), and stored in dark vials (Agilent Technologies, Santa Clara, CA, USA) at -20°C. Diluted solutions were used to volumetrically prepare working standard solutions of fentanyl in ultrapure water and serum, containing ACN 0.25% (v/v) and 5.0% (v/v), respectively. These concentrations were: 1000 µg·L^{-1} for DI-SPME studies, PFS experiments, and repeatability tests, and 5, 10, 50, 100, 200, 500, and 1000 µg·L^{-1} for calibration curves. Each solution was injected in triplicate. Serum was not treated nor diluted before use. No filtration of serum samples was needed because PAN does not bind hydrophobic groups, thus preventing the matrix from sticking onto the coating surface.

2.3. Instrumentation and equipment

The MOI-PFS-LEI-MS/MS apparatus and all tubing types and dimensions used in this study are shown in Fig. 1. The pivotal system parts are discussed in the following paragraphs.

2.3.1. Microfluidic open interface (MOI)

The original MOI design was modified to accommodate the nanoscale flow rates needed to accomplish LEI requirements. In

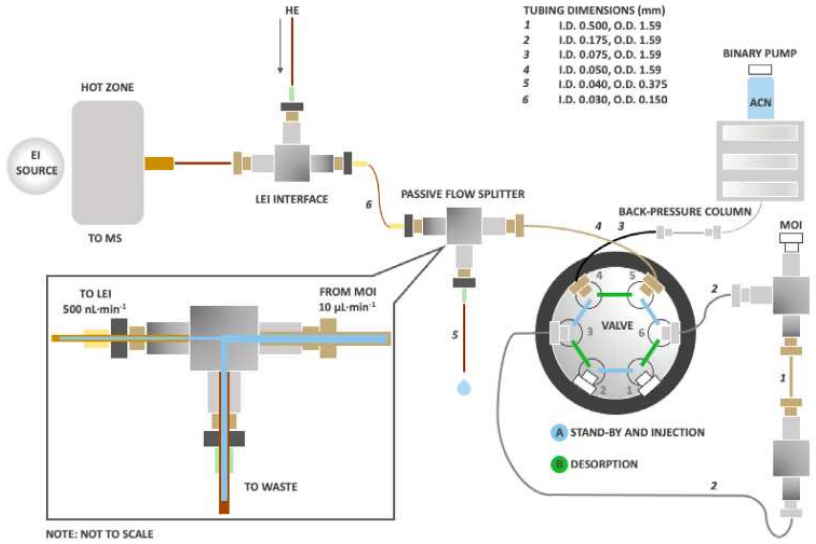


Fig. 1. Scheme of the MOI-PFS-LEI-MS/MS system.

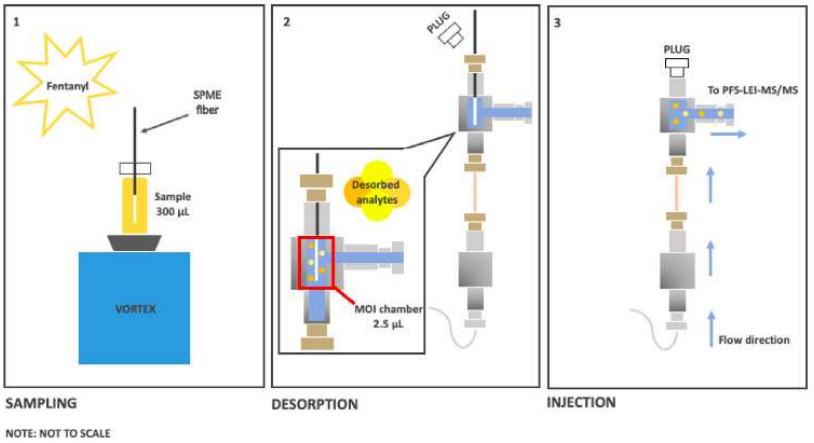


Fig. 2. Scheme of the MOI functioning. 1) 300 µL of serum are sampled by direct immersion (vortexed for 3 min) of a C18 SPME fiber; 2) quick desorption (1 min) in a flow isolated volume (MOI chamber, 2.5 µL) filled with 100% ACN; 3) the desorbed analytes are conveyed to the PFS-LEI-MS/MS for analysis.

particular, the desorption chamber volume was reduced to assemble a flow isolated volume of 9.8 µL. When the SPME fiber is inserted into the chamber, the internal volume is reduced to approximately 2.5 µL. Due to the restriction caused by the fiber diameter and connections, smaller volumes cannot be used. This chamber volume reduction has permitted to operate at a flow rate of 500 nL·min^{-1}. A thorough description of the modified MOI is reported elsewhere [26]. A detailed description of modified MOI is reported in Fig. 2. After each SPME analysis (sampling and desorption), the fiber was rinsed and conditioned in isopropanol for 5 minutes, then dried. Blank extractions and analyses were performed after each matrix extraction to avoid contamination and carry-over phenomena.

2.3.2. Passive-flow-splitter (PFS)

A rapid transfer of the desorbed analytes to the MS is mandatory to obtain a high signal-to-noise ratio, narrow peaks, and fast and sensitive results. It was observed that the optimal post-extraction flow rate to empty the MOI rapidly is in the µL·min^{-1} range. However, as discussed elsewhere [36], the optimal flow

rate for the proper LEI functioning is approximately 500 nL·min^{-1}. Therefore, the PFS (Fig. 1, zoomed box) was designed to reduce the high flow rate needed to empty the MOI and meet LEI requirements rapidly. In particular, the PFS reduces the flow from 10 µL·min^{-1} to 500 nL·min^{-1}, providing a 1:20 mobile phase split ratio. This device consists of a reduced internal volume (0.57 µL) stainless-steel tee (IDEX, Oak Harbor, WA, USA). One of the ports of the PFS is connected to the MOI outlet. The second port is connected to a 5 cm fused silica capillary (40 µm i.d. and 375 µm o.d.), where part of the flow is conveyed and goes to waste. The third port is connected to the LEI inlet capillary, where the reduced flow is transferred. At a constant incoming flow rate, different diameters and lengths of the waste-fused silica capillary can be selected to achieve the desired flow rate going to MS. The LEI inlet capillary was removed from the VMC for flow rate determinations and connected to a Sensirion liquid flowmeter (Sensirion SLG64-0075, Sensirion AG, Switzerland). After flow rate measurements, the LEI capillary was reinstalled in the VMC, and a stable liquid phase flow was assessed by monitoring the MS vacuum values.

2.3.3. MOI-PFS-LEI-MS/MS system

In addition to MOI and PFS described above, the MOI-PFS-LEI-MS/MS system is composed of a triple quadrupole Agilent 7010 B mass spectrometer (Agilent Technologies Inc., Santa Clara, CA, USA) equipped with an LEI interface, and an Agilent 1290 Infinity II binary pump (Agilent Technologies Inc., Santa Clara, CA, USA). An Agilent Zorbax Eclipse XDB C18 backpressure column (4.6×150 mm x 5 µm) stabilized the flow rate. During the procedure, 100% ACN was flowing through the system. As shown in Fig. 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 50 cm PEEKsil capillary (75 µm i.d. and 1.59 mm o.d.). Inlet and outlet MOI capillaries (30 cm) were of flexible stainless-steel (175 µm i.d. and 1.59 mm o.d.) and were connected to port 3 and 6, respectively. The PFS was connected to port 5 and MOI exit via a 20 cm PEEKsil capillary (50 µm i.d. and 1.59 mm o.d.), and to the LEI fused silica inlet capillary (30 cm, 30 µm i.d., and 150 µm o.d.). The EI source temperature was kept at 280°C. Fentanyl data acquisition was carried out in multiple reaction monitoring (MRM) using the following transitions and collision energies: Q = 245→189 (10 eV) and q = 245→146 (5 eV). A detailed description of the LEI interface is reported elsewhere [36]. In brief, with LEI, the vaporization of the liquid phase occurs at ambient pressure inside a VMC connected to the EI source. Gas-phase molecules are carried inside the ion source by a vacuum gradient and an inert gas (He) thrust. For all experiments, the VMC temperature was set at 350°C.

2.3.4. Hydrodynamic and operation of the MOI-PFS-LEI-MS/MS apparatus

The procedure starts in a stand-by position for MOI filling (A). As shown in Fig. 1, in this position, the solvent flows from the pump to port 4 at 10 µL·min^{-1}, 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 the PFS through port 5. Here, the flow stream is split: the majority goes to the waste line, and only 500 nL·min^{-1} are allowed into the MS via LEI. After MOI filling, the valve is switched to desorption position (B). In this position, the solvent flows through PFS directly to LEI-MS and the MOI chamber, filled with ACN, is isolated and ready for fiber introduction and analytes desorption. Once the desorption step is completed, the valve is switched back to injection position (A) for MOI draining. The solvent was constantly flowing to the MS in positions A (stand-by and injection) and B (desorption).

In the previous setup, the MOI chamber was isolated for desorption, and the liquid flow rate from the pump was conveyed to waste, so there was no solvent entering the MS during the desorption step. In the present configuration, to preserve the MS performance and ensure a constant solvent incoming, the MOI inlet and outlet (port 3 and 6) were connected to two plugged ports (port 1 and 2) to isolate the chamber during desorption, while the liquid flow rate from the pump (port 4) was directly conveyed to PFS (port 5) and then to the MS. In this way, the ion source is continuously supplied with the same solvent used for desorption, offering the advantage of a more stable signal.

3. Results and discussion

3.1. Method validation

The method performance was evaluated using Bio-SPME fibers on water and serum samples with fentanyl as a model compound. Linearity range, accuracy, intraday, and interday repeatability were assessed. The results obtained are reported in Table 1. The calibration curves were plotted for both matrices, injecting the standard in triplicate at the following concentrations: 5, 10, 50, 100, 200, 500, 1000 µg·L^{-1}. Linearity was satisfactory in both matrices, with a determination coefficient (R^2) of 0.9995 in water and 0.9981 in serum. Limits of detection (LODs) and quantitation (LOQs) were calculated as 3X and 10X of the S/N, respectively. LODs were 1.5 µg·L^{-1} in water and 2.5 µg·L^{-1} in serum, and LOQs were 5 µg·L^{-1} in water and 10 µg·L^{-1} in serum. LODs and LOQs were calculated using experimental peaks obtained after splitting the total amount injected (1:20). Precision was assessed by calculating the relative standard deviation (RDS%) of intraday (n = 10) and interday (n = 5) repeatability measurements. Without internal standard correction, good repeatability was obtained for both matrices with RSD values lower than 25%.

3.2. Matrix effects evaluation

ME can limit a correct quantitative analysis and must be carefully evaluated because, in this work, sample and matrix components are introduced simultaneously into the MS ion source without chromatographic separation. Different approaches can be used in ME evaluation [37–41]. In our experiments, the ME values were calculated by comparing the calibration curves of fentanyl in water and serum using Eq. (1):

$$ME(\%) = (slope\ serum / slope\ water) \times 100 \quad (1)$$

The evaluation of ME indicates how much the result obtained differs from 100%, which indicates no ME. In the present work, a value of 84% was obtained meaning a variance of 16%, indicating a scarce matrix influence.

3.3. Passive-flow-splitter operation

A major drawback noticed in the first attempt of MOI-LEI coupling is the long MOI chamber emptying time due to the nano mobile phase flow rates required for the proper LEI functioning. It was observed that this time delay caused a broad peak with low S/N and a long analysis time. A further MOI chamber volume reduction is impossible because this must be large enough to accommodate the SPME fiber and contain sufficient desorption solvent. A possible solution to these limitations involves the rapid draining of MOI at a high flow rate followed by a flow reduction before LEI. For this purpose, a passive-flow-splitter (PFS) is proposed in this work. The PFS allowed the rapid transfer of the desorbed analytes into the MS, enabling a sharper peak with higher S/N and increased sensitivity compared with the original design. PFS split ratio was

Table 1
Calibration data, LODs, and LOQs for fentanyl in water and serum.

Matrix	Linearity range (μg·L^{-1})	Equation	R^2	LOQ(μg·L^{-1})	LOD (μg·L^{-1})	Intraday RSD% at 1000 μg·L^{-1}	Interday RSD% at 1000 μg·L^{-1}
Water	5-1000	y = 1.2730x + 192.04	0.9995	5	1.5	15	16
Serum	10-1000	y = 1.0667x + 143,5	0.9981	10	2.5	18	24

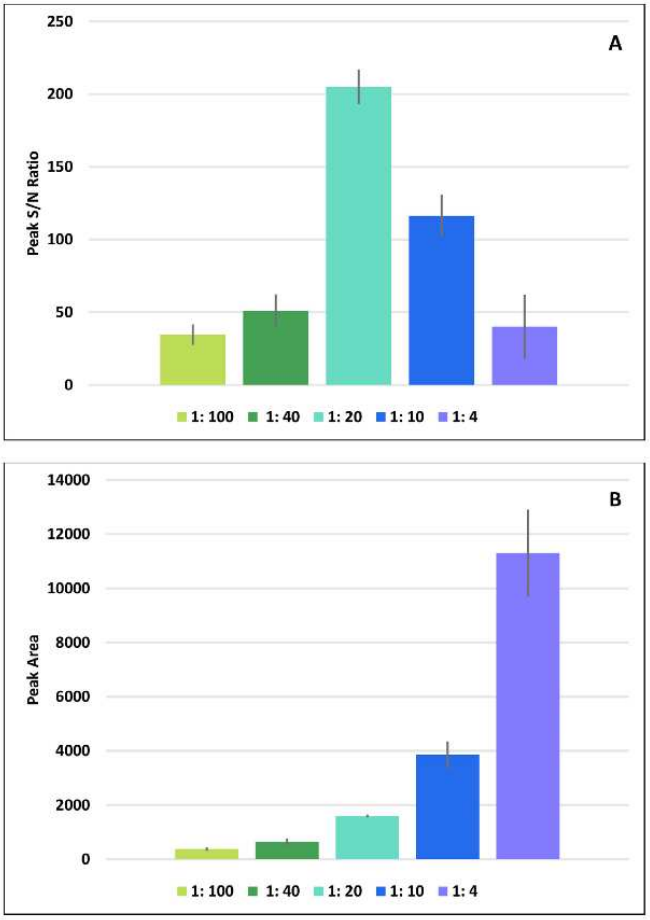


Fig. 3. Effect of different split ratios on peak S/N values (A) and peak areas (B). A solution of 1000 μg·L^{-1} of fentanyl in ACN was used for the tests.

optimized testing 1:100, 1:40, 1:20, 1:10, 1:4 values. As shown in Fig. 3A and B, at the highest split ratio (1:100), low peak S/N and area values were obtained because the sensitivity was penalized by the loss of analytes. At the lowest spit ratio (1:4), the highest area values were obtained because almost all the sample was sent to the MS, but the short transfer time affected the peak shape, which is too large, lowering the S/N response. Among all other split ratios tested (1:10, 1:20, 1:40), the value of 1:20 yielded the best compromise between peak area and S/N, thus enhancing the overall sensitivity. For this reason, MOI upstream flow rate was set at 10 μL·min^{-1} to empty the chamber rapidly, then the solution containing the desorbed analytes was conveyed to PFS, where the flow rate was reduced to 500 nL·min^{-1}.

Therefore, even if with PFS part of the sample is wasted, as demonstrated in Fig. 4, under the optimized conditions, a considerable increase of sensitivity is obtained compared to the previous configuration. A solution of fentanyl (100 μg·L^{-1}) in water was injected into the MS with (upper trace) and without (lower trace) PFS. In the upper trace, it can be observed that the peak is approximately 1 min large, whereas in the lower trace is approximately

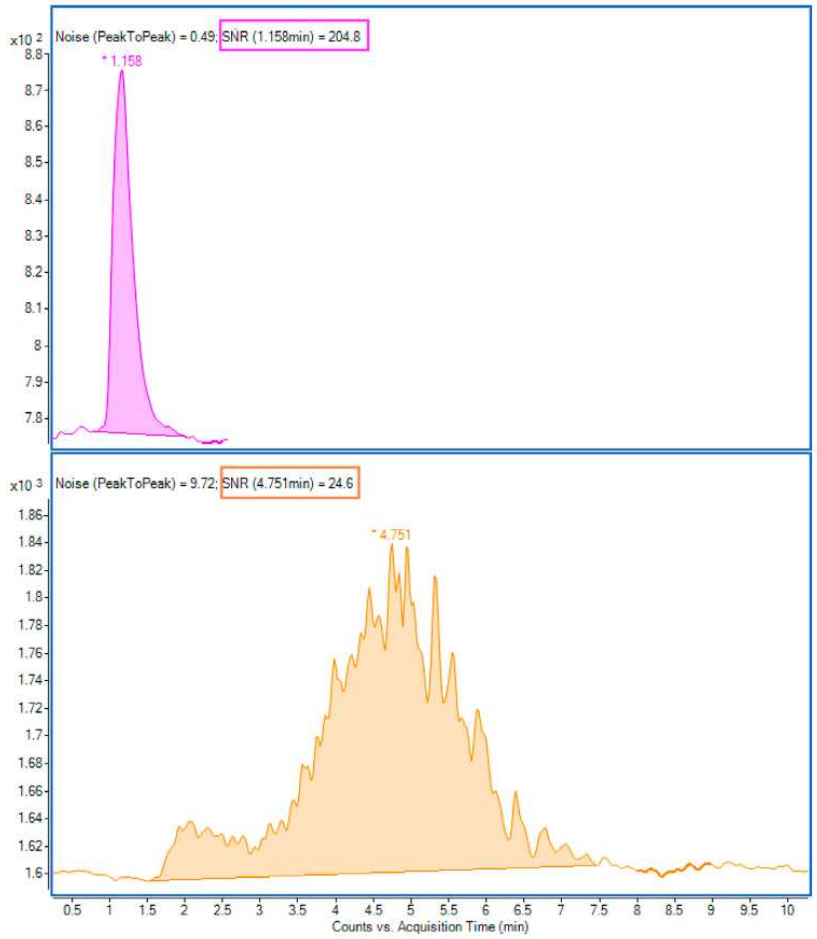


Fig 4. Fentanyl in water at 100 $\mu g \cdot L^{-1}$. Upper trace: with PFS, S/N = 204. Lower trace: without PFS, S/N= 24.

6 min large. The lower peak intensity obtained with the PFS is compensated by a much higher signal-to-noise ratio (S/N = 204) than recorded without PFS (S/N= 24).

All the tests were conducted using the same procedure described in detail in paragraph 2.3.4.

3.4. Greenness evaluation of the method

A further novel aspect of this analytical method is based on its greenness. In the last decade, many efforts have been made to spread the concept of green chemistry and make scientists conscious of their choices in this regard. Nowadays, the awareness of optimizing a method according to green analytical chemistry (GAC) criteria can play a crucial role in the success of the developed strategy. Sajid and Płotka-Wasylka [42] selected the Analytical GREEnness Metric Approach AGREE [43] as one of the most applicable to most analytical procedures, with Green Analytical Procedure Index (GAPI) [44] and Analytical Eco-Scale [45]. According to the reasons suggested in the literature [42], AGREE was selected as the most suitable metric for evaluating the greenness of this analytical procedure through the free downloadable software Analytical GREennEss Calculator. Fig. 5 shows the results obtained by applying the 12 criteria of AGREE. Fig. 6A represents the pictogram obtained using the default weights for all 12 parameters, which gave a score equal to 0.65. As shown in Fig. 6B, a score of 0.7 was obtained by modifying the default weight to fit better with the analysis conditions of this approach. The default weights of criteria 2, 7, 10, 11, and 12 were set equal to 3, 4, 1, 1, 1, respectively. The AGREE method foresees a default value of 2 for all the criteria listed in Fig. 5; however, AGREE developers assert that not all assessment criteria have to be of equal importance, to make the tool flexible according to the specification of the new analyti-

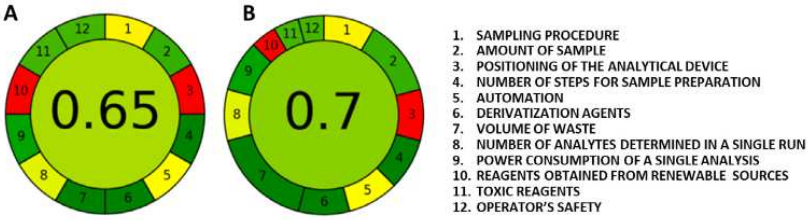


Fig. 5. Evaluation of the method greenness using AGREE [43]. In A, the pictogram is obtained using default weights (2). In B, the pictogram is obtained using modified weights (3, 4, 1, 1, 1 for criteria 2, 7, 10, 11, and 12; respectively). For more details, refer to Section 3.4.

cal method. A higher weight was assigned to criteria 2 and 7 (see Fig. 4) to highlight that a very low amount of sample is needed and few solvent wastes are generated. The impact of criteria related to the harmful effects of solvents (10-12) was reduced from 2 to 1, considering the low volume employed compared to conventional analytical methods. The final scores are very similar, with or without changes in the default parameters, and demonstrate in both cases a satisfactory result. These changes were made to emphasize the use of very low solvent volumes and attach less importance to the criteria based on their hazard. However, in both cases, the pictograms resulted in very satisfactory scores, which underline the green character of this analytical approach.

4. Conclusions

Compared to the previous setup, the proposed method, involving the new MOI-PFS-LEI-MS/MS configuration, presents the significant advantage of a fast sample transfer to MS. In addition, the new setup allows the continuous feeding of the mobile phase for a more stable MS response. The modifications proposed here were tested using fentanyl as a model compound in water and blood serum and demonstrated to improve the signal-to-noise ratio and peak shape, leading to fast, sustainable and sensitive results. Furthermore, the method proposed shows a low environmental footprint given by the satisfactory AGREE score.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Nicole Marittimo: Investigation, Visualization. **Giorgio Famiglini:** Conceptualization, Data curation. **Pierangela Palma:** Writing – original draft, Writing – review & editing, Data curation. **Adriana Arigò:** Validation. **Achille Cappiello:** Supervision, Conceptualization, Funding acquisition, Resources.

Acknowledgments

The authors are grateful to Agilent Technologies for providing the LC-MS/MS instrumentation. The authors thank Marco Agostini and Caterina Renzoni, Laboratorio di Tossicologia A.S.U.R. AV1, Via Lombroso 15, 61122 Pesaro, Italy for executing fentanyl extractions.

References

[1] A. Khaled, J.R. Belinato, J. Pawliszyn, Rapid and high-throughput screening of multi-residue pharmaceutical drugs in bovine tissue using solid phase microextraction and direct analysis in real time-tandem mass spectrometry (SPME-DART-MS/MS), Talanta (121095) (2020) 217, doi:10.1016/j.talanta.2020.121095.
[2] Z. Takats, J.M. Wiseman, B. Gologan, R.G. Cooks, Mass spectrometry sampling under ambient conditions with desorption electrospray ionization, Science 306 (5695) (2004) 471–473, doi:10.1126/science.1104404.
[3] R.G. Cooks, Z. Ouyang, Z. Takats, J.M. Wiseman, Ambient mass spectrometry, Science 311 (5767) (2006) 1566–1570, doi:10.1126/science.1119426.
[4] M.E. Monge, G.A. Harris, P. Dwivedi, F.M. Fernandez, Mass spectrometry: recent advances in direct open air surface sampling/ionization, Chem. Rev. 113 (4) (2013) 2269–2308, doi:10.1021/cr300309q.
[5] M.Z Huang, S.C. Cheng, Y.T. Cho, J. Shiea, Ambient ionization mass spectrometry: a tutorial, Analytica Chimica Acta 702 (1) (2001) 1–15, doi:10.1016/j.aca.2011.06.017.
[6] J. Deng, Y. Yang, X. Wang, T. Luan, Strategies for coupling solid phase microextraction with mass spectrometry, Trends Anal. Chem. 55 (2014) 55–67, doi:10.1016/j.trac.2013.12.004.
[7] A. Gałuszka, Z. Migaszewski, J. Namieśnik, The 12 principles of green analytical chemistry and the SIGNIFICANCE mnemonic of green analytical practices, Trends Anal. Chem. 50 (2013) 78–84, doi:10.1016/j.trac.2013.04.010.
[8] C.L. Arthur, J. Pawliszyn, Solid phase microextraction with thermal desorption using fused silica optical fibers, Anal. Chem. 62 (19) (1990) 2145–2148, doi:10.1021/ac00218a019.
[9] G.A. Gomez-Ríos, M.F. Mirabelli, Solid phase microextraction-mass spectrometry: Metanoia, Trends Anal. Chem. 112 (2019) (2019) 201–211, doi:10.1016/j.trac.2018.12.030.
[10] N. Reyes-Garcés, E. Gionfriddo, G.A. Gomez-Ríos, M.N. Alam, E. Boyacı, B. Bojko, V. Singh, J. Grandy, J. Pawliszyn, Advances in solid phase microextraction and perspective on future directions, Anal. Chem. 90 (1) (2018) 302–360, doi:10.1021/acs.analchem.7b04502.
[11] J.S. Herrington, G.A. Gomez-Ríos, C. Myers, G. Stidsen, D.S. Bell, Hunting molecules in complex matrices with SPME arrows: a review, Separations 7 (1) (2020) 12, doi:10.3390/separations7010012.
[12] M.J. Trujillo-Rodríguez, I. Pacheco-Fernández, I. Taima-Mancera, J.H. Ayala Díaz, V. Pino, Evolution and current advances in sorbent-based microextraction configurations, Journal of Chromatography A 1634 (2020) 461670, doi:10.1016/j.chroma.2020.461670.
[13] L. Fang, J. Deng, Y. Yang, X. Wang, B. Chen, H. Liu, H. Zhou, G. Ouyang, T. Luan, Coupling solid-phase microextraction with ambient mass spectrometry: Strategies and applications, TrAC Trends Anal Chem 85 (2016) 61–72, doi:10.1016/j.trac.2016.05.025.
[14] V. Jalili, A. Barkhordari, A. Ghiasvand, A comprehensive look at solid-phase microextraction technique: a review of reviews, Microchem. J. 152 (2020) 104319, doi:10.1016/j.microc.2019.104319.
[15] M. Tascon, M. Alam, G. Gómez-Ríos, J. Pawliszyn, development of a microfluidic open interface with flow isolated desorption volume for the direct coupling of SPME devices to mass spectrometry, Anal. Chem. 90 (2018) 2631–2638, doi:10.1021/acs.analchem.7b04295.
[16] E. Nazdrajić, M. Tascon, D.A. Rickert, G.A. Gómez-Ríos, V. Kulasingam, J.B. Pawliszyn, Rapid determination of tacrolimus and sirolimus in whole human blood by direct coupling of solid-phase microextraction to mass spectrometry via microfluidic open interface, Anal. Chim. Acta 1144 (2021) 53–60, doi:10.1016/j.aca.2020.11.056.
[17] N.T. Looby, M. Tascon, V. Acquaro, N. Reyes-Garcés, T. Vasiljevic, M. Wasowicz, J. Pawliszyn, Solid phase microextraction coupled to mass spectrometry via microfluidic interface for rapid therapeutic drug monitoring, Analyst 144 (2019) 3721e3728, doi:10.1039/C9AN00041K.
[18] M. Tascon, V. Singh, M. Huq, J. Pawliszyn, Direct coupling of dispersive extractions with magnetic particles to mass spectrometry via microfluidic open interface, Anal. Chem. 91 (2019) 4762e4770, doi:10.1021/acs.analchem.9b00308.
[19] A. Cappiello, G. Famiglini, P. Palma, E. Pierini, V. Termopoli, H. Trufelli, overcoming matrix effects in liquid chromatography-mass spectrometry, Anal. Chem. 80 (2008) 9343–9348, doi:10.1021/ac8018312.
[20] F. Gosetti, E. Mazzucco, D. Zampieri, M.C. Gennaro, Signal suppression/enhancement in high-performance liquid chromatography tandem mass spectrometry, J. Chromatogr. A 1217 (2010) 3929–3937, doi:10.1016/j.chroma.2009.11.060.
[21] I. Marchi, V. Viette, F. Badoud, M. Fathi, M. Saugy, S. Rudaz, J.L. Veuthey, Characterization and classification of matrix effects in biological samples analyses, J. Chromatogr. A 1217 (2010) 4071–4078, doi:10.1016/j.chroma.2009.08.061.

[22] H. Trufelli, P. Palma, G. Famiglini, A. Cappiello, An overview of matrix effects in liquid chromatography-mass spectrometry, Mass Spectrom. Rev. 30 (2011) 491–509, doi:10.1002/mas.20298.
[23] J. Rossmann, R. Gurke, L.D. Renner, R. Oertel, W. Kirch, Evaluation of the matrix effect of different sample matrices for 33 pharmaceuticals by post-column infusion, J. Chromatogr. B: Anal. Technol. Biomed. Life Sci. 1000 (2015) 84–94, doi:10.1016/j.jchromb.2015.06.019.
[24] J.H. Ye, L.H. Pao, Using visualized matrix effects to develop and improve LC-MS/MS bioanalytical methods, taking TRAM-34 as an example, PLoS One 10 (4) (2015) e0118818, doi:10.1371/journal.pone.0118818.
[25] RSC Analytical Methods CommitteeGuide to Achieving Reliable Quantitative LC-MS Measurements, LGC Limited, London, U.K., 2013 Sargent, M., Ed.ISBN 978-0-948926-27-3.
[26] P. Rocío-Bautista, G. Famiglini, V. Termopoli, P. Palma, E. Nazdrajić, J. Pawliszyn, A. Cappiello, direct coupling of bio-SPME to liquid electron ionization-MS/MS via a modified microfluidic open interface, J. Am. Soc. Mass Spectrom. 32 (2021) 262–269, doi:10.1021/jasms.0c00303.
[27] G. Famiglini, P. Palma, V. Termopoli, A. Cappiello, The history of electron ionization in LC-MS, from the early days to modern technologies: a review, Anal. Chim. Acta 1167 (2021) 338350, doi:10.1016/j.aca.2021.338350.
[28] G. Famiglini, P. Palma, A. Siviero, M.A. Rezai, A. Cappiello, Determination of endocrine disrupting compounds in marine water by nanoliquid chromatography/direct-electron ionization mass spectrometry, Anal. Chem. 77 (2005) 7654–7661, doi:10.1021/ac050842m.
[29] O. Granot, A. Amirav, LC-MS with electron ionization of cold molecules in supersonic molecular beams, Int. J. Mass Spectrom. 244 (2005) 15–28, doi:10.1016/j.ijms.2005.04.003.
[30] B. Seemann, T. Alon, S. Tsizin, A.B. Fialkov, A. Amirav, Electron ionization LC-MS with supersonic molecular beam the new concept, benefits and applications, J. Mass Spectrom. 50 (2015) 1252–1263, doi:10.1002/jms.3695.
[31] A. Amirav, A.B. Fialkov, A. Gordin, Improved electron ionization ion source for the detection of supersonic molecular beams, Rev. Sci. Instrum. 73 (2002) 2872–2876, doi:10.1063/1.1488683.
[32] F. Rigano, M. Russo, A. Arigò, P. Dugo, L. Mondello, Combining linear retention index and electron ionization mass spectrometry for a reliable identification in nano liquid chromatography, J. Chromatogr. A 1610 (2020) 460581, doi:10.1016/j.chroma.2019.460581.
[33] F. Rigano, A. Albergamo, D. Sciarrone, M. Beccaria, G. Purcaro, L. Mondello, Nano liquid chromatography directly coupled to electron ionization mass spectrometry for free fatty acid elucidation in mussel, Anal. Chem. 88 (7) (2016) 4021–4028, doi:10.1021/acs.analchem.6b00328.
[34] G. Famiglini, P. Palma, E. Pierini, H. Trufelli, A. Cappiello, Organochlorine pesticides by LC-MS, Anal. Chem. 80 (2008) 3445–3449, doi:10.1021/ac8000435.
[35] V. Termopoli, G. Famiglini, P. Palma, M. Piergiovanni, A. Cappiello, Atmospheric pressure vaporization mechanism for coupling a liquid phase with electron ionization mass spectrometry, Anal. Chem. 89 (2017) 2049–2056, doi:10.1021/acs.analchem.6b04646.
[36] V. Termopoli, G. Famiglini, P. Palma, M. Piergiovanni, P. Rocío-Bautista, M.F. Ottaviani, A. Cappiello, M. Saeed, S. Perry, Evaluation of a liquid electron ionization liquid chromatography-mass spectrometry interface, J. Chromatogr. A 1591 (2019) 120–130, doi:10.1016/j.chroma.2019.01.034.
[37] I. Marchi, V. Viette, F. Badoud, M. Fathi, M. Saugy, S. Rudaz, J.L. Veuthey, Characterization and classification of matrix effects in biological samples analyses, J. Chromatogr. A 1217 (25) (2010) 4071–4078, doi:10.1016/j.chroma.2009.08.061.
[38] A. Cappiello, G. Famiglini, P. Palma, H. Trufelli, Matrix effects in liquid chromatography-mass spectrometry, J. Liq. Chromatogr. Relat. Technol. 33 (2010) 1067–1081, doi:10.1080/10826076.2010.484314.
[39] H. Trufelli, P. Palma, G. Famiglini, A. Cappiello, An overview of matrix effects in liquid chromatography-mass spectrometry, Mass Spectrom. Rev. 30 (2011) 491–509, doi:10.1002/mas.20298.
[40] M. Cortese, M.R. Gigliobianco, F. Magnoni, R. Censi, P. Di Martino, Compensate for or minimize matrix effects? Strategies for overcoming matrix effects in liquid chromatography-mass spectrometry technique: a tutorial review, Molecules 25 (13) (2020) 3047, doi:10.3390/molecules25133047.
[41] W. Zhou, S. Yang, P.G. Wang, Matrix effects and application of matrix effect factor, Bioanalysis 23 (2017) 1839–1844, doi:10.4155/bio-2017-0214.
[42] M. Sajid, J. Płotka-Wasylka, Green analytical chemistry metrics: A review, Talanta 238 (2022) 123046, doi:10.1016/j.talanta.2021.123046.
[43] F. Pena-Pereira, W. Wojnowski, M. Tobiszewski, AGREE—analytical GREEnness metric approach and software, Anal. Chem. 92 (2020) 10076–10082, doi:10.1021/acs.analchem.0c01887.
[44] J. Płotka-Wasylka, A new tool for the evaluation of the analytical procedure: green Analytical Procedure Index, Talanta 181 (2018) 204–209, doi:10.1016/j.talanta.2018.01.013.
[45] A. Gałuszka, Z.M. Migaszewski, P. Konieczka, J. Namieśnik, Analytical eco-scale for assessing the greenness of analytical procedures, TrAC Trends Anal. Chem. (Reference Ed.) 37 (2012) 61–72, doi:10.1016/J.TRAC.2012.03.013.

scientific reports

Check for updates

OPEN

Extractive-liquid sampling electron ionization-mass spectrometry (E-LEI-MS): a new powerful combination for direct analysis

Adriana Arigò[1✉], Giorgio Famiglini[1], Nicole Marittimo[1], Marco Agostini[2,4], Caterina Renzoni[2,4], Pierangela Palma[1,3] & Achille Cappiello[1,3]

One of modern analytical chemistry main challenges is providing as fast as possible results in different application fields. In this view, real-time analysis techniques are experiencing ever-increasing success as they can provide data quickly, almost without sample preparation steps. Most of real-time approaches are based on direct mass spectrometry (DMS), a method of analyzing samples without the need for separation or pre-treatment steps. Instead, the sample is directly introduced into the mass spectrometer for analysis. In this context, ambient ionization mass spectrometry (AIMS) techniques are widely represented and successfully used. Extractive-liquid sampling electron ionization-mass spectrometry (E-LEI-MS) represents a different analytical strategy that allows coupling ambient sampling with electron ionization (EI), avoiding any sample preparation step and providing identification based on the comparison with the National Institute of Standards and Technology (NIST) library spectra. E-LEI-MS consists of a dispositive for solvent release and sampling at ambient conditions coupled with an EI source of a single quadrupole mass spectrometer. A micromanipulator allows fine (x,y,z) positioning of a sampling tip. MS can operate in scan or SIM modes depending on the application goals and requirements. Several preliminary successful results were already obtained due to the highly informative EI mass spectra generation. The system was applied to the analysis of active ingredients in pharmaceutical tablets, pesticides on fruit peel, a drug of abuse (cocaine) determination in banknotes, and analysis of unknown components on painting surfaces. Both forensic and artwork applications allowed determining the spatial distribution of the analytes. Here we present a proof-of-concept of E-LEI-MS for targeted/non-targeted analysis and semi-quantitative detection.

Extractive-liquid sampling electron ionization-mass spectrometry (E-LEI-MS) is a prototype developed to combine the advantage of ambient sampling with the high identification power provided by electron ionization (EI). E-LEI-MS technology is strictly related to the direct electron ionization (DEI) LC–EI–MS interface presented by Cappiello et al., which allows the conversion of a liquid nanoflow rate to gas phase directly inside the ion source[1]. DEI was successfully employed in many applications[2]; therefore, the same setup was exploited to develop E-LEI-MS, inspired also by the liquid micro-junction surface sampling probes (CF-LMJ-SSPs) to directly sample soluble materials from surfaces for subsequent atmospheric pressure ionization sources[3,4].

Despite E-LEI-MS being an innovative analytical approach, it refers somehow to ambient mass spectrometry, at least from the sampling point of view. Ambient MS is nowadays a fast-growing approach for real-time sampling and analysis in the native environment of various matrices without either sample preparation or chromatographic separation. With ambient MS, sample manipulation is needless or reduced to a minimum, samples can be handled in the open air and directly analyzed, thus preventing possible alterations or contaminations. Ambient MS was initiated with the development of desorption electrospray ionization (DESI)[5,6] and direct analysis in real-time (DART)[7]. Since their first introduction, DESI and DART have been continuously improved to enhance performance and suitability in many application fields, spanning from bioanalytical to environmental, forensic, reaction monitoring, and others[8]. Atmospheric pressure ionization techniques (API), such as ESI, APCI, and

[1]LC-MS Lab, Department of Pure and Applied Sciences, University of Urbino Carlo Bo, Piazza Rinascimento 6, 61029 Urbino, Italy. [2]Laboratorio di Tossicologia, A.S.T. AV1, Via Lombroso 15, 61122 Pesaro, Italy. [3]Department of Chemistry, Vancouver Island University, B-360-R306, 900 Fifth St., Nanaimo, BC, Canada. [4]These authors contributed equally: Marco Agostini and Caterina Renzoni. ✉email: adriana.arigo@uniurb.it

APPI, are typically used in ambient MS, and jointly, various techniques based on different physical-chemical mechanisms, were developed for the direct ionization of the analytes in their original environment[9,10]. API techniques mainly produce protonated or deprotonated molecules, with or without adduct ions, thus requiring MS-MS or high-resolution MS for analyte identification and characterization. These ionization techniques, in particular ESI, are characterized by high sensitivity and robustness, however, their response is often compound-specific and can be affected by ion suppression or enhancement[6]. Unlike the above-mentioned techniques, this paper proposes a new real-time approach for analytes introduction into a standard single quadrupole mass spectrometer equipped with an electron ionization (EI) source, to increase the identification power in direct analysis. To the best of our knowledge, this is the first real-time MS technique using EI for compound ionization. Sampling occurs in ambient conditions and neither sample preparation nor manipulation is required, contrary to what is necessary for separation techniques. A suitable solvent is deposited onto the sample surface where analytes are dissolved and transferred into the EI ion source of a single quadrupole mass spectrometer by the effect of the high vacuum using a sampling tip. Right after, the MS data acquisition begins. E-LEI-MS operates at atmospheric pressure and ground potential, analytes entering the ion source in a liquid phase, are sprayed into the ion source where high-temperature and high-vacuum conditions promote their gas-phase conversion. A 70-eV electron beam effects the typical EI ionization, providing highly informative and National Institute of Standards and Technology (NIST) searchable EI mass spectra for identifying unknown compounds or detecting targeted molecules in SIM acquisition mode. Lack of chromatographic separation may lead to overlapping ion formation, but, in most cases, background ions interfere to a limited extent without preventing the identification of targeted or untargeted compounds. Gas-phase ionization provides limitless small molecule applications scarcely influenced by the matrix composition or polarity of the compounds or the extraction solvent. Being a proof of concept, preliminary data are very encouraging and demonstrate that E-LEI-MS provides robust real-time data, an essential requirement for fast screening and high throughput.

Results and discussion

Several prototype adjustments were tried before achieving the final configuration. Several operating parameters were considered, such as capillary dimensions, aspiration rate, solvent delivery mode, type of valve used as an on/off device, and sampling mode. The optimized system configuration, described in the following section, was tested with different matrices to demonstrate its performance and versatility. Working parameters were set depending on the sample nature and application field. The application workflow was designed to demonstrate the system's capability to operate in several conditions and modes: targeted and untargeted screening, two dimensions (2D) and three dimensions (3D) analyses.

Configuration of the E-LEI-MS system. The entire E-LEI-MS apparatus consists of a single quadrupole mass spectrometer Agilent Technologies 5975 inert Mass Selective Detector (Agilent Technologies, Santa Clara, CA, USA) equipped with an EI source, a sampling tip (fused silica capillary; 30 µm I.D; 375 µm O.D.; Polymicro Technologies (Phoenix, USA), an MV201 manual microfluidic 3-port valve, 2 positions (+ closed), "L" pattern flow, valve volume 170 nL (LabSmith, Livermore, CA, USA), a tee connector, a capillary for solvent delivery (peek tube; 450 µm I.D.; O.D. 660 µm; 10 cm length), and a micromanipulator (Standa, Vilnius, Lithuania). The solvent is delivered by a KD Scientific syringe pump (KD Scientific Inc., Holliston, MA, USA) equipped with a 1-mL syringe (Hamilton, Bonaduz, Switzerland), directly connected to the tee through a Teflon tubing. Figure 1 and Fig. S1 show the system configuration. The sampling tip is the E-LEI-MS core and consists of two coaxial tubings. The inner tubing (orange) is connected to the EI source passing through an on–off valve and crossing a tee up to the sampling spot. The outer tubing (red) delivers the appropriate solvent surrounding the inner tub-

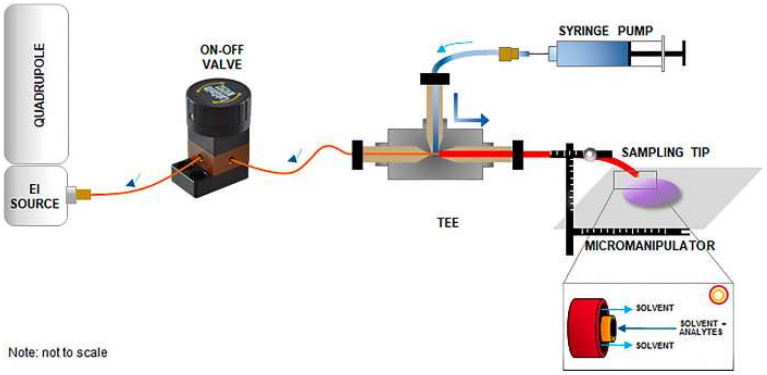


Figure 1. Configuration of the E-LEI-MS system.

ing from the tee to the sampling spot. A syringe pump filled with the solvent is connected to the tee. When the syringe pump is activated, the solvent flows between the two tubings up to the sampling spot, where it precisely mixes with the analytes. The system vacuum effect immediately delivers the solution to the ion source through the inner tubing. The on–off valve regulates access to the ion source. The sample signal appears after approximately 1 min after valve opening. MS acquisition is turned on before valve actuation. The micromanipulator can operate on x-y-z axes and angle degrees to finely adjust the sample and tip relative position with an accuracy of 0.1 mm. The final configuration was tested by analyzing spots of caffeine and chlorpyrifos solution to test repeatability and absence of carryover phenomena with satisfactory results (Figs. S2–S4).

Applications. *Pharmaceuticals.* This application was focused on the untargeted identification of the main active ingredients in selected medicinal tablets. Tiaprofenic acid contained in Surgamyl tablets was detected and correctly identified by the NIST library in scan acquisition mode, despite the simultaneous presence of several excipients in the pharmaceutical formulation, which could adversely affect spectral similarity, therefore, compound identification. Figure 2a shows the total ion current (TIC) signal acquired during the E-LEI-MS analysis of Surgamyl, showing a signal increase at 3.00 min when the aspirated solution in acetonitrile (ACN) reached the EI source; Fig. 2b shows the tiaprofenic acid identification by the NIST library search, with a spectral match of 93.6%. The experimental spectrum was recorded at 3.7 min. A few background ions (m/z 153 and 230) coming from the matrix do not interfere with the compound identification.

A similar experiment was carried out using Brufen tablets (Fig. 3): the TIC signal increased significantly after the sampling, and the spectral match with the NIST library resulted in the undoubted identification of the active ingredient ibuprofen dissolved in ACN. E-LEI-MS capability to identify active ingredients in commercial drugs without sample preparation was demonstrated for both types of tablets. For convenience, the MS acquisition starts before the actual sample analysis. The arrows in the figures indicate the moment the tip is positioned on the sample surface.

The analysis of NeoNisidina tablets (Fig. 4), containing acetylsalicylic acid (250 mg), acetaminophen (200 mg), and caffeine (25 mg) was carried out in scan acquisition mode, using ACN as solvent. Due to the simultaneous

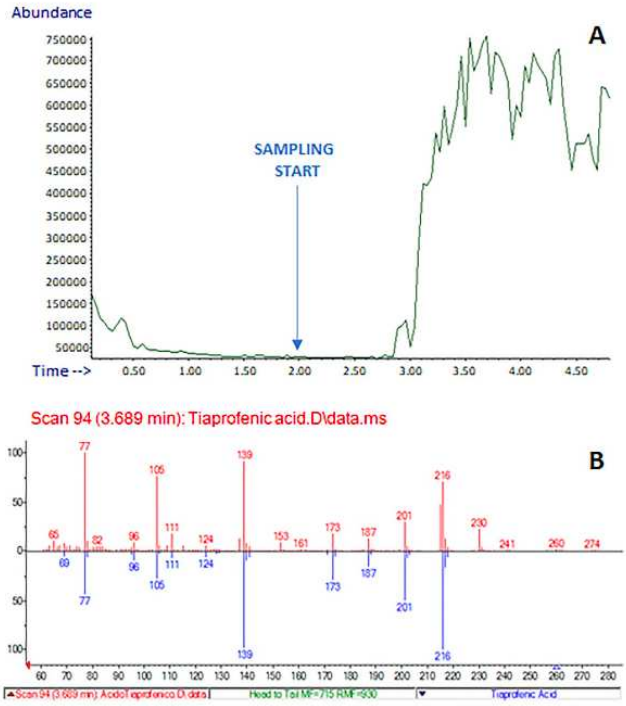


Figure 2. (**A**) Analysis of a Surgamyl tablet: TIC; (**B**) NIST library spectrum matching: red, experimental spectrum; blue, library spectrum. The arrow indicates the sampling start.

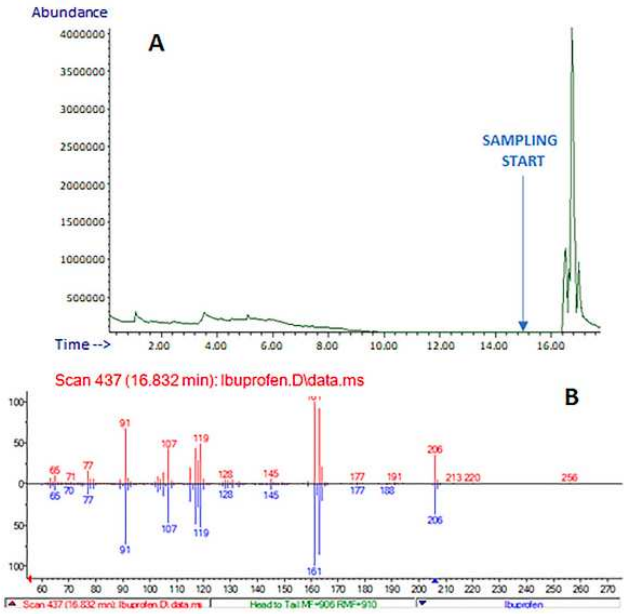


Figure 3. (**A**) Analysis of a Brufen tablet: TIC; (**B**) NIST library spectrum matching: red, experimental spectrum; blue, library spectrum. The arrow indicates the sampling start.

presence of three different targeted compounds in the same formulation, selected ions were monitored to visualize each specific signal. The selected ions were *m/z* 92, 120, and 138 for acetylsalicylic acid; *m/z* 109 and 151 for acetaminophen; *m/z* 109 and 194 for caffeine. All fragments were properly detected in SIM, demonstrating E-LEI-MS capability of identifying multiple target compounds in the same untreated matrix. These applications demonstrate E-LEI-MS potential to identify different types of pharmaceuticals, underlining its utility in quality control and forensic applications when a rapid identification of substances is needed. Compared to other efficient real-time MS technologies[7,11,12], E-LEI-MS offers a higher identification capability because of the reproducible fragmentation pattern. This application suggests also that the future perspective of coupling E-LEI-MS to high-resolution mass spectrometry (HRMS) could be resolutive for identifying targets with the same fragments, such as *m/z* 109 for acetaminophen and caffeine, which currently limits the specific attribution of fragments. E-LEI-MS can be considered somehow a selective approach considering that salts, heavy or non-volatile molecules are not ionized in the EI source and then detected. Adducts are not formed compared with API sources, and the chosen solvent can dissolve selectively target analytes. Despite the complexity of the matrix, these experiments show clearly that EI ionizes the main component of the pharmaceutical formulations, generating spectra not affected by excipients and other interferent components. Spectra deconvolution can further increase the library match score.

Pesticides analysis and food quality control. A targeted determination of pesticides in fruit peels was carried out to demonstrate the applicability of E-LEI-MS in food and environmental analysis. Pesticides were selected based on their specific use on orange and banana fruits; in particular, chlorpyrifos and imazalil were used to fortify orange peels, and benomyl was used on banana peels. An orange peel specimen (3 × 3 cm, 0.5 cm thickness) was fortified with 20 µL of chlorpyrifos solution at a concentration of 1000 mg/L. The orange specimen was stored for 24 h at room temperature to allow the complete standard adsorption on the surface and solvent evaporation before analysis. The same procedure was repeated using the same volume of an imazalil solution at a concentration of 1000 mg/L. The concentrations of the two pesticide solutions were calculated to match approximately those present in common commercial formulations used in agriculture. The experimental results are shown in Figure 5. TIC signals were clearly defined, and chlorpyrifos and imazalil EI spectra were correctly identified by the NIST library with a spectral similarity of 90.3 and 97.0%, respectively, despite the presence of the matrix. A banana peel specimen (3 × 3 cm, 0.5 cm thickness) was fortified with 20 µL of a benomyl solution at a concentration of 1000 mg/L. In this case, the identification resulted in a match equal to 75.2% (Fig. S5). Following solvent evaporation, the contaminants were distributed onto an area of the peel surface of approximately one square centimeter, resulting in a pesticide distribution of approximately 20 µg/cm^2. Moreover, the amount of

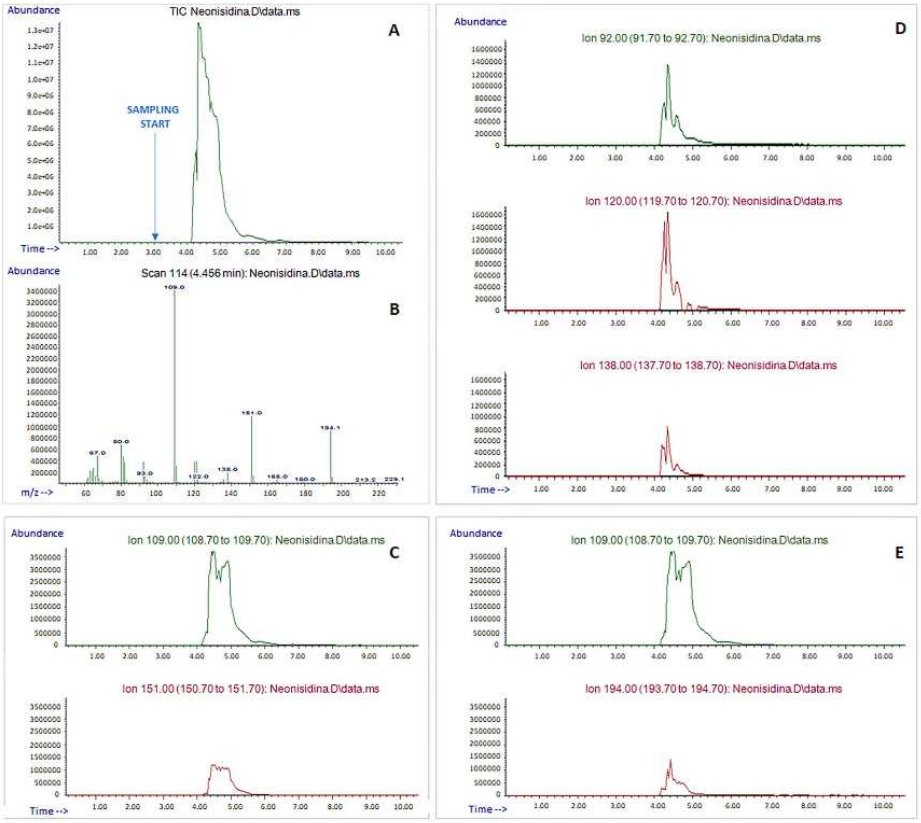


Figure 4. (**A**) Analysis of a NeoNisidina tablet: TIC; (**B**) EI experimental full spectrum; (**C**) acetaminophen selected ions: SIM; (**D**) acetylsalicylic acid selected ions: SIM; (**E**) caffeine selected ions: SIM. The arrow indicates the sampling start.

standard reaching the MS is related to the sampling tip aspiration. Based on these considerations, the obtained signal and the spectral match were satisfactory.

A quantitative approach was attempted by calculating a calibration curve for chlorpyrifos. Solutions of chlorpyrifos at five concentration levels (50, 100, 500, 1000, and 2000 mg/L) were used to fortify orange peel specimens. The analyses were carried out by sampling each peel for 1 minute, and the curves were plotted based on the concentration levels (x-axis) and the recorded areas of the signals corresponding to m/z *314* ion (y-axis) (Fig. S6). The resulting equation was $y = 48{,}302.6x - 2{,}371{,}974.6$ with a coefficient of determination R^2 of 0.9950 and completely satisfactory, considering the wide range of concentrations tested, the total absence of sample preparation, and, especially, the prototype stage of E-LEI-MS. No carryover was observed, and repeatability resulted in a coefficient of variability (CV%) within 8% for all concentration levels.

Forensic. Applications based on the rapid detection of illicit drugs via direct analysis in real-time mass spectrometry are enormously increasing since the last decade[13–17]. Law enforcement needs analytical devices to enable the fast identification of drugs of abuse, due to the rapid introduction of new molecules with psychotropic effects on the illicit market that may bypass conventional control systems and protocols. Following this trend, E-LEI-MS was applied to the analysis of a cocaine-spotted banknote. These experiments aimed to provide 2-D tests to verify if the system can fast recognize specific substances on selected surface spots, compared to controls, thus rendering analytes spatial distribution. The banknote was spotted with the target solution, then dried at ambient conditions before analysis. Figure 6 shows the signals corresponding to the sampling of three negative

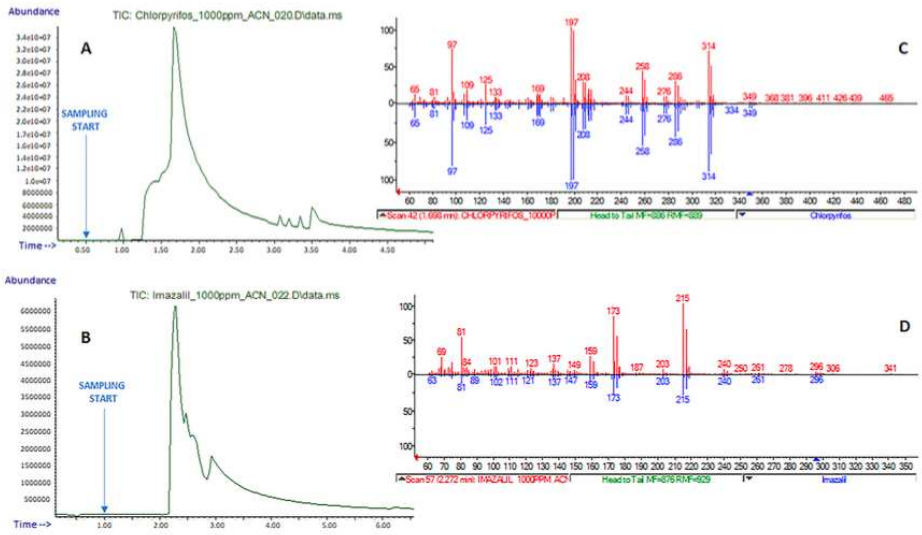


Figure 5. (**A**) Analysis of an orange peel fortified with chlorpyrifos: TIC; (**B**) analysis of an orange peel fortified with imazalil: TIC; NIST library spectra matching: red, experimental spectra for chlorpyrifos (**C**) and imazalil (**D**); blue, library spectra. The arrows indicate the sampling start.

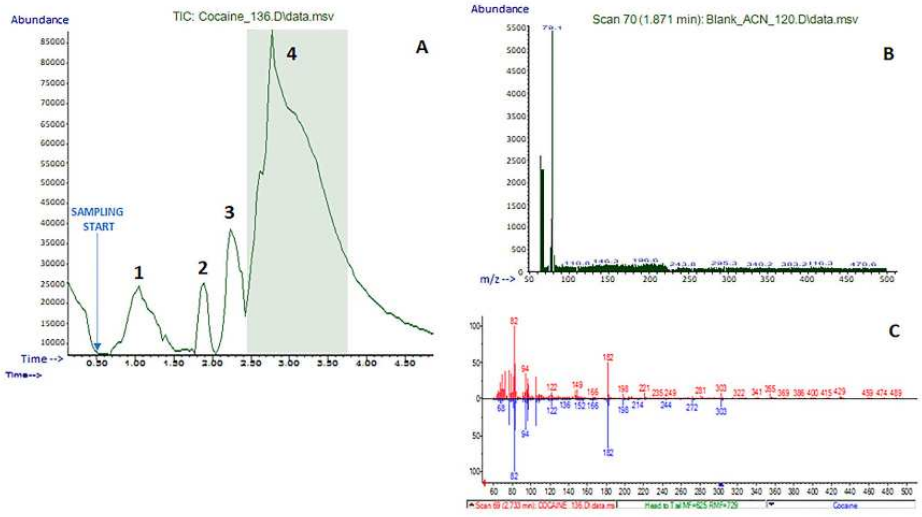


Figure 6. (**A**) Analysis of spots 1–3 (intact) and 4 (cocaine) on a banknote: TIC; (**B**) EI experimental full spectrum of a negative spot (matrix); (**C**) NIST library spectrum matching: red, experimental spectrum; blue, library spectrum. The arrow indicates the sampling start.

control spots (1–3) and one (4) positive control spot corresponding to the stain of 20 µL of a cocaine solution at a concentration of 100 mg/L on a 1 cm^2 spot.

The signal intensity corresponding to the cocaine-fortified spot (4) increased immediately after the aspiration, decreasing after 2 min, highlighting the system's capability to provide fast results without carryover. The cocaine EI spectrum was unambiguously identified by matching the NIST library with a high spectral similarity score (> 90%). In these experiments, E-LEI-MS demonstrated an unparalleled identification power which is mandatory in forensic applications and represents the main point of strength of this approach.

Artwork analysis—paintings. The potential of E-LEI-MS to identify completely unknown components in a complex matrix was tested in artwork analysis, particularly in detecting untargeted compounds in paintings for compounds attributable to the type of colors or fixative used, which helps select the proper materials in restoration interventions. An accurate understanding of the materials used in a particular painting is also fundamental for attributing the artwork to an artist or a historical period. Acrylic paintings on canvas were provided by the "Scuola di Conservazione e Restauro" laboratories of the University of Urbino.

The study aimed to identify painting surface components (e.g., synthetic or natural fixatives); this information plays a fundamental role in preparing the proper restoration procedure. A system capable of providing these data by analyzing a small painting surface demonstrates great potential in this application field. For these reasons, several solvents were tested on small squares of painting samples: water, ACN, MeOH, dichloromethane (CH_2Cl_2), hexane, and octanol. ACN was selected for the combination of proper dissolving power and viscosity. This study aimed to investigate also E-LEI-MS's ability to perform unknown and multilayer analyses permitting the sample mapping in three dimensions, where the third dimension corresponds to the deeper layers. For this reason, the sample was exposed to ACN for a longer time allowing the in-depth extraction of the compounds. Sampling started after 2 min of solvent release from the tip. As shown in Fig. 7, the first spectrum at 5.91 min corresponded to isopropyl myristate, with a NIST spectral similarity of 93.4%. This result is satisfactory, considering the matrix complexity, which could affect the spectrum quality by limiting unknown compounds' identification. The identified compound was concordant with the sample nature; isopropyl myristate is an esterified fatty acid,

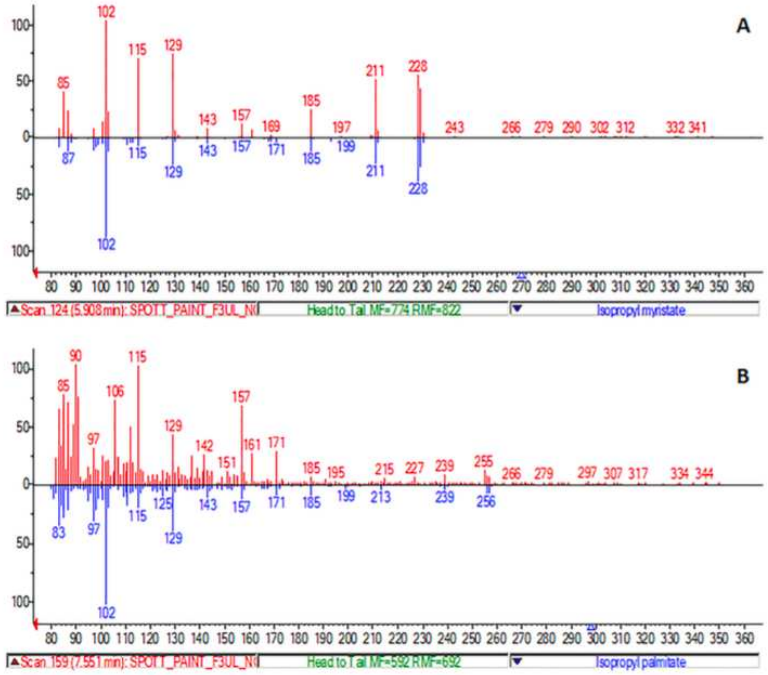


Figure 7. (**A**) Spectrum obtained from the analysis of the painting surface: TIC; (**B**) spectrum obtained from the analysis of the painting deeper layer: TIC. NIST library spectra matching: red, experimental spectra for isopropyl myristate (**C**) and isopropyl palmitate (**D**); blue, library spectra.

potentially a component of fixative varnish applied on paintings. After another three minutes of sampling in the same surface area, another spectrum was acquired corresponding to isopropyl palmitate (spectral similarity 43.2%), also used as a fixative component. Isopropyl palmitate was detected later than isopropyl myristate, probably because present in a deeper layer of the painting; therefore its extraction required a longer time. This application clearly shows the E-LEI-MS identification power of unknown compounds since it was conducted using a matrix whose surface components were totally unknown. As a micro-invasive technique, E-LEI-MS is particularly suitable for this kind of application when preserving prestigious matrices is mandatory.

Greenness evaluation. The Greenness evaluation of E-LEI-MS was performed using the Analytical GREEnness Metric Approach, AGREE[18], which was recognized as one of the most applicable to many analytical procedures[19]. AGREE is based on 12 criteria at which is attributed a default value equal to 2, which represents the impact of the single parameter on the entire procedure. The default weight value can be changed from 1 to 4, to make the evaluation tool flexible and suitable for each technology. AGREE evaluation of E-LEI-MS was based on the different experiments performed. Figure S7 shows the results obtained, maintaining the default value for all criteria. A very satisfactory score of 0.79 (out of 1) was obtained, showing the greenness of the new system. As clearly visible in Fig. S7, the lowest score, in red, was attributable to the positioning of the analytical device, which at the present, does not allow on-site analysis. In this view, because E-LEI-MS is a simple device, further developments will be aimed to make it applicable to a portable device, thus increasing enormously its ecological potential.

White analytical chemistry (WAC)[20] is a new concept that is worth to be mentioned here. In brief, WAC is based on the idea that increasing the greenness of an analytical method or technology must not undermine its functionality. E-LEI-MS was proved to be a fast and environmentally friendly ambient MS approach, with a high identification power guaranteed by EI, compared to conventional ambient MS techniques, in the view to meeting the principle of WAC mentioned above.

Methods

Materials. LC grade solvents, methanol (MeOH), acetonitrile (ACN), dichloromethane (CH_2Cl_2), hexane, and octanol were purchased from VWR BDH Chemicals HiPerSolv Chromanorm (Milan, Italy). Milli-Q water was produced using a Millipore Direct-Q 3 UV purification system (Millipore Corp., Milan, Italy). Fused silica capillaries of various internal (I.D.) and external (O.D.) diameters were purchased from Polymicro Technologies (Phoenix, USA).

Pesticides: chlorpyriphos (CAS 2921-88-2; PESTANAL, analytical standard 99.9% purity), imazalil (CAS 35554-44-0; PESTANAL, analytical standard 99.8% purity), and benomyl (CAS 17804-35-2; PESTANAL, analytical standard, ≥ 98.0% purity) were supplied by Sigma Aldrich. Imazalil and benomyl stock solutions were prepared gravimetrically at 1000 mg/L concentration in ACN. Chlorpyrifos stock solution was prepared gravimetrically at a concentration of 1000 mg/L in ACN. Solutions of chlorpyrifos at 50, 100, and 500 mg/L were prepared by diluting the stock solution with the same solvent.

Cocaine (CAS 50-36-2; Cerilliant, Certified Reference Material) stock solution at a concentration of 1000 mg/L in ACN was provided and manipulated by the Toxicology Laboratory A.S.T. AV1, (Pesaro, Italy).

Caffeine (CAS 58-08-2; analytical standard > 99.0% purity) solution was prepared gravimetrically at 1000 mg/L concentration in ACN.

Samples. The anti-inflammatory drugs, purchased at a local pharmacy, were the following: Surgamyl produced by Scharper S.p.a (Milano, Italy) containing tiaprofenic acid (300 mg); Brufen produced by Mylan S.p.a. (Milano, Italy) containing ibuprofen lysine salt acid (200 mg); and NeoNisidina produced by Pharmaidea S.r.l. (Travagliato, BS, Italy), containing acetylsalicylic acid (250 mg), acetaminophen (200 mg), and caffeine (25 mg). All the tablets also contained several excipients.

Oranges and bananas were bought at a local market and were fortified by adding pesticide standards on the peel at a specific concentration. The banana peel was fortified with benomyl (20 µL of a 1000 mg/L standard solution). In two different experiments, the orange peel was fortified with 20 µL of chlorpyrifos and 20 µL of imazalil, both at a concentration of 1000 mg/L. Chlorpyrifos was added to the orange peel at increasing concentrations spanning from 20 to 1000 mg/L.

A 5-euro banknote (62 × 120 mm) was signed with circles to perform 2D and 3D experiments. Some circles were wetted with ACN and represented the negative control, whereas others were spotted with 20 µL of a cocaine solution at 100 mg/L as positive controls.

Paintings were kindly provided by the "Scuola di Conservazione e Restauro" laboratories, of the University of Urbino. Water, ACN, MeOH, CH_2Cl_2, hexane, and octanol were tested to select the most suitable solvent for dissolving the painting sample surface.

The flow of solvent (ACN) delivered by the syringe pump on the sample surfaces was set at 3 µL/min for all the experiments.

Analytical conditions. ACN was used in most applications to dissolve the analytes on the sample surface and was delivered by the syringe pump at a flow rate of 3 µL/min. The timing of sampling was 1 min for all experiments and the time between two consecutive analyses or analyses of two consecutive spots was set based on the experimental time needed for reducing the signal to the baseline.

MS tuning was performed daily at an ion source temperature of 280 °C, using perfluorotributylamine (PFTB) as a reference compound, monitoring its characteristic ions. No mobile phase was admitted into the source during this procedure. The ion source can operate up to 350 °C depending on the nature, molecular weight, and boiling

point of the selected compounds. The single quadrupole mass spectrometer was operated at 150 °C. The solvent and analytes aspirations were ensured by the instrument's high vacuum pumps.

MS data acquisition was carried out in scan and SIM modes. Scan analyses were conducted in an m/z range of 60–500, depending on the solvent used, with a sampling rate of 0.43 scan/s, and a threshold of 10. The low sampling rate is needed for obtaining a continuous signal that does not impose a fast scan speed, as it is required in the case of chromatographic peak acquisition. Depending on the targeted molecules, SIM analyses were conducted by selecting two or three specific ions. Typical signal stabilization after sampling lasted less than a minute and did not require fast scanning rates.

The background was subtracted from all spectra before the search in the NIST library.

Blank analyses were performed after each acquisition.

Enhanced ChemStation MSD E.02.00.493 (Agilent Technologies) software was used for data acquisition and processing.

Greenness evaluation. AGREE green assessment tool[21] was used to evaluate the environmentally friendly character of E-LEI-MS.

Plant sample statement. The authors declare that the collection of plant material complies with relevant institutional, national, and international guidelines and legislation. No plants and seeds were collected and used in this study. No wild species and species at risk of extinction were used in this study. Bananas and oranges were bought in a local market and only their peels were used for the experiments.

Conclusions

E-LEI-MS, a new system for direct analysis in EI mode, is presented herein for the first time.

E-LEI-MS is an innovative and competitive real-time technique based on ambient sampling and EI-MS, which ensures a fast and reliable analytes identification.

The system allows the rapid analysis of targeted and unknown compounds without sample manipulation or preparation, as required for separation techniques, demonstrating great potential and versatility in several application fields. Satisfactory results were obtained with different matrices and samples, in 2D and multilayer analyses, highlighting the possibility of using E-LEI-MS in surface/spatial analysis. E-LEI-MS is also an environmentally friendly approach, as demonstrated by AGREE evaluation and further studies will be aimed at increasing E-LEI-MS performance and making it a portable device, with consequent improvements in the system greenness and applicability.

Data availability

Raw data used in the generation of Figs. 2–7, S2–S4 were submitted as separate files but are not publicly available due to the prototypal nature of the system herein presented and the specificity of software needed for data processing (Enhanced ChemStation, MSD ChemStation E.02.00.493 Copyright 1989-2008 Agilent Technologies, Inc.) but are available from the corresponding author upon reasonable request.

Received: 1 February 2023; Accepted: 17 April 2023
Published online: 20 April 2023

References

1. Cappiello, A., Famiglini, G., Mangani, F. & Palma, P. New trends in the application of electron ionization to liquid chromatography–mass spectrometry interfacing. *Mass Spec. Rev.* **20**, 88–104. https://doi.org/10.1002/mas.1004 (2001).
2. 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.* **1167**, 338350. https://doi.org/10.1016/j.aca.2021.338350 (2021).
3. Van Berkel, G. J. & Kertesz, V. Continuous-flow liquid microjunction surface sampling probe connected on-line with high-performance liquid chromatography/mass spectrometry for spatially resolved analysis of small molecules and proteins. *Rapid Commun. Mass Spectrom.* **27**, 1329–1334. https://doi.org/10.1002/rcm.6580 (2013).
4. Asano, K. G., Ford, M. J., Tomkins, B. A. & Van Berkel, G. J. Self-aspirating atmospheric pressure chemical ionization source for direct sampling of analytes on surfaces and in liquid solutions. *Rapid Commun. Mass Spectrom.* **19**, 2305–2312. https://doi.org/10.1002/rcm.2062 (2005).
5. Takáts, Z., Wiseman, J. M., Gologan, B. & Cooks, R. G. Mass spectrometry sampling under ambient conditions with desorption electrospray ionization. *Science* **306**, 471–473. https://doi.org/10.1126/science.1104404 (2004).
6. Cooks, R. G., Ouyang, Z., Takáts, Z. & Wiseman, J. M. Detection Technologies. Ambient mass spectrometry. *Science* **311**, 1566–1570. https://doi.org/10.1126/science.1119426 (2006).
7. Cody, R. B., Laramee, J. A. & Durst, H. D. Versatile new ion source for the analysis of materials in open air under ambient conditions. *Anal. Chem.* **77**, 2297–2302. https://doi.org/10.1021/ac050162j (2005).
8. Feider, C. L., Krieger, A., DeHoog, R. J. & Eberlin, L. S. Ambient ionization mass spectrometry: Recent developments and applications. *Anal. Chem.* **91**, 4266–4290. https://doi.org/10.1021/acs.analchem.9b00807 (2019).
9. Venter, A. R., Douglass, K. A., Shelley, J. T., Hasman, G. & Honarvar, E. Mechanisms of real-time, proximal sample processing during ambient ionization mass spectrometry. *Anal. Chem.* **86**, 233–249. https://doi.org/10.1021/ac4038569 (2014).
10. Mangraviti, D., Rigano, F., Arigò, A., Dugo, P. & Mondello, L. Differentiation of Italian extra virgin olive oils by rapid evaporative ionization mass spectrometry. *LWT Food Sci. Technol.* **138**, 110715. https://doi.org/10.1016/j.lwt.2020.110715 (2021).
11. Ayodeji, I., Vazquez, T., Bailey, R. & Evans-Nguyen, T. Rapid pre-filtering of amphetamine and derivatives by direct analysis in real-time (DART)-differential mobility spectrometry (DMS). *Anal. Methods.* **9**, 5044–5051. https://doi.org/10.1039/C7AY00892A (2017).
12. Chen, H., Talaty, N. N., Takáts, Z. & Cooks, R. G. Desorption electrospray ionization mass spectrometry for high-throughput analysis of pharmaceutical samples in the ambient environment. *Anal. Chem.* **77**, 6915–6927. https://doi.org/10.1021/ac050989d (2005).

13. Sisco, E., Verkouteren, J., Staymates, J. & Lawrence, J. Rapid detection of fentanyl, fentanyl analogues, and opioids for on-site or laboratory based drug seizure screening using thermal desorption DART-MS and ion mobility spectrometry. *Forensic Chem.* **4**, 108–115. https://doi.org/10.1016/j.forc.2017.04.001 (2017).
14. Musah, R. A., Domin, M. A., Walling, M. A. & Shepard, J. R. E. Rapid identification of synthetic cannabinoids in herbal samples via direct analysis in real time mass spectrometry. *Rapid Commun. Mass Spectrom.* **26**, 1109–1114. https://doi.org/10.1002/rcm.6205 (2012).
15. Gwak, S. & Almirall, J. R. Rapid screening of 35 new psychoactive substances by ion mobility spectrometry (IMS) and direct analysis in real time (DART) coupled to quadrupole time-of-flight mass spectrometry (QTOF-MS). *Drug Test. Anal.* **7**, 884–893. https://doi.org/10.1002/dta.1783 (2015).
16. França, H. S. *et al.* Experimental and ab initio investigation of the products of reaction from Δ9-tetrahydrocannabinol (Δ9-THC) and the fast blue BB spot reagent in presumptive drug tests for cannabinoids. *Forensic Chem.* **17**, 100212. https://doi.org/10.1016/j.forc.2019.100212 (2020).
17. Borden, S. A. *et al.* Mass spectrometry analysis of drugs of abuse: Challenges and emerging strategies. *Mass Spec. Rev.* **39**, 1–42. https://doi.org/10.1002/mas.21624 (2020).
18. Pena-Pereira, F., Wojnowski, W. & Tobiszewski, M. AGREE–analytical GREEnness metric approach and software. *Anal. Chem.* **92**, 10076–10082. https://doi.org/10.1021/acs.analchem.0c01887 (2020).
19. Sajid, M. & Płotka-Wasylka, J. Green analytical chemistry metrics: A review. *Talanta* **238**, 123046. https://doi.org/10.1016/j.talanta.2021.123046 (2022).
20. Nowak, P. M., Wietecha-Posłuszny, R. & Pawliszyn, J. White Analytical Chemistry: An approach to reconcile the principles of green analytical chemistry and functionality. *Trends Anal. Chem.* **138**, 116223. https://doi.org/10.1016/j.trac.2021.116223 (2021).
21. Płotka-Wasylka, J. A new tool for the evaluation of the analytical procedure: Green analytical procedure index. *Talanta* **181**, 204–209. https://doi.org/10.1016/j.talanta.2018.01.013 (2018).

Acknowledgements
The authors are grateful to Agilent Technologies for providing the MS instrumentation.

Author contributions
A.A.: Validation, Formal analysis, Investigation, Methodology, Writing-original draft, Project administration. G.F.: Methodology, Writing—review & editing, Project administration. N.M: Investigation. 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.

Competing interests
The authors declare no competing interests.

Additional information
Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-023-33647-5.

Correspondence and requests for materials should be addressed to A.A.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.