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Non-targeted analysis of co-formulants in antifungal pesticide formulations by gas chromatography-tandem high resolution mass spectrometry

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ABSTRACT

In the present study, six commercial pesticide formulations with antifungal activity were characterized. Thus, two complementary injection methods based on gas chromatography were employed: direct injection (DI) and headspace (HS), both coupled to high resolution mass spectrometry (GC-Q-Orbitrap-MS). The combination of both injection modes allowed the tentatively identification of potential co-formulants. Available analytical standards were acquired for their confirmation, and 21 compounds were successfully confirmed. Finally, the concentration of these co-formulants was calculated, finding the highest value in one of the pesticide formulation, at 218.22 g L^{-1} for cyclohexanone.

Results clearly show that this methodology is suitable for the reliable identification of co-formulants in pesticide formulations, offering high sensitivity, and highlighting that five co-formulants were detected by both injection techniques (DI and HS). Moreover, one of the main advantages of the proposed methods was the great capacity for the elucidation of compounds with similar molecular formula, bearing in mind that up to 8 co-formulants with the same molecular formula $C_{10}H_{14}$, were well differentiated by retention times between 8.46 (1-methyl-3-propylbenzene) and 10.98 min (1,2,3,4- tetramethylbenzene) in one of the pesticide formulation.

Toxicity to human health and the environment has been evidenced for the co-formulants detected, finding compounds with relatively high toxicity, as naphthalene and cyclohexanone.

1. Introduction

An increase in the demand for pesticides is being observed worldwide for effective crop protection, because high agricultural yields is being prioritized [1]. Pesticides might be found in a variety of formulations depending on the physical and chemical properties of their active ingredients, intended use, ease of storage, transport and application, or even production costs [2,3]. The greatest success of pesticide formulations or plant protection products (PPPs) is due to their broad application spectrum against multiple pests, and thus they can be classified as: insecticides (for insect control), herbicides (for weed control) or fungicides (for fungi control), among others [4,5].

PPPs might contain one or more active substances (pesticide) and other ingredients called co-formulants, which may act as carriers, emulsifiers, odorants, buffers, solvents, stabilisers or preservatives [2,6]. Co-formulants usually improve PPP properties, as to enhance the activity, mixing, application or effectiveness of the pesticide formulations and to reduce the adverse effect of active ingredients [7,8]. Furthermore, PPPs may also contain safeners to eliminate or reduce phytotoxic effects of pesticides on certain plants, or synergists to give enhanced activity of the active substance in a PPP [2,6].

All pesticide formulations should undergo an exhaustive procedure to be approved for commercial use, controlled by the updated Regulation (EC) No 1107/2009 [6], and Regulation (EU) 2021/383 sets a list of co-formulants which are not accepted for inclusion in PPPs [9].

Abbreviations: GC, Gas chromatography; Q, Quadrupole; MS, Mass spectrometry.

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As discussed previously, there is an extensive variety of formulations that differ in the composition, both active substances and other ingredients. Among the most common, highlights: emulsifiable concentrate (EC), which is composed by blends of pesticide, emulsifiers and adjuvants dissolved in a volatile oil; emulsion in water (EW), similar mixture than EC but using water instead of oil; water dispersible granule (WG), which contains active ingredient in spray form of the constituents insoluble in water; and suspension concentrate (SC), based on suspensions of micronized active pesticide in water [10].

Several methods have been developed for the determination of compounds contained in PPPs [11], and gas-chromatography (GC) coupled to several detection systems were used. For instance, GC coupled with a flame ionization detector (GC-FID) was employed for the determination of fifteen co-formulants (among them 1,3,5-trimethyl-benzene) in EC, SC, EW and SL (soluble concentrate) formulations [12]. GC was also coupled to mass spectrometry (MS) with ion trap as analyzer to monitor some organic co-formulants in two wettable powder (WP) pesticide technical formulations [8]. In the investigation carried out by Qi et al. [7], the determination of different co-formulants (toluene, p-xylene, o-xylene, m-xylene, N,N-dimethylformamide and dimethyl sulphoxide) was performed in different crops (lily bulb, millet, and Chinese yam) through single quadrupole mass analyzer.

However, high-resolution mass spectrometry (HRMS) in combination with GC has scarcely been used for the analysis of pesticide formulations. In this context, two studies have employed Q-Orbitrap coupled to GC. The research carried out by López-Ruiz et al. [13] allowed the determination of nine co-formulants (benzene or naphthalene derivates) by direct injection (DI) in three EC PPPs. In another investigation, fourteen different types of PPPs were characterized, using DI, detecting benzene and naphthalene derivates in co-formulants [14]. The PPPs belonged to 4 types of formulations (EC, DC, SC and ZC) and contained a fungicide (difenoconazole) or an insecticide (chlorantraniliprole). Both studies demonstrated that GC-Q-Orbitrap-MS can be used to characterize co-formulants in PPPs.

Despite headspace (HS)-GC-MS is a powerful tool for the analysis of volatile and semi-volatile compounds in complex matrices [15,16], few studies are focused on the determination of these compounds in PPPs using low-resolution mass spectrometry (LRMS). In this context, a research was performed to identify benzene and toluene in pesticide EC formulations using a single quadrupole as analyzer [17]. Petha et al. developed a method for the quantitative determination of dimethyl sulfate in a hexaconazole commercial fungicide by HS, using triple quadrupole (QqQ) as analyzer [18].

The novelty of this study was the application of HRMS, especially GC-Q-Orbitrap-MS, through two complementary injection techniques (DI and HS) for the identification of co-formulants (mainly volatile compounds) in six different antifungal commercial pesticide, containing as active substance one triazole compound (fenbuconazole, tebuconazole, penconazole, myclobutanil or flutriafol). For that, a suspect screening was carried out first, and then an unknown analysis was performed to identify other volatile co-formulants when HS injection mode was used. Both techniques were complementary and they provided comprehensive information, increasing the feasibility and sensitivity of the proposed methods for the identification and quantification of compounds with toxic activity in PPPs. To our knowledge, this is the first study where two different injection techniques (DI and HS) were combined and coupled to HRMS to monitor volatile co-formulants, allowing the confirmation of those compounds for which there were available commercial analytical standards, as well as their concentrations were estimated, providing a reliable tool to identify and quantify co-formulants present in PPPs.

2. Materials and methods

2.1. Equipment, material and reagents

Assays were performed using six fungicide formulations in different forms: FLINT® MAX (50% tebuconazole, WG); MASSOCUR 12.5 EC (12.5% myclobutanil, EC); LATINO (formerly known as MITRUS, 12.5% myclobutanil, EC); IMPACT® EVO (12.5% flutriafol, SC); TOPAS® (19.4% penconazole, EW); and IMPALA® STAR (2.5% fenbuconazole, EW). These pesticide formulations were acquired from different vendors, and they are described in Table S1 (see Supplementary information).

Analytical standards of 1,3,5-trimethylbenzene, ethylbenzene, naphthalene, n-propylbenzene, 1,2,3,4-tetramethylbenzene, 1,2,3,5-tetramethylbenzene, 1,2-diethylbenzene, 1,3-diethylbenzene, 1,4- diethylbenzene, 1-methyl-2-propylbenzene, 1-methyl-3-propylbenzene and 3-ethyltoluene were supplied from Dr. Ehrenstorfer (Augsburg, Germany). Analytical standards of 2-methylbiphenyl, 3-methylbiphenyl, 4-ethyltoluene, biphenyl, cyclohexanone, indane, d-limonene and pentamethylbenzene were acquired by Sigma-Aldrich (St. Louis, MO, USA), and 2-ethyltoluene and 2-ethyl-p-xylene were purchased from Tokyo Chemical Industry (Chuo-ku, Tokyo, Japan).

Water, LC-MS grade, was obtained from J.T. Baker (Deventer, The Netherlands) and acetone, HPLC grade, 99.8%, was supplied by Sigma-Aldrich.

Perfluorotributylamine from Thermo Fisher Scientific (Waltham, MD, USA) was employed for the mass calibration of the GC-Q-Orbitrap analysis.

2.2. Sample treatment

For DI analysis, individual solutions of each commercial formulation were initially prepared by dissolving 40 μL of each one in 40 mL of acetone (in case of MASSOCUR 12.5 EC, LATINO and IMPALA® STAR) or water (in case of FLINT® MAX, IMPACT® EVO and TOPAS®). The mixture of each commercial formulation was well-shaken and 100 μL was transferred to a chromatographic vial and then diluted with 900 μL of acetone, obtaining a dilution 1:10,000 (*v/v*). Finally, 100 μL of each new individual dilution was added to a vial and then diluted with 900 μL of acetone, obtaining a final dilution 1:100,000 (*v/v*).

For HS procedure, a 15-mL vial was filled with 10 µL of commercial formulation and 10 mL of water and immediately sealed with a PTFE-silicone septum. Finally, the mixture was shaken for 1 min.

2.3. Direct injection (DI) analysis

For DI, the analysis was performed using a Trace 1310 GC system with a TriPlus RSH autosampler (Thermo Scientific™) coupled to a Q-Exactive Orbitrap mass analyzer (Thermo Fisher Scientific, Waltham, MA).

The chromatographic and spectrometric optimization were carried out in a previous study [14]. For the chromatographic separation, a nonpolar column Varian VF-5ms (30 m × 0.25 mm; 0.25 μm), provided by Agilent Technologies (Santa Clara, CA, USA) was chosen. The parameters were as follows: the injector temperature was set at 280°C, and 2 μL of the sample were introduced into the system in splitless mode (split flow of 50 mL min^{-1}) using a splitless time of 2 min. Initially, the GC oven temperature was set at 40°C, and it was held for 1 min; then it was increased to 300°C at a rate of 15°C min^{-1}. Finally, it was remained for 7 min at 300°C. The total running time was 25.3 min. Ultra-high purity helium (99.9999%), as a carrier gas, was set at a constant flow rate of 1 mL min^{-1}.

For the HRMS acquisition, the electron ionization (EI) was applied at 70 eV, and data acquisition was performed at both full scan mode and data dependent acquisition (dd-MS^2). Resolution power was set at 60,000 full width at half-maximum (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-MS^2 acquisition, resolution was 30,000 FWHM at *m/z* 200 and AGC target value was set at 1e5. Ion source and MS transfer line temperatures were set at 250°C.

2.4. Headspace (HS-GC-MS) analysis

Additionally, HS was employed with the same equipment, column and spectrometric conditions reported above (see Section 2.3), but with different injection and chromatographic conditions. Regarding injection's system, optimum parameters were set as follows in splitless mode: incubation time 20 min, agitator temperature 60°C each 10 s, and injection volume was set at 100 µL. Inlet temperature was set at 250°C and split injection mode (split flow of 50 mL min^{-1}) was applied at 33:3 ratio for the first minute. For chromatographic conditions, oven temperature started at 60°C, and it was kept for 2 min, then it was increased at 6°C min^{-1} rate to 220°C, held there for 20 min, and finally increased with a 20°C min^{-1} rate to 280°C, and it was kept for 4 min. Helium, which was used as carrier gas, was set at a constant flow rate of 1 mL min^{-1}. The total running time was 60.0 min.

2.5. Data treatment and processing

The acquired chromatograms, from external calibration mode, were processed using Xcalibur version 4.3.73, with Quan Browser and Qual Browser (Thermo Fisher Scientific, Les Ulis, France). NIST MS spectral library - 2014, Search 2.2 library (National Institute of Standards and Technology, MS, USA) was used for the identification of compounds.

The raw files obtained from each analysis by DI were processed with an in-house database built by Maldonado et al. [14]. This database involved the name of the compounds and their molecular formula, theoretical exact mass of the characteristic ion and theoretical exact mass of two fragments. Moreover, full-scan data of each PPP was carefully studied with Xcalibur Qual Browser to monitor the spectra of the detected compounds.

An unknown analysis was carried out to identify other compounds. Raw files obtained by HS were processed with Xcalibur Qual Browser and NIST library. The identification criteria were defined according to SANTE guidance [19], including: suitable peak shaped signals; in case noise was absent, a signal should be present in at least 5 subsequent scans per peak of each ion; mass error $\leq$ 5 ppm; and at least two fragment ions of each co-formulant were selected.

The data of toxicity of some co-formulants were performed by the T.E.S.T (Toxicity Estimation Software Tool) software [20].

3. Results and discussion

For the identification of potentially expected compounds in the studied pesticide formulations, a non-targeted approach (suspect screening and unknown analysis) was performed. For that purpose, the diluted PPPs were injected into the GC-Q-Orbitrap-MS system by DI for suspect screening, and by HS for unknown analysis. Suspect screening was achieved using a database by DI [14]. Nevertheless, due to HS has not been carried out previously, an unknown analysis was considered appropriate to detect further co-formulants and volatile-compounds.

3.1. Tentative identification of co-formulants by suspect screening

Firstly, for DI analysis, a suspect screening was carried out using an in-house database. [14]. This tool allowed the search of all compounds included in the database in the studied samples, selecting a mass tolerance of 5 ppm. In this sense, nine benzene and naphthalene derivatives (Table 1) were tentatively identified in the samples as potential co-formulants (1,3,5-trimethylbenzene, 2-methylbiphenyl, 3-methylbiphenyl, 4-ethyltoluene, biphenyl, ethylbenzene, naphthalene, n-propylbenzene and pentamethylbenzene) and a cyclic ketone, cyclohexanone. Thus, ten compounds were tentatively identified by DI.

The most recurrent co-formulant was 2-methylbiphenyl, which was detected in four different PPPs (in all but MASSOCUR 12.5 and LATINO). Naphthalene, 1,3,5-trimethylbenzene and biphenyl were found in three samples. In two out of six PPPs, 3-methylbiphenyl, 4-ethyltoluene, cyclohexanone and pentamethylbenzene were detected, and finally ethylbenzene and n-propylbenzene in only one, FLINT® MAX. The retention times of the compounds ranged from 5.04 (cyclohexanone) to 10.54 min (3-methylbiphenyl), as it can be observed in Table 1. In all cases, the mass error was lower than 5 ppm for characteristic ions.

Two fragment ions were acquired for each one of the tentatively detected co-formulants, with mass error $\leq$ 5 ppm and matching with those provided by NIST database (Table 1**)**. Moreover, NIST database allowed to compare the ratios among molecular and fragment ions to suitably select the most similar ones. According to the results, it has been highlighted that 4-ethyltoluene, ethylbenzene and n-propylbenzene compounds had similar fragment ions: *m/z* 91.05422, which corresponded to the loss of the ethyl group bonded to the benzene, and *m/z* 105.06983, which corresponded to the loss of the methyl group of the ethylene. The fragment *m/z* 105.06983 was also found in 1,3,5-trimethylbenzene, with a mass error of -3.21 ppm (Table 1).

Due to some of the candidates had the same formula, it might be fully considered the presence of isomers in the samples, which means similar characteristics: theoretical mass and peaks, but different retention time, thus involving one of the biggest challenges of this study. This was particularly the case for the 4-ethyltoluene and 1,3,5-trimethylbenzene co-formulants, whose molecular formula was C_9H_{12} (theoretical mass *m/z* 120.09335), with retention times of 5.89 and 6.21 min, respectively. The same situation was observed for the 2-methylbiphenyl and 3-methylbiphenyl ($C_{13}H_{12}$) compounds with retention times of 10.25 and 10.54 min, respectively (Table 1). Furthermore, it should be emphasized that the pair of isomers of 2-methylbiphenyl and 3-methylbiphenyl was observed in the same PPP (IMPALA® STAR). The fragmentation pattern of these both co-formulants was similar, detecting common fragment ions at *m/z* values 152.06205 and 167.08552, with mass error ranging from -4.37 to -0.54 ppm. The first fragment ion corresponded to the loss of the methyl group in the two benzenes linked in position 1,1, and the second one has changed the methyl position from 3 to 2 in the two benzenes, which were linked in position 1,1.

Once the tentative identification of possible co-formulants was achieved, commercially available analytical standards of co-formulants were acquired to confirm their presence in the samples. The reliable confirmation was carried out by comparing experimental MS spectra (obtained in the step of tentative identification) and retention times with MS spectra of each analytical standard. For that purpose, the ten proposed compounds were purchased and injected by DI. Finally, it was observed that all possible co-formulants were satisfactory confirmed in the analyzed PPPs at the retention time indicated in Table 1.

Table 1
Characteristic parameters for tentatively identified compounds by suspect screening (DI-GC-MS)

Compound	RT (min)	Molecular formula	Theoretical mass (*m/z*)	Mass error (ppm)	Fragment ions			Commercial formulation
					Molecular formula	Theoretical mass (*m/z*)	Mass error (ppm)	
1,3,5-Trimethylbenzene (mesitylene)[a]	6.21	C_9H_{12}	120.09335	-2.77	C_8H_9	105.06983	-3.21	MASSOCUR 12.5 EC, TOPAS® and
					C_9H_{11}	119.08552	-1.92	
2-Methylbiphenyl	10.25	$C_{13}H_{12}$	168.09335	-3.82	$C_{12}H_8$	152.06205	0.32	FLINT® MAX, IMPACT® EVO,
					$C_{13}H_{11}$	167.08552	-4.37	TOPAS® and IMPALA® STAR
3-Methylbiphenyl	10.54	$C_{13}H_{12}$	168.09335	-3.66	$C_{12}H_8$	152.06205	0.54	LATINO and IMPALA® STAR
					$C_{13}H_{11}$	167.08552	-4.16	
4-Ethyltoluene[a]	5.89	C_9H_{12}	120.09335	-3.58	C_7H_7	91.05422	-4.53	FLINT® MAX and IMPACT® EVO
					C_8H_9	105.06983	-4.26	
Biphenyl	10.11	$C_{12}H_{10}$	154.07770	-2.98	$C_{12}H_8$	152.06205	-3.41	TOPAS®, LATINO and IMPALA® STAR
					$C_{12}H_9$	153.06987	-4.15	
Cyclohexanone[a]	5.04	$C_6H_{10}O$	98.07262	-0.81	C_3H_3O	55.01784	0.36	MASSOCUR 12.5 EC and LATINO
					C_6H_6O	72.05697	0.98	
Ethylbenzene	5.17	C_8H_{10}	106.07770	-3.66	C_7H_7	91.05422	-3.81	FLINT® MAX
					C_8H_9	105.06983	-4.46	
Naphthalene[a]	8.30	$C_{10}H_8$	128.06205	-3.95	C_8H_6	102.04640	-3.91	TOPAS®, LATINO and IMPALA® STAR
					$C_{10}H_5$	125.03857	-3.47	
n-Propylbenzene[a]	5.78	C_9H_{12}	120.09335	-4.18	C_7H_7	91.05422	-2.01	FLINT® MAX
					C_8H_9	105.06983	-4.53	
Pentamethylbenzene	9.19	$C_{11}H_{16}$	148.12465	-4.11	$C_{10}H_{13}$	133.10117	-3.24	LATINO and IMPALA® STAR
					$C_{11}H_{15}$	147.11682	-3.41	

[a] Compounds also detected by unknown analysis.

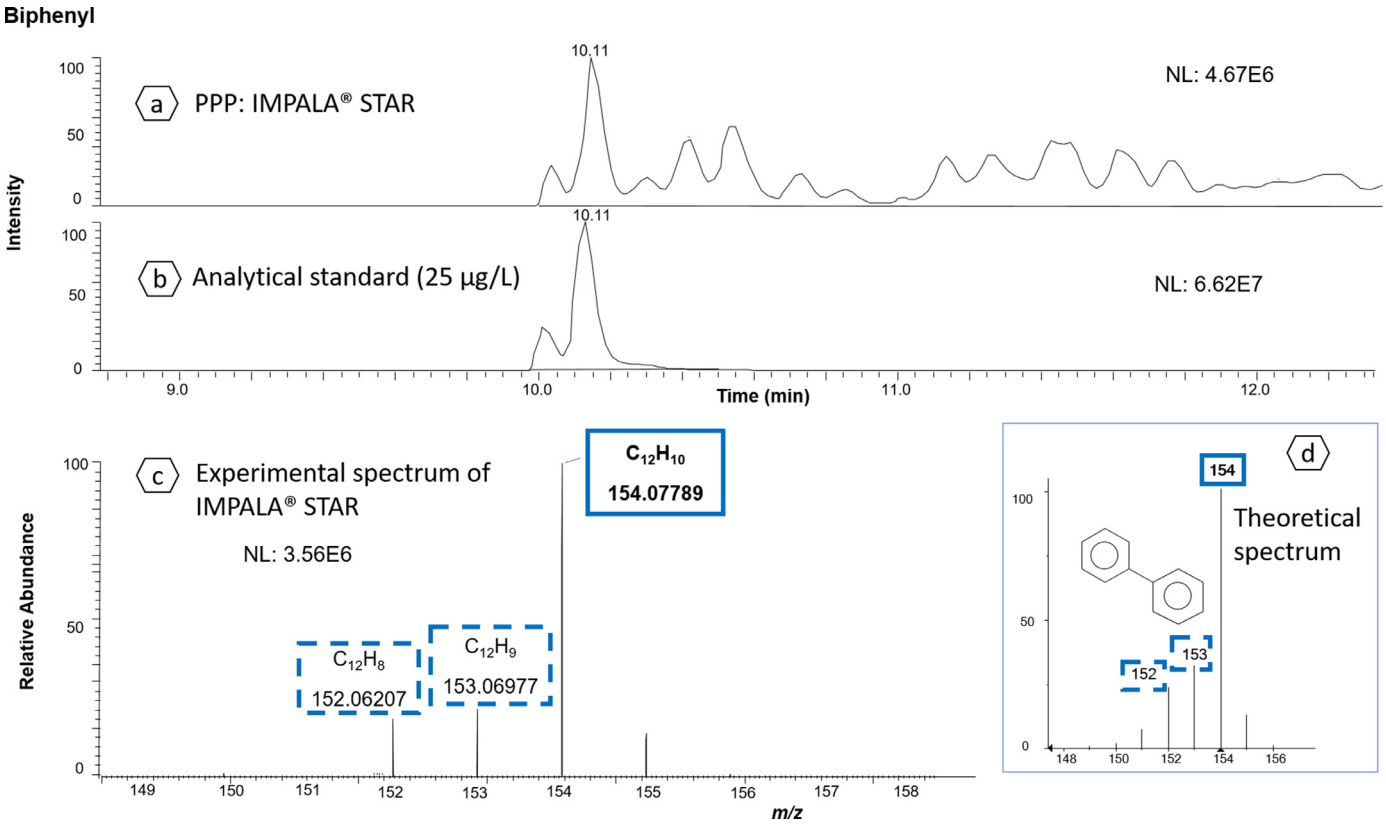

Fig. 1. Extracted Ion Chromatograms of biphenyl: a) IMPALA® STAR commercial product and b) analytical standard (at 25 µg L^{-1}); c) full Scan MS experimental spectrum of IMPALA® STAR commercial product at 10.11 min; d) theoretical spectrum obtained from the NIST database.

One of the confirmed co-formulants was biphenyl, and Fig. 1a shows its Extracted Ion Chromatogram (EIC) in one commercial product IMPALA® STAR, detected at 10.11 min. The confirmation of this compound was carried out by comparing this retention time with the EIC of the analytical standard (Fig. 1b), and by matching the full Scan MS experimental spectrum acquired (Fig. 1c) with the theoretical one obtained from the NIST database (Fig. 1d), showing in both cases the *m/z* 154.07779 and the fragment ions (*m/z* 152.06205 and 153.06987) and suitable mass errors (lower than 5 ppm).

3.2. Tentative identification of co-formulants by unknown analysis

So far, ten co-formulants have been identified during suspect screening and suitably confirmed by DI in the studied pesticide formulations. Therefore, in order to identify further compounds,

Table 2
Characteristic parameters for tentatively identified compounds (**in bold confirmed by analytical standards**) by unknown analysis (HS-GC-MS)[a]

Compound	RT (min)	Molecular formula	Theoretical mass (*m/z*)	Mass error (ppm)	Fragment ions			Commercial formulation
					Molecular formula	Theoretical mass (*m/z*)	Mass error (ppm)	
1,2,3,4- tetramethylbenzene	10.98	$C_{10}H_{14}$	134.10900	0.37	C_7H_7	91.05422	0.65	MASSOCUR 12.5, LATINO and
					C_9H_{11}	119.08552	-4.19	IMPALA® STAR
1,2,3,5-Tetramethylbenzene	10.24	$C_{10}H_{14}$	134.10900	-0.29	C_7H_7	91.05422	0.54	MASSOCUR 12.5, IMPACT® EVO,
					C_9H_{11}	119.08552	-3.70	TOPAS®, LATINO and IMPALA® STAR
1,2-Diethylbenzene	8.75	$C_{10}H_{14}$	134.10900	0.69	C_7H_7	91.05422	0.33	MASSOCUR 12.5, IMPACT® EVO,
					C_9H_{11}	119.08552	-3.89	LATINO and IMPALA® STAR
1,3-Diethylbenzene	8.48	$C_{10}H_{14}$	134.10900	-0.14	C_8H_9	105.06983	-5.61	MASSOCUR 12.5, TOPAS®, LATINO
					C_9H_{11}	119.08552	-4.78	and IMPALA® STAR
1,4- Diethylbenzene	8.63	$C_{10}H_{14}$	134.10900	0.22	C_8H_9	105.06983	-4.66	MASSOCUR 12.5, LATINO and
					C_9H_{11}	119.08552	-4.11	IMPALA® STAR
1-Methyl-2-propylbenzene	8.90	$C_{10}H_{14}$	134.10900	0.39	C_7H_7	91.05422	3.62	MASSOCUR 12.5, LATINO and
					C_8H_9	105.06983	-4.32	IMPALA® STAR
					C_9H_{11}	119.08552	-4.56	
1-Methyl-3-propylbenzene	8.46	$C_{10}H_{14}$	134.10900	0.22	C_7H_7	91.05422	-4.05	MASSOCUR 12.5, TOPAS®, LATINO
					C_8H_9	105.06983	-4.04	and IMPALA® STAR
					C_9H_{11}	119.08552	-4.13	
1-Methyl-4-propylbenzene (4-n-propyltoluene)	7.75	$C_{10}H_{14}$	134.10900	0.29	C_7H_7	91.05422	0.10	TOPAS® and IMPALA® STAR
					C_8H_9	105.06983	-4.75	
					C_9H_{11}	119.08552	-4.03	
2-Ethyl-p-xylene (1,4-dimethyl-2-ethylbenzene)	9.17	$C_{10}H_{14}$	134.10900	-0.29	C_7H_7	91.05422	-0.54	MASSOCUR 12.5, IMPACT® EVO,
					C_9H_{11}	119.08552	-4.53	LATINO and IMPALA® STAR
2-Ethyltoluene (1-ethyl-2-methylbenzene)	6.75	C_9H_{12}	120.09335	0.58	C_8H_7	103.05422	-3.23	MASSOCUR 12.5, IMPACT® EVO,
					C_8H_9	105.06983	-4.85	LATINO and IMPALA® STAR
2,4-Diethyltoluene	9.40	$C_{11}H_{16}$	148.12465	0.20	C_9H_7	115.05422	-1.25	IMPALA® STAR
					$C_{10}H_{13}$	133.10117	0.22	
3-Ethyltoluene	6.40	C_9H_{12}	120.09335	1.57	C_8H_7	103.05422	-2.13	MASSOCUR 12.5, IMPACT® EVO,
					C_8H_9	105.06983	-4.21	TOPAS®, LATINO and IMPALA® STAR
4-Ethenyl-1,2-dimethylbenzene	10.74	$C_{10}H_{12}$	132.09335	-3.25	C_9H_7	115.05422	0.94	TOPAS®
					C_9H_9	117.06987	0.25	
d-Limonene	8.05	$C_{10}H_{16}$	136.12465	0.88	C_6H_7	79.05422	0.12	IMPACT® EVO and LATINO
					C_7H_9	93.06987	0.21	
Indane	8.13	C_9H_{10}	118.07770	-0.08	C_9H_7	115.05422	-0.26	MASSOCUR 12.5, TOPAS® and
					C_9H_9	117.06987	0.17	IMPALA® STAR

Abbreviations: HS-GC-MS: headspace-gas chromatography-mass spectrometry RT: Retention time.
[a] Compounds also detected by suspect screening: n-propylbenzene, 1,3,5-trimethylbenzene (mesitylene), 4-ethyltoluene and naphthalene.

paying special attention to volatile co-formulants, which could be lost during the DI analysis, an unknown approach was performed by HS using the experimental conditions described in Section 2.4. Raw files obtained for each PPP were manually studied using Xcalibur Qual Browser, and features were identified as candidates by matching their corresponding MS spectra (processed with Qual Browser) with those provided by the NIST spectral library. Therefore, the following criteria were considered to identify potential co-formulants: appropriate peak shaped signals, R Match value higher than 700, 10% probability threshold, a signal present in at least 5 subsequent scans per peak of each ion, and mass error $\leq$ 5 ppm for characteristic ions.

Finally, fifteen compounds were selected as potential co-formulants and their characteristic parameters were described in Table 2**.** Furthermore, these compounds were included in our in-house database.

According to the results, similar characteristics were achieved between the 2-ethyltoluene and 3-ethyltoluene co-formulants, whose molecular formula was C_9H_{12} (*m/z* 120.09335) and retention times were 6.75 and 6.40 min, respectively (Table 2), assuming adequate mass errors below 2 ppm.

For each one of the fifteen co-formulants, at least two fragment ions were monitored (Table 2**)**. The fragment ions were sorted according to the following criteria: most abundant ion; retention time, which had to be equal to the corresponding precursor ion; and mass error (lower than 5 ppm). Considering the results, the common fragments found in the ten co-formulants with $C_{10}H_{14}$ as molecular formula were the following: *m/z* 91.05422 (C_7H_7), *m/z* 105.06983 (C_8H_9) and *m/z* 119.08552 (C_9H_{11}), noticing that at least two out of three fragment ions were found in all this group of compounds (Table 2). Therefore, for the ion *m/z* 134.10900, whose molecular formula was $C_{10}H_{14}$, various peaks were detected. This could correspond to the presence of different isomers, due to they were identified at different retention times, as it can be observed in Fig. 2, where that ion was monitored in MASSOCUR 12.5 commercial product. These co-formulants were: 1-methyl-3-propylbenzene (retention time was 8.46 min), 1,3-diethylbenzene (8.48 min), 1,4-diethylbenzene (8.63 min), 1, 2-diethylbenzene (8.75 min), 1-methyl-2-propylbenzene (8.90 min), 2-ethyl-p-xylene (9.17 min), 1,2,3,5-tetramethylbenzene (10.24 min) and 1,2,3,4 tetramethylbenzene (10.98 min) (Fig. 2). Furthermore, suitable mass error was detected between -0.29 (2-ethyl-p-xylene) and 0.69 ppm (1, 2-diethylbenzene), as displayed in Table 2.

Regarding the confirmation of co-formulants, indane was confirmed in MASSOCUR 12.5 commercial product at 8.13 min (Fig. 3a), by matching the EIC with that from the purchased analytical standard (Fig. 3b) at the same retention time. Moreover, full Scan MS experimental spectrum acquired (Fig. 3**c**) was compared with the theoretical one obtained from the NIST database (Fig. 3**d**), where it was shown the similarities between both spectra (*m/z* 115.05422 and 117.06987, which corresponded to C_9H_7 and C_9H_9, respectively). Thus, it was confirmed the presence of indane in one of the analyzed samples (MASSOCUR 12.5).

Among the PPPs, the highest number of co-formulants was observed in MASSOCUR 12.5, achieving thirteen, followed by LATINO and IMPALA® STAR, with eleven compounds each one.

Once the tentative identification of co-formulants was achieved, commercially available analytical standards of co-formulants (Table 2) were acquired to confirm their presence in the samples.

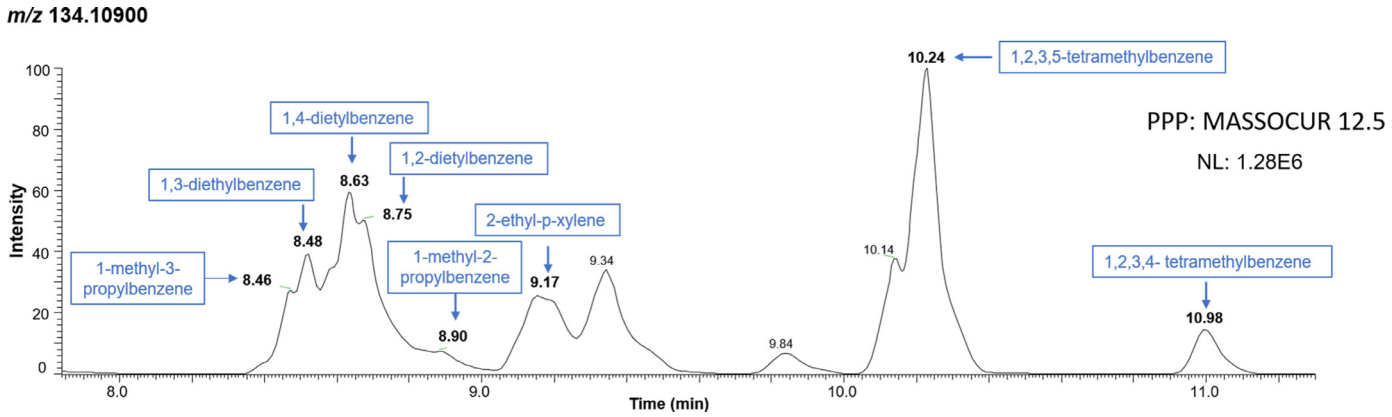

Fig. 2. Extracted Ion Chromatogram of compounds with molecular formula $C_{10}H_{14}$ (theoretical mass *m/z* 134.10900) in MASSOCUR 12.5 commercial product.

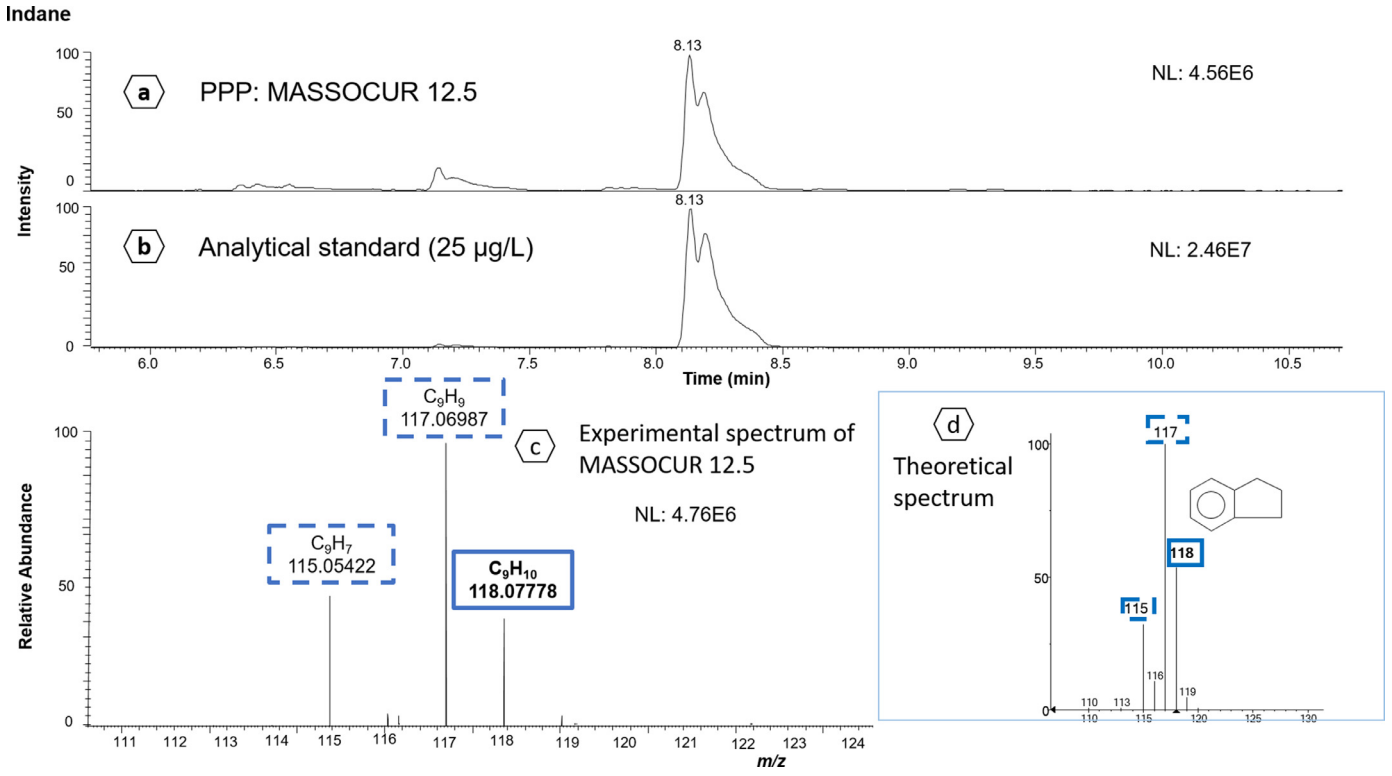

Fig. 3. Extracted Ion Chromatograms of indane: a) MASSOCUR 12.5 commercial product and b) analytical standard (at 25 µg L^{-1}); c) full Scan MS experimental spectrum of MASSOCUR 12.5 commercial product at 8.13 min; d) theoretical spectrum obtained from the NIST database.

Confirmation was carried out by comparing experimental MS spectra (obtained in the step of tentative identification) to MS spectra of each analytical standard. For that purpose, twelve of the fifteen proposed compounds were purchased and injected (Table 2**)**. The other compounds (1-methyl-4-propylbenzene, 2,4-diethyltoluene and 4-ethenyl-1,2-dimethylbenzene), whose standards could not be obtained, were only tentatively identified (level 2) [21] in the analyzed samples.

Finally, it was noticed that by unknown analysis (HS-GC-MS) eleven candidate co-formulants were satisfactory confirmed in the tested PPPs, but d-limonene, which was a false positive (Table 2). Moreover, five of these confirmed co-formulants (n-propylbenzene, 1,3,5-trimethylbenzene, 4-ethyltoluene, cyclohexanone and naphthalene) were also detected and confirmed by suspect analysis (DI-GC-MS), empathizing the capability of the proposed methods to monitor co-formulants.

As an example, Fig. 4 shows the chromatograms and spectra of the compound 1,2,3,5-tetramethylbenzene. This co-formulant was tentatively identified in the LATINO commercial product by HS at 10.24 min (Fig. 4**a**) and confirmed by injection of analytical standard at 250 μg L^{-1} (Fig. 4b). The confirmation of this compound was performed by matching the full Scan MS experimental spectrum acquired (Fig. 4c) with the spectrum of analytical standard (Fig. 4d) and with the theoretical spectrum obtained from the NIST database (Fig. 4e), showing a similar fragmentation pattern of 1,2,3,5-tetramethylbenzene in each case. Consequently, it could be confirmed that the compound was contained in LATINO commercial product.

Nevertheless, some false positives were considered after analytical standard injection. This was particularly the case of d-limonene, which was tentatively identified by HS in TOPAS® and LATINO. However, when the EIC was compared with the corre-

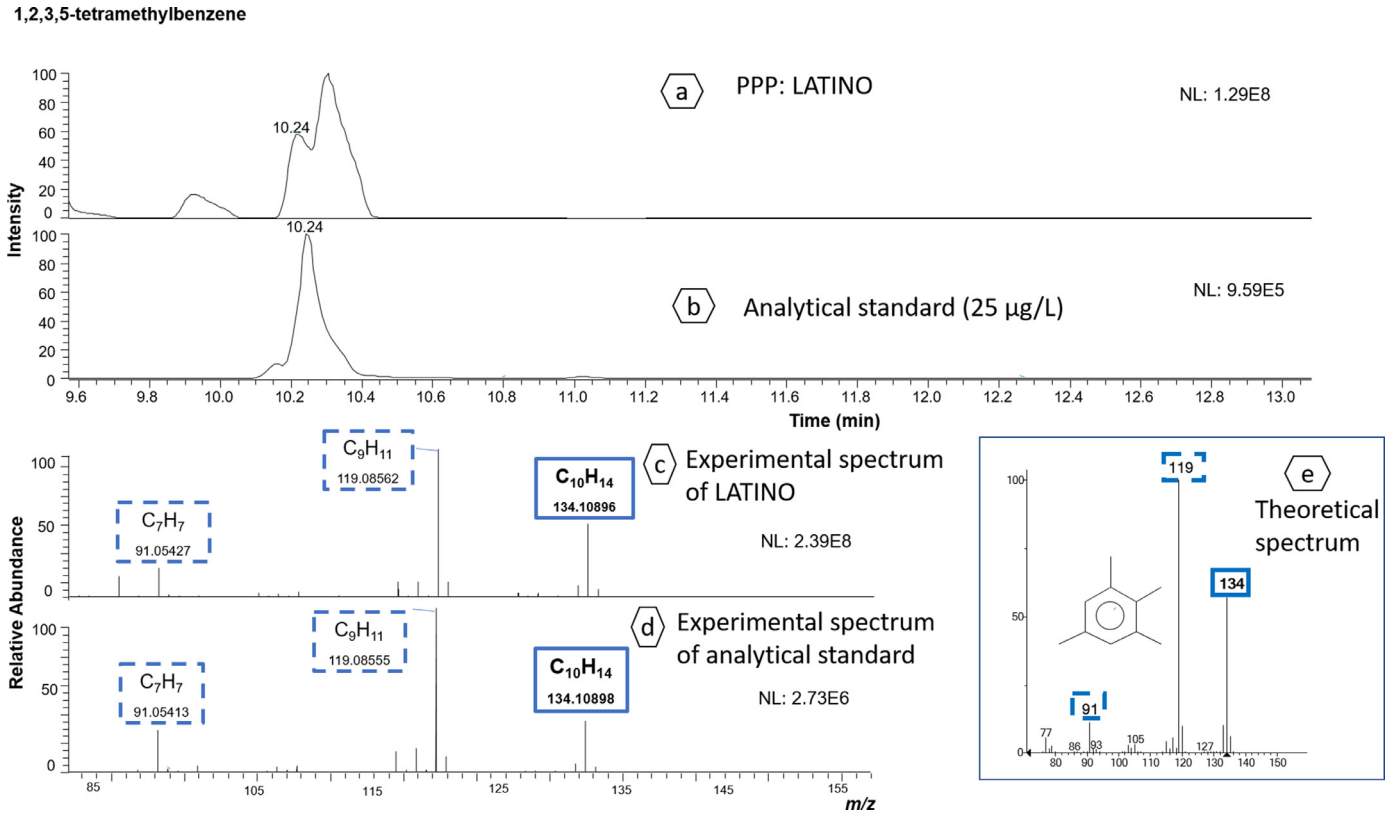

Fig. 4. Extracted Ion Chromatograms of 1,2,3,5-tetramethylbenzene: a) LATINO commercial product identified by headspace and b) analytical standard (at 25 µg L^{-1}); c) full Scan MS experimental spectrum of LATINO commercial product by headspace; d) full Scan MS experimental spectrum at 10.24 min of analytical standard; and e) theoretical spectrum obtained from the NIST database.

sponding analytical standard, no signals appeared at that retention time (8.13 min). Therefore, d-limonene was not detected in any of these samples.

3.3. Quantification of co-formulants in the commercial samples

The concentration of the twenty-one confirmed compounds, both by suspect screening (ten) and unknown (eleven) analyses, was calculated by preparing calibration curves (ranged from 1 to 100 μg L^{-1}) of standard solutions, which contained all the studied compounds (**Table S2**). The results in the analyzed PPPs, expressed in g L^{-1}, are shown in Table 3.

The most recurrent compounds were 1,2,3,5-tetramethylbenzene and 3-ethyltoluene, because they were detected in five PPPs at concentration range between 0.02 and 1.02 g L^{-1} for 1,2,3,5-tetramethylbenzene, and 0.05-13.52 g L^{-1} for 3-ethyltoluene. The 1,2-diethylbenzene, 1-methyl-3-propylbenzene and 1,3-diethylbenzene compounds were detected in all pesticide formulations except in one, as shown in Table 3.

IMPALA® STAR was the pesticide formulation which showed the greatest number of co-formulants, especially seventeen out twenty-one compounds, whose concentration was ranged from 0.03 (1,2,3,4-tetramethylbenzene) and 3.53 g L^{-1} (pentamethylbenzene). As those values were not relatively high, it can be assumed that there is not a significant relationship between the number of compounds detected in the PPPs with their corresponding concentrations.

It should be noted that the highest concentration of all co-formulants was detected in the LATINO commercial product, especially in the cyclohexanone compound (at 218.22 g L^{-1}). Moreover, MASOCCUR 12.5 showed the highest value on cyclohexanone (at 134.09 g L^{-1}). Followed by 1,3,5-trimethylbenzene and 3-ethyltoluene, with values of 28.30 g L^{-1} and 13.52 g L^{-1}, respectively. Nevertheless, the majority of co-formulant concentrations were found below 1 g L^{-1} (Table 3).

In terms of type of formulation, differences among WG, EC, SC and EW could be observed, being EW (IMPALA® STAR) and EC (LATINO) the formulations that contained a greater number of co-formulants, especially seventeen and fifteen, respectively. EC formulation (MASSOCUR 12.5) and EW (TOPAS®) were composed by thirteen and nine different compounds each one, respectively. As displayed in Table 3**,** WG commercial formula (FLINT® MAX) was the one with the lowest number of co-formulants, only four. In spite of EW formulations contained the greater number of co-formulants in comparison with other types of PPPs, higher concentration levels were found in the EC formulation (LATINO).

Among EC formulations, thirteen and fifteen compounds were found in MASSOCUR 12.5 and LATINO, respectively. In relation to MASSOCUR 12.5, this formulation showed values ranged between 0.14 (1,2,3,4-tetramethylbenzene) and 134.09 g L^{-1} (cyclohexanone), while LATINO achieved concentrations between 0.04 (1,2,3,4-tetramethylbenzene) and 218.22 g L^{-1} (cyclohexanone). Nevertheless, as it is shown in Table 3, the concentrations of these co-formulants were rather uneven. It can be observed that both compounds were the co-formulants with the highest and lowest concentrations in both formulations.

Furthermore, significant differences were noted between EW formulations in relation to concentrations, due to indane achieved the maximum value in TOPAS® at 9.73 g L^{-1}, whereas the higher value in IMPALA® STAR was 3.53 g L^{-1}, corresponding to pentamethylbenzene. These differences could be associated with the manufacturer of each pesticide formulation, as it was previously mentioned.

Table 3
Concentration of co-formulants in the tested plant protection products (g compound L^{-1} formulation)

Compound	FLINT® MAX (WG)	MASSOCUR 12.5 (EC)	LATINO (MITRUS, EC)	IMPACT® EVO (SC)	TOPAS® (EW)	IMPALA® STAR (EW)
1,2,3,4-Tetramethylbenzene	NQ	0.14	0.04	ND	ND	0.03
1,2,3,5-Tetramethylbenzene	NQ	1.02	0.06	0.02	0.07	0.08
1,3,5-Trimethylbenzene	ND	28.30	ND	ND	1.91	2.56
1,2-Diethylbenzene	NQ	1.18	0.11	0.03	ND	0.06
1,3-Diethylbenzene	NQ	1.41	0.12	ND	9.52	0.16
1,4- Diethylbenzene	NQ	4.17	0.05	ND	ND	0.06
1-Methyl-2-propylbenzene	NQ	0.43	0.05	ND	ND	0.06
1-Methyl-3-propylbenzene	NQ	1.50	0.06	ND	0.01	0.08
2-Ethyl-p-xylene	NQ	1.10	0.11	0.02	ND	0.06
2-Ethyltoluene	NQ	8.43	0.08	0.06	ND	0.24
2-Methylbiphenyl	0.06	ND	ND	0.21	0.01	0.48
3-Ethyltoluene	NQ	13.52	0.09	0.12	0.05	0.65
3-Methylbiphenyl	ND	ND	2.91	ND	ND	0.90
4-Ethyltoluene	0.08	ND	ND	0.01	ND	ND
Biphenyl	ND	ND	2.23	ND	0.01	0.94
Cyclohexanone	ND	134.09	218.22	ND	ND	ND
Ethylbenzene	0.64	ND	ND	ND	ND	ND
Indane	NQ	4.86	ND	ND	9.73	0.24
Naphthalene	ND	ND	0.25	ND	0.53	1.95
n-Propylbenzene	0.11	ND	ND	ND	ND	ND
Pentamethylbenzene	ND	ND	7.67	ND	ND	3.53

In relation to the investigation carried out by Maldonado et al. [14], in which co-formulants were studied in eleven PPPs (seven EC and four SC), the highest concentrations were achieved for pentamethylbenzene (at 9.63 g L^{-1}) and ethylbenzene (at 4.81 g L^{-1}) in EC and SC formulations, respectively. Nevertheless, in the current research, 1,3,5-trimethylbenzene was found at 28.30 g L^{-1}, and 2-methylbiphenyl at 0.21 g L^{-1}, in EC and SC formulations, respectively, which means a difference of more than 3 times in EC formulations, and almost 4 times in SC formulations. Despite these values, the majority of the other co-formulants were detected below 0.94 g L^{-1} in both studies. These results revealed that there was a clear difference between the amount of co-formulants and the number of them found in each formulation, what allows to be considered as a result of the difference of suppliers used in each study.

In another study, nine co-formulants (benzene or naphthalene derivates) were detected in three EC pesticide formulations, achieving a wide range of concentration for 1,3,5-trimethylbenzene, from 0.01 to 56.31 g L^{-1}, and for 4-ethyltoluene, from 0.01 to 82.75 g L^{-1} [13]. In contrast, in the current research, 1,3,5-trimethylbenzene was only detected in one of the two EC PPPs at 28.30 g L^{-1}, and 4-ethyltoluene was not found in none of EC formulations. As it was reported previously, differences could be explained because PPPs were produced by diverse manufacturers using different conditions, materials and range of quality of the raw materials [22].

Other studies focused on analysis of benzene and toluene present in PPPs revealed toluene residue levels of 0.015-0.035 mg kg^{-1} [7] and concentrations of benzene and toluene in pesticide EC achieved the levels 3.20-16.0 g L^{-1} [17], while in the present study the concentration range of toluene was between 0.01 (in 4-ethyltoluene in IMPACT® EVO) and 13.52 g L^{-1} (in 2-ethyltoluene in MASSOCUR 12.5), and benzene was found at 0.02 (in 2-ethyl-p-xylene in IMPACT® EVO) and 4.17 g L^{-1} (in 1,4-diethylbenzene in MASSOCUR 12.5), observing clear differences between both studies.

Finally, and bearing in mind the information indicated at the Material Safety Data Sheet (MSDS) of the PPPs, a specific analysis of total aromatic hydrocarbons (C8-C13) was also performed to determine this fraction, using a DB-624 column [23].The results are shown in Table S3 and it can be observed that the experimental concentrations are similar to those indicated in the MSDS.

3.4. Toxicity of co-formulants

Toxicity of active substances in PPPs has been investigated for many decades, although it was also reported that co-formulants can be hazardous and cause risks to human health and environment. Furthermore, some volatile co-formulants might contribute to ground-level ozone pollution, which means that adverse effects on environment could be increased [24].

The data of Reference Dose for Oral Exposure (RfDs) in rats has been found for some of the studied co-formulants: for 1,3,5-trimethylbenzene, it was set at 0.01 mg kg^{-1} per day, for naphthalene at 0.02 mg kg^{-1} per day, for ethylbenzene and n-propylbenzene at 0.1 mg kg^{-1} per day, for biphenyl at 0.5 mg kg^{-1} and cyclohexanone at 5 mg kg^{-1} per day [25].

For other compounds, oral rats LD_{50} (Lethal Dose, 50%) was found at 4850 mg kg^{-1} for 4-ethyltoluene, at 5157 mg kg^{-1} for 1,2,3,5-tetramethylbenzene, at 5628 mg kg^{-1} for pentamethylbenzene and at 6408 mg kg^{-1} for 1, 2, 3, 4-tetramethylbenzene. Regarding LD_{Lo} (lethal dose low) in rats, it was established at 5 g kg^{-1} for 2-ethyltoluene, indane and 1,3-diethylbenzene, and the LC_{50} (Concentration Dose, 50%) at 1.5 mg L^{-1} in fat-head minnows for 2-methylbiphenyl [26].

Despite the toxicity data of some co-formulants are still lacking in literature [27], their corresponding oral rats LD_{50} was predicted using the T.E.S.T (Toxicity Estimation Software Tool) software for the following compounds: 3-methylbiphenyl (2653 mg kg^{-1}), 1,2-diethylbenzene (4790 mg kg^{-1}), 1,4-diethylbenzene (4462 mg kg^{-1}), 1-methyl-2-propylbenzene (4812 mg kg^{-1}), 1-methyl-3-propylbenzene (4716 mg kg^{-1}), 2-ethyl-p-xylene (4356 mg kg^{-1}) and 3-ethyltoluene (3868 mg kg^{-1}).

Among co-formulants detected in the analyzed PPP, naphthalene is classified as the most toxic, due to it is a volatile organic compound and exits in the atmosphere in both vapor and particulate phases [28].

Therefore, there is an urgent need to assess environmental and human health risks presented by co-formulants in pesticide formulations.

4. Conclusions

The results of the presented approach indicate that the technique used (based on GC-Q-Orbitrap-MS) is a useful tool to mon-

itor co-formulants contained in PPPs. The suitable combination of both injection methods (DI and HS) allowed the wide identification of volatile compounds in the samples, and 21 compounds were confirmed injecting analytical standards. Suspect and unknown analysis provided enough information to emphasize the high reliability and sensitivity of the proposed methodology. It should be highlighted that five co-formulants (1,3,5-trimethylbenzene, 4-ethyltoluene, cyclohexanone, naphthalene and n-propylbenzene) were common in both techniques (DI and HS).

One of the main challenges was the identification of isomers, especially $C_{10}H_{14}$ that involved eight co-formulants in MASSOCUR 12.5 commercial product. In spite of the fact that same compounds have been analyzed in the same type of formulation, remarkable differences were observed, and this can be explained because different manufacturers were considered.

Finally, the concentration of these co-formulants was calculated, finding values between 0.01 (1-methyl-3-propylbenzene) and 218.22 g L^{-1} (cyclohexanone) in MASSOCUR 12.5 and LATINO commercial products, respectively.

Moreover, the toxicity of each one was studied, finding values relatively high that produce negative impacts on the environment and human health. Thus, further toxicological evaluation of co-formulants is essential for proper environmental and human health risk assessment of pesticide formulations used in agriculture.

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

María Elena Hergueta-Castillo: Formal analysis, Investigation, Validation, Writing – original draft, Visualization. **Rosalía López-Ruiz:** Investigation, Software, Data curation, Methodology, Supervision, Writing – review & editing. **Roberto Romero-González:** Methodology, Data curation, Software, Supervision, Writing – review & editing. **Antonia Garrido Frenich:** Conceptualization, Resources, Writing – review & editing, Funding acquisition, Project administration.

Data Availability

Data will be made available on request.

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Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2022.463588.

References

[1] European Parliament, The use of pesticides in developing countries and their impact on health and the right to food, (2021). https://www.europarl.europa.eu/thinktank/en/document/EXPO_STU(2021)653622.
[2] M. Płonka, S. Walorczyk, M. Miszczyk, Chromatographic methods for the determination of active substances and characterization of their impurities in pesticide formulations, TrAC - Trends Anal. Chem. 85 (2016) 67–80, doi:10.1016/j.trac.2016.03.011.
[3] L. Alder, K. Greulich, K. Günther, V. Bärbel, Residue analysis of 500 high priority pesticides: better by GC–MS or LC–MS/MS? Mass Spectrom. Rev. 25 (2006) 838–865, doi:10.1002/mas.20091.
[4] M.O. Pogăcean, M. Gavrilescu, Plant protection products and their sustainable and environmentally friendly use, Environ. Eng. Manag. J. 8 (2009) 607–627, doi:10.30638/eemj.2009.084.
[5] A. Murawska, P. Migdał, A. Roman, Effects of plant protection products on biochemical markers in honey bees, Agric (2021) 11, doi:10.3390/agriculture11070648.
[6] European Commission, Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC, Off. J. Eur. Union. (2021) 1–84.
[7] Y. Qi, C. Ma, M. Wan, Z. Liu, L. Zheng, F. Jin, M. Jin, Y. She, J. Wang, S. Wang, Multiresidue determination of six pesticide adjuvants in characteristic minor crops using QuEChERS method and gas chromatography-mass spectrometry, ChemistrySelect 4 (2019) 66–70, doi:10.1002/slct.201803328.
[8] E. Pose-Juan, R. Rial-Otero, E. Martínez-Carballo, E. López-Periago, J. Simal-Gándara, Determination of metalaxyl and identification of adjuvants in wettable powder pesticide technical formulas, Anal. Bioanal. Chem. 394 (2009) 1535–1544, doi:10.1007/s00216-009-2633-z.
[9] European 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, Off. J. Eur. Union. (2021) 1–20.
[10] D.K. Hazra, A. Purkait, Role of pesticide formulations for sustainable crop protection and environment management: A review, J. Pharmacogn. Phytochem. 8 (2019) 686–693.
[11] H. Karasali, G. Balayannis, A. Hourdakis, A. Ambrus, Development and single-laboratory validation of a new gas chromatographic multi-pesticide method of analysis of commercial emulsifiable concentrate formulations containing alachlor, chlorpyrifos methyl, fenthion and trifluralin as active ingredients, J. Chromatogr. A. 1129 (2006) 300–303, doi:10.1016/j.chroma.2006.08.040.
[12] J. Siebers, A. Besinger-Riedel, C. Vinke, Determination of active substances, co-formulants and impurities in plant protection products using high performance liquid chromatography and gas chromatography, J. Fur Verbraucherschutz Und Leb. 9 (2014) 137–144, doi:10.1007/s00003-014-0873-0.
[13] R. López-Ruiz, E. Belmonte-Sánchez, R. Romero-González, J.L. Martínez Vidal, A. Garrido Frenich, Identification of adjuvants in plant protection products applying a suspect screening workflow based on orthogonal techniques, Anal. Bioanal. Chem. 412 (2020) 4301–4311, doi:10.1007/s00216-020-02662-0.
[14] A.J. Maldonado-Reina, R. López-Ruiz, A. Garrido Frenich, F.J. Arrebola, R. Romero-González, Co-formulants in plant protection products: An analytical approach to their determination by gas chromatography–high resolution mass accuracy spectrometry, Talanta 234 (2021), doi:10.1016/j.talanta.2021.122641.
[15] I. San Román, M.L. Alonso, L. Bartolomé, R.M. Alonso, R. Fañanás, Analytical strategies based on multiple headspace extraction for the quantitative analysis of aroma components in mushrooms, Talanta 123 (2014) 207–217, doi:10.1016/j.talanta.2014.01.021.
[16] S.X. Zhang, X.S. Chai, D.G. Barnes, Determination of critical micelle concentration of surfactants by headspace gas chromatography, J. Surfactants Deterg. 20 (2017) 1395–1400, doi:10.1007/s11743-017-1983-3.
[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, doi:10.1155/2013/121783.
[18] N.H. Petha, R.S. Lokhande, D.T. Seshadri, R.M. Patil, Determination of residual dimethyl sulfate in hexaconazole technical (fungicide) by head space gas chromatography mass spectrometry, Microchem. J. 133 (2017) 506–509, doi:10.1016/j.microc.2017.04.016.
[19] European Commission, Guidance document on analytical quality control and method validation for pesticide residues analysis in food and feed SANTE 11312/2021, Sante/11312/2021. (2021) 1–57. https://ec.europa.eu/food/system/files/2022-02/pesticides_mrl_guidelines_wrkdoc_2021-11312.pdf.
[20] United States Environmental Protection AgencyToxicity Estimation Software Tool (TEST), 2022 https://www.epa.gov/chemical-research/toxicity-estimation-software-tool-test.
[21] E.L. Schymanski, J. Jeon, R. Gulde, K. Fenner, M. Ruff, H.P. Singer, J. Hollender, Identifying small molecules via high resolution mass spectrometry: Communicating confidence, Environ. Sci. Technol. 48 (2014) 2097–2098, doi:10.1021/es5002105.
[22] A. Ambrus, D.J. Hamilton, H.A. Kuiper, K.D. Racke, Significance of impurities in the safety evaluation of crop protection products (IUPAC technical report), Pure Appl. Chem 75 (2003) 937–973, doi:10.1351/pac200375070937.
[23] AgilentAnalysis of drinking water with the Agilent 8860 GC and 7697A Headspace Sampler, 2019 https://www.agilent.com/cs/library/applications/application-drinking-water-8860-gc-7697a-headspace-5994-1239en-agilent.pdf.
[24] 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, doi:10.1016/j.atmosenv.2011.02.015.
[25] United States Environmental Protection AgencyIRIS Advanced Search, 2016 https://iris.epa.gov/AdvancedSearch/.

[26] National Institutes of Health (NIH)PubChem, 2022 https://pubchem.ncbi.nlm.nih.gov/.
[27] J. Zhang, W. Wang, Z. Pei, J. Wu, R. Yu, Y. Zhang, L. Sun, Y. Gao, Mutagenicity assessment to pesticide adjuvants of toluene, chloroform and trichloroethylene by ames test, Int. J. Environ. Res. Public Health. 18 (2021) 1–13, doi:10.3390/ijerph18158095.
[28] I. Uria-Tellaetxe, M. Navazo, M. de Blas, N. Durana, L. Alonso, J. Iza, Gas-phase naphthalene concentration data recovery in ambient air and its relevance as a tracer of sources of volatile organic compounds, Atmos. Environ. 131 (2016) 279–288, doi:10.1016/j.atmosenv.2016.01.047.