1 **Unravelling plant protection product analysis: use of chromatography**
2 **techniques (GC and LC) and high resolution mass spectrometry**

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Abstract

This study proposes a methodology for the characterization of plant protection products (PPPs) based on suspect and unknown analyses. This was divided in three main stages: sample preparation, separation and detection, and data analysis. Sample preparation was based on dilute and shoot strategies employing different solvents depending on both the type of compounds and the type of PPPs to be analyzed. Chromatographic techniques, as liquid (LC) and gas chromatography (GC), coupled with high resolution mass spectrometry (HRMS) analyzers are used for the separation and detection stage. HRMS allowed a huge number of possibilities in terms of data acquisition, and this work reveals the most suitable options and the principal parameters to maximize the number of features and to perform an accurate detection. Finally, tips and recommendations to perform data analysis are indicated, providing the pre-processing and processing strategies to perform suspect screening and unknown analysis.

Keywords: Chromatographic techniques, high resolution mass spectrometry, plant protection products, additives, methodology, suspect screening, unknown analysis

57 **1. Introduction**

58 The use of plant protection products (PPPs) in agriculture has been one of the most
59 important factors leading to increased yields [1]. The use of PPPs is still growing,
60 reaching an estimated market value of nearly $130.7 billion by 2023 (from $84.5 billion
61 in 2019) [2]. The overall cost of discovery and development of new PPPs has increased
62 by 88% during the last twenty years. It should be noted that green chemistry issues have
63 become increasingly important during the last years, due to the rise in environmental
64 safety data required by regulatory bodies [3].

65 PPPs are established by Regulation (EU) No 1107/2009 [4], which sets that they are
66 composed of the active substance (pesticide), that has the property of protecting the
67 plants, killing or attacking pests, improving the conservation, destroying the unintended
68 parts, or controlling the unwanted growth, and of other compounds called impurities
69 and additives (Figure 1). Impurities are any other component, including all the isomers
70 not being part of the active substance definition, which are present in the PPPs
71 originated from the manufacturing process or from degradation during storage.
72 Additives are intentionally added to the PPPs to enhance their characteristics, and they
73 can be classified as safeners, synergists, co-formulants or adjuvants.

74 The application of PPPs in the field inevitably leads to the presence of active substance
75 residues on the treated crops, as well as residues from additives and/or impurities. In the
76 European Economic Area, PPPs regulation [4] provided a full description about
77 definitions, additives, quality control, packaging, labelling and methods of analysis.
78 This Regulation defines residues as *one or more substances present in or on plants or*
79 *plant products, edible animal products, drinking water or elsewhere in the environment*
80 *resulting from the use of a PPP, including their metabolites, breakdown or reaction*
81 *products.* However, despite efforts to control PPPs, risk assessment and the regulation
82 of residues are limited to the pesticide (active substance) and selected metabolites, for
83 which maximum residue levels in or on food are established. Impurities and additives
84 are not mentioned.

85 Considering the commercialization of PPPs, Regulation (EU) No 284/2013 [5] lists the
86 steps to follow, whereas Regulation (EU) No 546/2011 [6] sets a series of general and
87 specific principles that should be met by PPPs for their evaluation and authorization,
88 such as the application conditions, or their impact on humans, plants, and the
89 environment. Labelling is one of the most important parts of the commercialization and
90 authorization of PPPs. For this reason, all substances included in a PPP must be listed

(included impurities and additives). However, in the Regulation (EU) No 547/2011 [7], which provides the requirements that are necessary for the labelling of PPPs, impurities and additives are not considered and consequently, very little information is available concerning their nature and magnitude [8]. Regulation (EU) No 547/2011 [7] indicates that the information should be clear and indelible in the packaging and contain information like commercial name, name of active substance, quantity, lot number and production date, security, product action, type of product, uses and instruction, security term, toxicity, and storage [8].

In the last years, scarce studies have focused on the study of additives and impurities in foods despite the importance of this problem. For instance, in the study carried out by Balmer et al. [8], co-formulants half-lives were determined in treated crops such as vegetables. It seems that their half-live is higher than one day, so they can be an underestimated health problem. Consequently, a few studies have analysed these compounds in commercial products [9] as well as in the aqueous environment [10,11] or marine sediments [12]. Despite that, PPPs impurities and additives are not normally monitored in fruits or vegetables, so the real extent of their hazard remains unknown.

In the past, analytical methods that used liquid chromatography (LC) [13–16] and gas chromatography (GC) [17–21] coupled with mass spectrometry (MS) were employed, predominating the use of low-resolution mass spectrometry analyzers (LRMS) versus high resolution MS (HRMS) [9,22]. In addition, classic detectors as ultraviolet-visible (UV) or flame ion detector (FID) were also used [20,23]. However, to achieve a full characterization, HRMS must be employed.

Therefore, the main objective of this article is to propose an analytical workflow to perform an integral evaluation of the composition of PPPs using HRMS approaches, including sample treatment, chromatographic separation, and data analysis. Including data acquisition (quality control (QC)) and data processing (identification, validation and quantification).

2. PPPs framework

As previously mentioned, PPPs are composed of active substances, impurities, and additives (Figure 1). Impurities can be formed during the manufacturing process or by degradation of the active substance during the manufacturing process and storage, or even during PPPs preparation process, when they are dissolved in water before their application in crops. For example, in the analysis of the commercial product Equation

Pro®, a famoxadone PPP, the metabolite IN-KF015 was also found, so, it is expected that it could be originated during the manufacturing process or in the process of PPP dissolution with water [24].

Additives are composed of several types of compounds that are defined as [4]:

(a) substances or preparations referred to as 'safeners', which are added to eliminate or reduce the phytotoxic effects of the PPP on certain plants. e. g. flurazole, oxabetrinil or cloquintocet-mexyl [25,26].

b) substances or preparations known as 'synergists', which may increase the activity of the active substances of a PPP. e.g., piperonyl butoxide or diethyl maleate [27].

(c) substances or preparations known as 'co-formulants', which are used or intended for their use in a PPP, but which are neither active substances, nor safeners, nor synergistic. e.g., naphtha derivatives, sodium alkyl sulfates or alkyl ethoxylates.

d) substances or preparations called 'adjuvants', which consist of co-formulants, or preparations containing one or more co-formulants, in the form in which they are supplied to the user and marketed, so that the user mixes them with a PPP, to improve the efficacy or other properties of the pesticides.

The most important one of these four groups are co-formulants. They are a heterogeneous group of molecules involving solvents, wetting or antifoaming agents, stabilizers, emulsifiers, etc., that can be present at high concentrations, and they are added to PPPs to increase the effectiveness of the pesticide, as well as to increase the dissolution of the active substance on account of their low solubility in water [22,28]. Due to the variety of compounds and the wide range of properties and toxicities, European Commission has recently established Regulation (EU) No 2021/383 [29], which involves a list of co-formulants forbidden for their use in PPPs, such as amines, tallow alkyl or ethoxylates, due to their potential negative effects on human health or the environment.

Moreover, PPPs are commercialized in a wide range of formulations and each one involves different co-formulants. They are emulsifiable concentrates (EC), wettable powders (WP), soluble concentrates (SC), water dispersible granules (WG), dispersible concentrates (DC), suspension concentrates (SC), capsule suspensions (CS), or a blend of a CS in an SC (ZC) [30]. The most common formulation is EC, in which hydrophobic pesticides are dissolved in nonpolar solvents, which forms an emulsion when the PPP is dissolved in water. The principal advantages are a higher concentration of active substances, easy processing, handling and storage, or even a higher biological

159 activity. Nevertheless, EC formulations have several drawbacks derived from their co-
160 formulants, such as flammability, possible instability after dilution or phytotoxic effects,
161 and increased dermal toxicity of the active substance [22,31].
162 Co-formulants can also cause environmental and health adverse effects as toxicity to
163 aquatic life, skin, eyes and respiratory irritation or narcotic and toxic effects [22].
164 Among the different types of co-formulants, the most important one is surfactants.
165 These compounds have hydrophobic and hydrophilic moieties in their chemical
166 structure. Therefore, they show both lipophilic and hydrophilic properties [32,33] and it
167 can enhance the efficiency of formulations by increasing the water solubility,
168 bioavailability, and biological activity of the active ingredients. Surfactants may
169 improve the solubility, adsorption, or penetration of the active ingredient in these
170 formulations, but they also enhance environmental stability, bioavailability, and
171 capability to reach the site of action. Surfactants are generally classified according to the
172 type of their hydrophilic part. Therefore, anionic, cationic, non-ionic, and amphoteric
173 surfactants can be distinguished [34].
174 The toxicity of PPPs is normally associated to the active substances, and for this reason
175 Regulation (EU) 283/2013 [35] requires comprehensive mammalian toxicity testing for
176 acute, chronic, and sub-chronic effects only for the active substance, but not for the
177 other components of the PPPs [36]. Thus, additives used in PPPs do not require any
178 toxicological evaluation as part of PPP Regulation (EU) 1107/2009 [4]. Instead, they
179 are commonly subjected to the REACH regulation [37] and, hence, toxicologically
180 tested and assessed depending on their annual production volume. However, the
181 unintended side effects of PPPs can be caused by additives in these preparations.
182 Additives in PPP formulations may have adverse effects on the environment and on
183 non-targeted organisms. The toxic effect of co-formulants in PPPs has been clearly
184 demonstrated by several studies in which formulated pesticide products were proven to
185 be more toxic than their active ingredient alone [38–40]. As an example, exposure to 4-
186 ethyltoluene and 2-ethyltoluene, two isomers present in EC products, which are made
187 with naphtha derivatives as solvent, have been proved to trigger acute narcotic effects,
188 in addition to a lower survival in mice [22,39]. In addition, the recent investigation of
189 the combined toxicity of the most used herbicide active ingredient worldwide,
190 glyphosate, and polyoxyethylene tallow amine (POEA), as its most common co-
191 formulant, indicated higher individual toxicity of the surfactant or combined synergistic
192 effects between POEA and the active ingredient [40]. The effects of POEA and a

glyphosate-based herbicide formulation (Roundup) on different tested organisms were compared, and POEA proved to be more toxic. The acute toxicity of glyphosate, a glyphosate-based formulation, and the surfactant applied in given formulation, on aquatic invertebrates and fish species were investigated, and POEA was found to be the most toxic component, compared to the effects of technical grade glyphosate and the investigated formulation [41]. In a later study, ethoxylated co-formulants used in glyphosate-based formulations were confirmed to be nearly ten thousand times more toxic than the active ingredient [42]. In fact, in-vitro studies indicate that co-formulants significantly increase the cytotoxicity of some PPPs, and they can also induce an enhancement of cellular permeation, enhancing the bioavailability of the respective active substances [36]. They have also been shown to be a hazard for the environment, and for instance, the co-formulants present in the Amistar® fungicide can cause negative effects in bumblebees [43].

3. Sample treatment methods for the analysis of PPPs

The comprehensive characterization of PPPs using analytical techniques has been scarcely studied. There are only a few articles involved in this topic, and even less addressing the evaluation of the additives in PPPs (Table 1).

In terms of sample treatment, dilute and shoot can be employed as a good method using different solvents. Solvent selection will depend on the polarity of the compounds and the injection and/or separation technique employed (Figure 2). For example, acetone, dimethyl sulfoxide (DMSO) or ethyl acetate can be used for the analysis of non-polar analytes by GC [9,20,22], whereas water, acetonitrile, isopropyl alcohol, or methanol can be utilized to dissolve the PPPs prior LC analysis [9,13,15] (Figure 2). This step is critical because some PPPs, as EC, SC, ZC or WG, can only be dissolved in water, or in the case of SC and ZC also in DMSO, as it was previously indicated [22]. Therefore, the solubility of PPPs in the selected solvents should be carefully studied in order to check that PPP is properly dissolved prior to analysis.

Other methods can also be employed for the extraction of polar analytes, e.g., pyrrolidone derivatives, as QuEChERS [16], which was applied for the analysis of co-formulants in plant-derived agro-products (apple, cabbage, tomato, cucumber, rice, and wheat), or solid-phase extraction (SPE) [21], which was used in liquid pesticide formulations as a clean-up step. These methods are more adequate when the determination of the active substance and the impurities and/or additives is performed in

227 matrices as food, water, soils and sediments, for which a exhaustive extraction is
228 required to eliminate the matrix effect, and some information about them is known [10–
229 12]. Finally, for non-polar analytes, automatic extraction techniques including solid-
230 phase microextraction (SPME) and headspace (HS) (used together or separately), could
231 be employed (Figure 2), taking into account that they are less time-consuming, allowing
232 for the preconcentration of the compounds, and automatization. Moreover sample
233 contamination can be reduced due to sample treatment is minimized, and higher
234 repeatability can be achieved, since they reduce experimental errors [44]. In this case,
235 samples can be easily dissolved in a suitable solvent as water, eliminating the
236 dissolution problems indicated above, previous the extraction procedure based on HS-
237 SPME is performed. For instance, HS was used for the determination of volatile
238 compounds as toluene or benzene [18,19]. HS-SPME was used for the monitoring of
239 co-formulants in a myclobutanil PPP (Mitrus) by GC-HRMS, allowing the detection of
240 seven benzene derivate compounds [45]. In this work, HS-SPME was compared with
241 liquid injection, demonstrating being more adequate for the analysis of volatile
242 compounds, due to the low detection limits obtained compared to liquid injection.
243
244 *3.1. Sample treatment and quality control*
245 It is important to note that external contamination could take place during sample
246 preparation, or even during sample analysis, if the analytes are present in the used
247 laboratory material or even in the analytical equipment. For that, as a QC measure, the
248 employment of extraction blanks (followed the same dissolution/extraction as samples),
249 can be useful and mandatory in the case of non-targeted analysis (Figure 2). If
250 contamination was observed, the source of that contamination should be found
251 monitoring the solvent in which samples are diluted (solvent blanks) as well as the
252 equipment, injecting methanol (LC) or ethyl acetate (GC) to evaluate the mass analyzer
253 and the chromatographic module. In addition, they must be injected throughout the
254 batch to control external contamination or carryover effect. For example, glyceryl
255 monostearate, a co-formulant identified in PPPs [9], is also used as an antistatic agent in
256 centrifuge tubes [46] and might migrate to the sample during the extraction step.
257 Another example is sodium lauryl sulfate, present in HRMS calibration solutions and
258 PPPs, which would interfere unless the signal in the sample is noticeable higher
259 compared to the signal in the blank, so that they can be properly distinguished.
260 Additionally, caution should be taken when PPPs are being analyzed, as many additives

261 (co-formulants) have assorted industrial applications, such as the manufacture of
262 cleaning products or paints, so an extraction blank should always be useful, allowing the
263 distinction between compounds present in the samples and those from contamination
264 source. Finally, washing laboratory material with no soap is also advised to avoid
265 contamination stemming from detergents, especially anionic surfactants, such as sodium
266 lauryl sulfate, another co-formulant previously identified in PPPs [9].
267 To avoid carry-over contamination between samples and detector saturation, PPPs
268 samples should always be diluted before chromatographic-MS analysis [22], since they
269 usually contain over 100 g/L of active substance, which would surely leave residues in
270 both the chromatographic system, and the analyzer. Therefore, a dilution of at least
271 1:100,000 (*v/v*), or even 1:500,000 (*v/v*), is suggested, avoiding contamination in the
272 equipment (Figure 2). For example, in the analysis of Lexor 25, an EC PPP, dilutions
273 ranging from 1:1,000 (*v/v*) to 1:2,000,000 (*v/v*) were tested for GC-HRMS analysis,
274 selecting the dilution 1:500,000 as the optimum one, as it provided an acceptable
275 number of chromatographic peaks without signal saturation. By LC-HRMS, dilution
276 tested ranged from 1:1,000 (*v/v*) to 1:1,000,000 (*v/v*), choosing dilution 1:10,000 (*v/v*)
277 due to the high number of results obtained in unknown analysis compared to other ratio
278 dilutions.
279 In addition, when non-targeted analysis is being carried out, sample replicates are
280 necessary to check whether the compounds detected are in all the replicates or not, that
281 can help distinguish between false positives or possible compounds present in the
282 samples. For that, at least 3 replicates of each sample should be analyzed to obtain
283 reliable results.
284 Another additional QC activity is the addition of internal standards (ISs) to ensure the
285 sample analysis is being properly performed, and to control compound ionization and
286 fragmentation throughout the batch (Figure 2). ISs should have similar properties to
287 expected compounds and at least one IS for each compound group (i.e. pesticides,
288 plasticisers, etc) should be added, taking into account their commercial availability [47].
289 Isotopically labelled ISs can be an alternative due to their properties and advantages,
290 since in unknown or suspect screening analysis the previous knowledge about the
291 compounds present in the samples is scarce. For example, in this case, the use of
292 isotopically labelled triphenylphosphate (TPP-d15), which can be detected in both LC-
293 HRMS and GC-HRMS could be a good option.
294

295 **4. Separation and detection techniques for the analysis of PPPs**

296 In terms of separation, and due to the complexity of the compounds to be analysed, LC
297 and GC are necessary for a comprehensive analysis of PPPs. LC is indicated for
298 relatively polar, ionic, thermolabile or non-volatile compounds, such as alkyl
299 ethoxylates (e.g., nonaethylene glycol monododecyl ether), alkyl benzene sulfonates or
300 alkyl naphthalene sulfonates.

301 For LC, common columns as C18 (Table 1) are employed, although specific stationary
302 phases, as HILIC, were used for the determination of four adjuvants in PPPs [16].
303 However, C18 columns do not provide optimal selectivity for the simultaneous analysis
304 of anionic, non-ionic, and cationic surfactants using the same mobile phase system. In
305 those cases, other columns specially developed for the separation of surfactants as
306 ThermoFisher Acclaim™ Surfactant or Shodex ODP2 HP-2D, a polymer-based
307 reversed phase chromatography column, may improve the separation of these
308 compounds.

309 In addition, conventional mobile phases containing water, methanol or acetonitrile with
310 formic acid or acetic acid are used (Table 1). The elution time and gradient applied are
311 slightly longer than conventional LC methods due to the variability of the compounds
312 with different molecular formula and polarities. Some of them have long hydrocarbon
313 or ethoxylates chains that are strongly retained in the analytical columns, so retention
314 time is higher. Therefore, run times of more than 30 min and keeping 100 % of organic
315 solvent for 10 min (at least) is highly recommended to allow the complete elution of the
316 non-polar compounds.

317 On the other hand, GC is suitable for volatile, thermally stable, and less polar
318 compounds, such as benzene or naphthalene derivatives, which can be found in most
319 PPPs [22]. For GC, 5MS ((5%-phenyl)-methylpolysiloxane) capillary columns are the
320 most employed (Table 1), although other stationary phases, as HP-1 column (100%
321 dimethylpolysiloxane) were used to determine volatile organic compounds [18].

322 It is important to highlight that some additives are isomers which means similar
323 characteristics as the same theoretical mass and fragments. However, GC has the
324 advantage of separating them in the chromatographic run and providing different
325 retention times, involving one of the biggest challenges of this technique. For instance,
326 4-ethyltoluene and 1,3,5-trimethylbenzene co-formulants, whose molecular formula was
327 C_9H_{12}, have different retention times at 5.89 and 6.21 min, respectively, so they can be
328 determined individually when GC is used [48].

329 Finally, for the detection of active substances, impurities and/or additives, MS is the
330 most suitable technique. LRMS analyzers were previously used, as simple quadrupole
331 (Q), triple quadrupole (QqQ) or Qtrap (Table 1). For the use of these analyzers involved
332 the performance of targeted analysis, as the mass of the analytes to be monitored should
333 be known prior analysis. When the active substance is being monitored, this is a simple
334 step, but in terms of additives, it is a complex issue since they are normally not included
335 in the PPP label. Only a few of them are indicated in either the label or in the safety
336 sheet, and for this reason, there is very little literature on this topic. To deal with this
337 problem, HRMS, as (Q)-Orbitrap or (Q)-TOF are the most suitable instruments.
338 However, these tools are complex, and additional concern rises, as data analysis or data
339 processing is required, as it is shown in the next section.
340 In addition to MS, nuclear magnetic resonance spectroscopy (NMR) may seem as an
341 alternative to chromatographic and MS techniques, but it turns out not to be as helpful
342 as expected, due to the complexity of PPPs [9]. These formulations usually contain an
343 active substance at high concentrations, even up to 8.000 times more concentrated than
344 co-formulants, and therefore, their signals may be overlapped by those from the active
345 substance, low peak height may be obtained, or even they cannot be distinguished
346 among the baseline noise due to the low sensitivity of this technique to analyze
347 compounds at low concentrations. Therefore, it is rather a supporting confirmation
348 technique, especially if information from several spectra (^{1}H, ^{13}C, or bi-dimensional)
349 can be evaluated.
350

351 **5. Data acquisition and data processing advances for the analysis of PPPs**

352 Both data analysis and data processing are the most important stages in the analysis of
353 suspect or unknown compounds when HRMS is used. The methodology involves the
354 use of complex analytical software developed for that use and requires some previous
355 knowledge about the techniques employed. For that, in this section a typical workflow
356 for the analysis of PPPs has been proposed (Figure 3), taking into account the last
357 studies focused on this problem [9,22].
358

359 *5.1. Data acquisition*

360 The main issue regarding data acquisition in HRMS is the acquisition mode. When GC
361 is coupled to HRMS, the acquisition mode is simple, being full scan in positive
362 ionization mode. Due to the high hard ionization of electron impact ionization mode, a

363 subsequent fragmentation step is not required (Table 2). Otherwise, when LC-HRMS is
364 used, full scan mode in electrospray (ESI), positive and negative ionization, is employed
365 for the acquisition of the compounds, and in order to obtain the fragments, there are
366 several fragmentation modes, being data independent analysis (DIA) the most useful
367 one in this kind of analysis. It is similar than all-ion-fragmentation (AIF) mode but
368 employing *m/z* ranges set by the user. For instance ranges of *m/z* 100, involves a
369 fragmentation from 100 to 200, 200 to 300, 300 to 400, etc. It was really useful because
370 spectra are simpler and contain less ions compared to AIF [49,50], and it is helpful
371 when compounds presenting a low intensity in the sample are analyzed (i.e., pesticides
372 present in fruit or vegetables) [51], improving the search possibilities for fragments of
373 unknown compounds. The DIA acquisition mode, with a previous full scan step, was
374 the best option for characterization of PPPs using non-targeted analysis, because no
375 previous information about the sample is required and a total information about
376 precursor ions and fragments is provided, reducing the number of undesirable fragment
377 ions thanks to the selection of *m/z* ranges. The other parameters mentioned in the Table
378 2 are characteristics of Q-Orbitrap analysers, so they can only be replicated in the same
379 type of instrument, but it can serve as a guide for HRMS users.
380 The critical points related to GC-HRMS acquisition are the use of full scan acquisition
381 in a wide *m/z* window, starting from the lowest *m/z* value detectable by the equipment
382 until the maximum *m/z* according to the resolution power, so that it can be able to detect
383 as many compounds as possible (i.e. at 60000 Full Width at Half Maximum (FWHM)
384 of resolution, between *m/z* 50-500) (Table 2). For LC-HRMS, when full scan (positive
385 and negative ionization mode to detect compounds at both polarities) is used, a wide *m/z*
386 window from the lower *m/z* detectable until the maximum *m/z* allowed by resolution (i.e
387 at 70000 FWHM, *m/z* 50-750) should be selected; however, in some cases, the
388 compounds with a *m/z* higher than 750 cannot be detected at suitable resolution if
389 Orbitrap is used as analyzer. For this reason, when compounds at higher *m/z* are
390 expected, additional full scan can be added to the method introducing another *m/z* range
391 (i.e. m/z 150-2000).
392 Then, the fragmentation step is critical, because it is necessary to choose the correct
393 range of *m/z* windows to ensure that MS/MS spectra are cleanest as possible, especially
394 when DIA was selected. For the characterization of PPPs, *m/z* window is the best
395 suitable choice (Table 2), even though a large number of fragmentation spectra for each
396 sample are obtained, which makes raw data heavier. Nonetheless, current software tools

397 are allowed to interpret each one and those MS/MS data can be linked to the full scan
398 spectra. If Orbitrap is used as analyzer, when resolution, an important parameter, is set,
399 it affects to the number of scans obtained, so, a compromise between resolution and
400 number of scans is necessary. Nevertheless we consider that the best option is a
401 resolution of 60000 FWHM (at *m/z* 200) in Full Scan for GC-HRMS and 70000 FWHM
402 (at *m/z* 200) in Full Scan and 35000 FWHM (at *m/z* 200) when DIA was used in LC-
403 HRMS. So once the acquisition mode had been defined, the next step was the analysis
404 of the sample followed by data processing.
405
406 *5.2. Data processing*
407 Data processing can be carried out using various analytical software provided by the
408 commercial brands of the mass analyzers, as Metaboscape® by Bruker, MassHunter
409 Mass Profiler® and MassHunter® Unknowns Analysis by Agilent or Compound
410 Discoverer® and TraceFinder® by ThermoFisher Scientific. Also, there are open-
411 source programs as FOR-IDENT (https://water.for-ident.org/#!home), MS-FINDER
412 (http://prime.psc.riken.jp/compms/msfinder/main.html) or patRoon [52] All of them
413 allowed performing suspect screening or unknown analysis (Figure 3).
414 Before data analysis, raw data must be pre-processed to generate a practicable data
415 matrix in a variety of ways, eliminating the noise and reducing data weight. The key
416 step is minimizing the variance and bias in the data analysis to reduce the complexity
417 and to enhance significant signals of interest. Consequently, several algorithms have
418 been developed and implemented in the software mentioned above and multiple open-
419 source programs have also been applied to process raw MS data acquired on LC-MS or
420 GC-MS [53], as XCMS (https://xcmsonline.scripps.edu/) [54], MZmine
421 (http://sourceforge.net/projects/mzmine/) [55], OpenMS (http://open-
422 ms.sourceforge.net/) [56], and MetAlign (http://www.metalign.nl) [57]. The pre-
423 processing steps have drawn particular attention for their practicability and
424 effectiveness. The first step in LC-HRMS and GC-HRMS raw data pre-processing is the
425 normalization to remove confounding variations attributed to experimental sources,
426 such as analytical noise or experimental bias. The second step is RT alignment of
427 detected features in different samples, which aims to remove shifts among samples for a
428 given signal, in order to guarantee downstream extraction of useful information (Figure
429 3). To make them applicable to the large amounts of two-dimensional data generated by
430 chromatographic systems coupled with HRMS instruments, the dimensionality must be

431 reduced [53,58]. Additionally, in pre-processing data, there is a third step, the
432 deconvolution that must be done to induce a high probability of obtaining a good match
433 in library search, as compared to non-deconvoluted spectra (Figure 3). The
434 deconvolution algorithm can generate hundreds or even thousands of detected peaks.
435 Therefore, it is important to appropriately select the deconvolution parameters to
436 minimize the number of false positives and negatives [59]. This step is more useful in
437 GC-HRMS data than in LC-HRMS data, but it can be used for both. To sum up, the
438 procedure consists of normalization, followed by RT alignment and deconvolution.
439
440 *5.2.1. Suspect screening analysis*
441 Once the data is pre-processed, in the case of suspect screening analysis, a database is
442 usually employed (Figure 3). It can be either home-made, commercially available or
443 provided by a group of researchers, such as the NORMAN digital sample freezing
444 platform (DSFP). This last tool involves databases of a wide range of compounds,
445 created exclusively for retrospective suspect screening. The NORMAN DSFP combined
446 information on (i) exact mass, (ii) predicted retention time window in the
447 chromatogram, (iii) isotopic fit and (iv) qualifier fragment ions [60].
448 To build a home-made database, as Maldonado Reina et al. [22] and Hergueta-Castillo
449 et al. [61] performed for co-formulants in PPPs, a bibliographic revision must be done,
450 to obtain the maximum numbers of potential compounds. In addition, information such
451 as name, molecular formula, structure, precursor ion, fragment ions and ionization mode
452 must be collected in a table to complete the database (See Table S2 home-made
453 database of GC-HRMS additives of Maldonado-Reina et al [22] and Table S2 home-
454 made database of LC-HRMS additives of Hergueta-Castillo et al. [61]). Once the
455 database is built, the samples are processed using analytical software mentioned above,
456 as MS-FINDER, MassHunter® Unknowns Analysis, or TraceFinder. The criteria
457 selected for the identification of precursor ions and fragments are mass error lower than
458 5 ppm, monitoring at least one fragment ion, which must be detected at the same RT of
459 the precursor ion, and extraction blank subtraction in a ratio 5/1 sample area/blank area
460 [62]. Additionally, when compounds have a characteristic isotopic pattern because of
461 the presence of Cl, Br o S, it is also evaluated, and a fit of at least 70% [63], compared
462 to the theoretical spectrum, must be achieved (Figure 3).
463
464 *5.2.2. Unknown analysis*

465 For unknown analysis, the workflow is slightly different (Figure 3). Here, the
466 compounds are "unknown" and the structure of the candidate needs to be elucidated. In
467 that workflow, the software is used and characterized by a first step consisting in the
468 annotation of a probable molecular formula and then suggesting a tentative structure
469 [64]. Both steps rely on knowing the exact mass of the component and its fragments,
470 which highlights the importance of HRMS and high quality of the fragment spectrum
471 for structural elucidation. Tentative structure elucidation is complex and it is generally
472 approached by searching in a database of chemical structures that can be provided by
473 the analytical software as ChemSpider databases, NIST library or *m/z* cloud. However,
474 this approach limits possible candidates to those listed in the chemical database, which
475 are all known structures, and will cause problems if the true compound is not known.
476 Compounds with unknown structure that are not listed in chemical databases, e.g., most
477 transformation products, have been referred to as "unknown unknowns". In this case,
478 orthogonal approaches, including analytical techniques like NMR or infrared
479 spectroscopy (IR), are powerful tools for the improvement of the identification
480 performance of "unknown unknowns", which, however, have limited application
481 possibilities [64–66], as it has been previously mentioned in *Section 4.2*. For the
482 characterization of PPPs, studied compounds must be "known" and be included in NIST
483 library and some ChemSpider databases. However, some precautions must be adopted
484 to obtain the data in a simple way to finally evaluate them.
485 The data processing workflow performs unknown compounds detection, predicts
486 elemental composition, fix a minimum threshold value, which is low in the case of PPPs
487 characterization (1e4) due to some impurities and additives are present in a minor
488 proportion, and removes background features using extraction blank samples in a ratio
489 5/1 sample area/blank area (Figure 3) [62]. In addition, as mentioned above, the
490 processing software automatically identifies compounds using in the case of LC-HRMS
491 data, *m/z* cloud, ChemSpider (exact mass and formula), and local database as Mass Lists
492 searches [63]. For GC-HRMS data, the compounds are identified using Mass List
493 searches and NIST spectral library. For that last one, it is important to mention that
494 compounds identified by NIST are more reliable than results provided by *m/z* cloud or
495 Chemspider database in LC. NIST library provided theoretical spectra of the precursor
496 ion and the fragments, meanwhile in *m/z* cloud MS^2 spectra is not available for some
497 compounds, and in the case of Chemspider, spectra are not available and only the
498 precursor ion is provided. In addition, another criterion, that introduces more confidence

499 in the case of GC-HRMS, is the tentative identification using Kovats Index (KI), by
500 comparison of the experimental KI calculated for each tentative compound and the KI
501 included in the NIST library, considering a maximum KI deviation of ± 20 units for a
502 reliable tentative identification [67] (Figure 3).
503

504 *5.2.3. Annotation, validation, and quantification*

505 In both cases, either using LC or GC, the processing software allocates several potential
506 features to the tentative compounds detected in the samples, which need to be analyzed
507 by the operator (Figure 3). Depending on the compliance of the identified substances
508 with the identification criteria, novel levels of identification confidence were defined in
509 the present review considering previous works as Schymanski et al. [68] and Miralles et
510 al. [63,69] as well as our knowledge in this topic. These parameters are shown in Table
511 3 and indicate the criterion and threshold values to ensure a correct identification of the
512 compound of interest. They range from just a preliminary tentative identification, based
513 on molecular formula and mass error lower than 5 ppm (level 5), levels 4a and 4b that
514 refer to tentative identification as they do not take MS^2 data into account, but more
515 reliable than level 5, since they include isotope pattern fit. Levels 3 and 2 includes a
516 confident confirmation based on a wide number of requirements, including molecular
517 formula, mass error lower than 1 ppm, isotope pattern fit greater than 70 % and MS^2 *m/z*
518 cloud or MS^2 NIST fit greater than 50% or 90 %, and three matching fragments.
519 Finally, level 1 is the confirmation of the compound using analytical standards.
520 However, in some cases, the MS^2 match cannot be done because the potential
521 compound is not available in *m/z* cloud database or because the concentration of the
522 suspect compound is too low to clearly obtain identified fragments. If fragments are not
523 available in databases, the use of in-silico tools as MassFrontier (Thermo Fisher), CFM-
524 ID or MetFrag [70] can be useful. That software gives a potential list of fragments
525 generated using the precursor structure and a theoretical list of fragmentations. With
526 that list of in-silico fragments, the MS^2 spectra can be analysed in a qualitative way to
527 search the fragmentation of the tentative compound, which will ensure a correct
528 elucidation (Figure 3).
529 Finally, to confirm the tentative compounds by using the proposed methodology,
530 commercially available analytical standards can be purchased. They are used to confirm
531 their identity and they can be also used for validation and quantification purposes.
532 Standard solutions containing the tentative analytes will be prepared and analyzed under

533 the same conditions of the samples. The tested compounds will be evaluated in terms of
534 the acquired retention time, exact mass, and MS spectrum [69] to confirm their
535 presence. However, a lot of compounds are not commercially available, so this step
536 cannot always be performed (Figure 3).
537 To validate the confirmed compounds, according to SANTE 11312/2021 [71], if the
538 analytical method does not allow the determination of recoveries because the samples
539 are directly analysed or because liquid samples are diluted with a suitable solvent, only
540 linearity, limit of quantification (LOQ) and precision (intra- and inter-day) must be
541 checked. To study linearity, calibration curves in the same solvent of the sample
542 dilution could be prepared due to the high dilution of the samples restricts the matrix
543 effect. If a high dilution is not required because sensitive issues, and if there are not
544 matrix blanks, linearity should be studied by standard addition. Linearity is evaluated in
545 terms of R^2, obtaining satisfactory results in the case of R^2 greater than 0.99 and also by
546 the deviation of back-calculated concentration from true concentration $\leq \pm 20\%$. For
547 precision, five samples fortified with the standard at a known concentration (lowest
548 level of the calibration curve) can be tested in 5 different days (inter-day precision) and
549 in the same day (intra-day precision). If all results fall under the threshold required for
550 an acceptable precision (RSD $\leq$ 20%), the method will be validated. Finally, LOQ is
551 evaluated by calibration points in solvent at low concentrations, selecting as LOQ, the
552 concentration that achieve acceptable results in terms of precision and linearity.
553 To quantify the confirmed compounds, due to the high dilution of the samples and the
554 absence of sample blanks, calibration curves in solvent can be used as it was indicated
555 above. Therefore, the calibration curves in the PPPs solvent dilution should be
556 performed in a wide range of concentrations i.e., from 1 μg/L to 250 μg/L, to ensure
557 that analyte concentrations are within this range. However, in some cases compound
558 concentrations are higher, and additional sample dilution is required to perform a
559 correct quantification.
560 Finally, when analytical standards of the detected compounds are not available a semi-
561 quantification using analytical standards from compounds of the same family can be
562 carried out. For example, in the case of impurities, as pesticide metabolites present in
563 the PPPs, their quantification could be carried out using the parent compound
564 (pesticide) as standard and their concentrations were calculated and expressed as a
565 function of the pesticide [72].

566 As an example of the described methodology, the study of Lexor 25 PPP was described.
567 This PPP was analysed by LC-HRMS and raw data was processed by Compound
568 Discoverer® to perform a comprehensive unknown analysis through 14 ChemSpider
569 libraries, including FDA UNII-NLM, which encompasses a large number of co-
570 formulants, and a list of compounds was obtained. Myreth-6, an alkyl ethoxylate also
571 known as hexaethylene glycol monotetradecyl ether, was one of the several co-
572 formulants found. It was manually searched in extraction blanks in Xcalibur Qual
573 Browser, which provided a negative result. Therefore, myreth-6 was subjected to in-
574 silico fragmentation in MassFrontier, and generated theoretical fragments, which were
575 searched in the experimental DIA MS^2 spectra. As Figure 4 shows, theoretical
576 fragments 269.24751, 261.13326 and 255.23186 were found in the *m/z* 350-400 DIA
577 MS^2 spectra, with a maximum mass error of 3.38 ppm (absolute value).
578 Analytical standard of myreth-6 was acquired, it was injected and the presence of this
579 co-formulant in Lexor 25 was confirmed (Figure 4), achieving a level of confidence 1
580 for the identification of unknown compounds, as reported in Table 3. Figure 4 depicts
581 confirmation of myreth-6 via matching retention time (0.02 min shift), similar peak
582 shape and concordant characteristic ion with both the analytical standard and the
583 predicted isotopic pattern, with a mass error of -3.83 ppm. Then, the method was
584 successfully validated according to SANTE 11312/2021 [71]. A matrix effect lower
585 than 20 % (12 %), allowed the use of solvent calibration standards for quantitation
586 purposes, and linearity and precision values were $R^2>0.99$ and RSD < 8% respectively.
587 Finally, quantification was also performed obtaining a concentration value of 0.03 g/L
588 in the PPP.
589
590 **7. Conclusions and future outlooks**
591 This study proposed a methodology to characterize PPPs using chromatographic
592 techniques (GC and LC) coupled to HRMS. Despite the fact that conventional stages of
593 the analytical method were described, as sample treatment, separation and detection,
594 and data analysis, different tips were provided. Thus, a suitable dilution of the sample
595 should be ensured to maximize the number of features avoiding saturation of the
596 system. Whereas the separation of the compounds can be performed using well-known
597 stationary phases, as C18 or HILIC, specific ones developed for surfactants as
598 ThermoFisher Acclaim™ Surfactant or Shodex ODP2 HP-2D could be suitable
599 alternatives. In addition to LC or GC, which have been commonly used so far, capillary

electrophoresis (CE) coupled to HRMS offers great promises for the analysis of highly charged and polar metabolites, and thus ensure a complete characterization of PPPs [73], combining the information provided by these separation techniques.

Regarding the detection techniques, a new dimension could be added to the information provided by HRMS, and ion mobility spectrometry (IMS) can be implemented since its main advantage is isomers or isobars resolution, improving the information provided by current LC-HRMS equipment [74]. This is relevant in this field, since most of co-formulants are isomers or compounds with a very similar structure, and the use of the collision cross section parameter would enhance the identification of detected compounds. Moreover, new advances in the data processing are required to solve the weakest points that they currently have, as processing time or human supervision. Furthermore, solution of bugs in the software development are needed and each year commercial brands introduce new changes in the software to improve them. In addition, deconvolution should be improved, especially for overlapped peaks, that cannot be discriminated or a reliable blank subtraction, bearing in mind that sometimes compounds present in blanks are not suppressed in the samples and a manual revision is required to minimize the number of false positives.

Finally, this study enhances the need to control co-formulants in PPPs during pesticide monitoring and the need of improving the regulation for these substances, considering the wide variety of molecules present in this category, their properties and their toxicologic aspects.

Declaration of competing interest

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

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637

638 **Authorship contribution statement**

639 **Rosalía López-Ruiz**: Methodology, Software, Data Curation, Writing – Original Draft,
640 Visualization, Editing. **Antonio Jesús Maldonado-Reina**: Methodology, Writing –
641 Review and Editing. **Jesús Marín-Saez**: Methodology, Writing – Review and Editing.
642 **Roberto Romero-Gonzalez**: Methodology, Writing - Review and Editing, Supervision.
643 **Jose Luis Martinez-Vidal**: Methodology, Writing - Review and Editing, Supervision.
644 **Antonia Garrido Frenich**: Conceptualization, Resources, Writing - Review and
645 Editing, Project administration, Funding acquisition.

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Figure captions

Figure 1. Plant Protection Products composition.

Figure 2. Sample treatment steps for the characterization of PPPs.

Figure 3. Workflow applied to characterize PPPs using HRMS.

Figure 4. Myreth-6 identification by LC-HRMS and confirmation in Lexor 25 when unknown analysis was peformed: A) extracted ion chromatograms of myreth-6 in Lexor 25 and in methanol (100 µg/L); B) experimental full scan spectra of Myreth-6 in Lexor 25, in methanol (100 µg/L) and predicted isotopic pattern, and C) experimental MS^2 spectrum of myreth-6 in Lexor 25.

Table 1. PPPs analyzed in the last years using chromatographic and spectrometric techniques.*

Analytes	Activity/ Type of PPPs	Extraction	Separation	Detection	Reference
Piperonyl butoxide and N-octylbicycloheptene dicarboximide (MGK264)	Synergist/ NI	Dilute and shoot: Isopropyl alcohol contained 5 mL of the 0.8% of 2,2-dimethylpropiophenone	LC: Zorbax Eclipse XDB C_{18} (150 x 4.6 mm, 5 µm). MP: Water/methanol/acetonitrile (35:10:55, *v/v/v*), where the water/methanol contained 1.0 mM formic acid	MS: Q	[13]
Non-ionic surfactants	Surfactants//NI	NI	LC: Grom Sil 120 Butyl-1 ST microbore cartridge (6 x 2 mm, 5 µm) MP: Methanol and water	MS: Qtrap	[14]
Adjuvants	Adjuvants/WP	Dilute and shoot: ethyl acetate	LC: Luna C18 (150 × 4.6 mm, 5.0 µm) MP: Methanol and water GC: PB-5 fused-silica (30 m×0.25 mm, 0.25 µm)	UVD MS: ion trap	[23]
Polyoxyethylene tallow amine	Surfactant/ Pesticide formulations of glyphosate	Dilute and shoot: acetonitrile: water (50:50, *v/v*)	LC: Shodex MSpak GF-310 4D (150 × 4.6 mm, 5.0 µm) Luna C18 (150 × 3 mm, 3.0 µm) Atlantis T3 (150 × 3 mm, 3.0 µm) Kinetex C18 (150 × 3 mm, 2.6 µm) XSelect HSS C18 (150 × 3 mm, 2.5 µm) BEH C18 (150 × 2.1 mm, 1.7 µm) HSS T3 (150 × 2.1 mm, 1.8 µm) MP: water 0.3% acetic acid and acetonitrile	MS: QqQ	[15]
2-pyrrolidone,N-methyl-2-pyrrolidone,and N-ethyl-2-pyrrolidone	Adjuvants/NI	Original QuEChERS	LC: XBridge HILIC (150 × 2.1mm, 5µm) MP: Water 0.1% formic acid and acetonitrile 0.2% formic acid	MS: QqQ	[16]
Surfactants and solvents	Adjuvants/ EC	Dilute and shoot: Methanol, acetonitrile, and water for LC	LC: Zorbax Eclipse Plus C18 (100 × 4 mm, 5 µm)	MS: Orbitrap	[9]

		Ethyl acetate, *n*-hexane and dichloromethane for GC	MP: water 0.1% formic acid, and methanol GC: VF-5 ms (30 m × 0.25 mm, 0.25µm)	MS: Q-Orbitrap	
Volatile organic compounds	Co-formulants/ EC	HS-SPME: Fiber Carboxen™/polydimethylsiloxane	GC: HP-1 (30 m, 0.32 mm, 1.0 µm)	MS: MSD	[18]
Toluene and benzene	Co-formulants/ EC	HS	GC: Rtx-5 capillary column (30 m×0.20 mm, 0.25 µm)	MS: Q	[19]
15 co-formulants and 8 impurities	Co-formulants and impurities/SC, EC, EW, ZC	SLE: acetone and filtration step (0.2 µm filter)	GC: Zebron ZB (30 m × 0.32 mm, 0.25 µm)	FID	[20]
2-pyrrolidone, N-Methyl-2-pyrrolidone, and N-Ethyl-2-pyrrolidone	Adjuvants/EC, EW and SC	SPE: Oasis HLB	GC: DB5-MS (30 m × 0.25 mm, 0.25 µm)	MS: Q	[21]
Volatile benzene and naphthalene derived co-formulants	Co-formulants /EC, SC, DC and ZC	Dilute and shoot: ethyl acetate or dimethyl sulfoxide	GC: VF-5ms (30 m × 0.25 mm, 0.25 µm)	MS: Q-Orbitrap-MS	[22]

*Abbreviations: DC, dispersible concentrates; EC, emulsifiable concentrates; EW, emulsion, oil in water FID, flame ionization detector; GC, gas chromatography; HS, head space; HS-SPME, head space solid phase microextraction; LC, liquid chromatography; MP, mobile phase; MS, mass spectrometry; MSD, mass single detector; NI, not indicated; Q, quadrupole; QqQ, triple quadrupole; Q-trap, quadrupole- ion trap; SC, soluble concentrates; SLE, solid-liquid extraction; SPE, solid phase extraction; WP, wettable powders; ZC, blend of a capsule suspension in an SC.

Table 2. Suitable acquisition parameters for the analysis of PPPs by GC and LC-Q-Orbitrap-MS*

Scan mode	Full Scan	DIA
	GC-HRMS	
Ionization mode	Electron ionization	NA
Resolution (at m/z 200)	60000 FWHM	NA
AGC target	1e6	NA
Max IT	100 ms	NA
Scan range (*m/z*)	50-500	NA
	LC-HRMS	
Ionization mode	Electrospray (Positive/ Negative)	Positive/ Negative
Resolution (at *m/z* 200)	70000 FWHM	35000 FWHM
AGC target (adimensional)	1e6	2e5
Max IT (microscans)	250	Automatic
Scan range (*m/z*)	50-750	Ranges of m/z 50 from m/z 50 to 750, i.e. m/z 50-100, m/z 100-150, etc.
Isolation window (*m/z*)	NA	50
Fixed first mass (*m/z*)	NA	50
Collision energy (eV)	NA	30

* Abbreviations: AGC: automatic gain control; FWHM: Full Width at Half Maximum; IT: injection time; NA: Not applicable

Table 3. Criteria established and levels of confidence for the identification of unknown compounds (adapted from: [63,68]).

Parameter	**Criteria**	**Level of confidence**
Molecular formula Mass error lower than 5 ppm	Molecular formula proposal is consistent according to the exact mass. No compound identification was successful from ChemSpider and Mass List databases	Level 5
Molecular formula Mass error lower than 5 ppm Isotope pattern fit (%) >70 %	Molecular formula coincidence with a proposed feature from ChemSpider or Mass List databases, including exact mass and isotope pattern	Level 4b
Molecular formula Mass error lower than 1 ppm Isotope pattern fit (%) >90 %		Level 4a
Molecular formula Mass error lower than 1 ppm Isotope pattern fit > 90 % MS^2 *m/z* cloud fit // MS^2 NIST fit > 50 % In-silico match (at least one theoretical fragment)	Coincidence between acquired MS^2 spectra and MS^2 *m/z* cloud or MS^2 NIST spectra. In-silico fragmentation is considered when *m/z* cloud does not provide good results	Level 3
Molecular formula Mass error lower than 1 ppm Isotope pattern fit > 90 % MS^2 *m/z* cloud fit // MS^2 NIST fit > 90 % In-silico match (at least three theoretical fragment)		Level 2
Analytical standard confirmation	Coincidence between the RT of the analytical standard and the compound propose. Shift time allowed 0.1 min	Level 1

Highlights

- Development of a workflow for plant protection products characterization by HRMS
- A non-targeted methodology was proposed using chromatography-HRMS
- Sample treatment, separation, detection, and data analysis were discussed
- Different levels of confidence were described for unknown compound identification
- An overview of the current analytical methods was presented

Active substance
(pesticide)

Impurities
- Manufacturing process
- Transformation products (from pesticide)

Additives
- Safeners
- Synergists
- Co-formulants
- Adjuvants

PPPs sample treatment

QC recommendations
- ✓ Blanks
- ✓ Internal standard (IS)
- ✓ 3 Replicates per sample

Adequate dilution
at least 1:100,000 (*v*/*v*)

Dilute and shoot

Head Space SPME

Polar solvent

Water
ACN
MeOH

LC-HRMS

Non-volatile compounds
Semi-volatile compounds

Non-Polar solvent

AcOEt
DMSO
Acetone

GC-HRMS

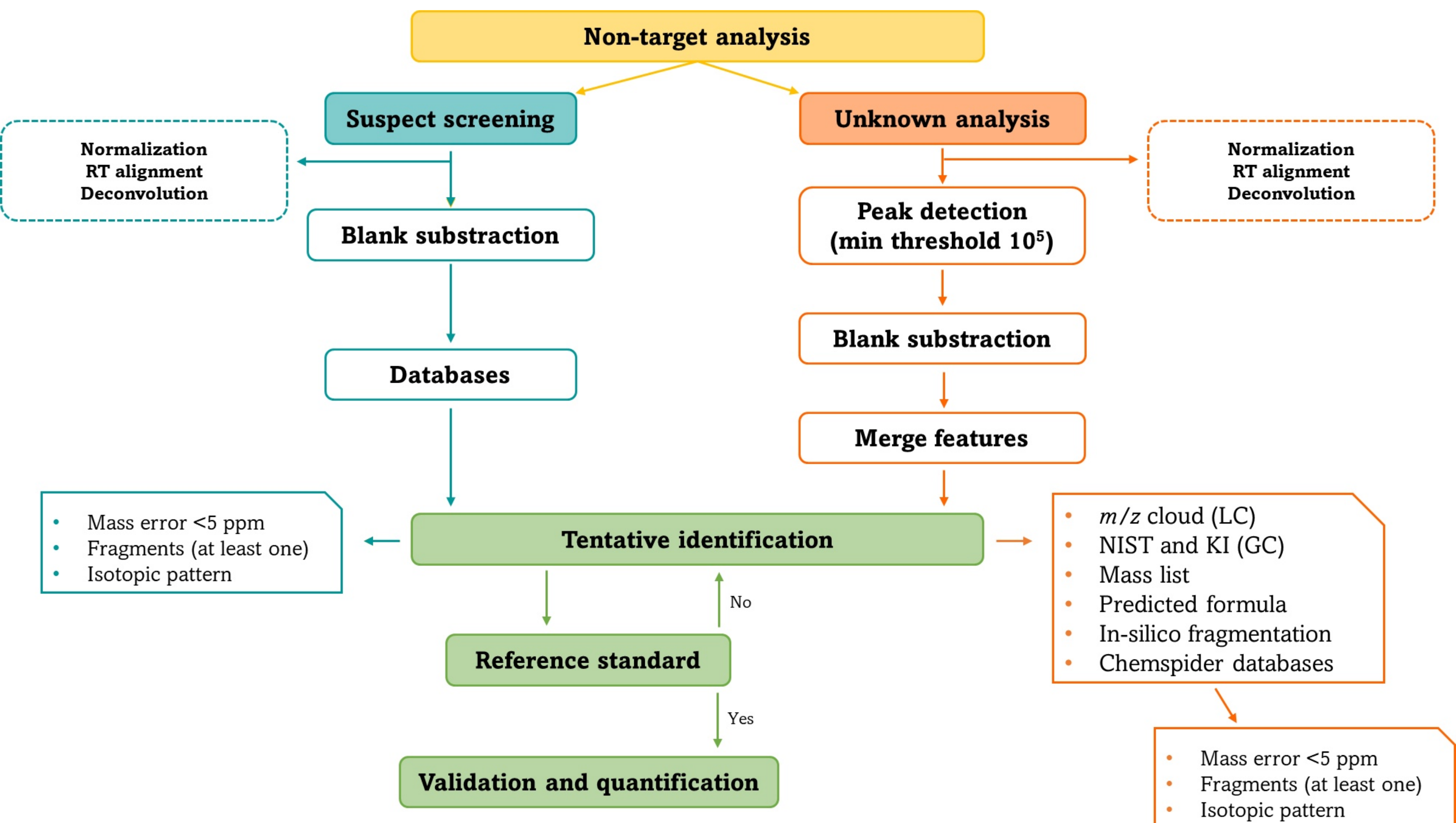
Volatile compounds
Semi-volatile compounds

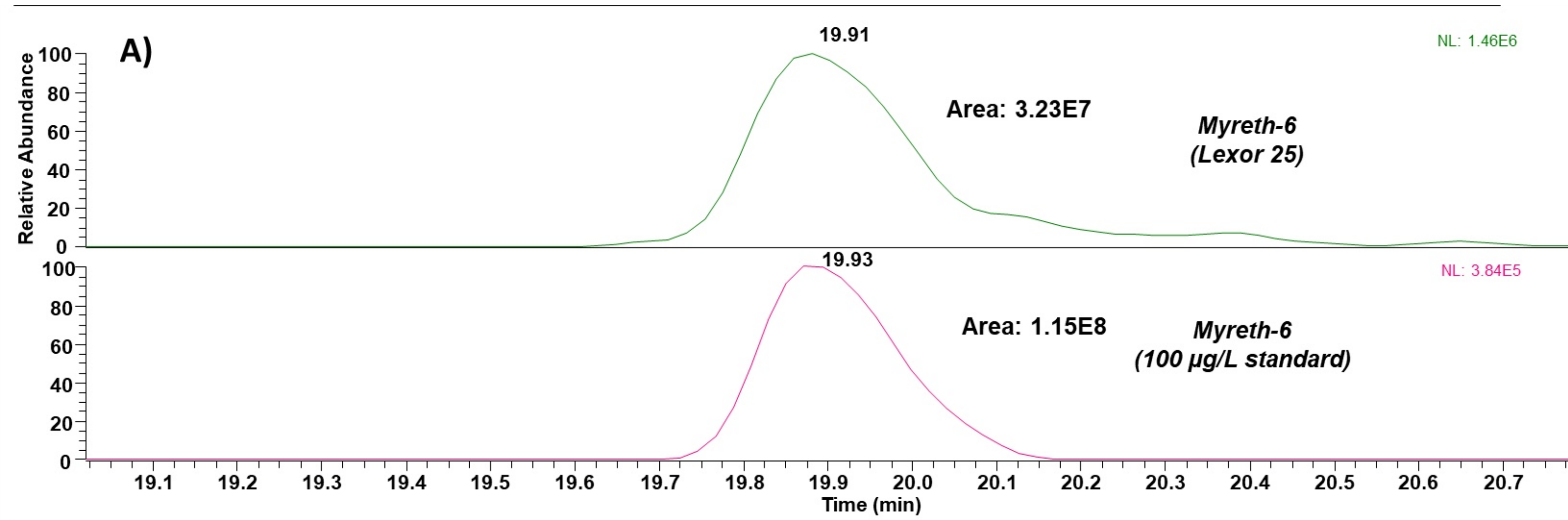
Polar solvent

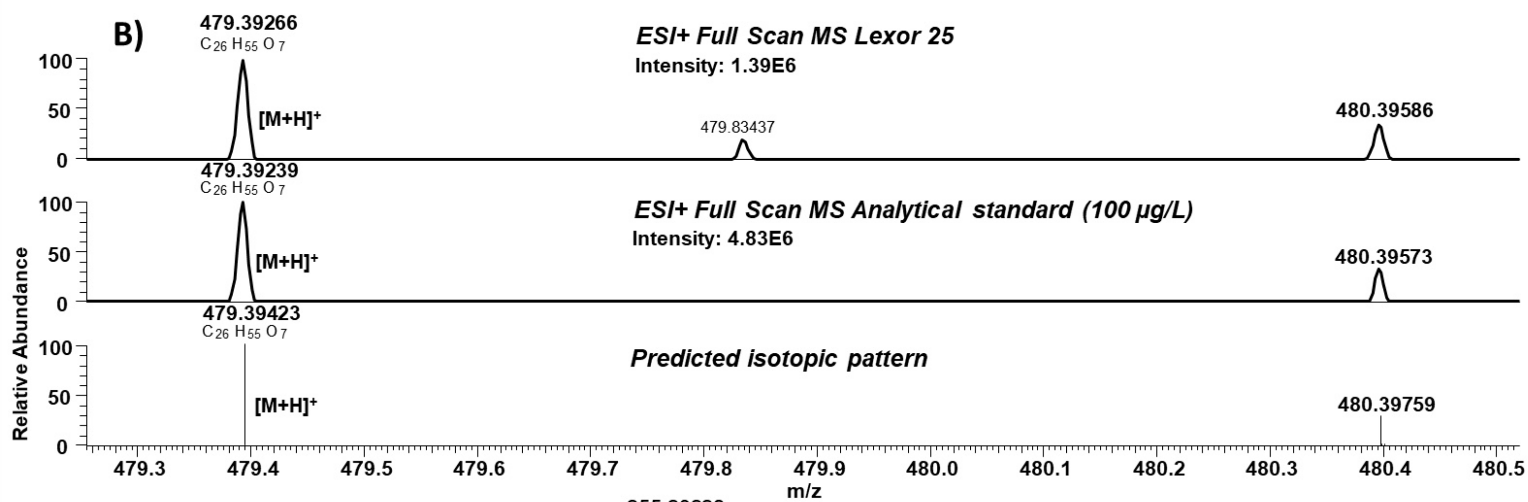
Water
(sample dilution)

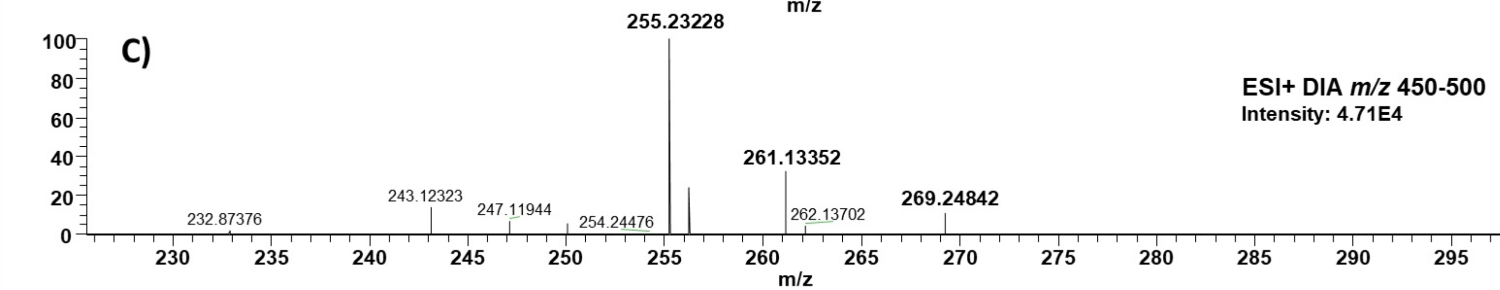
GC-HRMS

Volatile compounds


Non-target analysis
Suspect screening
Unknown analysis
Normalization
RT alignment
Deconvolution
Blank substraction
Databases
Peak detection
(min threshold 10^5)
Normalization
RT alignment
Deconvolution
Blank substraction
Merge features
Mass error <5 ppm
Fragments (at least one)
Isotopic pattern
Tentative identification
m/z cloud (LC)
NIST and KI (GC)
Mass list
Predicted formula
In-silico fragmentation
Chemspider databases
No
Reference standard
Yes
Validation and quantification
Mass error <5 ppm
Fragments (at least one)
Isotopic pattern


A)
19.91
NL: 1.46E6
Area: 3.23E7
Myreth-6
(Lexor 25)
19.93
NL: 3.84E5
Area: 1.15E8
Myreth-6
(100 µg/L standard)
Relative Abundance
Time (min)


B)
479.39266
ESI+ Full Scan MS Lexor 25
Intensity: 1.39E6
[M+H]+
479.83437
480.39586
479.39239
ESI+ Full Scan MS Analytical standard (100 µg/L)
Intensity: 4.83E6
[M+H]+
480.39573
479.39423
Predicted isotopic pattern
[M+H]+
480.39759
Relative Abundance
m/z


C)
255.23228
ESI+ DIA m/z 450-500
Intensity: 4.71E4
261.13352
269.24842
232.87376
243.12323
247.11944
254.24476
262.13702
m/z