Suspect screening of pesticide co-formulants in fruits, vegetables and leaves by liquid and gas chromatography coupled to high resolution mass accuracy spectrometry: potential impact on human health

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Abstract

Vegetables can contain co-formulants derived from the use of plant protection products (PPPs) in crops. Thus, in the current study co-formulants were determined in different fruits and vegetables and their leaves by gas and liquid chromatography coupled to Q-Orbitrap high-resolution mass spectrometry (GC-Q-Orbitrap and LC-Q-Orbitrap-MS). A total of 37 co-formulants were tentatively identified, and among them, 12 compounds were quantified by LC-Q-Orbitrap-MS and 9 by GC-Q-Orbitrap-MS. The mean co-formulant levels in fruit and vegetable samples was 92% lower than in leaf samples. Selected samples showed a high concentration of 1-ethyl-2-pyrrolidone among the co-formulants detected. This compound ranged from 22 µg/kg (strawberry) to 722 µg/kg (red grape), whereas in the case of leaves, its concentration was up to 6513 µg/kg in cucumber leaf. In addition, it has an LD_{50} equal to 1.440 g/kg. Therefore, this type of PPP co-formulants should be controlled in fruits and vegetables to avoid adverse health effects.

Keywords: Co-formulants; LC-Q-Orbitrap-MS; GC-Q-Orbitrap-MS; suspect analysis; food commodities

1. Introduction

Co-formulants are part of the mixture in the commercial plant protection products (PPPs), and they are employed to improve the efficiency and the stability of active ingredients. These are mainly classified in surfactants, foam inhibitors, solvents and wetting agents, and they may constitute more than 50% of the formulation product (Schaller & Balmer, 2018). Some of these substances have shown a high toxicity and some of them may increase the toxicity of the PPPs (Adler-Flindt & Martin, 2019; Karaca et al., 2021; Zahn et al., 2018). In 2021, the EU Commission established a list of "unacceptable co-formulants", which contains 144 substances that must be banned due to their inherent dangerous properties (Official Journal of the European Union, 2021). This list includes substances such as nonyl-phenols and octyl-phenols, their ethoxylated form, which have properties such as endocrine disruptors, and other solvents derived from petroleum that have carcinogenic properties. In addition, there are certain substances, such as ethoxy and methoxy ethanol, and ethyl and methyl pirrolidin-2-one, which have toxic properties for reproduction (Official Journal of the European Union, 2021). Even so, some co-formulants used in PPPs are unknown as they are not always published in the PPP label.

When PPPs are applied to agricultural crops, the treated plants should be expected to contain toxic substances derived from the co-formulants and active ingredients. There are studies that evaluated the dissipation of selected co-formulants in fruits or vegetables. For instance, one study developed a method for measuring the dissipation of tristyrylphenol ethoxylates in lettuce by liquid chromatography (LC) coupled to single quadrupole (Q) (Li et al., 2020). Another study selected four co-formulants (di-2-ethylhexyl sulfosuccinate, sodium dodecyl sulfate, dimethyl naphthalene sulfonate sodium salt and N,N-dimethyldecanamide), and evaluated their degradation in vegetables and apples under field conditions by LC–tandem mass spectrometry (LC–MS/MS) system (Balmer

et al., 2020). In addition, a recent study investigated co-formulant residues from anionic surfactants and solvents in parsley and oak leaf lettuce in three different cropping systems (Balmer et al., 2023). Nevertheless, there are not studies focused on the identification of the possible co-formulants by non-targeted analysis in fruits and vegetables after harvest. Recent studies have used gas chromatography (GC) and LC coupled to high resolution mass spectrometry (HRMS) to carry out non-targeted analyses (suspect or unknown) as a powerful tool to determine a wide range of co-formulants present in PPPs (Hergueta-Castillo, López-Ruiz, Frenich, et al., 2022; López-Ruiz et al., 2023; Maldonado-Reina et al., 2022). For instance, one study tentatively detected 26 co-formulants by GC-quadrupole (Q)-Orbitrap HRMS by using a suspect screening in 14 PPPs corresponding to several types of formulations (Maldonado-Reina et al., 2021a). These compounds were mainly benzyltoluene, ethyltoluene, methyl and ethyl naphthalene and biphenyl derivatives. Another study identified 9 co-formulants, using both LC and GC techniques coupled to Orbitrap HRMS, in three emulsifiable concentrates applying the suspect screening approach (López-Ruiz et al., 2020). In addition, other studies used the suspect screening strategy, confirming the presence of six co-formulants using standards (Hergueta-Castillo, López-Ruiz, Frenich, et al., 2022) and 12 compounds in PPPs (Maldonado-Reina et al., 2022). Among these co-formulants, the ionic surfactant sodium dodecyl benzene was the most concentrated substance, reaching to 3.23% in an emulsifiable concentrate product (Maldonado-Reina et al., 2022). In addition, non-ionic surfactants, including glyceryl monostearate and monopalmitin, represented to 1.9% and 1.4% in PPPs (Hergueta-Castillo, López-Ruiz, Frenich, et al., 2022). Based on these previous studies that have identified a wide variety of co-formulants in PPPs, there is a lack of studies on detecting them in fruits and vegetables with the aim of determining the amounts adsorbed after their application in crops. Therefore, a more exhaustive study of

the co-formulants derived from PPPs to fruits, vegetables as well as leaves would be needed in order to know the quantities of these substances that can be reached in these samples and evaluate their toxicity. For that reason, this study aims to identify and quantify possible co-formulants in harvested fruit, vegetable and leaf samples. Thus, the application of previous methods used in co-formulant analyses in PPPs based on the use of both GC and LC-HRMS was applied in the indicated matrices. In addition, for the LC methodology, two stationary phases (Shodex and C18) have been used in order to increase the scope of the analysis, considering the physico-chemical characteristics of the co-formulants.

2. Materials and methods

2.1. Chemicals and reagents

Regarding analytical grade standards for LC-MS, sodium dodecyl benzene sulfonate (CRM, 100 %), aniline (≥ 99.5 %), monopalmitin (≥ 99%), glyceryl monostearate and N,N-dimethyldecanamide (99%) were supplied by Sigma Aldrich (St. Louis, MO, USA). 4-sec-butyl-2,6-di-tert-butylphenol (>98.0%), 1-ethyl-2-pyrrolidone and N-lauryldiethanolamine (99%) were acquired from TCI (Zwijndrecht, Belgium). Naphthalene-1-sulfonic acid sodium salt and 2,6-dimethylaniline were supplied by Alfa Aesar (99 %) (Haverhill, Massachusetts, USA), whereas lauramide DEA (≥ 95.0 %) and palmitamide (>95%) were purchased from Fluorochem (Hadfield, United Kingdom). Analytical standards for GC-MS, 1,2,4-trimethylbenzene, 1,3,5-trimethylbenzene, isopropylbenzene, n-butylbenzene, naphthalene, n-propylbenzene, tert-butylbenzene, 2-isopropyltoluene and 4-ethyltoluene, which were acquired from Dr. Ehrenstorfer (Augsburg, Germany) (>95%).

Methanol (LC-MS Chromasolv™, ≥ 99.9 %) purchased from Honeywell (Charlotte, NC, USA), water (LC-MS LiChromasolv®) obtained from Merck (Darmstadt, Germany) and

acetonitrile (LC-MS Chromasolv™, ≥ 99.9 %) supplied by Honeywell, were used. Ammonium acetate, ammonium hydroxide (LC-MS, 99.0 %) and formic acid (LC-MS, 99.0%) were acquired from Fischer Scientific (Waltham, MD, USA). The internal standard, triphenyl phosphate, was purchased from Supelco 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, ≥ 99.8%) obtained from Chem-Lab (Zedelgem, Belgium) was also used. Ethyl acetate (HPLC grade, ≥ 99.8%), obtained from Chem-Lab (Zedelgem, Belgium) and nylon syringe filters Econofltr Nyln 13mm 0.2 μm, purchased from Agilent technologies (Santa Clara, CA, USA), were also used.

2.2. Sample processing

Thirty different samples (fruits, vegetables and leaves) were randomly purchased from local supermarkets (**Table S1**). Samples collected were chosen based on these fruits and vegetables are widely consumed in Spain. The samples are classified in 18 fruits and vegetables, which were chili pepper, cucumber, red grape, tangerine, clementine, strawberry, orange (two samples), cucumber plant, blueberry, papaya, tomato (two samples), chard, raspberry, white grape, raisin and spinach. In addition, 12 leaf samples were from eggplant, pistachio, clementine, tomato (two samples), strawberry, cucumber (two samples), zucchini, cured tobacco, orange, and pepper. The extraction method was the well-known Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) multimethod for pesticide residue analysis (Anastassiades & Lehotay, 2003). This extraction method has been used to analyze 4 target co-formulants in vegetables and apples (Balmer et al., 2020), proving to be effective in the co-formulant recovery with short extraction time. The fruit and vegetable samples were previously crushed, and an aliquot of the homogenate (10 g) was extracted with 10 mL of acetonitrile for 1 minute. In the case of leaf samples, 5 g was extracted with 10 mL of acetonitrile for 1 minute. All samples were

diluted 1:10 and when it was necessary, a higher dilution was carried out. Samples were evaporated and dissolved in ethyl acetate for the GC-MS analysis, whereas for LC-MS, the extract was injected directly. All samples were injected into the LC and GC equipment after being filtered using syringe filters.

2.3. LC-Q-Orbitrap-MS conditions

The LC method was previously developed by our research group, and it was used for the characterization of co-formulants in PPPs (Martín-García, Romero González, Martínez-Vidal, & Garrido Frenich, n.d.). Briefly, Shodex ODP2 HP-2D (2 x 150 mm, 5 μm) (Symta, Madrid, Spain) was the stationary phase used for the separation of co-formulants in the selected samples, and it was composed by a polyhydroxy methacrylate. The mobile phase was an aqueous solution of ammonium hydroxide (0.1%) as aqueous phase (A) and acetonitrile as organic phase (B) respectively. The flow rate was 0.2 mL/min and the injection volume was 10 μL. The gradient conditions were: 20% B from 0 to 5 min, increased up to 90% B from 5 min to 19 min and remained constant for 5 min, decreasing to 20% B for one minute. Therefore, the total running time was 25 min.

In addition, Hypersil GOLD aQ column (100 mm × 2.1 mm, 1.9 μm) was another stationary phase previously used in the detection of co-formulants (Hergueta-Castillo, López-Ruiz, Frenich, et al., 2022; Maldonado-Reina et al., 2022). For that reason, this stationary phase has also been employed to cover other co-formulants or extend the range of co-formulants present in the selected samples (Maldonado-Reina et al., 2022).

The LC equipment employed was a Vanquish Flex Quaternary LC from Thermo Fisher Scientific (Waltham, MA, USA), coupled to a Q Exactive™ Orbitrap (Thermo Fisher Scientific) mass spectrometer. The mass calibration of Q-Orbitrap analyzer was carried out by using a mixture of acetic acid, caffeine, Met-Arg-Phe-Ala-acetate salt and

160 Ultramark 1621 (ProteoMass LTQ/FT-hybrid ESI positive and negative) from Thermo-
161 Fisher.
162 The detection was carried out using an HRMS analyzer (Q-Exactive Orbitrap, Thermo
163 Fisher Scientific, Bremen, Germany) with a heated electrospray interface (ESI; HESI-II,
164 Thermo Fisher Scientific, USA) in positive and negative ionization mode. ESI conditions
165 were: capillary temperature (300 ºC), heater temperature (305 ºC), sheath gas (N_2, 95%),
166 35 (arbitrary units), auxiliary gas (N_2, 95%), 10 (arbitrary units), spray voltage (4 kV) and
167 S-lens radio frequency (RF) level, 50 (arbitrary units). Full-scan MS was selected to
168 acquire the total ion chromatogram (TIC). In addition, fragment ions were obtained by
169 data-dependent acquisition ($ddMS^2$). Full Scan MS data was acquired in the *m/z* range
170 from 90 to 1300, at a resolution of 70,000 full width at half-maximum (FWHM) at *m/z*
171 200, and an AGC target of 10^6; $ddMS^2$ was performed with a resolution of 35,000 FWHM
172 at *m/z* 200 and an AGC target value of 10^5, loop count 5 and an isolation window of *m/z*
173 5.0. The software Xcalibur Sequence Setup was used to collect all the data.
174 **2.4. GC-Q-Orbitrap MS conditions**
175 Co-formulants were also analyzed in a Trace 1310 GC system with a TriPlus RSH
176 autosampler (Thermo Scientific) coupled to a Q-Exactive Orbitrap mass analyzer
177 (Thermo Fisher Scientific, USA). The method employed for the analysis of co-formulants
178 was previously developed for the characterization of these compounds in PPPs
179 (Maldonado-Reina et al., 2021b). The column used was a nonpolar stationary phase
180 Varian VF-5ms (30 m × 0.25 mm; 0.25µm), from Agilent Technologies (Santa Clara, CA,
181 USA). The injector temperature was 280 °C, and 1 µL was the volume injected by splitless
182 mode (split flow of 50 mL/min), using a splitless time of 2 min. The temperature program
183 of the column was as follows: initial temperature was set at 40 °C and it was held for 1
184 min; then it was increased at 15 °C/min to 300 ºC. Finally, it was remained for 7 min at

300 °C. The total running time was 25.3 min. The carrier gas was ultra-high purity helium (99.9999%) with a constant flow rate of 1 mL/min.

The detection was carried out using an HRMS analyzer, using electron ionization (EI) at 70 eV, and data acquisition was performed at both full scan mode and dd-MS^2. Full scan MS was performed with a resolution power set at 60,000 FWHM at *m/z* 200, and an AGC target of 10^6, from 50 to 500 *m/z* regarding dd-MS^2, resolution was 30,000 FWHM at *m/z* 200 and AGC target value was set at $1e^5$. Ion source and MS transfer line temperatures were set at 250°C.

2.5. Data treatment

TraceFinder™ version 4.0 (Thermo Fisher Scientific) was employed for the identification of co-formulants by suspect screening analysis. The acquired chromatograms were processed using Xcalibur version 3.0, employing Qual Browser and Quan Browser. Mass Frontier 8.0 (Thermo Fisher Scientific, Les Ulis, France) was used for in-silico fragmentation.

2.5.1. Identification of co-formulants by GC and LC-Q-Orbitrap-MS

Data obtained from LC-Q-Orbitrap-MS and GC-Q-Orbitrap-MS were processed with TraceFinder™ software. Regarding the LC-Q-Orbitrap-MS, the raw files were processed by using an extensive co-formulant homemade database of 264 compounds obtained from previous studies (Hergueta-Castillo, López-Ruiz, Frenich, et al., 2022; Maldonado-Reina et al., 2022) and Data collection on co-formulants in the context of the EFSA peer review (EFSA, 2022) and Regulation (EU) 2021/383 (Official Journal of the European Union, 2021). In the case of GC-Q-Orbitrap-MS, the analysis by direct injection were processed with TraceFinder™ software using an in-house database with 97 compounds (Maldonado-Reina et al., 2021b). 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 when they are known. The criteria that lead to a tentative identification of compounds were mass error lower than 5 ppm for characteristic ions, mass error lower than 5 ppm for fragment ions, when these fragments are found, 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 for fragment ions. Moreover, full-scan data of each sample was carefully studied with Xcalibur Qual Browser to monitor the spectra of the detected compounds.

2.5.2. Quantification of co-formulants by GC and LC-Q-Orbitrap-MS

The quantification of co-formulants was carried out by using the Thermo Xcalibur Processing Setup window to create a processing method. For that purpose, the Quan view of the Processing Setup window was used to set up the information for the target components in an analysis corresponding to a mixture of standards at 100 µg/L. This information included the name, retention time and *m/z* of the precursor ion. After a processing method was created, it was added to the sequence used to acquire the data set and the data system created a result file for each raw file. Then, Quan Browser was used for the integration of each compound, analysing samples and standards.

2.6. Multivariate data analysis

The data were analysed by means of principal component analysis (PCA) to determine the systematic variation and underlying relationships between co-formulants in the fruits and vegetables with leaves. The quantification data of all co-formulants was imported to SIMCA® version 17 software (Sartorius, Umeå, Sweden) for unsupervised statistical analysis PCA.

3. Results ad discussion

3.1. Identification of co-formulants in samples of vegetal origin

3.1.1. LC-Q-Orbitrap-MS analysis

For the separation of LC-amenable compounds, two columns were used, one based on a stationary phase of polyhydroxy methacrylate (Shodex) and a conventional C18 reverse phase. The Shodex-based approach was originally developed for the purpose of identifying co-formulants that were not separated by conventional reversed phase, C18. A total of 28 co-formulants were tentatively detected by suspect screening analysis when the two stationary phases (Shodex and C18) were used (**Table 1**). The Schymanski criteria, which are based on setting several levels of confidence, were used during the identification process (Schymanski et al., 2014). Nevertheless, the MS/MS could be uninformative, contain interferences or not even exist. Therefore, the co-formulants identified in the samples were classified into the following levels: 8 belong to the level 2, 3 compounds to the level 3, 4 compounds were identified at level 4, whereas the rest were confirmed with their standards (12 compounds) (level 1).

The co-formulants detected are mainly classified as anionic surfactants, including alkyl benzene sulfonates (dodecylbenzenesulfonic acid, 4-undecylbenzenesulfonic acid and 4-decylbenzenesulfonic acid) and alkyl sulfonates (1-naphtalenesulfonic acid and sodium decyl sulfate) (**Table 1**). Besides, non-ionic surfactants, such as alkyl phenols and ethoxylates (2-[2-[4-(1,1,3,3-tetramethylbutyl)phenoxy]ethoxy]-ethanol and 2-(4-nonylphenoxyethanol)), triethylene glycol alkyl decyl ethers, fatty amides (palmitamide, lauramide DEA and cocamide monoethanolamide), amines (triethanolamine, 2,6-dimethylaniline, aniline and N-lauryldiethanolamine), alkyl alcohols (3,6,9,12-tetraoxapentacosan-1-ol), amphoteric surfactant (cocamidepropyl betaine) and aprotic solvents (ethyl-pirrolidin-2-one and N,N-dimethyldecanamide) were also identified in the

samples (**Table 1**). Other non-ionic co-formulants, such as butyl linoleate, monopalmitin glyceryl monostearate and 4-sec-butyl-2,6-di-tert-butylphenol, were also detected in the vegetable samples. **Table 1** shows the typical parameters found by the two stationary phases (Shodex and C18) when suspect analysis was applied. All of these tentative compounds were previously detected in PPPs by using C18 and Shodex columns (Maldonado-Reina et al., 2022; Martín-García et al., n.d.). Comparing the compounds found with the two stationary phases, it is important to note that 5 out of the total 28 compounds (1-ethyl-2-pyrrolidone, triethanolamine, laureth-2 sulfate, cocamide monoethanolamide, and 2-(4-nonylphenoxyethanol)) were only detected when the Shodex column was used, whereas the others could be detected using either Shodex or C18 (Maldonado-Reina et al., 2022). This fact could be explained because Shodex column is more suitable for the separation of polar compounds. Besides, glyceryl monostearate and monopalmitin were only detected using the C18 stationary phase. These results are in concordance with the previous study, where these substances where not found using Shodex stationary phase (Martín-García et al., n.d.).

The component most frequently found in the fruit, vegetable, and leaf samples was ethylpirrolidin-2-one (**Table S2**). 1-Ethyl-2-pyrrolidone at *m/z* 114.0913 $[M+H]^+$ possess an abundant fragment at *m/z* 112.0757 ($C_6H_{10}NO^+$) and **Figure 1** shows its confirmation. In the **Figure 1a,** the extracted ion chromatogram (EIC) of 1-ethyl-2-pyrrolidone in clementine sample is shown. This compound was confirmed by the analytical standard (**Figure 1b**), and by matching the full Scan MS spectrum obtained at *m/z* 114.0913 from the sample (**Figure 1c**) with its standard and theorical one (**Figure 1d and 1e**). This substance had been previously identified in 8 PPPs (Martín-García et al., n.d.), so it is commonly used in PPPs.

A common fragment at *m/z* 79.9574 was found for the anionic surfactants, including dodecylbenzene sulfonic acid, 4-undecylbenzenesulfonic acid, naphthalenesulfonic acid and 4-decylbenzenesulfonic acid, which corresponded with radical sulfate anion ($SO_3^{\cdot-}$) (Pawlak & Wojciechowski, 2021). In addition, a common fragment at *m/z* 183.0121 ($C_8H_7O_3S^-$) was found for the alkylbenzene sulfonates (dodecylbenzenesulfonic acid, 4-undecylbenzenesulfonic acid, and 4-decylbenzenesulfonic acid), which corresponded with the ethylene substituted benzenesulfonate ion (Andreu et al., 2004). Among these anionic surfactants, it should be noted that dodecylbenzenesulfonic acid was identified in a greater number of samples. This result agrees with previous studies that usually identified this co-formulant in PPPs (Maldonado-Reina et al., 2022; Martín-García et al., n.d.). Glyceryl monostearate was commonly detected in most samples, except for the cucumber in its fruit and leaf, and in leaves of strawberry, tomato and blueberry and orange, and some fruit samples including red grape, tomato, and raspberry (**Table S2**). This possess an abundant fragment ion at *m/z* 341.3050 ($C_{21}H_{41}O_3^+$), which is derived from the loss of hydroxyl group. This compound was also previously identified in 13 PPPs (Maldonado-Reina et al., 2022) and in 6 PPPs (Hergueta-Castillo, López-Ruiz, Frenich, et al., 2022) respectively (**Table 1**). Finally, lauramide DEA (N,N-Bis(2-hydroxyethyl)dodecanamide) was also detected in most of the samples, except in clementine, clementine leaf and cured tobacco (**Table S2**). This compound was identified at *m/z* 288.2533 and the most abundant fragment ion at *m/z* 106.08626 ($C_4H_{12}O_2N^+$) corresponded with N,N-bis(2-hydroxyethyl)amine obtained from the breakage of amide C-N bonds. This component was previously detected in the Altacor PPP (Maldonado-Reina et al., 2022) and in other 12 PPPs (Martín-García et al., n.d.).

3.1.2. GC-Q-Orbitrap-MS

A total of 9 co-formulants were identified by GC-Q-Orbitrap-MS, and six were benzene derivatives, including isopropylbenzene, propylbenzene, 1,3,5-trimethylbenzene, 1,2,4-trimethylbenzene, tert-butylbenzene and n-butylbenzene. The remaining three detected compounds corresponded with naphthalene, 4-ethyltoluene and isopropyltoluene **(Table 2)** (Maldonado-Reina et al., 2021a). These co-formulants were previously detected in PPPs (Hergueta-Castillo, López-Ruiz, Romero-González, et al., 2022; Maldonado-Reina et al., 2021b). Naphthalene and 1,2,4-trimethylbenzene were the most frequently detected co-formulants, being found in all the assayed samples. The identification parameters of these compounds are shown in **Table 2**. The mass error of the characteristic ions was lower than 5 ppm in all cases. Two fragment ions were acquired for each one of the tentatively detected co-formulants, with mass error lower than 5 ppm and matching with those provided by NIST database. Furthermore, the NIST database made it possible to compare the ratios of molecular and fragment ions and choose those that were most similar according to a match factor. Results showed that propylbenzene, 4-ethyltoluene, and sec-butylbenzene all shared the same fragment ions at *m/z* 91.05422, which corresponded to the loss of the ethyl group bonded to the benzene, and *m/z* 105.06983, which was produced by ethyl ethylene's loss of the methyl group (Hergueta-Castillo, López-Ruiz, Romero-González, et al., 2022).

Due to the fact that some of the candidates had the same formula, the presence of isomers in the samples could be considered, meaning that they had identical features, such as theoretical mass and peaks, but they could have different retention time. For instance, 1,3,5-trimethylbenzene and 1,2,4-trimethylbenzene were isomers, thus, both included the same fragment ion with the molecular weight at *m/z* 105.0698, but they have different retention time (5.79 and 6.06 min). Another example is 1,3,5-trimethylbenzene and 4-ethytoluene that possessed a similar retention time (5.70 and 5.79 min). For that reason,

their identification must be confirmed by the use of standards. Therefore, commercially analytical standards of co-formulants were acquired to confirm their presence in the samples. By comparing experimental MS spectra and retention times with MS spectra of each analytical standard, satisfactory confirmation was accomplished. Nine suggested chemicals were purchased and injected by DI achieving the retention time shown in **Table 2**. All of them were satisfactorily confirmed in the analyzed samples. Therefore, these compounds belong to the level 1 of confidence (Schymanski et al., 2014).

Figure 2 shows one of the confirmed co-formulants using analytical standards. In **Figure 2a** the EIC of 2-isopropyltoluene in clementine leaf at 6.40 min is shown. This compound was confirmed by the EIC of the analytical standard (**Figure 2b**), and by matching the full Scan MS experimental spectrum acquired (**Figure 2c**) with the theoretical one obtained from the NIST database (**Figure 2d**)

3.2. Quantification of co-formulants in samples of vegetal origin

3.2.1. Quantification by LC-Q-Orbitrap-MS analysis

Table S3 lists the analytical parameters of the method used, including calibration curves, determination coefficients and limit of quantification (LOQ). A quantification of the confirmed co-formulants in samples was carried out by the calibration curves of each standard, and these were prepared in acetonitrile in a concentration range from LOQ to 100 μg/L. No matrix effect was appreciated ($< |\pm 20|$) and this could be explained because the dilution of the extract prior injection. The selection of co-formulants for quantification in the target samples was based on those co-formulants that were detected in most of the samples as well as previously detected in PPPs. Most of co-formulants were quantified by the method that used the Shodex stationary phase approach. However, the C18 column was used for the quantification of the glyceryl monostearate and monopalmitin compounds due to they were not identified with the Shodex column. Triphenyl phosphate

was used as internal standard at 50 µg/L in solvent and the calibration curves were carried out by using the ratio of the area of the analyte standard/area of internal standard against the concentration of each standard. All calibration curves showed good linearity and the determination coefficients were higher than 0.991. The instrumental LOQ was the lowest concentration of each compound that was possible to determine in the samples after diluton. The LOQ was assessed by reference points in the solvent at low concentrations, choosing as the LOQ the concentration that achieves acceptable results in terms of precision and linearity.

Table S4 shows the concentrations of co-formulants found in each sample, and the sum of quantified co-formulants ranged from 58 µg/kg in blueberry to 98379 µg/kg in tomato leaf 1. Amon them, 1-ethyl-2-pyrrolidone was one of the most concentrated, whose content represented more than 58% of the total amount of the co-formulants quantified by LC-Q-Orbitrap-MS in fruits. **Table 3** shows the minimum, maximum and mean content of co-formulants in the samples. 1-Ethyl-2-pyrrolidinone ranged from 22 µg/kg in strawberry to 6513 µg/kg in cucumber leaf (**Table S4**). Comparing the content of this compound in fruits and vegetables with the leaves, the mean concentration of 1-ethyl-2-pyrrolidinone in fruits and vegetables was 426 µg/kg, which was 88% lower than the mean value obtained in leaves (3642 µg/kg) (**Table 3**). This substance had been previously detected by our research group in 8 PPPs by using the Shodex column, being one of the most concentrated, whose content ranged from 1 to 113 mg/L (Martín-García et al., n.d.).

Regarding the most concentrated co-formulants in fruits and vegetables, glyceryl monostearate was the most concentrated one in orange 1, whose content was 90% lower than the obtained in clementine leaf (33226 µg/kg) (**Table 3**). This substance was previously detected in six PPPs at concentrations between 1.78 to 19 g/L (Hergueta-

Castillo, López-Ruiz, Frenich, et al., 2022). Furthermore, in tomato 2 and raspberry, the most concentrated co-formulant was 4-sec-butyl-2,6-di-tert-butylphenol (1637 and 1688 µg/kg), whose content was 92% lower in comparison with tomato leaf 1 (**Table S4**).

In the case of leaf samples, in tomato leaf 1 the most concentrated co-formulant was N-lauryldiethanolamine (56143 µg/kg), and this content was 99% higher than the maximum obtained in vegetables and fruits (783 µg/kg). Therefore, according to the results, it could be indicated that the co-formulant levels in leaf samples are higher than the obtained in fruits and vegetables. This fact could be explained due to the large leaf surface areas. Thus, leafy vegetables with high leaf area-to-leaf weight ratios tend to accumulate higher levels of pesticide residue than fruits or non-leafy vegetables (Noh et al., 2019), so co-formulants should have the same behavior. In addition, a small quantity of residual pesticides may be absorbed into the fruit flesh. In leaf samples, pesticide residue content was found to be higher in outer leaves compared to in inner ones (Bajwa & Sandhu, 2014). Considering that the pulp constitutes the largest portion of the entire fruit and vegetables it is expected that the total residue content in whole fruits and vegetables would be considerably lower than in leaf samples.

According to previous studies, the most concentrated co-formulants obtained in the vegetable, fruit and leaf samples coincided with the most concentrated compounds in PPPs (Hergueta-Castillo, López-Ruiz, Frenich, et al., 2022).

There is a recent study that evaluated co-formulants from PPPs monitored in parsley and lettuce crops (Balmer et al., 2023). These PPPs are mainly composed by co-formulants (N,N-dimethyldecanamide, octyl-pirrolidin-2-one, sodium dodecyl sulfate, docusate, linear alkylbenzene sulfonates). Among them, N,N-dimethyldecanamide and alkyl benzenesulfonates were also determined in the present study. Concentrations for linear alkylbenzene sulfonates in the day 0 were 2.3-4.5 mg/kg, which was similar than the mean

obtained in leaves (1.35 mg/kg). Nevertheless, the mean value obtained in vegetables and fruits (0.125 mg/kg) was lower than that reported by Balmer et al. Regarding N,N-dimethyldecanamide, it was reported an initial quantity of 3.8 and 20 mg/kg in parsley and lettuce (Balmer et al., 2023), but this co-formulant was only detected in leaves in a low quantity in tomato leaf 1 and 2 (0.036 and 0.01 mg/kg).

3.2.2. Quantification by GC-Q-Orbitrap-MS analysis

Nine compounds were confirmed and quantified with their standards using their calibration curves (**Table S5**), showing their concentrations in **Table S6**. Instrumental LOQ was from 1 to 4 µg/L, and linear range was from LOQ to 100 µg/L. Among them, the sum of co-formulants in fruit and vegetable samples was from 13 µg/kg in raisin to 379 µg/kg in orange 1, whereas in leaves, the sum was from 21 µg/kg in cucumber leaf 1 to 539 µg/kg in pistachio leaf. Among these co-formulants, 1,2,4-trimethylbenzene and naphthalene were quantified in all samples of the study. In the case of 1,2,4-trimethylbenzene, its mean content in fruits and vegetables was 45% lower than the mean content in the leaves (**Table 3**). In addition, the mean content of naphthalene in vegetables and fruits was 52% lower than the mean concentration in leaves (**Table 3**). These co-formulants detected in vegetables and fruit samples by GC-MS were also detected in PPPs (Maldonado-Reina et al., 2021a).

4-Tertbutylbenzene was one of the most concentrated co-formulant, founding in orange 1 the highest concentration (182 µg/kg). This compound was also one of the most concentrated in PPPs, and its content in orange 1 represented around 0.05-0.6 % of the reported in PPPs (0.03-0.36 g/L) (Maldonado-Reina et al., 2021a). 2-Isopropyltoluene was also another abundant co-formulant, reaching concentrations up to 179 µg/kg in fruits (orange 1) and 316 µg/kg in pistachio leaf. This co-formulant was also detected at high concentrations (0.09-0.41 g/L) in PPPs (Maldonado-Reina et al., 2021a).

Comparing the results of co-formulants with the obtained in the literature, Balmer et al. reported the quantification of 1- and 2-methylnaphthalene in parsley and lettuce, which were analysed by GC-MS/MS (Balmer et al., 2023). The content of these co-formulants were 0.043-0.12 mg/kg in parsley, whereas in lettuce the concentration was lower than the LOQ. This quantity was similar that the content of naphthalene in fruit, vegetable, and leaf samples. Another investigation determined the naphthalene content in leaves of cabbage and of rose/hibiscus, and its content was 1.71 µg/kg and 1.95 µg/kg respectively (Mohammed et al., 2019). This concentration was lower than the mean value obtained in the leaves in the present study (15 µg/kg).

3.3. Principal Component Analysis (PCA) of the co-formulants content in samples

Principal component analysis (PCA) is one of the most common multivariate data analyses, and most widely used. It is a method for lowering the dimensionality of such datasets, improving interpretability while minimizing information loss (Jolliffe et al., 2016). For that reason, principal component analysis (PCA) was applied to better understand the trends and relationships between the variables of different samples.

An overview of the concentrations of co-formulants obtained by LC and GC in the samples classified in two types (fruits and vegetables and leaves) is shown in **Figure S1**. Firstly, the PCA was carried out by taking into account all samples. Then, the samples clementine leaf and tomato leaf 1 were exclude of the model because these were outliers due to these values were outside the usual range of a particular variable**.** Therefore, a total of 28 samples were included to the PCA (**Figure S1**). PCA contributed to a further profiling of the accessions considered, and it was applied to the data set containing the concentrations of the 21 co-formulants. Two components were chosen based on the variability, where the first and second principal components (PC1 x PC2) described 67.8% and 27% of the analysis data variability for the samples under study. The types of

458 samples fruits and vegetables and the leaves were well-separated and only one overlap
459 was found between cured tobacco and fruit and vegetables. This could be due to the fact
460 that the PPP doses applied to the tobacco plant may be lower than those applied to other
461 types of crops since the tobacco leaf is a consumer product. On the other hand, the rest of
462 leaves, from the fruits and vegetables under study, are not consumed and it may be that
463 the doses used are higher than the employed in tobacco since the concentrations of
464 pesticide residues from adsorption to the fruits are lower than in the leaves.

465 **3.4. Toxicity**

466 To assess whether these chemical substances have an impact on human health,
467 toxicological data on co-formulants quantified in fruit, vegetables, and their leaves are
468 needed. The oral reference dose (RfD) of co-formulants quantified in the samples under
469 study is shown in **Table 4**. Regarding the co-formulants analysed by LC-Q-Orbitrap-MS,
470 alkylbenzene sulfonates possess an RfD value of 0.5 mg/kg/day. This compound has been
471 reached up to 7.94 mg/kg in clementine leaf. Therefore, this dose per day is 94% higher
472 in comparison with the RfD. Nevertheless, in the case of vegetables and fruits, the mean
473 dose of dodecylbenzenesulfonic acid was 0.125 mg/kg, which is 75% lower than its RfD
474 value. Consequently, the consumption of this substance at these doses would not pose a
475 health risk. Moreover, N,N-dimethylaniline is more toxic than dodecylbenzenesulfonic
476 acid RfD (0.002 mg/kg/day). Despite this, N,N-dimethylaniline has been found in the
477 samples at mean quantities of 0.025 mg/kg in fruits and vegetables and 0.16 mg/kg in
478 leaves (**Table S4**). Therefore, with the ingestion of vegetables and fruits in amounts larger
479 than 100 g, this substance may pose a health concern. In addition, the most toxic
480 substances detected by GC-Orbitrap-MS, in accordance with their RfD values, were
481 1,2,4-trimethylbenzene and 1,3,5- trimethylbenzene, which possess a RfD of 0.01
482 mg/kg/day. In the case of 1,2,4-trimethylbenzene, its mean concentration in fruits and

vegetables was 0.005 mg/kg, which was below its RfD. Naphthalene possess an RfD of 0.02 mg/kg/day and the RfD for tert-butylbenzene, isopropylbenzene, isopropyltoluene, n-butylbenzene were 0.1 mg/kg/day (*United States Environmental Protection Agency IRIS Advanced Search*, 2023). The amounts of these substances were only slightly greater than RfD in leaves, but as leaves are not consumed, eating fruits and vegetables containing the levels found would not be harmful to the health (**Table S6**). Finally, for 1-ethyl-2-pyrrolidone, lauramide DEA, and N,N-dimethyldecanamide, no information regarding their RfD was discovered.

Additionally, the predicted oral LD_{50} values, obtained by Toxicity Estimation Software Tool (T.E.S.T), are shown in **Table 4**. Among them, aniline was the most toxic compound with a low value of LD_{50} (0.372 g/kg), followed by N,N-dimethylaniline, dodecylbenzenesulfonic acid, naphthalene and 1-ethyl-2-pyrrolidone (LD_{50} of 0.78, 1.412 and 1.440 g/kg).

Toxic hazard estimation via decision tree approach (Toxtree), in accordance with Cramer criteria, was also used to assess the toxicity of co-formulants (**Table 4)**. According to this method, organic substances are divided into one of three classes (I, low, II, and high, or Cramer classes), each of which explicitly reflected the probability of low, moderate, and high toxicity (Roberts et al., 2015). It should be noted that active ingredients in PPPs belong to the class III due to their high toxicity. Aniline, naphthalenesulfonic acid, 1-ethyl-2-pyrrolidone, N,N-dimethyldecanamide, lauramide DEA and naphthalene had also a high toxicity (class III). Therefore, it is critical to control the concentration of these co-formulants in PPPs to avoid their dispersion in fruit and vegetable samples, which may have a detrimental effect on people's health. Aniline, 2-pyrrolidone and naphthalene were actually declared to be unsuitable co-formulants for inclusion in PPP by the Commission's Regulation (EU) 2021/383 of March 3, 2021, because they are carcinogenic and

hazardous to reproduction (Spanish Ministry of Health, 2021). Therefore, it would also be interesting to analyze these compounds in fruits and vegetables.

4. Conclusion

The use of analytical methodologies based on LC-HRMS and GC-HRMS has shown to be effective for the tentative identification of a total 37 co-formulants in fruits, vegetables and leaves. Nine of them were identified by GC-HRMS, whereas 28 were detected by LC-HRMS. Regarding the compounds detected by GC-HRMS, these were mainly benzene derivatives and other co-formulants, such as naphthalene, 4-ethyltoluene and isopropyltouene. In addition, the co-formulants detected by LC-HRMS using two stationary phases were mainly anionic surfactants, including alkylbenzene sulfonates and alkylsulfonates, and non-ionic surfactant among other non-ionic co-formulants.

Furthermore, 21 compounds of the 37 identified were confirmed by standards, which were quantified by LC or GC with HRMS. The mean levels of co-formulants in leaves was 92% higher than in fruit and vegetable samples. Therefore, PCA showed a clear separation between fruits and vegetables with leaves based on the high concentrations of co-formulants in leaves, except for cured tobacco. According to the toxicity of substances, ethylpirrolin-2-one, aniline and naphthalene possessed a high toxicity and a LD_{50} lower than 1.440 g/kg. One of the most concentrated co-formulants was 1-ethyl-2-pyrrolidone, which was found in all targeted samples and had concentrations up to 722 µg/kg in fruits and vegetables and 6513 µg/kg in leaves. As a result, this method could be applicable for the subsequent study of the possible residues and degradation products in vegetable or fruit samples derived from these types of co-formulants.

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Conflict of Interest

Authors declare no conflict of interest.

References

Adler-Flindt, S., & Martin, S. (2019). Comparative cytotoxicity of plant protection products and their active ingredients. *Toxicology in Vitro*, *54*(July 2018), 354–366. https://doi.org/10.1016/j.tiv.2018.10.020

Anastassiades, M., & Lehotay, S. J. (2003). Fast and Easy Multiresidue Method Employing Acetonitrile Extraction/Partitioning and "Dispersive Solid-Phase Extraction" for the Determination of Pesticide Residues in Produce. *Journal of AOAC International*, *86*, 412–431.

Andreu, V., Pico, Y., & Vale, U. De. (2004). *Determination of Linear Alkylbenzenesulfonates and Their Degradation Products in Soils by Liquid Chromatography-Electrospray-Ion Trap Multiple-Stage Mass Spectrometry*. *251*(6), 2878–2885.

Bajwa, U., & Sandhu, K. S. (2014). Effect of handling and processing on pesticide residues in food- A review. In *Journal of Food Science and Technology* (Vol. 51, Issue 2, pp. 201–220). Springer. https://doi.org/10.1007/s13197-011-0499-5

Balmer, M. E., Janser, D., Schaller, U., Krauss, J., Geiser, H. C., & Poiger, T. (2023). Residues of pesticide co-formulants in lettuce and parsley : Identification of decline processes using field trials in different cropping systems. *Pest Management Science*. https://doi.org/10.1002/ps.7455

Balmer, M. E., Janser, D., Schaller, U., Krauss, J., Geiserc, H. C., & Poiger, T. (2020). Magnitude and decline of pesticide co-formulant residues in vegetables and fruits: Results from field trials compared to estimated values. *Pest Management Science* , *77*, 1187–1196.

EFSA. (2022). *Data collection on co-formulants used in representative plant protection product formulations in the context of the EFSA peer review process for approval/renewal of approval of active substances*. https://doi.org/10.2903/sp.efsa.2022.EN-7547 Data

Hergueta-Castillo, M. E., López-Ruiz, R., Frenich, A. G., & Romero-González, R. (2022). Characterization of the composition of plant protection products in different formulation types employing suspect screening and unkown approaches. *Journal of The Science of Food and Agriculture*. https://doi.org/10.1002/jsfa.11952

Hergueta-Castillo, M. E., López-Ruiz, R., Romero-González, R., & Garrido Frenich, A. (2022). Non-targeted analysis of co-formulants in antifungal pesticide formulations by gas chromatography-tandem high resolution mass spectrometry. *Journal of Chromatography A*, *1685*. https://doi.org/10.1016/j.chroma.2022.463588

Jolliffe, I. T., Cadima, J., & Cadima, J. (2016). Principal component analysis: a review and recent developments. *Philos Trans A Math Phys Eng Sci*, *374*.

Karaca, M., Fischer, B. C., Willenbockel, C. T., Tralau, T., Marx-Stoelting, P., & Bloch, D. (2021). Effects of co-formulants on the absorption and secretion of active substances in plant protection products in vitro. *Archives of Toxicology*, *95*(10), 3205–3221. https://doi.org/10.1007/s00204-021-03140-x

Li, C., Zhang, P., He, Q., Shao, H., Zheng, L., Wang, J., & Jin, F. (2020). Dissipation Profiles of Tristyrylphenol Ethoxylate Homologs in Lettuce under Greenhouse and Field Conditions.

582 *Journal Agricultural and Food Chemistry*, *68*, 1507–1513.
583 https://doi.org/10.1021/acs.jafc.9b03678

584 López-Ruiz, R., Belmonte-Sánchez, E., Romero-González, R., Martínez Vidal, J. L., & Garrido
585 Frenich, A. (2020). Identification of adjuvants in plant protection products applying a
586 suspect screening workflow based on orthogonal techniques. *Analytical and Bioanalytical*
587 *Chemistry*, *412*(18), 4301–4311. https://doi.org/10.1007/s00216-020-02662-0

588 López-Ruiz, R., Maldonado-Reina, A. J., Marín-Sáez, J., Romero-González, R., Martínez-Vidal, J.
589 L., & Garrido Frenich, A. (2023). Unravelling plant protection product analysis: Use of
590 chromatography techniques (GC and LC) and high resolution mass spectrometry. *Trends*
591 *in Environmental Analytical Chemistry*, *37*. https://doi.org/10.1016/j.teac.2022.e00191

592 Maldonado-Reina, A. J., López-Ruiz, R., Garrido Frenich, A., Arrebola, F. J., & Romero-González,
593 R. (2021a). Co-formulants in plant protection products: An analytical approach to their
594 determination by gas chromatography–high resolution mass accuracy spectrometry.
595 *Talanta*, *234*, 122641. https://doi.org/10.1016/j.talanta.2021.122641

596 Maldonado-Reina, A. J., López-Ruiz, R., Garrido Frenich, A., Arrebola, F. J., & Romero-González,
597 R. (2021b). Co-formulants in plant protection products: An analytical approach to their
598 determination by gas chromatography–high resolution mass accuracy spectrometry.
599 *Talanta*, *234*, 122641. https://doi.org/10.1016/j.talanta.2021.122641

600 Maldonado-Reina, A. J., López-Ruiz, R., Romero-González, R., Martínez Vidal, J. L., & Garrido-
601 Frenich, A. (2022). Assessment of Co-Formulants in Marketed Plant Protection Products
602 by LC-Q-Orbitrap-MS: Application of a Hybrid Data Treatment Strategy Combining
603 Suspect Screening and Unknown Analysis. *Journal of Agricultural and Food Chemistry*, *70*,
604 7302–7313. https://doi.org/10.1021/acs.jafc.2c01152

605 Martín-García, B., Romero González, R., Martínez-Vidal, J. L., & Garrido Frenich, A. (n.d.).
606 Unveiling co-formulants in plant protection products by LC-HRMS using a polyhydroxy
607 methacrylate stationary phase. Under reviewing process. *Journal of Agricultural and Food*
608 *Chemistry*.

609 Mohammed, S., Obiri, S., Ansa-Asare, O. D., Dartey, G., Kuddy, R., & Appiah, S. (2019).
610 Assessment of concentration of polycyclic aromatic hydrocarbons (PAHs) in vegetables
611 from farms in Accra, Ghana. *Environ Monit Assess*, *191*.

612 Noh, H. H., Lee, J. Y., Park, H. K., Lee, J. W., Jo, S. H., Lim, J. B., Shin, G., Kwon, H., & Id, K. S. K.
613 (2019). Dissipation, persistence, and risk assessment of fluxapyroxad and penthiopyrad
614 residues in perilla leaf (Perilla frutescens var. japonica Hara). *Plos One*, 1–15.

615 Official Journal of the European Union. (2021). *Commission regulation (EU) 2021/383 of 3*
616 *March 2021 amending Annex III to Regulation (EC) No 1107/2009 of the European*
617 *Parliament and of the Council listing co-formulants which are not accepted for inclusion in*
618 *plant protection products*. *74*.

619 Pawlak, K., & Wojciechowski, K. (2021). Precursor ion approach for simultaneous
620 determination of nonethoxylated and ethoxylated alkylsulfate surfactants. *Journal of*
621 *Chromatography A*, *1653*, 462421. https://doi.org/10.1016/j.chroma.2021.462421

Roberts, D. W., Aptula, A., Schultz, T. W., Shen, J., Api, A. M., Bhatia, S., & Kromidas, L. (2015). A practical guidance for Cramer class determination. *Regulatory Toxicology and Pharmacology*, *73*(3), 971–984. https://doi.org/10.1016/j.yrtph.2015.09.017

Schaller, U., & Balmer, M. (2018). Co-formulants in plant protection products: Initial study on the risk assessment of co-formulants in plant protection products. In *Federal Food Safety and Veterinary Office*.

Schymanski, E. L., Jeon, J., Gulde, R., Fenner, K., Ruff, M., Singer, H. P., & Hollender, J. (2014). Identifying small molecules via high resolution mass spectrometry: Communicating confidence. *Environmental Science and Technology*, *48*(4), 2097–2098. https://doi.org/10.1021/es5002105

Spanish Ministry of Health. (2021). *Co-Formulants Unacceptable for Inclusion in PPP in Spain. Spanish Ministry of Health*.

United States Environmental Protection Agency IRIS Advanced Search. (2023). https://iris.epa.gov/AdvancedSearch/

Zahn, E., Wolfrum, J., Knebel, C., Heise, T., Weiß, F., Poetz, O., Marx-Stoelting, P., & Rieke, S. (2018). Mixture effects of two plant protection products in liver cell lines. *Food and Chemical Toxicology*, *112*(December 2017), 299–309. https://doi.org/10.1016/j.fct.2017.12.067

Table 1. Identification of co-formulants in samples by two stationary phases (Shodex and C18) by LC-Q-Orbitrap-MS. Compounds in bold were confirmed with their analytical standard.[a]

Nº	Compound	Molecular formula	Retention time (min)		Adduct	Characteristic ions			Fragment ions				LoC
			Shodex	C18		Theoretical mass	Mass error (ppm)		Theoretical mass	Molecular formula	Mass error (ppm)		
							Shodex	C18			Shodex	C18	
1	**1-Ethyl-2-pyrrolidone**	$C_6H_{11}NO$	2.56	N.D.	$[M+H]^+$	114.0913	0.696		112.0757	$C_6H_{10}NO$	-0.807		1
2	**Dodecylbenzenesulfonic acid**	$C_{18}H_{30}O_3S$	1.24	20.92	$[M-H]^-$	325.1843	3.684	3.407	79.9574 183.0121	SO_3 $C_8H_7O_3S$	-4.078 -1.646	-3.203 0.703	1
3	4-Undecylbenzenesulfonic acid	$C_{17}H_{28}O_3S$	1.24	20.8	$[M-H]^-$	311.1686	3.336	3.625	79.9574 183.0121	SO_3 $C_8H_7O_3S$	-3.828 0.976	0.174 0.102	2
4	**Palmitamide**	$C_{16}H_{34}NO$	2.10	20.91	$[M+H]^+$	256.2635	-3.946	-1.214	88.0757 102.0913	$C_4H_{10}NO$ $C_5H_{12}NO$	0.369 0.778	0.489 2.051	1
5	**N-Lauryldiethanolamine**	$C_{16}H_{35}NO_2$	19.72	18.28	$[M+H]^+$	274.2735	-2.245	-1.917	256.2635	$C_{16}H_{34}NO$	-2.034	0.932	1
6	**1-Naphthalenesulfonic acid**	$C_{10}H_8O_3S$	1.33	11.10	$[M-H]^-$	207.0121	1.443	3.995	79.9574 143.0502	O_3S $C_{10}H_7O$	0.829 -1.688	1.039 -5.079	1
7	**N, N-dimethyldecanamide**	$C_{12}H_{25}NO$	1.95	18.87	$[M+H]^+$	200.2009	-2.502	-1.403	102.0913 116.1070 198.1852	$C_5H_{12}ON$ $C_6H_{14}ON$ $C_{12}H_{24}ON$	2.541 -0.005 -2.174	2.345 0.081 -1.468	1
8	**4-Sec-butyl-2,6-di-tert-butylphenol**	$C_{18}H_{30}O$	2.20	20.55	$[M+H]^+$	263.2369	-2.325	-1.984	245.2264 207.1742	$C_{18}H_{29}$ $C_{14}H_{23}O$	-3.048 -1.795	-1.498 -1.457	1
9	**Lauramide DEA (N,N-Bis(2-hydroxyethyl)dodecanamide)**	$C_{16}H_{33}NO_3$	2.18	18.95	$[M+H]^+$	288.2533	-3.575	-2.083	88.0766 106.0863	$C_4H_{10}NO$ $C_4H_{12}O_2N$	3.741 0.800 3.483	4.876 2.496 -0.845	1
10	**2-Amino-1,3-dimethylbenzene (2,6-Dimethylaniline)**	$C_8H_{11}N$	2.35	4.63	$[M+H]^+$	122.0964	-0.704	-0.376	105.0699 107.0731	C_8H_9 C_7H_9N	0.791 0.832	3.075	1
11	**Aniline**	C_6H_7N	2.26	1.57	$[M+H]^+$	94.0651	2.064	3.765					1
12	**Glyceryl monostearate**	$C_{21}H_{42}O_4$	N.D.	21.59	$[M+H]^+$	359.31559		-2.216	267.2682 341.3050	$C_{18}H_{35}O$ $C_{21}H_{41}O_3$		-1.019 -1.060	1

13	**Monopalmitin**	$C_{19}H_{38}O_4$	N.D.	20.97	$[M+H]^+$	331.28429		-2.705	313.2737 257.24751	$C_{19}H_{37}O_3$ $C_{16}H_{33}O_2$		-1.505 -0.648	1
14	4-Decylbenzenesulfonic acid	$C_{16}H_{26}O_3S$	1.31	19.55	$[M-H]^-$	297.1530	3.460	3.157	79.9574 183.0121	SO_3 $C_8H_7O_3S$	-2.453 0.280	-3.828 0.266	2
15	4-Phenylcyclohexanone	$C_{12}H_{14}O$	2.02	17.48	$[M+H]^+$	175.1117	-4.178	-2.008	157.10118 145.10118	$C_{12}H_{13}$ $C_{11}H_{13}$	-0.936 -2.047	-0.490 -0.944	2
16	Lauryldiemthylamine oxide	$C_{14}H_{31}NO$	16.39	18.75	$[M+H]^+$	230.2478	-2.437	-1.699	133.1010 60.0443	$C_{10}H_3$ C_2H_6ON	-0.278 3.150	0.849 2.488	2
17	Cocamidepropyl betaine	$C_{19}H_{38}N_2O_3$	14.50	18.91	$[M+H]^+$	343.2955	-2.271	-1.834	240.2315	$C_{15}H_{30}NO$	-1.586	-0.629	2
18	Triethanolamine	$C_6H_{15}NO_3$	3.46	N.D.	$[M+H]^+$	150.1125	-1.598		132.1019	$C_6H_{14}NO_2$	-1.554		2
19	Sodium decyl sulfate	$C_{10}H_{22}O_4S$	1.23	18.13	$[M-H]^-$	237.1166	3.938	-1.690	79.9574	SO_3	2.320	-4.579	2
20	Triethylene glycohexamonohexadecyl ether (Ceteth-3)	$C_{22}H_{46}O_4$	2.12	21.79	$[M+H]^+$	375.3469	-3.001	-1.536	89.0597	$C_4H_9O_2$	4.760	-3.998	2
21	Laureth-2 sulfate	$C_{16}H_{34}O_6S$	12.11	N.D.	$[M-H]^-$	353.2003	2.362		79.9574 97.0659	SO_3 C_6H_9O	-6.330 -2.179		2
22	Dodecyl 4-hydroxybenzoate	$C_{19}H_{30}O_3$	1.90	18.99	$[M+H]^+$	307.2268	-3.357	-2.055					3
23	Triethylene glycol monotetradecyl ether (Myreth-3)	$C_{20}H_{42}O_4$	18.77	21.47	$[M+H]^+$	347.3156	-2.725	-2.811					3
24	2-[2-[4-(1,1,3,3-tetramethylbutyl)phenoxy]ethoxy]-ethanol	$C_{18}H_{30}O_3$	1.67	19.41	$[M+H]^+$	295.2268	-2.579	-1.122					3
25	Cocamide monoethanolamide	$C_{14}H_{29}NO_2$	2.91	N.D.	$[M+H]^+$	244.2271	-2.357						4
26	Butyl linoleate	$C_{22}H_{40}O_2$	1.87	21.06	$[M+H]^+$	337.3101	-3.489	-2.689					4
27	2-(4-Nonylphenoxyethanol)	$C_{17}H_{28}O_2$	1.60	N.D.	$[M+H]^+$	265.2162	-2.137						4
28	3,6,9,12-tetraoxapentacosan-1-ol	$C_{21}H_{44}O_5$	1.90	20.42	$[M+H]^+$	377.3262	-2.785	-2.706					4

[a]LoC: level of confidence

Table 2. Identification of co-formulants in samples by GC-Q-Orbitrap-MS. Compounds in bold were confirmed with their analytical standard.[a]

Nº	Compound name	Molecular formula	Retention time	Characteristic ions		Fragment ions			Samples
				Theoretical mass	Mass error (ppm)	Theoretical mass	Molecular formula	Mass error (ppm)	
1	Isopropylbenzene (cumene)	C_9H_{12}	5.29	120.0939	-0.266	105.07043 103.05478	C_8H_9 C_8H_7	-0.541 -0.454	S7, S27
2	Propylbenzene	C_9H_{12}	5.63	120.0939	-0.016	105.07043 91.05478	C_8H_9 C_7H_7	0.220 -0.294	All except for S7, S11-S16 and S29
3	4-Cymene (4-ethyltoluene)	C_9H_{12}	5.79	120.0939	-0.183	105.07043 91.05478	C_8H_9 C_7H_7	-0.256 0.035	All except for S12-S16, S23
4	1,3,5-Trimethylbenzene	C_9H_{12}	5.79	120.0939	0.483	105.07043 119.08608	C_8H_9 C_9H_{11}	0.315 1.033	All except for S12-S16, S23
5	1,2,4-Trimethylbenzene	C_9H_{12}	6.06	120.0939	-0.266	105.07043 119.08608	C_8H_9 C_9H_{11}	-0.256 0.278	All samples
6	Tert-butylbenzene	$C_{10}H_{14}$	6.23	134.1095	-0.090	119.08607 91.05478	C_9H_{10} C_7H_7	-0.562 -0.514	S1-S7-S10, S13, S18, S21-S24, S27, S30

7	2-Isopropyltoluene	$C_{10}H_{14}$	6.40	134.1095	-0.612	91.05478 119.08608	C_7H_7 C_9H_{10}	-0.844 -1.15	All expecpt for S2-S6
8	n-Butylbenzene	$C_{10}H_{14}$	6.74	134.1095	-0.761	92.06260 91.05478	C_7H_8 C_7H_7	-1.649 -0.953	All except for S7, S9-S13, S17-S18, S20, S27, S28
9	Naphthalene	$C_{10}H_8$	8.25	128.0626	-0.639	126.04695 102.04641	$C_{10}H_6$ C_8H_6	0.303 0.277	All samples

[a] Abbreviation: S1: Chili pepper; S2: Cucumber; S3: Red grape; S4: Tangerine; S5: Clementine; S6: Strawberry; S7: Pistachio leaf; S8: Clementine leaf; S9: Orange 1, S10; Tomato leaf 1; S11: Cucumber plant, S12: Strawberry leaf; S13: Tomato leaf 2; S14: Cucumber leaf 1; S15: Blueberry; S16: Cucumber leaf 2, S17: zucchini leaf; S18: Orange 2; S19: Papaya; S20: Tomato 1; S21: Cured tobacco; S22: Orange leaf, S23: Tomato 2; S24: Eggplant leaf; S25: Pepper leaf; S26: Chard, S27: Raspberry, S28: White grape; S29: Raisin and S30: Spinach

Table 3. Minimum, maximum and mean content of co-formulants quantified in samples by LC-Q-Orbitrap-MS and GC-Q-Orbitrap-MS.

Compound	Minimum-Maximum (µg/kg)	Samples (Minimum-Maximum)	Mean (µg/kg)	Mean value (fruits and vegetables, µg/kg)	Mean value (leaves, µg/kg)
Dodecylbenzenesulfonic acid	1-7936	Cucumber-Clementine leaf	572	125.34	1353
1-Naphtalenesulfonic acid	2-35	Blueberry-Tomato leaf 1	11	2.71	23
N,N-dimethyldecanamide	10-36	Tomato leaf 2- tomato leaf 1	23	-	23
4-sec-butyl-2,6-di-tertbutylphenol	4-20151	Blueberry-Tomato leaf 1	2756	756.73	5256
Lauramide DEA	1-94	Cucumber plant-Tomato leaf 1	7	0.92	18
1-Ethyl-2-pyrrolidone	22-6513	Blueberry-Cucumber leaf 1	1712	426.13	3642
2,6-Dimethylaniline	10-283	Orange 2-Eggplant leaf	76	24.97	161
Aniline	6	Orange 1	6	6	-
Palmitamide	165-5888	Orange 1-Tomato leaf 1	1588	817	2359
N-Lauryldiethanolamine	19-56143	Tomato 2-Tomato leaf 1	6772	503	11788
Monopalmitin	3-12837	White grape-Clementine leaf	1498	111	4619
Glyceryl monostearate	2-33226	Raisin-Clementine leaf	3180	533	7338
Isopropylbenzene (cumene)	1-8	Raspberry-Pistachio leaf	4	1	8
Propylbenzene	2-15	White grape-Eggplant leaf	6	5.0	8
Sum (4-Ethyltoluene + 1,3,5-Trimethylbenzene)	1-46	Tomato 1-Strawberry	9	9	7
1,2,4-Trimethylbenzene	2-40	Tomato 2-Pistachio leaf	8	5	12
4-Tert-butylbenzene	5-182	Tomato 2-Orange 1	38	39	37

2-Isopropyltoluene	2-316	Spinach-Pistachio leaf	36	17	58
n-Butylbenzene	1-20	Cucumber leaf 2-Strawberry	5	6	4
Naphthalene	4-49	Chard-Strawberry leaf	10	7	15
Total	72-98490	Blueberry-Tomato leaf 1	8001	1434	17851

Table 4. Toxicological information of confirmed co-formulants.[a]

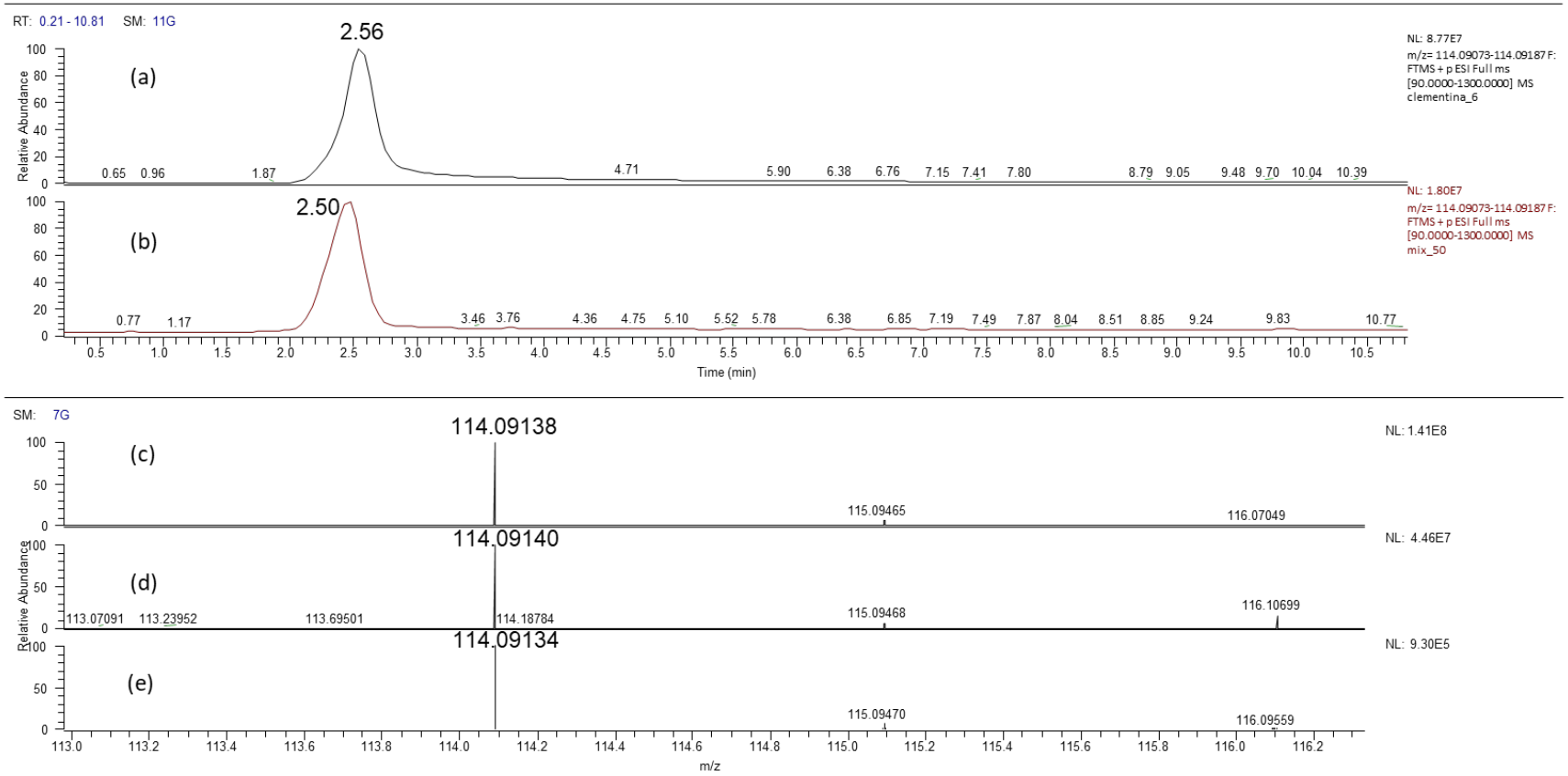
Co-formulant	LD_{50} (T.E.S.T. g /kg)	Class (Toxtree)	RfD (mg/kg/day)
Aniline	0.372	III	NA
1-Naphtalenesulfonic acid	4.873	III	0.500
Dodecylbenzenesulfonic acid	1.297	I	0.500
1-Ethyl-2-pyrrolidone	1.440	III	NA
N,N-Dimethylaniline	0.780	I	0.002
Lauramide DEA	8.175	III	NA
N, N-Dimethyldecanamide	4.395	III	NA
4-sec-butyl-2,6-di-tert-butylphenol	15.850	II	NA
N-lauryldiethanolamine	6.599	I	NA
Palmitamide	3.682	I	NA
Monopalmitin	11.292	I	NA
Glyceryl monostearate	16.729	I	NA
Isopropylbenzene	2.507	I	0.100
Propylbenzene	4.226	I	0.100
4-Ethyltoluene	4.419	I	NA
1,3,5-trimethylbenzene	3.281	I	0.010
1,2,4-Trimethylbenzene	3.395	I	0.010
Tert-butylbenzene	2.559	I	0.100
2-isopropyltoluene	2.943	I	0.100
n-butylbenzene	5.110	I	0.100
Naphthalene	1.412	III	0.020

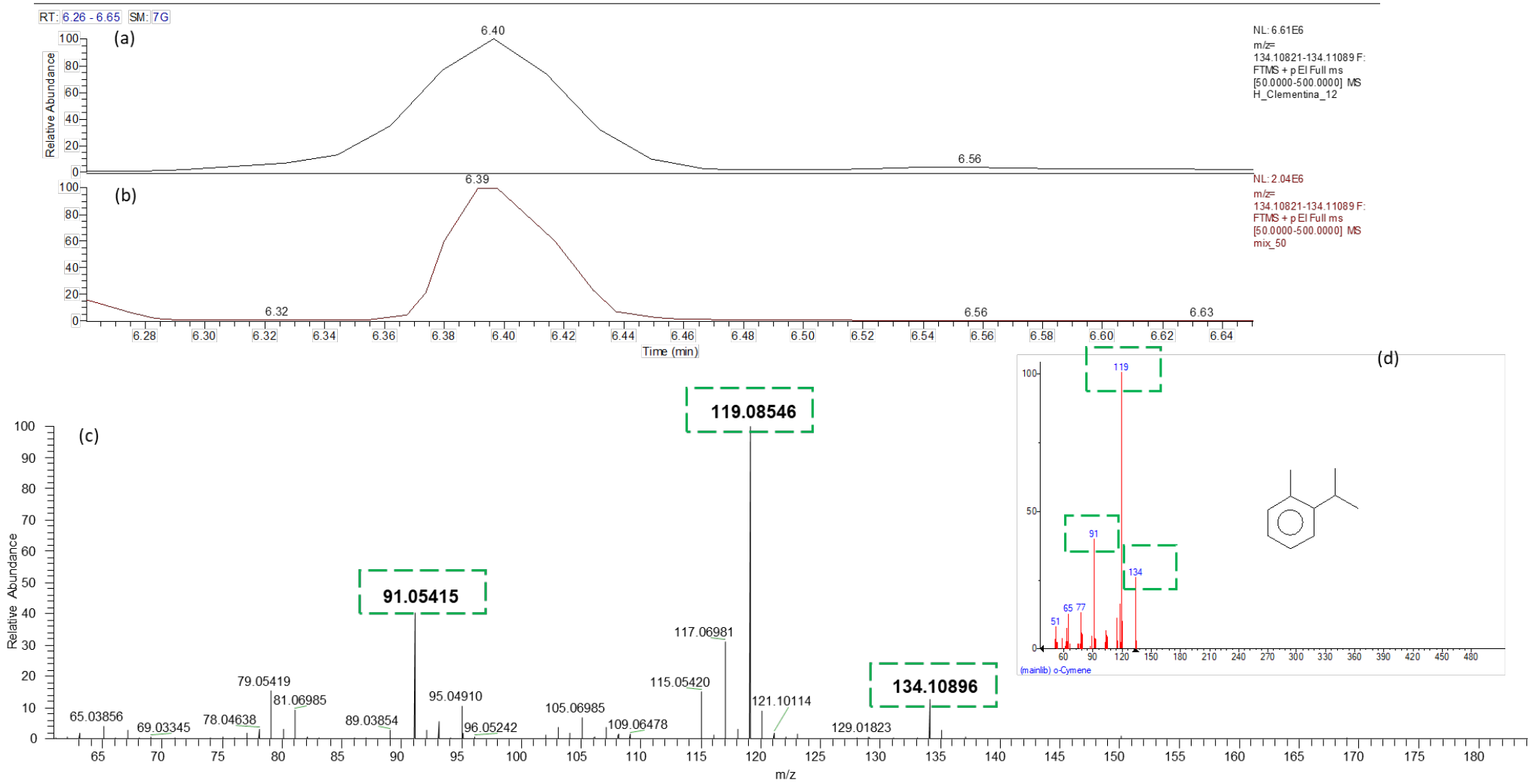
[a] Abbreviation: LD_{50}: Median lethal dose; Toxtree: Toxic hazard estimation by decision tree approach (Toxtree); RfD: Reference Dose; NA: Not available.

Figure captions

Figure 1. Extracted ion chromatogram of 1-ethyl-2-pyrrolidone by LC-Q-Orbitrap in: (a) clementine (210 μg/kg); (b) analytical standard (50 μg/L); (c) full-scan MS spectrum of the sample; (d) full-scan MS spectrum of the analytical standard, and (e) full-scan MS spectrum of the theorical one.

Figure 2. Extracted ion chromatogram of 2-isopropyltoluene by GC-Q-Orbitrap in: (a) clementine leaf (238.36 μg/kg); (b) analytical standard at 50 μg/L; (c) full Scan MS spectrum of the sample and (d) Full Scan MS spectrum from the NIST.


RT: 0.21 - 10.81 SM: 11G
2.56
(a)
Relative Abundance
0.65 0.96 1.87 4.71 5.90 6.38 6.76 7.15 7.41 7.80 8.79 9.05 9.48 9.70 10.04 10.39
NL: 8.77E7
m/z= 114.09073-114.09187 F: FTMS + p ESI Full ms [90.0000-1300.0000] MS clementina_6
2.50
(b)
0.77 1.17 3.46 3.76 4.36 4.75 5.10 5.52 5.78 6.38 6.85 7.19 7.49 7.87 8.04 8.51 8.85 9.24 9.83 10.77
NL: 1.80E7
m/z= 114.09073-114.09187 F: FTMS + p ESI Full ms [90.0000-1300.0000] MS mix_50
Time (min)
SM: 7G
114.09138
(c)
115.09465
116.07049
NL: 1.41E8
114.09140
(d)
113.07091 113.23952 113.69501 114.18784 115.09468 116.10699
NL: 4.46E7
114.09134
(e)
115.09470 116.09559
NL: 9.30E5
m/z


RT: 6.26 - 6.65 SM: 7G
(a)
Relative Abundance
6.40
6.56
NL: 6.61E6
m/z= 134.10821-134.11089 F: FTMS + p EI Full ms [50.0000-500.0000] MS H_Clementina_12
(b)
6.39
6.32
6.56
6.63
NL: 2.04E6
m/z= 134.10821-134.11089 F: FTMS + p EI Full ms [50.0000-500.0000] MS mix_50
Time (min)
(c)
119.08546
91.05415
134.10896
117.06981
115.05420
121.10114
79.05419
81.06985
95.04910
105.06985
109.06478
65.03856
69.03345
78.04638
89.03854
96.05242
129.01823
Relative Abundance
m/z
(d)
119
91
134
65 77
51
(mainlib) o-Cymene