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

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

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

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1. Introduction

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

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

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

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

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

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

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

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

2. Materials and Methods

2.1. Materials and Supplies

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

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

2.2. Standard Solution Preparation

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

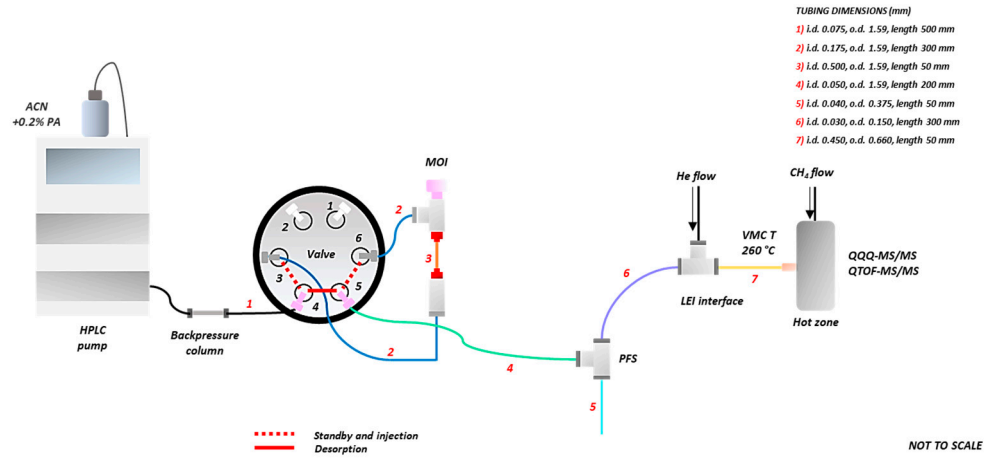

Figure 1. Scheme and operation of the MOI-PFS-LEI-QQQ and MOI-PFS-LEI-QTOF systems. Step 1: MOI is filled in the standby position. The solvent flows from the pump to port 4 of the valve at 10 µL/min, which is in line with port 3 (MOI inlet). Once the MOI chamber is filled, the liquid flows to port 6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

2.3. Instruments and Equipment

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

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

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

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

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

2.4. Direct Immersion-SPME Method Optimization

SPME was used in direct immersion mode (DI-SPME) for dicamba and tefluthrin sampling in CF and ultra-pure water. Several parameters were optimized for improving extraction and desorption efficiency and process speeding, such as the amount of organic solvent in the sample, sampling and desorption time, and agitation methods. Since it is independent of the MS technique, DI-SPME method optimization was carried out with the low-resolution instrumentation (MOI-PFS-LEI-QQQ), using 1 mL aliquots of CF and water fortified with 100 ng/mL of dicamba and tefluthrin. The optimized parameters were selected considering the highest integrated peak area values of the most intense transitions (Q). The DI-SPME optimized procedure was then applied to high-resolution experiments. Before use, the SPME fiber was preconditioned in H_2O/ACN (50/50, v/v) for 10 min using a magnetic stir bar. Sampling was conducted by completely immersing the fiber in 1 mL of the sample. Before desorption, a 5-s rinsing step in water was performed using a vortex to clean the fiber from any matrix components adhering to the coating surface. Desorption was performed by inserting the fiber inside the MOI chamber, filled with ACN acidified with 0.2% PA.

3. Results and Discussion

3.1. Sampling and Desorption Steps

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

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

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

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

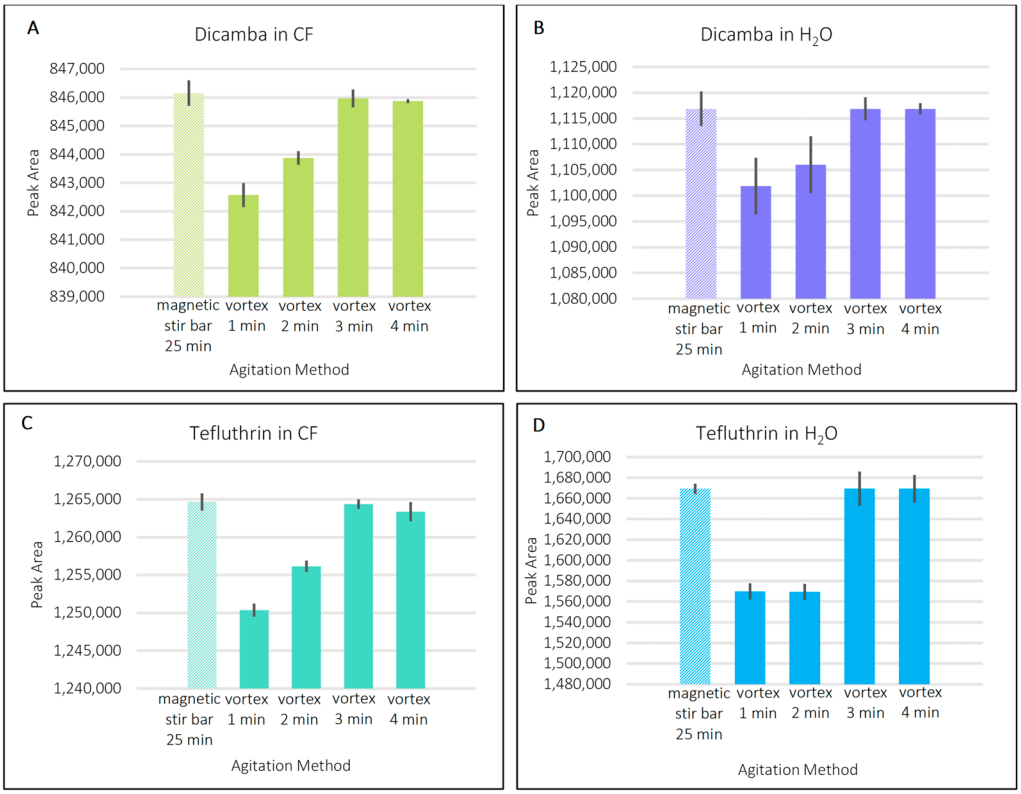

Figure 2. Effects of different extraction times on the integrated peaks area values of dicamba in (**A**) CF and (**B**) H_2O and tefluthrin in (**C**) CF and (**D**) H_2O using vortex as agitation method compared with magnetic stir bar. Analytes concentration: 100 ng/mL.

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

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

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

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

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

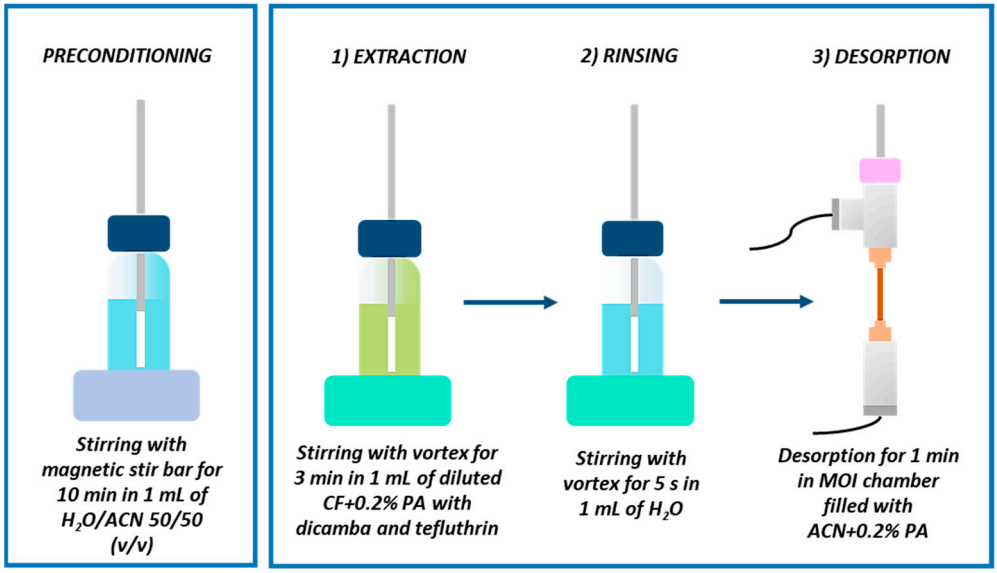

Figure 3. DI-SPME optimized workflow. The procedure consists of (1) extraction, (2) rinsing and (3) desorption. The C18 fiber was preconditioned before use.

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

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

Matrix Effects (ME) Evaluation

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

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

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

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

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

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

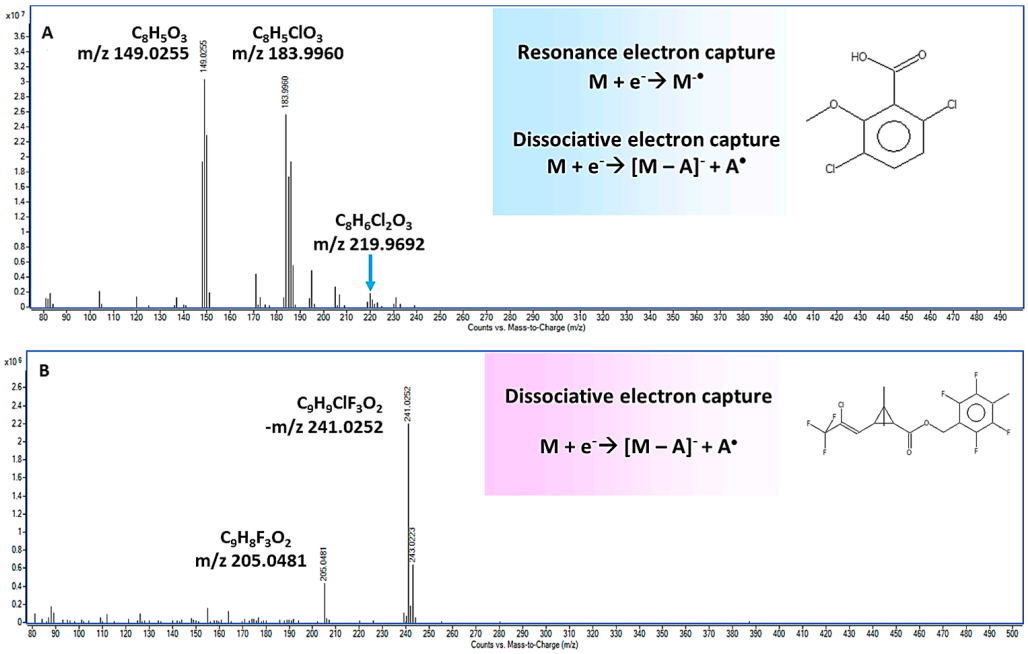

Figure 4. High-resolution mass spectra and NCI fragmentation pathways of (**A**) dicamba and (**B**) tefluthrin at 100 ng/mL in H_2O recorded with the MOI-PFS-LEI-QTOF-MS/MS system.

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

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

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

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

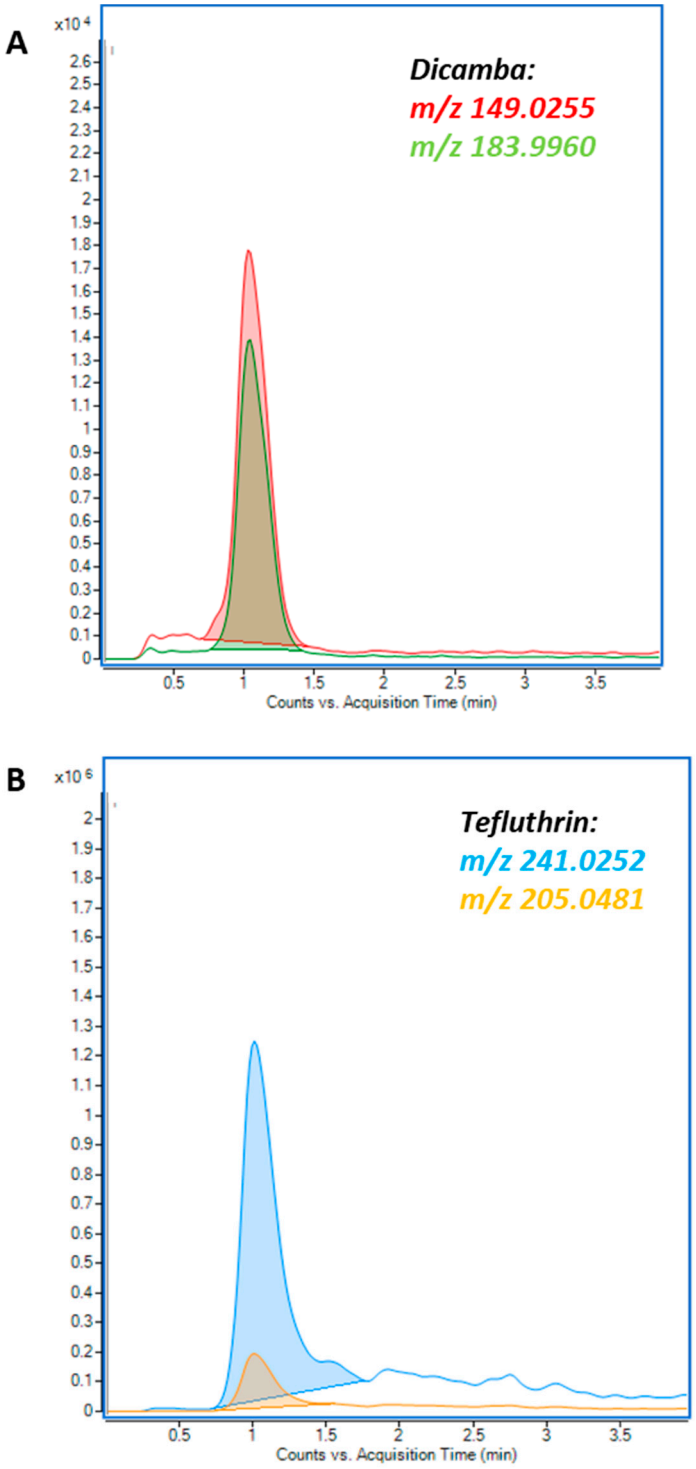

Figure 5. EIC chromatogram of dicamba (**A**) and tefluthrin (**B**) at 100 ng/mL in CF obtained with the MOI-PFS-LEI-QTOF system in NCI. Dicamba peaks: m/z 149.0255 (red), m/z 183.9960 (green). Tefluthrin peaks: m/z 241.0252 (blue), m/z 205.0481 (orange). Since chromatography has not been used, the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

3.4. Greenness Evaluation

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

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

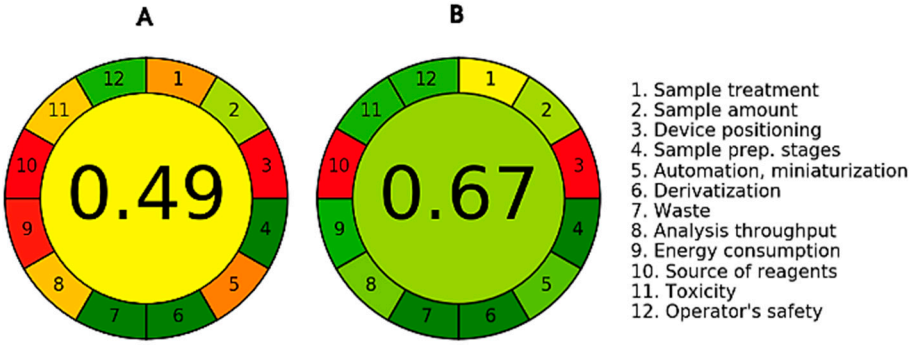

Figure 6. AGREE comparison between LC-LEI-QQQ (**A**) and MOI-PFS-LEI-QQQ (**B**) methods for detecting dicamba and tefluthrin in CF.

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

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

4. Conclusions

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

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

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

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/separations10060325/s1, Sustainable and Rapid Determination of Two Halogenated Pesticides in a Commercial Formulation by Solid Phase Microextraction and Liquid Phase Chemical Ionization Mass Spectrometry. Figure S1, A–D Peak area values at different percentages of ACN in (A) dicamba in CF, (B) dicamba in H2O, (C) tefluthrin in CF, (D) tefluthrin in H_2O. Analytes concentration: 100 ng/mL.; Figure S2, A,B Effects of different extraction times on the integrated peaks area values of dicamba and tefluthrin at 100 ng/mL using magnetic stir bar as agitation method in (A) CF and (B) H_2O.; Figure S3, A,B Effects of different desorption times on the integrated peaks area values of dicamba and tefluthrin at 100 ng/mL using magnetic stir bar as agitation method in (A) CF and (B) H_2O.; Figure S4, A,B Calibration curves (0.5, 2.5, 5, 25, 50, and 100 ng/mL) of (A) dicamba and (B) tefluthrin in CF and H_2O.

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