



Co-formulants in plant protection products: An analytical approach to their determination by gas chromatography–high resolution mass accuracy spectrometry

Antonio Jesús Maldonado-Reina, Rosalía López-Ruiz, Antonia Garrido Frenich, F. Javier Arrebola, Roberto Romero-González*

Research Group "Analytical Chemistry of Contaminants", Department of Chemistry and Physics, Research Centre for Mediterranean Intensive Agrosystems and Agri-Food Biotechnology (CIAMBITAL), University of Almería, Agri-Food Campus of International Excellence, ceiA3, Ctra. Sacramento s/n, 04120, La Cañada de San Urbano, Almería, Spain

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ABSTRACT

In the present study, 12 volatile benzene and naphthalene derived co-formulants were identified by suspect screening and unknown analysis in 14 plant protection products (PPPs) corresponding to several types of formulations, as emulsifiable concentrates (EC), suspension concentrates (SC), dispersible concentrates (DC) and ZC, which is a mixture of a capsule suspension (CS) in an SC, containing either difenoconazole or chlorantraniliprole as main active ingredients. The selected technique was gas chromatography coupled to Q-Orbitrap high resolution mass accuracy spectrometry (GC-Q-Orbitrap-MS), providing efficient separation and detection of all identified compounds. Finally, 42 compounds were tentatively identified, and 12 of them were confirmed and quantified using analytical standards. Results showed that the applied methodology was able to detect these co-formulants at concentrations as low as 0.03 g/L (tert-butylbenzene), encompassing a wide concentration range, up to 9.63 g/L (pentamethylbenzene). Pentamethylbenzene was the only compound detected in all studied samples.

1. Introduction

Pesticides are a broad group of compounds used in agriculture as crop protection substances. According to the United States Environmental Protection Agency (EPA), a pesticide is any substance or mixtures of them whose aim is the prevention, destruction, repulsion or mitigation of any pest [1]. They are marketed as plant protection products (PPPs), which are commercialised in a wide range of formulations, including emulsifiable concentrates (EC), wettable powders (WP), water dispersible granules (WG), soluble concentrates (SL), suspension concentrates (SC), dispersible concentrates (DC), capsule suspensions (CS), or a blend of a CS in an SC (ZC) [2]. The most common formulation is an EC, in which hydrophobic pesticides are dissolved in nonpolar solvents, which forms an emulsion when the PPP is dissolved in water. Their main advantages, in comparison to other formulations, are a higher concentration of active substances, easy processing, handling and storage, or even a higher biological activity [3]. Nevertheless, EC formulations have several drawbacks such as flammability,

possible instability after dilution or phytotoxic effects, and increased dermal toxicity of the active substance [4].

In addition to the active substances (one or more pesticides), PPPs also contain other compounds called co-formulants, a heterogeneous group of molecules encompassing solvents, adjuvants, wetting or anti-foaming agents, stabilizers, emulsifiers, etc., which will later on be named as co-formulants in this study. They can be present at high concentrations and they are added to PPPs to increase their effectiveness by means of a lower pesticide volatilisation, enhanced activity of the pesticide, higher spreadability, wettability and penetration, as well as to dissolve the active substance on the basis of their low solubility in water [5,6]. In fact, these co-formulants will ultimately shape the properties and applicability of the PPP.

Co-formulants for agricultural purposes had a market share of \$3.100 million in 2020, and this value is expected to increase up to \$4.400 million in 2026 [7]. In spite of that, the relevance of co-formulants in both PPPs and treated agricultural commodities has been largely disregarded, as the majority of previous analytical studies

* Corresponding author.

E-mail address: rromero@ual.es (R. Romero-González).

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focused on the determination of pesticides, the active ingredients, whereas only a few of them determined co-formulants in these matrices [8–10]. European Union (EU) legislation mandates the declaration of active substances and their concentration, but the notification of any other compound is not compulsory in a bid to protect business confidentiality (*Commission Regulation (EU) No 547/2011* [11]). Moreover, Regulation EC No 540/2011 establishes the allowed active substances in PPPs [12], whereas Regulation EC No 2021/383 lists the co-formulants considered unacceptable for inclusion in PPPs [13].

Nonetheless, some additives may pose a threat to both human and animal health, as well as to the environment. For instance, exposure to 4-ethyltoluene and 2-ethyltoluene, two isomers present in EC products, which are made with naphtha derivatives as solvent [14], have been proved to trigger acute narcotic effects, in addition to a lower survival in mice [15]. Therefore, it is necessary to count on analytical methods for the determination of co-formulants in different PPP formulations to ensure human, animal and environmental safety, taking into consideration the fact that methods are scarcely available. Most authors selected gas (GC) or liquid (LC) chromatography as the separation technique, as it is a suitable approach for the separation of compounds. However, different detection techniques as flame ionisation detection (FID) [16] and mass spectrometry (MS) [8,17] were used. It is important to notice that, while there are several low-resolution mass spectrometry (LRMS) studies available [18], very few of them use high resolution accurate mass spectrometry (HRAMS) [19], increasing the capabilities of performing a reliable unknown study required to determine the composition of PPPs. Some of the main strengths of HRAMS compared to LRMS are higher sensitivity in full scan mode (a key feature in the analysis of highly diluted samples), the availability of multiple MS² experiments supported by mass accuracy, and the use of mass error, which allows accurate molecular formula assignments and improves the identification process. As a result of it, HRAMS also allows for suspect screening and unknown analysis, which is an appealing feature in terms of health alerts, as already acquired data can be used to search for any compound, whereas LRMS is mainly used in targeted analysis. However, HRMS does not provide any advantage over LRMS regarding isomeric distinction, as both techniques are unable to distinguish them. Therefore, isomers must be determined by chromatographic parameters, namely their retention time or retention index.

Concerning previously described studies, Pose et al. [8] tentatively identified several co-formulants in two different metalaxyl WP technical formulae, by GC-MS with an ion trap (IT) analyser operating in full scan mode. Aldehydes, ketones, alkyl amides, carboxylic acids, methyl esters, monoalkyl ethers and monoaromatic hydrocarbons were tentatively identified, all of them with a low molecular weight. Zeinali et al. [9] characterised various volatile organic co-formulants in two fungicides, two insecticides and two herbicides by GC-MS, using single quad as analyser. Compounds were tentatively identified with NIST library, and then confirmed with analytical standards. These co-formulants were alcohols, benzene derivatives, indane derivatives, naphthalene derivatives and biphenyl derivatives. Finally, a method was developed by López-Ruiz et al. [19], which combined LC-HRAMS and GC-HRAMS techniques, with Exactive Orbitrap and Q-Exactive Orbitrap, respectively, for the determination of co-formulants in three EC quizalofop-P-ethyl PPPs. In the LC-MS analysis, 3 surfactants were identified whereas in the GC-MS analysis, 6 compounds were determined. All tentative identifications were confirmed with analytical standards.

To sum up, the aim of this study is to provide an innovative and thorough characterisation of different types of pesticide formulations. Unknown and suspect screening were carried out by a GC-HRAMS methodology, as most consulted studies did not address neither HRAMS analysis nor the differences between different types of formulations, or even the quantification of co-formulants. Hence, one of the cornerstones of this study is the analysis of 14 different PPPs belonging to 4 types of formulations (EC, DC, SC and ZC), containing a fungicide

(difenoconazole) or an insecticide (chlorantraniliprole), being the latter a novel pesticide. The comprehensive analysis of a large number of assorted samples allowed us to assess whether the composition of PPPs and their type of formulation can be related. This is of interest, since different PPPs could show different properties or toxicity that are ultimately determined by their composition. Furthermore, the combination of mass accuracy and high resolution facilitated the application of both unknown and suspect screening workflows for a reliable identification.

2. Experimental and methods

2.1. Materials, equipment and reagents

Fourteen different difenoconazole and chlorantraniliprole PPPs of 4 types of formulations, were purchased from several suppliers. These PPPs included Kabuto JED (EC), Ceremonia 25 (EC), Mavita 250 (EC), Cidely Top (DC), Dynali (DC), Lxor 25 (EC), Score 25 (EC), Dagonis (SC), Nomad (EC), Duaxo (EC) and Ortiva Top (SC) for difenoconazole, and Voliam Targo (SC), Coragen 20 (SC) and Ampligo 150 (ZC) for chlorantraniliprole, as displayed in Table S1.

Analytical standards of 1,2,4-trimethylbenzene, 1,3,5-trimethylbenzene, 4-isopropyltoluene (4-cymene), ethylbenzene, isopropylbenzene, n-butylbenzene, naphthalene, n-propylbenzene, sec-butylbenzene, styrene, tert-butylbenzene and toluene were acquired from Dr. Ehrenstorfer (Augsburg, Germany). Analytical standards of 2,4-dimethylstyrene, 4-ethyltoluene, 1,3-diisopropylbenzene, pentamethylbenzene, biphenyl, 2-methylbiphenyl, 3-methylbiphenyl, 4-methylbiphenyl and diphenylmethane were supplied by Sigma-Aldrich (St. Louis, MO, USA). Perfluorotributylamine from Thermo Fisher Scientific (Waltham, MD, USA) was used as mass calibrant for GC-Q-Orbitrap analysis.

Ethyl acetate (HPLC grade, $\geq 99.8\%$), obtained from Chem-Lab (Zedelgem, Belgium), *n*-hexane ($\geq 97.0\%$) purchased from Honeywell (Charlotte, NC, USA), acetone (Sigma-Aldrich, HPLC grade, 99.8%) and dimethyl sulfoxide (DMSO, Honeywell, HPLC grade, $\geq 99.7\%$) were also used.

2.2. Sample treatment

For GC-HRAMS analysis, 40 μL of each PPP were dissolved in 4 mL of ethyl acetate or DMSO, homogenizing the mixture for 1 min by means of a vortex mixer. SC samples were only dissolved in DMSO, and then sonicated for 10 min at room temperature, as they were not soluble in any other tested solvent. Then, 100 μL of this mixture were dissolved in 900 μL of ethyl acetate or DMSO in a chromatographic vial. This last step was repeated twice to yield a 1:100.000 (v/v) dilution. Lastly, 200 μL of this dilution were mixed with 800 μL of ethyl acetate or DMSO to obtain a final dilution of 1:500.000 (v/v), which was injected into the GC. The aim of this dilution was to prevent any contamination in the mass spectrometer, as the active substances, either difenoconazole or chlorantraniliprole, were highly concentrated in the studied samples.

2.3. Gas chromatography coupled to mass spectrometry

The selected technique for the separation of volatile and nonpolar compounds was GC. A Trace 1310 GC system equipped with a TriPlus RSH autosampler (Thermo Scientific™) was used. The selected column was a Varian VF-5ms (30 m \times 0.25 mm, 0.25 μm), composed of polydimethylsiloxane as nonpolar stationary phase, provided by Agilent Technologies (Santa Clara, CA, USA). A precolumn (1.5 m \times 0.25 mm) provided by Supelco (Bellefonte, PA, USA) was attached to the chromatographic column. Ultra-high purity helium (99.9999%) was used as carrier gas, at a steady flow rate of 1 mL/min. Injection port temperature was 280 °C, whereas injection volume was set to 2 μL in splitless mode. Initial column temperature was 40 °C, which was held for 1 min, and then it was increased up to 300 °C at a rate of 15 °C/min, and held for

another 7 min [19]. The total run time was 25.3 min.

A Q-Exactive Orbitrap high resolution mass accuracy spectrometer was used for detection. Ionisation was carried out in positive electron ionisation mode (EI) at 70 eV, with a filament delay of 5 min, setting MS transfer line at 250 °C. Data acquisition was executed in both full scan mode and data dependent acquisition (dd-MS²), at a resolution of 60,000 FWHM at m/z 200 and an AGC target value of 1e6, from m/z 50 to 500 in the case of the full scan mode. Regarding dd-MS² acquisition, resolution was 30,000 FWHM at m/z 200 and AGC target value was set at 1e5. Acquired raw data was processed using Xcalibur™ 3.0 Qual Browser and Quan Browser. Unknown and suspect screening were performed by Qual Browser. Compounds were identified with the NIST MS Search 2.2 library (National Institute of Standards and Technology, MS, USA).

2.4. Data processing

Raw data obtained after GC-Q-Orbitrap analysis was manually processed with Xcalibur Qual Browser. Full Scan data of each sample was exhaustively examined to detect any peak belonging to a compound present in the sample. Benzene-based compounds were expected to elute approximately from 5 min to 10 min, and naphthalene-based compounds from 10 min to 14 min. Compounds were identified by matching their MS spectra with those available in the NIST library. The criteria that lead to a tentative identification of compounds were as follows: R Match value greater than 700, 20% probability threshold, mass error lower than 5 ppm for characteristic ions, mass error lower than 10 ppm for fragment ions and visual spectra comparison. Available analytical standards were then acquired and injected, and confirmation of these compounds was carried out by establishing a retention time tolerance of 0.1 min, comparing both spectra and peak shape, ion ratio, a mass error lower than 5 ppm for characteristic ions, and a mass error lower than 10 ppm for fragment ions.

3. Results and discussion

3.1. "Dilute and shoot" sample optimization for GC-MS

First, four different solvents were tested to determine which one offers the best possible solution of the co-formulants in the different PPPs. These solvents were ethyl acetate, acetone, *n*-hexane and DMSO. Peaks of the diluted samples were monitored in the total ion chromatogram (TIC) and compared in the different solvents. Peak area and sensitivity were in all cases greater in ethyl acetate, whereas peak widths were narrower in *n*-hexane. However, the chromatographic profile and the number of monitored signals remained identical in all solvents. During sample preparation it could be observed that many samples were not entirely dissolved in *n*-hexane, but in ethyl acetate. This evidence could explain why peak areas were larger in ethyl acetate. Additionally, SC and ZC formulations could not be dissolved in any solvent other than DMSO. Furthermore, acetone is a highly volatile solvent, which made it less suitable for sample preparation, as involuntary sample concentration could take place. A downside of carrying out extractions in *n*-hexane is that it is a non-green solvent, so other solvents should be used preferably. Therefore, the chosen solvents were ethyl acetate for EC/DC formulations, and DMSO for SC/ZC PPPs.

Pesticide formulations are highly concentrated products, especially considering their active substances, which in this case reached up to 25% (*w/v*). Hence, a large dilution must be applied prior to sample injection, with the aim of preventing contamination of the analytical equipment and minimizing its maintenance. Six dilutions were tested in ethyl acetate and DMSO: 1:1000 (*v/v*), 1:10,000 (*v/v*), 1:100,000 (*v/v*), 1:500,000 (*v/v*), 1:1,000,000 (*v/v*) and 1:2,000,000 (*v/v*). Dilution 1:500,000 (*v/v*) was selected, as it offered the best protection for the analytical equipment, due to a considerable decrease in the signals of the active ingredients to acceptable values, while providing satisfactory

results for unknown analysis. Fig. S1 (see Supplementary Material) represents the TIC for dilutions 1:1000 (*v/v*) and 1:500,000 (*v/v*) in a selected retention time window. A considerable reduction in peak signal can be noticed in the most diluted sample, especially in compounds eluting 5.82 and 6.17 min and some other peaks can barely be distinguished from the baseline.

3.2. GC-Q-Orbitrap analysis

Suspect screening was initially performed via a database built from literature research, including compounds found in previous studies [19] and shown in Table S2. This database included the name of the compounds, their molecular formula, theoretical exact mass of the characteristic ion and theoretical exact mass of two fragments. Suspect screening was performed by filtering theoretical exact masses in extracted ion chromatograms (EICs). Resulting peaks were then matched with the NIST reference spectra of the studied compounds. Seven benzene and naphthalene derivatives were tentatively identified by suspect screening in several PPPs, reported in Table 1. These compounds were: 1-methylnaphthalene, 2-methylnaphthalene, 1-ethylnaphthalene, 2-ethylnaphthalene, 1,3,5-trimethylbenzene, 1,2,4-trimethylbenzene and 4-ethyltoluene. Fig. S2 depicts the tentative identification of 1-methylnaphthalene and 2-methylnaphthalene by suspect screening. Two unresolved peaks appeared at 9.39 and 9.50 min in the EIC, corresponding to 2-methylnaphthalene and 1-methylnaphthalene, respectively, whose eluting order was described previously [19]. Their mass spectra revealed that methylnaphthalene ions 142, 141 and 115 were present in those peaks, as expected, with a matching relative abundance. Furthermore, mass error was -3.9 ppm for the characteristic ion 142.07769, and -4.0 ppm and -4.7 ppm for fragments 141.06987 and 115.05424, respectively.

Afterwards, tentative unknown identification was carried out. As it has been previously discussed, it was performed by NIST spectral library search according to the described procedure in the data processing section of this article. Thus, 30 compounds (13 naphthalene derivatives and 17 benzene derivatives) were tentatively identified in different samples by unknown analysis (Table 2) and added to the database (Table S2). Compounds previously identified by suspect screening were also detected by unknown analysis. Fig. S3 represents tentative identification of naphthalene in Voliam Targo by matching a sample spectrum and its NIST reference spectrum, with a mass error of 0.9 ppm for the characteristic ion, and -3.6 and 0.1 ppm for fragments 126.04695 and 102.04641, respectively. The biggest challenge involved in the confirmation of these compounds was the great number of isomers of a same molecular formula that could correspond to a certain peak. Reference spectra were so similar for most isomers, such as trimethylbenzenes or methylbiphenyls, that it was not feasible to elucidate them properly with no confirmation by analytical standards. Consequently, many of these compounds were reported as multiple isomers. For that reason, either all standards had to be acquired or only those suspected of being in the samples, as reported in prior literature research. However, few standards were commercially available and only 13 standards were purchased. Therefore, some tentative compounds could not be confirmed due to the lack of their standards.

Afterwards, acquired standards were injected and a total of 12 compounds (3 from suspect screening, and 9 from unknown analysis) were confirmed in 14 different samples of 4 varieties of fungicide formulations based on difenoconazole or chlorantraniliprole (DC, EC, SC and ZC), eluting from 5.17 min to 10.54 min. It is worth mentioning that most of the detected compounds were either benzene or naphthalene derivatives. These compounds are depicted in Table 3, including both confirmed and tentatively identified compounds. As an example, Fig. 1 shows the confirmation of pentamethylbenzene in Lector 25. As it can be observed in the chromatograms, standard and sample peaks had a similar shape and a retention time shift of barely 0.02 s. Concerning mass spectra, both theoretical and experimental spectra showed a

Table 1
Compounds tentatively identified by suspect screening.^a

	Kabuto JED (EC)	Ceremonia (EC)	Mavita 250 (EC)	Lexor 25 (EC)	Score 25 (EC)	Nomada (EC)	Duaxo (EC)	Cidely Top (DC)	Dynali (DC)	Voliam Targo (SC)	Dagonis (SC)	Coragen 20 (SC)	Ortiva Top (SC)	Ampligo 150 (ZC)
1-Methylnaphthalene	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2-Methylnaphthalene	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
1-Ethynaphthalene	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	No	No	No	Yes	Yes
2-Ethynaphthalene	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	No	No	No	Yes	Yes
4-Ethyltoluene	Yes	Yes	Yes	No	Yes	Yes	Yes	No	Yes	Yes	No	No	No	No
1,3,5-Trimethylbenzene	Yes	Yes	No	No	Yes	No	Yes	No	Yes	Yes	No	No	No	No
1,2,4-Trimethylbenzene	Yes	Yes	No	No	Yes	No	Yes	No	Yes	Yes	No	No	No	No
Benzyltoluenes ^b	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No	No	No	No	No	Yes
Dimethylbiphenyls ^c														

^a Abbreviations: DC: dispersible concentrate; EC: emulsifiable concentrate; SC: suspension concentrate; ZC: a mixture of capsule suspension (CS) in SC.

^b 2-Benzyltoluene, 3-benzyltoluene, 4-benzyltoluene.

^c 2,2'-Dimethylbiphenyl, 2,4'-dimethylbiphenyl, 1,1-diphenylethane, 4-ethylbiphenyl.

strongly similar pattern. Mass error was -4.12 ppm for characteristic ion m/z 148.12520, and -3.94 ppm and -4.36 ppm and for fragment ions m/z 147.11738 and m/z 133.10173, respectively. However, 1,3-diisopropylbenzene, initially identified by unknown screening, was determined to be a false positive after standard injection, as it can be observed in Fig. S4. In this case, while the standard eluted at 7.82 min, the alleged compound did so at 9.87 min. On top of that, relative abundances in the sample mass spectra did not match those in the standard mass spectra, despite the detection of characteristic and several fragment ions in both standard and sample with a low mass error. Thus, this false positive is likely to be caused by another isomer of 1,3-diisopropylbenzene, even though the sample spectra initially seemed to fit 1,3-diisopropylbenzene reference spectra pattern to a greater degree. Therefore, confirmed compounds were: 1,2,4-trimethylbenzene, 1,3,5-trimethylbenzene, 2-methylbiphenyl, 3-methylbiphenyl, 4-ethyltoluene, biphenyl, ethylbenzene, isopropylbenzene, naphthalene, n-propylbenzene, pentamethylbenzene and tert-butylbenzene.

After unknown analysis was completed, further literature review was made in a bid to confirm the likelihood of the presence of confirmed compounds. Several of these co-formulants were previously reported by some authors [9,20,21] which supports our results. All additional compounds reported in these articles were then searched in our samples to enhance the scope of discovered compounds, and 5 additional compounds were tentatively identified (dimethylbiphenyl and benzyltoluene isomers), displayed in Table 1. These compounds were subsequently included in the abovementioned library (Table S2). Therefore, the final number of tentatively identified compounds in the present study rose to 42, including all compounds identified by both suspect screening and unknown analysis and the provided database can be used in further analyses.

3.3. Presence of co-formulants in tested PPPs

The number of analysed formulations in which each compound was detected is represented in Fig. 2. In the light of the provided results, pentamethylbenzene was the most recurrent compound, being the only additive found in all samples. On the other hand, naphthalene was detected in 12 samples, except for 2 SC formulations, Dagonis and Coragen 20 (Table 4), whereas biphenyl was found in 11 samples, but again, it was not identified in the aforementioned SC formulations, plus a DC formulation (Cidely Top). Biphenyl and tert-butylbenzene were also recurrent components, as they were detected in 11 samples, while isopropylbenzene was discovered in 10 of them. 4-Ethyltoluene was found in 8 PPPs, 3-methylbiphenyl, 1,2,4-trimethylbenzene and n-propylbenzene were detected in 6 samples. On the other hand, 2-methylbiphenyl, 4-ethylbenzene and 1,3,5-trimethylbenzene were the sole co-formulants determined in less than half of the analysed commercial formulations, 4, 4 and 3 PPPs, respectively. It must be highlighted that none of the compounds included in Regulation EC No 2021/383 were identified in the tested samples.

In order to establish a correlation between the type of formulation and its composition, data regarding each compound in each formulation must be interpreted individually. As it can be seen in Fig. 3, EC formulations tended to have the greatest number of characterised compounds, with a mean value of 8 compounds per sample. Score 25 was the only formulation with the 12 characterised compounds detected in the present study. Other EC PPPs were Kabuto JED (10 compounds), Ceremonia (10 compounds), Mavita 250 (8 compounds), Duaxo (7 compounds), Nomada (7 compounds), and Lexor 25 (5 compounds). For DC formulations, Dynali had 9 compounds whereas Cidely Top contained 4 compounds. Regarding SC formulations, results were also dissimilar. Both Ortiva Top and Voliam Targo, from the same manufacturer, contained 7 compounds, whereas only 2 and 1 compounds were detected in Dagonis and Coragen 20, respectively, which were manufactured by different companies. Finally, Ampligo 150, a ZC formulation, contained 6 compounds.

Table 2
Compounds tentatively identified by unknown analysis.^a

	Kabuto JED (EC)	Ceremonia (EC)	Mavita 250 (EC)	Lexor 25 (EC)	Score 25 (EC)	Nomada (EC)	Duaxo (EC)	Cidely Top (DC)	Dynali (DC)	Voliam Targo (SC)	Dagonis (SC)	Coragen 20 (SC)	Ortiva Top (SC)	Ampligo 150 (ZC)
2,3-Dihydrodimethyl-1H-indenes ^b	No	Yes	Yes	Yes	Yes	Yes	No	No	No	No	No	No	No	Yes
Biphenyl	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	No	No	Yes	Yes
Diisopropylbenzenes ^c	No	No	Yes	Yes	Yes	Yes	No	No	No	No	No	No	No	No
Dimethylnaphthalenes ^d	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes
Ethylbenzene	No	Yes	No	No	Yes	No	No	No	Yes	No	No	No	Yes	No
Isopropylbenzene	Yes	Yes	Yes	No	Yes	No	Yes	Yes	Yes	Yes	No	No	Yes	Yes
Methylbiphenyls ^e	Yes	No	Yes	Yes	Yes	Yes	No	No	No	No	No	No	No	Yes
Naphthalene	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No	Yes	Yes
n-Propylbenzene	Yes	Yes	No	No	Yes	No	No	No	Yes	No	No	No	Yes	Yes
Pentamethylbenzene	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Tert-butylbenzene	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	No
Tetramethylbenzenes ^f	No	No	Yes	No	Yes	No	No	No	No	No	No	No	No	No
Ethylxylenes ^g														
2-Isopropyltoluene														
Trimethylnaphthalenes ^h	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No	No	No	No	No	Yes
2-Isopropyl-naphthalene														
4,6,8-Trimethylazulene														

^a Abbreviations: DC: dispersible concentrate; EC: emulsifiable concentrate; SC: suspension concentrate; ZC: a mixture of capsule suspension (CS) in SC.

^b 2,3-Dihydrodimethyl-4,7-dimethyl-1H-indene, dihydromethyl-1,6-dimethyl-1H-indene, 2,3-dihydrodimethyl-2,3-dimethyl-1H-indene, 2,3-dihydrodimethyl-1,2-dimethyl-1H-indene, 2,3-dihydrodimethyl-1,3-dimethyl-1H-indene.

^c 1,3-Diisopropylbenzene, 1,4-diisopropylbenzene.

^d 1,7-Dimethylnaphthalene, 1,2-dimethylnaphthalene, 1,3-dimethylnaphthalene, 1,6-dimethylnaphthalene, 1,4-dimethylnaphthalene, 2,6-dimethylnaphthalene, 2,3-dimethylnaphthalene, 2,7-dimethylnaphthalene, 1,5-dimethylnaphthalene or 1,8-dimethylnaphthalene.

^e 2-Methylbiphenyl, 3-methylbiphenyl, 4-methylbiphenyl, diphenylmethane.

^f 1,2,3,4-tetramethylbenzene, 1,2,4,5-tetramethylbenzene, 1,2,3,5-tetramethylbenzene.

^g 4-Ethyl-m-xylene, 5-ethyl-m-xylene, 2-ethyl-m-xylene.

^h 1,6,7-Trimethylnaphthalene, 2,3,6-trimethylnaphthalene, 1,4,5-trimethylnaphthalene, 1,4,6-trimethylnaphthalene, 2,4,5-trimethylnaphthalene.

Table 3
Characteristic parameters for confirmed (**in bold**) and tentatively identified compounds.

Confirmed compounds/Tentative compounds	Retention time (min)	CAS number	Molecular formula	Characteristic ion		Fragment ions	
				Theoretical mass (<i>m/z</i>)	Mass error (ppm)	Theoretical mass (<i>m/z</i>)	Mass error (ppm)
1,2,4-Trimethylbenzene	5.94	95-63-6	C ₉ H ₁₂	120.09390	-4.90	105.07043 119.08608	-5.80 -4.53
1,3,5-Trimethylbenzene (Mesitylene)	6.21	108-67-8	C ₉ H ₁₂	120.09390	-2.83	105.07043 119.08608	-3.62 -1.09
2-Methylbiphenyl	10.25	643-58-3	C ₁₃ H ₁₂	168.09390	-4.82	167.08608 165.07043	-4.13 -3.76
3-Methylbiphenyl	10.54	643-93-6	C ₁₃ H ₁₂	168.09390	-4.76	167.08608 165.07043	-4.07 -3.57
4-Ethyltoluene (p-Cymene)	5.89	622-96-8	C ₉ H ₁₂	120.09390	-4.75	105.07043 91.05478	-5.62 -6.15
Biphenyl	10.11	92-52-4	C ₁₂ H ₁₀	154.07825	-4.80	153.07043 152.06260	-4.25 -3.81
Ethylbenzene	5.17	100-41-4	C ₈ H ₁₀	106.07825	-5.56	105.07043 91.05478	-5.23 -6.48
Isopropylbenzene (Cumene)	5.47	98-82-8	C ₉ H ₁₂	120.09390	-3.25	105.07043 103.05478	-4.28 -3.88
Naphthalene	8.30	91-20-3	C ₁₀ H ₈	128.06260	-4.69	126.04695 102.04641	-3.94 -3.89
n-Propylbenzene	5.78	103-65-1	C ₉ H ₁₂	120.09390	-4.66	105.07043 91.05478	-5.33 -6.81
Pentamethylbenzene	9.19	700-12-9	C ₁₁ H ₁₆	148.12520	-4.32	147.11738 133.10173	-3.87 -4.51
Tert-butylbenzene	6.17	98-06-6	C ₁₀ H ₁₄	134.10955	-3.80	119.08607 91.05478	-4.79 -6.48
1-Methylnaphthalene	9.50	90-12-0	C ₁₁ H ₁₀	142.07825	-4.71	141.07018 115.05478	-2.34 -4.95
2-Methylnaphthalene	9.37	91-57-6	C ₁₁ H ₁₀	142.07825	-4.12	141.07018 115.05478	-3.27 -4.30
2,3-Dihydropentamethyl-1H-indene ^a	8.88 to 9.17 (3 peaks)	NA	C ₁₁ H ₁₄	146.10955	-4.17	145.10118 131.08549	-1.45 -0.08
Dimethylbiphenyl ^b Benzyltoluene ^c	11.08 to 11.45 (5 peaks)	NA	C ₁₄ H ₁₄	182.10955	-3.79	181.10112 167.08540	0.11 0.06
Dimethylnaphthalene ^d	10.32 to 10.63 (5 peaks)	NA	C ₁₂ H ₁₂	156.0939	-4.23	155.08608 141.07043	-3.80 -4.32
1-Ethyl-naphthalene 2-Ethyl-naphthalene	10.70, 10.83	NA	C ₁₂ H ₁₂	156.0939	-2.43	141.07043 115.05478	-2.06 -2.27
Tetramethylbenzene ^e Ethyl-m-xylene ^f 2-Isopropyltoluene	6.41 to 7.85 (5 peaks)	NA	C ₁₀ H ₁₄	134.10955	-3.58	119.08608 91.05478	-3.95 -5.71
Trimethylnaphthalene ^g 2-Isopropyl-naphthalene 4,6,8-Trimethylazulene	11.23 to 12.09 (7 peaks)	NA	C ₁₃ H ₁₄	170.10955	-3.59	169.10173 155.08608	-2.90 -4.00
1,3-Diisopropylbenzene 1,4-Diisopropylbenzene	9.87	99-62-7 100-18-5	C ₁₂ H ₁₈	162.14085	-4.07	147.11738 119.08608	-4.76 -6.05

^a 2,3-Dihydropentamethyl-4,7-dimethyl-1H-indene, dihydropentamethyl-1,6-dimethyl-1H-indene, 2,3-dihydropentamethyl-2,3-dimethyl-1H-indene, 2,3-dihydropentamethyl-1,2-dimethyl-1H-indene, 2,3-dihydropentamethyl-1,3-dimethyl-1H-indene.

^b 2,2'-Dimethylbiphenyl, 2,4'-dimethylbiphenyl, 2,3'-dimethylbiphenyl, 1,1-diphenylethane, 4-ethylbiphenyl.

^c 2-Benzyltoluene, 3-benzyltoluene, 4-benzyltoluene.

^d 1,7-Dimethylnaphthalene, 1,2-dimethylnaphthalene, 1,3-dimethylnaphthalene, 1,6-dimethylnaphthalene, 1,4-dimethylnaphthalene, 2,6-dimethylnaphthalene, 2,3-dimethylnaphthalene, 2,7-dimethylnaphthalene, 1,5-dimethylnaphthalene, 1,8-dimethylnaphthalene.

^e 1,2,3,4-Tetramethylbenzene, 1,2,4,5-tetramethylbenzene, 1,2,3,5-tetramethylbenzene, 4-ethyl-m-xylene, 5-ethyl-m-xylene, 2-ethyl-m-xylene.

^g 1,6,7-Trimethylnaphthalene, 2,3,6-trimethylnaphthalene, 1,4,5-trimethylnaphthalene, 1,4,6-trimethylnaphthalene, 2,4,5-trimethylnaphthalene.

As a conclusion, a correlation can be established between the type of formulation and its non-polar and volatile composition. According to these results, SC formulations are less likely to be composed of GC-amenable co-formulants, whereas EC PPPs, have a higher chance of containing more non-polar volatile compounds. This agrees with the fact that EC formulations are prepared from solvent naphtha. In spite of this, there is not enough data to establish any correlation for DC and ZC formulations. None of the abovementioned commercial formulations specifically declared any of the detected compounds in their labels, whereas some of them actually reported solvent naphtha (a complex mixture of aromatic hydrocarbons) as an ingredient, which is likely to be the source of these compounds. Nonetheless, no differences could be observed among the 3 chlorantraniliprole-based (Voliam Targo SC, Coragen 20 SC and Ampligo 150 ZC) and the 11 difenoconazole-based PPPs, beyond those appreciated among EC and SC/ZC formulations.

It can be concluded that these results match those previously reported by other authors involving volatile co-formulants in PPPs, as many of the compounds that have been determined in this study were also identified in other authors' studies by different methodologies other than HRMAS, in fewer PPPs [9]. This supports results previously reported in different studies and leads us to believe that the amount of these volatile compounds expected to be identified is mostly limited to certain benzene and naphthalene derivatives. Despite this, the composition of each PPP seems unique.

3.4. Quantification

Due to the complexity of the analysed samples, no blanks of any sample were available for the preparation of matrix-matched calibration. Therefore, the standard addition technique had to be applied, by

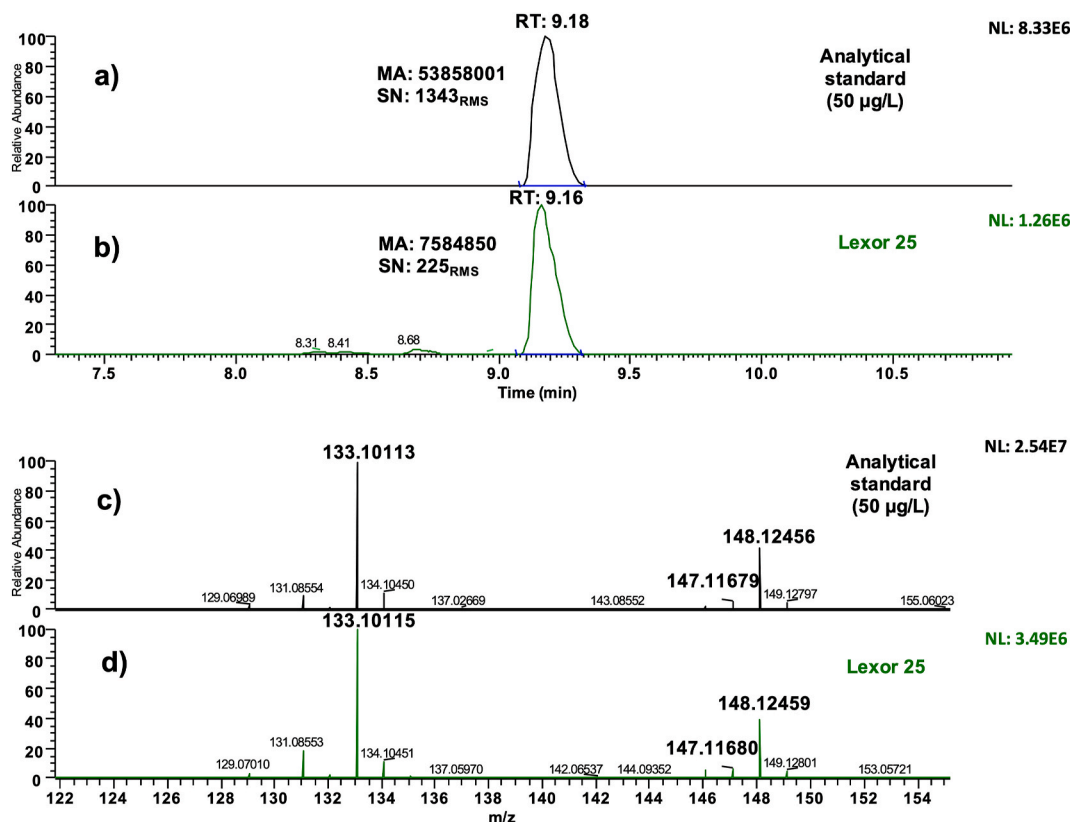


Fig. 1. Chromatograms and mass spectra of pentamethylbenzene in Lexor 25 and a standard solution: a) Extracted Ion Chromatogram (TIC) of an analytical standard (50 µg/L); b) chromatogram of Lexor 25; c) full Scan MS spectra of an analytical standard (50 µg/L), and d) full Scan MS spectra of Lexor 25.

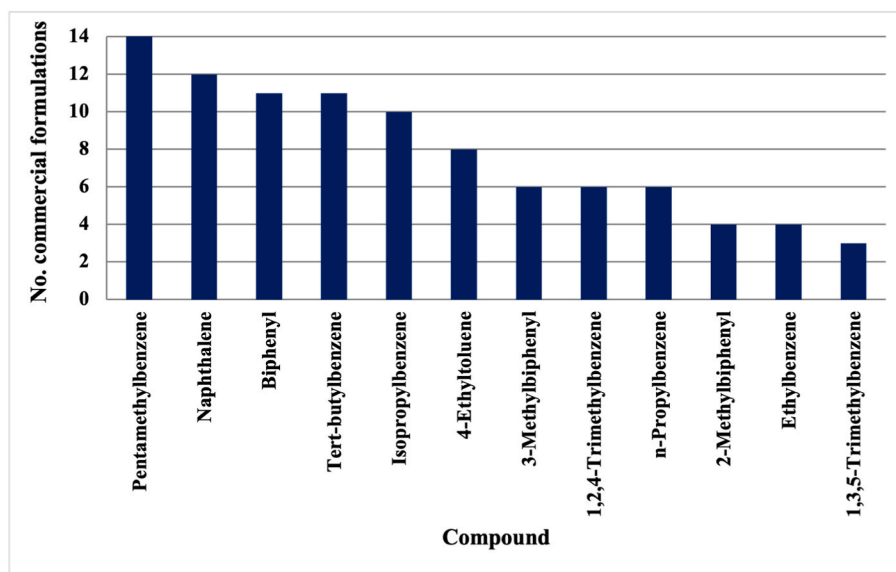


Fig. 2. Number of commercial formulations containing each detected compound.

spiking samples with several standard solutions containing all the studied analytes.

Standard-addition calibration standards were prepared from 0.1 µg/L to 100 µg/L. In all cases, the R^2 value was greater than 0.99. Results are expressed in g/L, and the 1:500.000 (v/v) dilution has been taken in consideration (Table 4). According to SANTE/12682/2019 [22], if the analytical method does not permit determination of recovery (direct analysis of liquid samples), only precision must be assessed from repeat

analysis of standards. Thus, five replicates were tested in 5 different days. All results fell under the threshold required for an acceptable precision ($RSD \leq 20\%$), ranging from 2% for n-propylbenzene, to 8% for pentamethylbenzene.

As it can be seen, concentration values in most formulations usually dropped below 1 g/L, with some exceptions. Overall, Lexor 25 was the PPP with the most concentrated analytes per number of detected compounds, which contained 2-methylbiphenyl (0.47 g/L), 3-

Table 4
Concentration results for tested plant protection products (g compound/L formulation)^a

	Kabuto JED (EC)	Ceremonia (EC)	Mavita 250 (EC)	Lexor 25 (EC)	Score 25 (EC)	Nomada (EC)	Duaxo (EC)	Cidely Top (DC)	Dynali (DC)	Voliam Targo (SC)	Dagonis (SC)	Coragen 20 (SC)	Ortiva Top (SC)	Ampligo 150 (ZC)
1,2,4-Trimethylbenzene	0.42	1.01			0.38		0.28		0.39	0.29				
1,3,5-Trimethylbenzene	0.69	9.61			0.69									
2-Methylbiphenyl	0.29		0.52	0.47	0.07	0.81								0.25
3-Methylbiphenyl	0.25		2.55	3.05	4.34	2.41	0.33		0.32	0.20				
4-Ethyltoluene	0.21	0.15	1.40	1.40	2.20	2.11	0.12		0.14	0.11			0.18	0.26
Biphenyl		2.19			2.46				3.48				4.81	
Ethylbenzene	0.32	0.41	0.09		0.35		0.25	0.11	0.32	0.34			0.32	0.15
Isopropylbenzene	0.64	1.32	3.43	4.32	3.45	3.81	0.40	0.34	0.55	0.40			0.54	0.54
Naphthalene	0.41	0.41			0.36				0.39				0.47	0.37
N-Propylbenzene	0.28	0.67	7.54	7.74	9.63	3.39	0.10	0.14	0.16	0.13		0.10	0.11	0.40
Pentamethylbenzene	0.30	0.14	0.04		0.36	0.03	0.12	0.26	0.24	0.13	0.28	0.29	0.22	
Tert-butylbenzene														

^a Abbreviations: DC: dispersible concentrate; EC: emulsifiable concentrate; SC: suspension concentrate; ZC: a mixture of capsule suspension (CS) in SC.

methylbiphenyl (3.05 g/L), biphenyl (1.40 g/L), naphthalene (4.32 g/L), and pentamethylbenzene (7.74 g/L). Score 25 also had a certain number of highly concentrated compounds, such as pentamethylbenzene (9.63 g/L), naphthalene (3.45 g/L), 3-methylbiphenyl (4.34 g/L), ethylbenzene (2.46 g/L), and biphenyl (2.20 g/L). Furthermore, Kabuto JED, Duaxo, Cidely Top, Voliam Targo, Dagonis, Coragen 20 and Ampligo 150 exclusively had values under 1 g/L. Non-EC formulations with an analyte at concentration greater than 1 g/L, were Dynali DC (ethylbenzene, 3.48 g/L), and Ortiva Top SC (Ethylbenzene, 4.81 g/L) (Table 4).

In terms of compounds, the lowest calculated concentration was 0.03 g/L (tert-butylbenzene in Nomada, EC), whereas the highest concentration was 9.63 g/L (pentamethylbenzene in Score 25). Regarding pentamethylbenzene, it gathers the majority of the highest values in several samples, such as Score 25 (9.63 g/L), Mavita 250 (7.54 g/L), and Lexor 25 (7.44 g/L). Most noticeably, while ethylbenzene was only detected in 4 formulations, its concentration was greater than 1 g/L in all cases: 2.19 g/L in Ceremonia, 2.46 g/L in Score 25, 3.48 g/L in Dynali, and 4.81 g/L in Ortiva Top. As a matter of fact, it was the only studied compound detected at high concentration in 3 different types of formulations (EC, DC and SC). This was not the case for the remaining compounds, which even though they were at 1 g/L in some PPPs, their concentration fell under 1 g/L in many formulations. In this case, it was not possible to establish a clear connection between the type of formulation, and the concentration of the co-formulants it contains.

3.5. Risk assessment of co-formulants

Toxicological information related to 10 confirmed compounds was reviewed with the purpose of determining their estimated toxicity (Table S3). Reference dose (RfD) assesses the daily oral human intake of a compound that is likely not to produce any appreciable harmful effect during a lifetime [23]. It stems from non-observed-adverse-effect level (NOAEL), which sets the highest tested dose of a certain compound that has been proved not to cause any appreciable adverse effects, in addition to several uncertainty factors [24]. RfD was intended to replace acceptable daily intake (ADI) values.

Oral RfD values in rats ranged from 0.01 mg/kg-day, for both 1,2,4-trimethylbenzene and 1,3,5-trimethylbenzene, to 0.5 mg/kg-day for biphenyl, as shown in Table S3. Therefore, the most toxic substances in accordance with their RfD values are 1,2,4-trimethylbenzene and 1,3,5-trimethylbenzene. On the other hand, difenoconazole has a RfD value of 0.01 mg/kg-day, like those for 1,2,4-trimethylbenzene and 1,3,5-trimethylbenzene, which points at a similar toxicity. The highest concentration of 1,2,4-trimethylbenzene in any analysed PPP was 1.0 g/L, whereas 1,3,5-trimethylbenzene was detected at 9.6 g/L. Co-formulants may enter food chain at unsafe concentrations, in the same way pesticides do in spite of the dilution of PPPs in water. This may pose a threat to human health, considering that co-formulants may show a relatively similar toxicity than pesticides, according to their respective RfD values.

Additionally, LD50 or LC50 values were reported for some compounds, rather than oral RfD values, which had not published. These parameters represent the dose or concentration required to provoke death in half the studied population. One of the most prominent remarks of this assessment is that, while pentamethylbenzene was the most recurrent molecule being identified in all studied samples, its toxicity data remain unreported. Therefore, the authors consider that further toxicological studies concerning pentamethylbenzene should be performed, as this compound has been quantified at high concentrations, thus being likely to be detected in agricultural foodstuff treated with these PPPs. By carrying out toxicological tests in diverse organisms, it was suggested that the toxicity of pesticide formulations may usually be greater than the active substances alone [25]. Thus, it can be inferred that co-formulants can show a synergistic effect that enhances toxicity, so future toxicological studies should tackle this possibility, and carry out a thorough assessment of not only the active substance or identified

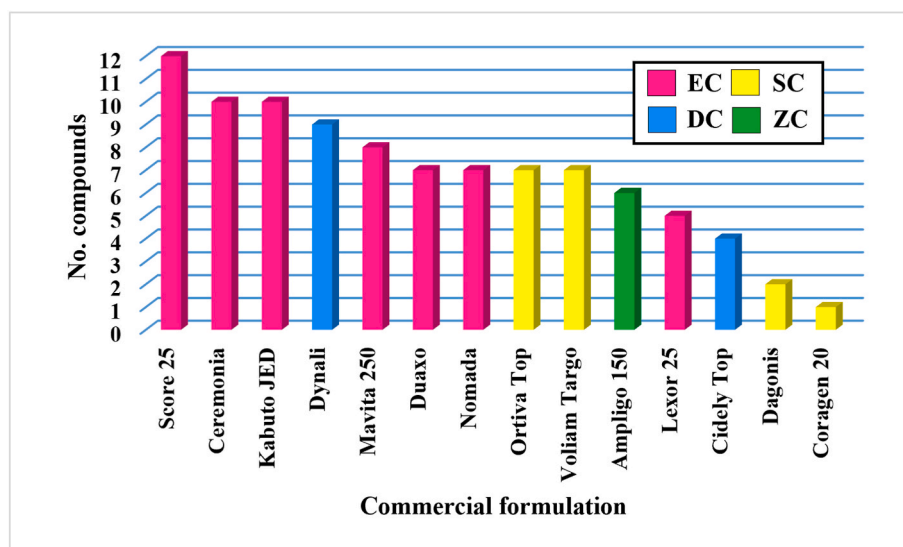


Fig. 3. Number of compounds found in every studied commercial formulation.

co-formulants, but the toxicological properties of the commercial formulations as well.

4. Conclusions

In this study, a novel technique was applied for the identification of co-formulants in 14 different PPPs of 4 types of formulations (SC, EC, ZC and DC) by GC-Q-Orbitrap. The compounds were tentatively identified according to several criteria such as their fragmentation pattern or mass error, and then confirmed and quantified by means of analytical standards. All the identified compounds were either benzene or naphthalene derivatives, and eluted from 5.17 min to 10.54 min. The main feature of this study is innovation, as it is one of the few available concerning the suspect and unknown determination of co-formulants by GC-HRMAS. As a novelty, this paper introduces the identification by HRMAS of a wide range of formulations containing difenoconazole and chlorantraniliprole, a fungicide and an insecticide on the rise, in contrast to previous studies that used targeted acquisition in low resolution mass spectrometers and a scarce number of formulations. HRMAS provided a satisfactory approach for compound elucidation in this study using both suspect and unknown approaches. Its use ensured that all characteristic and precursor ions were correctly identified, so a reliable determination of co-formulants could be attained by means of a spectral library.

Furthermore, none of the identified compounds were reported in the label by the manufacturer, a fact that highlights the importance of characterising these chemical products, since they are used on marketed agricultural foodstuff. The high concentration of these co-formulants in the analysed samples, and their possible toxic effects on humans and animal health, emphasise the importance of enforcing complete labeling for marketed PPPs, and developing analytical methods for the determination of these compounds in foodstuff treated with PPPs, as well as the environmental surroundings of crops, such as soils or air.

CRedit author statement

Antonio Jesús Maldonado-Reina: Methodology, Investigation, Software, Writing - Original Draft, Data curation, Visualization; **Rosalía López-Ruiz:** Methodology, Software, Data Curation, Supervision; Writing - Review & Editing; **Antonia Garrido Frenich:** Conceptualisation, Resources, Writing - Review & Editing, Funding acquisition, Project administration; **F. Javier Arrebola:** Conceptualisation, Methodology, Supervision, **Roberto Romero-González:** Methodology, Formal analysis, Supervision, Writing - Review & Editing, Funding

acquisition.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.talanta.2021.122641>.

References

- [1] U.S. Environmental, Protection agency (EPA), U.S. Code of federal regulations. Title 40 protection of environment, Chapter I. Subchapter E. Part 152. Subpart A. 152.3., 2008 <https://www.govinfo.gov/content/pkg/CFR-2012-title40-vol25/pdf/CFR-2012-title40-vol25-chap1.pdf>. (Accessed 7 March 2021).
- [2] D. Hazra, A. Purkait, Role of pesticide formulations for sustainable crop protection and environment management: a review, *J. Pharmacogn. Phytochem.* 8 (2019) 686–693.
- [3] J. Feng, Q. Zhang, Q. Liu, Z. Zhu, D.J. McClements, S.M. Jafari, Application of Nanoemulsions in Formulation of Pesticides, Elsevier Inc., 2018, <https://doi.org/10.1016/B978-0-12-811838-2.00012-6>. First.
- [4] D. Hazra, R. Karmakar, R. Poi, S. Bhattacharya, S. Mondal, Recent advances in pesticide formulations for eco-friendly and sustainable vegetable pest management: a review, *Arch. Agric. Environ. Sci.* 2 (2017) 232–237, <https://doi.org/10.13140/RG.2.2.30036.91527>.
- [5] H. De Ruiter, H.J. Holterman, C. Kempenaar, H.G.J. Mol, J.J. De Vlieger, J.C. van de Zande, Influence of Adjuvants and Formulations on the Emission of Pesticides to the Atmosphere, 2003.
- [6] R.L. Zimdahl, Herbicide formulation, in: *Fundam. Weed Sci.*, fifth ed., 2018, pp. 501–509, <https://doi.org/10.1016/B978-0-12-811143-7.00017-2>.
- [7] Markets and Markets, Agricultural Adjuvants Market by Function (Activator and Utility), Application (Herbicides, Insecticides, and Fungicides), Formulation

- (Suspension Concentrates and Emulsifiable Concentrates), Adoption Stage, Crop Type, and Region - Global Forecast 2026, 2020. <https://www.marketsandmarkets.com/Market-Reports/adjuvant-market-1240.html>. (Accessed 9 March 2021).
- [8] E. Pose-Juan, R. Rial-Otero, E. Martínez-Carballo, E. López-Periágo, J. Simal-Gándara, Determination of metalaxyl and identification of adjuvants in wettable powder pesticide technical formulas, *Anal. Bioanal. Chem.* 394 (2009) 1535–1544, <https://doi.org/10.1007/s00216-009-2633-z>.
- [9] M. Zeinali, L.L. McConnell, C.J. Hapeman, A. Nguyen, W.F. Schmidt, C.J. Howard, Volatile organic compounds in pesticide formulations: methods to estimate ozone formation potential, *Atmos. Environ.* 45 (2011) 2404–2412, <https://doi.org/10.1016/j.atmosenv.2011.02.015>.
- [10] D. Tush, K.A. Loftin, M.T. Meyer, Characterization of polyoxyethylene tallow amine surfactants in technical mixtures and glyphosate formulations using ultra-high performance liquid chromatography and triple quadrupole mass spectrometry, *J. Chromatogr. A* 1319 (2013) 80–87, <https://doi.org/10.1016/j.chroma.2013.10.032>.
- [11] Commission Regulation (EU), No 547/2011 of 8 June 2011 Implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as Regards Labelling Requirements for Plant Protection Products, European Commission, 2011.
- [12] Commission Implementing Regulation (EU), No 540/2011 of 25 May 2011 Implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as Regards the List of Approved Active Substances, European Commission, 2011.
- [13] Commission Regulation (EU), 2021/383 of 3 March 2021 Amending Annex III to Regulation (EC) No 1107/2009 of the European Parliament and of the Council Listing Co-formulants Which Are Not Accepted for Inclusion in Plant Protection Products, European Commission, 2021.
- [14] M.J. Pascual-Villalobos, P. Guirao, F.G. Díaz-Baños, M. Cantó-Tejero, G. Villora, Oil in Water Nanoemulsion Formulations of Botanical Active Substances, Elsevier Inc., 2019, <https://doi.org/10.1016/B978-0-12-815829-6.00009-7>.
- [15] G.K. Roberts, C.J. Willson, D.S. Olivera, D.E. Malarkey, D.L. Morgan, Comparative inhalation toxicity of ethyltoluene isomers in rats and mice, *Inhal. Toxicol.* 29 (2017) 577–585, <https://doi.org/10.1080/08958378.2018.1430190>.
- [16] M. Płonka, S. Walorczyk, M. Miszczyk, D. Kronenbach-Dylong, Simultaneous gas chromatographic determination of chlorpyrifos and its impurity sulfotep in liquid pesticide formulations, *J. Environ. Sci. Health Part B Pestic. Food Contam. Agric. Wastes* 51 (2016) 736–741, <https://doi.org/10.1080/03601234.2016.1198637>.
- [17] L. Cao, H. Jiang, J. Yang, L. Fan, F. Li, Q. Huang, Simultaneous determination of benzene and toluene in pesticide emulsifiable concentrate by headspace GC-MS, *J. Anal. Methods Chem.* 2013 (2013) 1–6, <https://doi.org/10.1155/2013/121783>.
- [18] M. Płonka, S. Walorczyk, M. Miszczyk, Chromatographic methods for the determination of active substances and characterization of their impurities in pesticide formulations, *Trends Anal. Chem.* 85 (2016) 67–80, <https://doi.org/10.1016/j.trac.2016.03.011>.
- [19] R. López-Ruiz, E. Belmonte-Sanchez, R. Romero-González, J. Martínez Vidal, A. Garrido Frenich, Identification of adjuvants in plant protection products applying a suspect screening workflow based on orthogonal techniques, *Anal. Bioanal. Chem.* (2020), <https://doi.org/10.1007/s00216-020-02662-0>.
- [20] M.A. Martínez, S. Ballesteros, Two suicidal fatalities due to the ingestion of chlorfenvinphos formulations: simultaneous determination of the pesticide and the petroleum distillates in tissues by gas chromatography-flame-ionization detection and gas chromatography- mass spectrometry, *J. Anal. Toxicol.* 36 (2012) 44–51, <https://doi.org/10.1093/jat/bkr014>.
- [21] G. Petrelli, G. Siepi, L. Miligi, P. Vineis, Solvents in pesticides, *Scand. J. Work. Environ. Health* 19 (1993) 63–65, <https://doi.org/10.5271/sjweh.1502>.
- [22] European Commission, Analytical Quality Control and Method Validation for Pesticide Residues Analysis in Food and Feed (SANTE/12682/2019), Sante/12682/2019, 2019, pp. 1–48. https://www.eurl-pesticides.eu/userfiles/file/EurlALL/AqcGuidance_SANTE_2019_12682.pdf.
- [23] U.S. Environmental Protection Agency (EPA), Reference Dose (RfD): description and use in health risk assessments background document 1A, 1993. March 15, 1993, <https://www.epa.gov/iris/reference-dose-rfd-description-and-use-health-risk-assessments>. (Accessed 26 March 2021).
- [24] M.A. Dorato, J.A. Engelhardt, The no-observed-adverse-effect-level in drug safety evaluations: use, issues, and definition(s), *Regul. Toxicol. Pharmacol.* 42 (2005) 265–274, <https://doi.org/10.1016/j.yrtph.2005.05.004>.
- [25] K. Nagy, R.C. Duca, S. Lovas, M. Creta, P.T.J. Scheepers, L. Godderis, B. Ádám, Systematic review of comparative studies assessing the toxicity of pesticide active ingredients and their product formulations, *Environ. Res.* 181 (2020) 108926, <https://doi.org/10.1016/j.envres.2019.108926>.