Angle

Analysis of highly polar pesticides in foods by LC-MS/MS

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Abstract

Highly polar pesticides (HPP) are a group of pesticides that are characterize as low Log Kow. Many high-throughput multi-residue analysis methods based on liquid chromatography-tandem mass spectrometry (LC-MS/MS) for the simultaneous determination of such polar pesticides have been proposed. In this article, we summarize the various sample preparation methods including quick polar pesticides (QuPPe), dispersive solid phase extraction (dSPE), solid phase extraction (SPE) and QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe), especially for QuPPe, which are mainly used for the determination of HPP in foods. In addition, we summarize LC-based separation methodologies that are currently used for the analysis of HPP in foods, including reversed-phase chromatography (RPC), hydrophilic interaction liquid chromatography (HILIC), ion chromatography (IC) and mixed-mode chromatography (MMC). Finally, the current mass spectrometry-based methodologies for the analysis of HPP are summarized with a specific focus on MS configurations and acquisition modes.

Keywords: Highly polar pesticides (HPP), QuPPe, HPLC-MS/MS

1. Introduction

Highly polar pesticides (HPP) are a group of pesticides that have non-QuEChERS-amenable characteristics and can generally be characterized as having Log Kow values less than 1. Included among these compounds are the cationic quaternary ammonium herbicides diquat (LogKow −4.6) and paraquat (LogKow −4.5), which a member of an extremely challenging group of pesticides due to their physical-chemical properties [1]. HPP, such as glyphosate have become one of the world's most widely used herbicides due to its relatively low cost and high efficiency [2]. The European Reference Laboratory-Single Residue Methods (EURL-SRM) is a method that specifically detects HPP residues in food matrices and 55 of members of this class have been determined using this method [3]. The ten most common HPP characteristics, including IUPAC Name, structural formula, type and solubility, are summarized in Table 1.

Considering method development, a suitable method for the simultaneous extraction of various HPP in one sample is needed. The QuEChERS methodology has been applied for the extraction of many different classes of pesticides. However, HPP are not efficiently recovered. Moreover, the analysis of HPP by a single LC-MS method is extremely challenging due to the complexities associated with their separation and detection behavior. Conventional approaches to the analysis of HPP often involve the use a single residue method or a small group of compounds with similar properties and the specific methods that are used time-consuming with limited throughput [4,5].

The development of an appropriate sample preparation method for residues is becoming crucial since HPP show poor recovery when typical stationary phases are used for QuEChERS multi-residue methods for processing low to middle polar pesticides. In 2008, the Quick Polar Pesticides (QuPPe) Method was developed by the European Reference Laboratory for Single residue methods (EURL-SRM) [3] for the simultaneous extraction of numerous HPP in foods. This method involves the use of acidified methanol and details regarding the
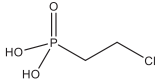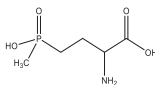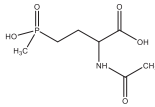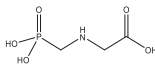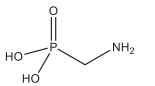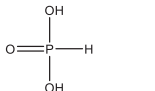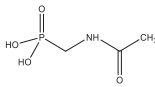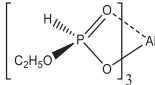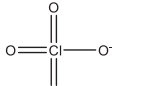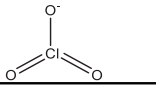
Table 1. *Ten most common HPP residues listed in EURL-SRM characteristics.*

Pesticide	IUPAC Name	Structural formula	Type	Solubility (mg/mL)
Ethephon	2-chloroethylphosphonic acid		Growth regulator	1239.0
Glufosinate	2-amino-4-[hydroxy (methyl)phosphoryl] butanoic acid		Herbicide	500.0 (Glufosinate ammonium)
N-Acetyl-Glufosinate	[tert-butyl (dimethyl)silyl] 2-[acetyl-[tert-butyl (dimethyl)silyl]amino]-4-[[tert-butyl (dimethyl)silyl]oxy-methylphosphoryl] butanoate		Metabolite (gluphosinate)	−
Glyphosate	2-(phosphonomethylamino)acetic acid		Herbicide	1050.0 (Glyphosate-trimesium)
AMPA	aminomethylphosphonic acid		Metabolite (glyphosate)	1467.0
Phosphonic acid	dihydroxy (oxo)phosphanium		Metabolite (fosetyl-Al)	310000.0
N-Acetyl-AMPA	((Acetylamino)methyl)phosphonic acid		Metabolite (glyphosate)	−
Fosetyl-Al	aluminum tris(O-ethylphosphonate)		Fungicide	120.0
Perchlorate	perchlorate		Herbicide and insecticide	99.6 (Magnesium perchlorate)
Chlorate	chlorate		Herbicide and insecticide	650.0

QuPPe procedure are shown in Fig. 1. To reduce the matrix effects of different types of foods, the QuPPe method needs to be optimized. HPP are frequently applied to crops, including vegetables [6], fruits [7], beans [8], cereals [5], and honey samples [9], and the pretreatment methods for such diverse different matrices can be complex. The common methods include liquid−liquid extraction (LLE) [7], solid-phase extraction (SPE) [9] and QuPPe of various versions [10]. The sample preparation methods introduced in this review are divided into three categories according to sample properties: (1) high water content materials; (2) low water content materials; (3) high oil content materials.

For HPP analysis, the QuPPe method is typically combined with liquid chromatography-tandem mass spectrometry (LC-MS/MS) for separating and detecting polar compounds [1]. The development of a method that permits different HPP compounds to be simultaneously analyzed since various pesticides are used in foods during their cultivation. Over the years, reversed-phase chromatography (RPC) [11,12], hydrophilic interaction liquid chromatography (HILIC) [13,14], ion chromatography (IC) [9,15] and mixed-mode chromatography (MMC) [16] have been studied for the separation of HPP compounds. In the case of reversed-phase chromatography, there are two approaches for the separation of HPP including HPP derivatization or stationary phase modification. The structure of the stationary phase in hydrophilic interaction liquid chromatography allows the targeted HPP to have a good peak shape and selectivity, but is not suitable for use with samples that are only partially soluble in organic solvents. The ion chromatography columns have
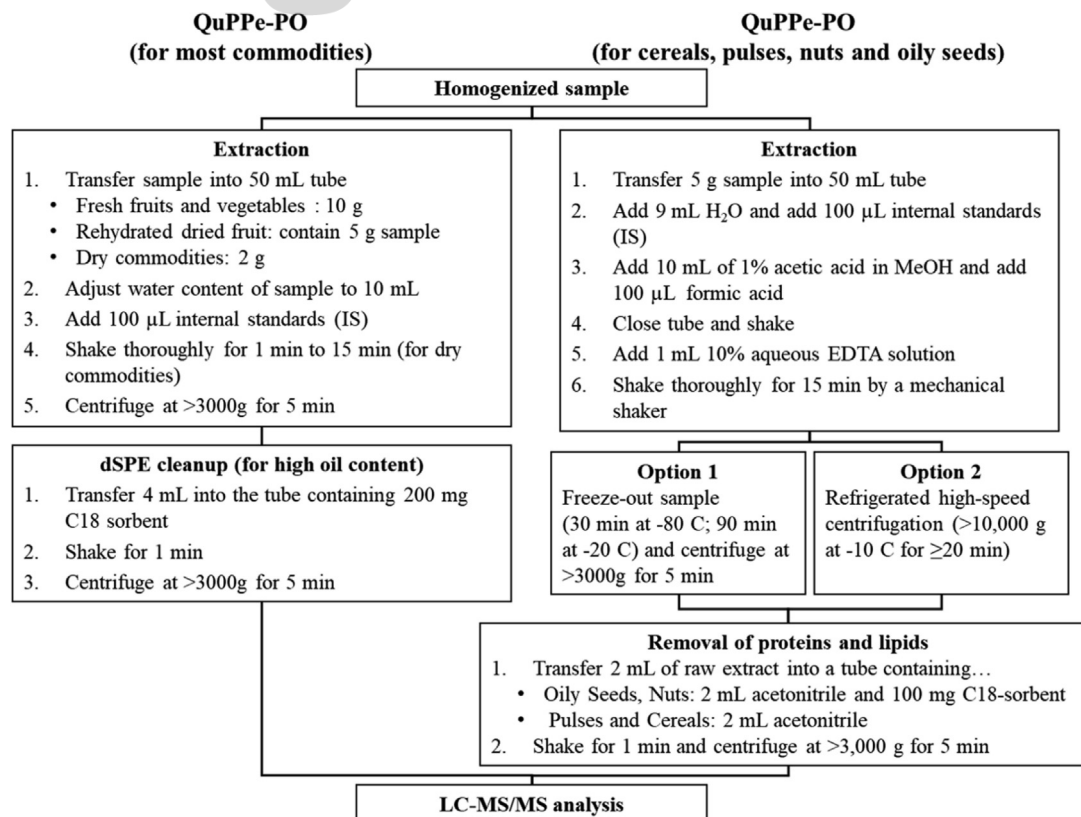
Fig. 1. Workflow of the EURL-SRM QuPPe methods.

good retention properties for highly polar compounds, but the disadvantage is that positive and negative ion analytes usually cannot be analyzed simultaneously.

For the subsequent detection of HPP, LC-MS/MS is the preferred option due to the high sensitivity and selectivity of this method, which allows multi-residue analysis. Moreover, MS can distinguish between pesticides with the same retention time based on their m/z values, which leads to increased specificity. Hence, LC-MS/MS is currently the most commonly used method for the analysis of HPP residues. The electrospray ionization - triple quadrupole mass spectrometer (ESI-QqQ-MS) using MRM acquisition is the gold standard for the quantification and determination of HPP [2]. In addition, high-resolution mass spectrometry (TOF and orbitrap) combined with PRM acquisition are used for quick determination of HPP residue in foods [9,17].

The main issues that are addressed in this review are: (i) integration of HPP characteristics, (ii) extraction methods for HPP in different foods, (iii) summary the LC-MS/MS-based methods used for HPP analysis (Fig. 2).

2. Sample preparation

The purpose of a sample pretreatment is to remove interferences from co-extracts or co-elutes, i.e., to reduce matrix effects. In recent years, the use of QuChERS has become the mainstream method for determining pesticides in food matrices [18]. The QuChERS procedure is demonstrated in Fig. 1S. In this method, target analytes are first extracted by liquid−liquid extraction with acetonitrile (ACN), and dSPE as a sorbent is used subsequently to remove the matrix. The advantage of QuChERS is the ability to clean up multi-residues at the same time, thus making it capable of treating different food matrices using various sorbents. However, the QuChERS method is unsuitable for use in cleaning up HPP since the target analyte prefers to remain in the aqueous phase, resulting in poor recovery.

The QuPPe method was first introduced by the European Reference Laboratory (EURL) in 2008, which allows for the simultaneous extraction of a variety of HPP, the detailed procedure of QuPPe is demonstrated in Fig. 1. The QuPPe method proceeds via water adjustment and the addition of acidified methanol to extract HPP residues in foods. However, samples with high oil contents including pulses, nuts,
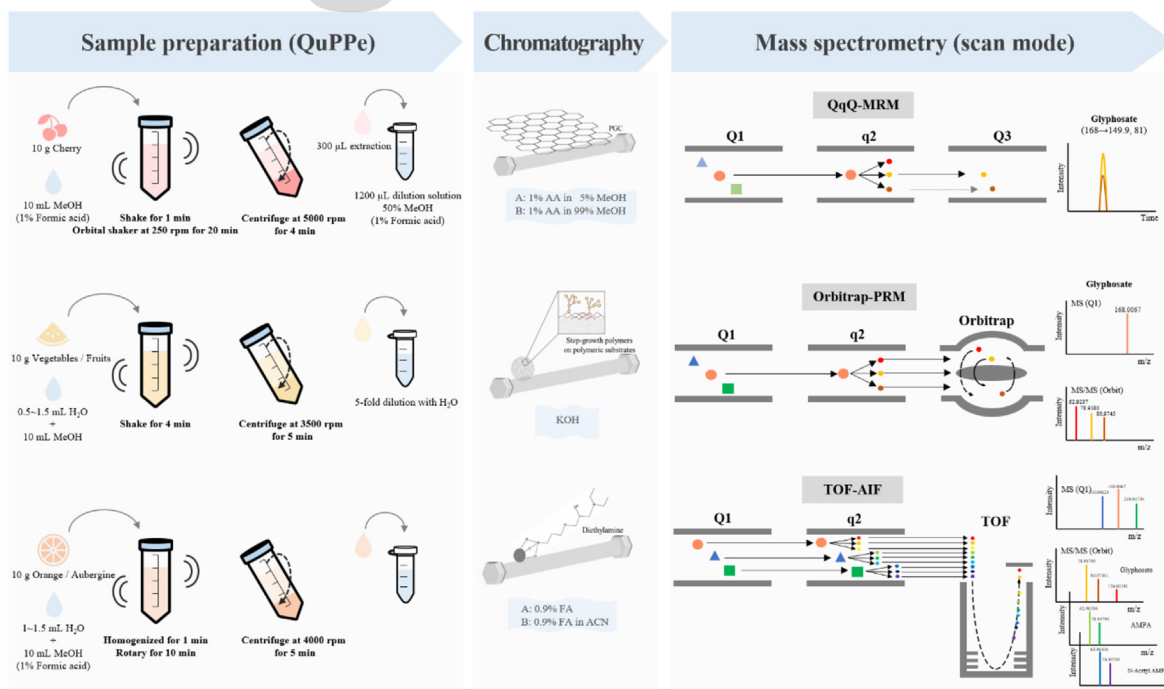
Fig. 2. Schematic depiction of HPP (AMPA, Glyphosate and N-Acetyl AMPA) analysis via 3 types of modified QuPPe sample preparation methods, and detected by liquid chromatographies combined with QqQ-MRM, Orbitrap-PRM and TOF-AIF MS [17,45,47].

and oils, are dissolved in acidified methanol and then cryogenically milled to prevent residues from degradation. The dSPE C18 sorbent is then added for sample clean-up. In recent studies, modified QuPPe methods were used to analyze specific sample matrices by optimizing extract solvent type and volume, extract time and dSPE sorbent [19]. In brief, the QuPPe method provides a method for the pretreatment for most food samples. The matrix effect can be reduced by modifying QuPPe individually [3].

In this section, the sample preparation methods are divided into three categories based on the properties of the matrices: (1) high water content commodities; (2) low water content commodities; (3) high oil content commodities.

2.1. High water-content commodities

QuPPe defines high water commodities as samples that contain more than 80% natural moisture and most fruits and vegetables therefore belong to this category [3]. The HPP residues in high water-content samples are extracted with methanol. HPP residues in a variety of orange species were successfully extracted with methanol [10]. Another study applied the QuPPe method coupled with LC-orbitrap-MS for the analysis of HPP residues in fruits, vegetables, and vegetable soups [20]. The addition of dSPE sorbents can reduce matrix interference. Five sorbents including C18, florisil,

graphitized carbon black (GCB), chitosan and graphene were evaluated for removing HPP residues from wheat, potatoes, and peas [13]. Among them, chitosan was found to be the best sorbent for the extraction of quaternary ammonium and phosphonic pesticides; the primary secondary amine (PSA) sorbent was suitable for removing the coextracts interfering with the analysis of amine and triazole pesticides [21]. In addition, a multi-walled carbon nanotube (MWCNT) dSPE sorbent was proposed to removed interfering gluphosinate and glyphosate pesticides coextracts [19].

Apart from QuPPe method, liquid–liquid extraction (LLE) has also been reported to be efficient for extracting HPP residues in high water-content commodities. Acidified methanol was used to extract HPP residues in fruits and vegetables [7]. An antioxidant, ascorbic acid, was found to give a better recovery for ethylene thiourea (ETU) and propylene thiourea (PTU) in comparison with other acid reagents [7]. In addition, a commercial SPE cartridge was also used in the extraction of glyphosate and other HPP in grapes [9]. Among methods mentioned in this article, the obtained recoveries were between 71% and 119%.

2.2. Low-water content/dry commodities

Low-water content/dry commodities are defined as samples that contain less than 80% moisture. The

QuPPe method suggests that milling the sample down into pieces smaller than 500 μm before adding water can shorten the extraction time. Adjustment of the water content followed by LLE using water and methanol was conducted on 10 g of milled samples. It is essential that 10% of EDTA was added to analytes with showed poor recovery. In addition, ACN was added to samples such as cereals for precipitate protein or lipid; in a recent study, residues in oat, wheat, and rice were reported to be extracted efficiently by QuPPe [3,8,22].

The same method for high water-content commodities, modification by additional dSPE and SPE were also applied to reduce the matrix effect associated with the analysis of HPP residues in low-water content commodities. For example, a supplemental clean-up method using dSPE as the sorbent was applied to clean up quaternary ammonium, phosphonic acid, and glufosinate pesticide residues [19]. The clean-up efficiency of 5 sorbents, including C18, CGB, florisil, chitosan, and graphene, were also evaluated for wheat samples [13]. A chitosan sorbent clean-up showed the lowest matrix effect; the MWCNT sorbent was applied to the extraction of HPP residue extracts [19]. For SPE, the performance of different commercial SPE cartridges was evaluated. The result indicates that combining mixed-mode cation-exchange (MCX) and enhanced matrix removal (EMR) can give the best recovery and the lowest matrix effect of HPP residues in oats [14,23,24]. Another easy way to precipitate unwanted coextracts is by freezing the extracts [15]. This method was used in a sample pretreatment procedure for the determination of multi-residues in cereal samples. In summary, with the optimized sample pretreatment for low-water commodities, the recoveries were within 70%–120%.

2.3. High oil/lipid content commodities

The sample pretreatment procedure for high oil-content commodities (e.g. nuts, oily seeds and avocado) is very different from those described above. These commodities tend to form a thick paste during milling at room temperature. To mill cryogenically, as suggested by QuPPe, can prevent this from happening [3]. Oil and nuts are representative food samples in this category [11]. The high oil/lipid content QuPPe sample pretreatment process is the same as the procedure for low-water content commodities but additional clean-up steps are needed in which the sample is extracted with n-hexane and then cleaned up with a C18 sorbent [11]. A hydrophilic-lipophilic-balanced (HLB) SPE cartridge was also used for the determination of glyphosate

residues in cereals [9]. This technique enables sample pretreatment and analysis to be completed at the same time. In addition, an ion exchange mini column can also be utilized as a sample clean-up method for quantifying fosetyl-Al and phosphonic acid in rice, wheat, and barley [23].

Glufosinate residues in soybean oil was extracted by MWCNT and analyzed by the derivatization LC-MS method with acceptable recovery [19]. The advantage of this method is that the use of dichloromethane is avoided in soy-based samples [25]. In another study, low water phase LLE with heptafluorobutyric acidified n-hexane was used to extract diquat and paraquat from olive oil [26]. The recoveries of analytes obtained in reported optimized sample pretreatment for high-oil commodities were within 70%–119%.

3. LC-MS/MS

3.1. LC

Polar pesticides show poor retention and peak shapes when separated using the C18 stationary phases that are commonly used for multi-residue methods for pesticides. For this reason, columns with different stationary phases other than C18 have been developed for use in the analysis of HPP. Reverse-phase chromatography (RPC), hydrophilic interaction liquid chromatography (HILIC), ion chromatography (IC) and mixed-mode chromatography (MMC) were used to retain and separate highly polar compounds based on the characteristics of each stationary phase. LC-based separation in the analysis of HPP residues in foods is summarized in Table 2.

3.1.1. Reversed-phase chromatography (RPC)

Reversed-phase chromatography is widely used to separate less polar compounds. Applying RP columns for the separation for polar compounds mainly relies on two approaches, i.e., target compound derivatization and stationary phase modification. The derivatization reagents consist of a reactive group that reacts with the analytes and a nonpolar modified group which can enhance the retention of the derivatized HPP in RP. For example, glufosinate residues in 12 foods of plant origin were determined by derivatizing the samples with a 9-fluorenylmethyl chloroformate (FMOC-Cl) derivation reagent and coupled with an RP-base LC-MS/MS analysis [19]. In another study, the FMOC-Cl derivatization method was applied after the SPE method to determine glyphosate and aminomethylphosphonic acid (AMPA) residues in 16

Table 2. Summary of the experimental conditions for HPP analyses for different types of chromatographic columns.

Column	Mobile Phase	Pesticides	Matrix	Ref.
Porous Graphitic Carbon				
Pros: It is stable throughout the entire pH range 1−14, and resists extreme acidic and alkaline conditions.				
Cons: The PGC column needs to take more time for stabilization or alter surface properties.				
Hypercarb™ porous graphitic carbon	A: H_2O/Methanol/Formic acid, 94/5/1 B: Methanol/Formic acid, 99/1	Chlorate, Ethephon, Fosetyl-Al, Glyphosate, Perchlorate, Phosphonic acid	Pomegranate	[12]
	A: H_2O/Methanol/Formic acid, 94/5/1 B: Methanol/Formic acid, 99/1	AMPA, N-acetyl-AMPA, Chlormequat, Ethephon, Glufosinate-Al, N-acetyl-glufosinate, Glyphosate, Maleic hydrazide	Cherry	[45]
	A: H_2O/Methanol/Formic acid, 95/5/1 B: Methanol/formic acid, 99/1	Chlorate, Perchlorate, Bromate	Strawberry, Grape, Apple, Red wine, Rye, Orange, Rice	[44]
	A: H_2O with 5 mL/L HFo B: Methanol with 5 mL/L HFo	1,2,4-Triazole, Triazole-alanine, Triazole-lactic acid, Triazole-acetic acid	Lettuce, Tomato, Broccoli, French bean, Soy bean, Turnip root, Carrot root, Barley grain, Orange, Grape, Flax seed	[48]
C18				
Pros: Derivatization with a derivatizing agent like FMOC-Cl enhances retention capabilities to improve retention for high polar compounds.				
Cons: The derivatization efficiency is indeterminate and the derivatization process is time-consuming.				
UltiMate XB-C18	A: ACN B: 5 mM Ammonium acetate	Glufosinate	Grape, Papaya, Banana, Apple, Celery, Eggplant, Leek, Tomato, Green tea, Maize, Soya beans, Soya bean oil	[19]
XBridge™ C18	A: Methanol B:10 mM Ammonium formate and 0.1% formic acid	Dinotefuran, MNG, UF, DN	Tea	[49]
Zorbax Eclipse Plus	A: 10 mM Ammonium formate (pH 4) B: ACN (isocratic, 95/5)	Maleic hydrazide	Onion, Potato, Grape, Citrus	[50]
Aquasil C18	A: H_2O with 5 mL/L HFo B: Methanol with 5 mL/L HFo	1,2,4-Triazole, Triazole-alanine, Triazole-lactic acid, Triazole-acetic acid	Lettuce, Tomato, Broccoli, French bean, Soy bean, Turnip root, Carrot root, Barley grain, Orange, Grape, Flax seed	[48]
Negative charge/hydrophobic linkages				
Positive charged/hydrophilic linkages				
Pros: It is a unique mixed-mode stationary phase with various mechanisms.				
Cons: Instability of retention time and high background.				
Obelisc R	A: ACN B: 0.5% Formic acid C: H_2O	Kasugamycin, Streptomycin	Tomato, Chard, Lettuce, Zucchini, Red Pepper	[6]
Obelisc N	A: 1% Formic acid B: ACN	AMPA, Bromide, Ethephon, Chlorate, Perchlorate, Fosetyl-Al, Glufosinate, Glyphosate, HEPA, MPPA, N-acetyl-AMPA, N-acetyl-glufosinate, N-acetyl-glyphosate, Phosphonic acid	Grapes, Lettuce, Orange, Oat, Soya beans	[14]
	A: 20 mM Ammonium formate and 1% Formic acid B: ACN	Chlormequat, Diquat, Glyphosate, AMPA, N-acetyl-AMPA, Trimethylsulfonium, Glufosinate, N-acetyl-glufosinate, Maleic hydrazide, Mepiquat, Paraquat	Onion, Wheat, Potato, Pea	[13]
	A: 1% Formic acid B: ACN	AMPA, Bromide, Chlorate, Ethephon, Fosetyl-Al Glufosinate, Glyphosate, HEPA, MPPA, N-acetyl-AMPA, N-acetyl-glufosinate, N-acetyl-glyphosate, Perchlorate, Phosphonic acid	Milk, Wine, Beer	[36]

Table 2. (continued)

Column	Mobile Phase	Pesticides	Matrix	Ref.
Hydroxide-selective anion-exchange				
Pros: High matrix loading withstands and provides reproducible chromatogram and retention times.				
Cons: Generally low LC resolution compared to others.				
IonPac™ AS19	KOH	Fosetyl-Al, Phosphonic acid, Ethephon, Ethephon hydroxy, Glufosinate, N-acetyl-glufosinate, MPPA, Glyphosate, N-acetyl-glyphosate, AMPA, N-acetyl-AMPA	Grape, Honey, Wheat	[9]
		Glyphosate, AMPA, N-acetyl-AMPA, Glufosinate, 3-MPPA, N-acetyl-glufosinate, Perchlorate, Chlorate, Ethephon, Clopyralid, Fosetyl-Al, Phosphonic acid, Cyanuric acid, Bialaphos	Cereal, Grape, Infant food	[15]

different food matrices. This result demonstrates that the derivatization is a reliable method for determining low concentrations of AMPA in foods [27].

The porous graphitic carbon (PGC) column is equipped with a flat graphite surface and thus has a better capability to retain polar compounds than the conventional C18 RP column. Since the flat surface adsorbs the polar compound better and is able to resist both extreme acidic and alkaline conditions (in the range from pH 1 to 14) [28]. The PGC column was successfully used to separate chlorate, perchlorate, ethephon, fosetyl-Al, glyphosate, and phosphonic acid in numerous pomegranate samples which had high water contents [12]. In other studies, the PGC column was used to retain perchlorate and maleic acid in low water soy nutraceutical products and chlorate [25] and phosphoric acid derivatives in oil and nuts [11]. The PGC column was also used to separate chlorate, perchlorate, glyphosate and AMPA in processed fruits and vegetables by isocratic elution [20]. These studies demonstrate the versatility of PGC columns in the analysis of HPP residues in different types of matrices.

3.1.2. Hydrophilic interaction liquid chromatography (HILIC)

Hydrophilic interaction liquid chromatography is widely used in the separation of medium to high polar compounds and are especially suited for the analysis of HPP. The HILIC column is used to separate chlorate, fosetyl-Al, maleic hydrazide and perchlorate in soy nutraceuticals [25]. The HILIC column was also applied to the analysis of 2-pyrrolidone, N-methyl-2-pyrrolidone and N-ethyl-2-pyrrolidone in fruits [29]. In addition, HILIC was applied to separate highly polar pesticides such as glyphosate, glufosinate, ethephon and fosetyl in highly complex feed materials [30]. The HILIC column was used to separate quaternary ammonium pesticides (QUATs) paraquat, diquat, chlormequat and mepiquat residues in barley and wheat by LC-MS/MS [31].

3.1.3. Ion chromatography (IC)

Ion chromatography can effectively retain highly polar compounds and has good selectivity for ionic compounds. The usefulness of this method was confirmed for the analysis of environmentally relevant micropollutants such as the herbicide glyphosate and its metabolite AMPA [32]. In addition, an IC column was selected for the detection of HEPA, ethephon, chlorate and perchlorate. The matrix effect of ethephon in apples was reduced by optimizing the gradient elution conditions, in which the mobile phase consists of %ACN and %ACN containing ammonium bicarbonate [33]. The IC procedure was also applied to the separation of ethephon, glufosinate, 3-(Methylphosphinico)propionic acid (MPPA) and AMPA, and 11 anionic HPP in honey using KOH as a mobile phase for gradient elution [9]. Compared to the reverse phase mode, the quantification of glyphosate, glufosinate, fosetyl-Al and related metabolites, the use of an IC column can reduce analysis time and mass-loss due to derivatization [34]. The IC was proven to be useful for the separation of glyphosate, glufosinate, chlorate, perchlorate and fosetyl-Al spiked in oat flour with a fat content and in high-water grapes [15].

3.1.4. Mixed-mode chromatography (MMC)

Mixed-mode chromatography (MMC) represents a novel and attractive option, in that the advantages of each separate mode, i.e., reverse phase, anion exchange and cation exchange can be combined. In addition, selected mixed-mode columns combine multiple chromatographic modes that are complementary or orthogonal to each other [35]. A mixed-mode column was successfully used in detecting pesticides in carrots and apples, containing amitrole, ETU, PTU, cyromazine and daminozide HPP residues. For example, increasing the concentration of ACN in the mobile phase which can shorten the retention time for cyromazine in the reversed-phase mode permitted daminozide to be retained in the ion-exchange mode. However, the mixed-mode column of HILIC/ion-exchange mode successfully reduced the retention time of amitrole and sharp peaks were observed for other analytes [7]. Moreover, it was also applied to the detection of AMPA, ethephon and fosetyl-Al in tomatoes and onion. Mobile phase adjustment can be crucial in the case of a mixed-mode mechanism. In this type of MMC, HILIC retention was adjusted by adjusting the ratio of ACN, but the ion-exchange mode was dependent on the percentage of buffer being used [16].

In addition, a stationary phase with functional group modifications is also an option for the separation of HPP residues. Adding certain polar functional groups such as cyano or amino groups at the end of the carbon chain can be used to adjust the affinity of the column for polar compounds. For example, a cyano column was applied to the separation of amitrole, chlormequat, ethephon, and glyphosate residues and an amino column was applied to the separation of diethanolamine, triethanolamine, and morpholine residues in orange samples [10]. In addition, a novel Obelisc R column, which has reversed-phase characteristics and cationic groups close to the silica surface separated from anionic groups by a hydrophobic chain was developed. It was used to separate ethephon, glyphosate and streptomycin, and a total of 26 HPP compounds in oranges [10]. An Obelisc R column was also used to separate kasugamycin and streptomycin in tomatoes, and the elution method involved simultaneously decreasing the ACN and increasing the formic acid concentration in the mobile phase [6]. Moreover, the Obelisc N column has been frequently used in the analysis of polar pesticides. A column equipped with positively charged and hydrophilic linkages that interact with high polar compounds was reported. Thus, phosphoric acid and its metabolites can be separated and analyzed in a single run [14]. Compared to the PGC column, the Obelisc N column showed better reproducibility and sensitivity when they were applied to the determination of AMPA, HEPA, MPPA and ethephon in fruits, vegetables and cereals [14]. The Obelisc N column was also used to separate anionic HPP constituents in milk, wine and beer [36].

3.2. MS

LC-MS is the most commonly used method for the analysis of HPP residues and LC tandem MS (LC-MS/MS) is the method of choice. LC-MS/MS has been applied to quantify HPP residues in food matrices in EURL-SRM. The standard method is electrospray ionization combined with a triple quadrupole mass spectrometer (QqQ MS) [22]. According to the QuPPe-PO method (V12), there are 55 HPP that are currently in use and 33 HPP compounds can be analyzed in the positive mode and 22 can be analyzed in the negative mode. In the positive ion mode, the majorities are azo compounds for which the N atoms are acidified by adding formic acid or acetic acid that easily form $[M+H]^+$ when ESI is applied as the ion source; In the negative ion mode, most of them are phosphoric acid derivatives that form $[M-H]^-$ when the ESI is used [3].

3.2.1. Ionization method and polarity

Pesticides that can be analyzed in the ESI positive ion mode mostly contain nitrogen functional groups, such as triazole, triazine, and nicotine. To cite an example, triazole is a 5-membered aromatic ring that contains 3 nitrogen atoms. These pesticides are highly polar and slightly alkaline, making them stable in the ESI interface [37]. Triazine groups such as cyromazine and melamine are 6-membered aromatic rings that contain 3 nitrogen atoms. These pesticides are also slightly alkaline, making their molecular ions stable in ESI [38]. Nicotine is relatively less polar, consequently, the atmospheric pressure chemical ionization (APCI) method can also be an alternative option [39]. This result was reported that both ESI and APCI can apply as ionization methods for nicotine in human plasma without difference. However, ESI is still the most popular ionization method in nicotine pesticide analysis. For instance, the nicotine in tea was determined using LC-ESI-MS/MS with lower LOD and LOQ [40]. Other HPP analyzed in ESI positive ion mode, including amine [31], aminoglycoside [6], hydrazide [30,41], matrine [42], quaternary ammonium [26], and thiourea [43] derivatives, have mostly been analyzed using ESI while coupled with LC in recent studies.

The pesticides that are analyzed in the ESI negative ion mode mostly contain phosphate and halogen. Phosphate-containing HPP can be categorized into two groups, phosphonic acid and glufosinate pesticides. The phosphonic acid pesticides including ethephon, hydroxyethyl phosphonic acid (HEPA), and amino-methyl phosphonic acid (AMPA) et al., have different functional groups attached to the phosphonic acid. Moreover, glufosinate pesticides including glufosinate, MPPA, and bialaphos., that are glufosinate pesticides in which a methyl group substitutes for the hydrogen on the phosphinic acid group. The ESI negative ion mode has been used successfully to observe and quantify $[M-H]^-$ ions derived from these HPP including ethephon, AMPA, glufosinate, and other HPP [34]. However, the structures of the halogen HPP are very diverse and includes inorganic oxyhalides and organohalogens. It has been reported that oxyhalides like chlorate, perchlorate, and bromate in water and food samples can be determined by LC-ESI-MS/MS [44]. In summary, although other ionization methods such as APCI have been applied to HPP analysis, ESI is still the most suitable ionization method for use in LC-MS/MS-based HPP analyses.

3.2.2. Scan function

Multiple reaction monitoring (MRM) or single reaction monitoring (SRM) is the most potent scan mode for the quantification of pesticides. For example, MRM was used to determine 8 HPP (AMPA, N-acetyl-AMPA, ethephon, glyphosate, chlormequat chloride, ammonium glufosinate, N-acetyl-glufosinate, and maleic hydrazide) in cherries. The validation results show excellent repeatability and reproducibility [45]. In recent years, parallel reaction monitoring (PRM) in quadrupole-orbitrap MS has become an alternative option for HPP quantification. It compensates for the analytical challenges of MRM/SRM scan functions, including low resolution and an impaired sensitivity. The PRM scan function was coupled with SPE and a dilution sample pretreatment method to quantify HPP residues in food samples in recent studies [9,17]. PRM appears to be a reliable scan function for HPP residue analysis; however, the narrower dynamic range makes it less used than MRM.

Apart from quantitative analysis, high-resolution mass spectrometry (HRMS), including orbitrap and time-of-flight (TOF), has been applied in qualitative studies of HPP. Due to the high mass accuracy of HRMS, the full scan mode can determine HPP residues by their accurate mass [46]. Other scan functions include data independence acquisition, which has been used for the qualitative analysis of HPP residue

in food samples. All ion fragmentation (AIF, Thermo), a type of DIA scan mode, was applied to obtain the production of 7 HPPs in fruit and vegetable samples. AIF scan provided a reliable result considering that at least 2 fragments were monitored per compound [47].

4. Conclusions

In this review, we summarize the analytical methods that are currently available for the detection of highly polar pesticides, including various versions QuPPe, LC separation and MRM/SRM-MS detection, with emphasis on measuring low residue concentrations. Mixed-mode chromatography has considerable potential for the analysis of residues in agricultural products using combining different stationary phases characteristics such as RPC/IC and RPC/HILIC stationary phases. In addition, high-resolution mass spectrometry (TOF and orbitrap) combined with the PRM scan mode provides an alternative method for MS detection in HPP analysis. This methodology possesses significant value regarding the sensitive and rapid determination of HPP residues in foods and further studies in various fields of application can be expected.

Acknowledgments

References

[1] Highly polar pesticide multi-residue analysis in food safety by LC-MS/MS (Shimadzu application news LC/MS No.C118). 2015. Available at: https://www.shimadzu.com/an/sites/shimadzu.com.an/files/pim/pim_document_file/applications/application_note/12240/ego115089.pdf.

[2] Zhang L, Rana I, Shaffer RM, Taioli E, Sheppard L. Exposure to glyphosate-based herbicides and risk for non-Hodgkin lymphoma: a meta-analysis and supporting evidence. Mutat Res Rev Mutat Res 2019;781:186−206.

[3] Quick method for the analysis of highly polar pesticides in food involving extraction with acidified methanol and LC- or IC-MS/MS measurement - I. In: Food of plant origin (QuPPe-PO-Method) − version 12 (published on EURL-SRM website on July 23, 2021); 2021. Available at: https://www.eurl-pesticides.eu/docs/public/tmplt_article.asp?CntID=887&LabID=200&Lang=EN.

[4] Bueno MJM, Díaz-Galiano FJ, Ł Rajski, Cutillas V, Fernández-Alba AR. A non-targeted metabolomic approach to identify food markers to support discrimination between organic and conventional tomato crops. J Chromatogr A 2018;1546:66−76.

[5] Goscinny S, Unterluggauer H, Aldrian J, Hanot V, Masselter S. Determination of glyphosate and its metabolite AMPA (aminomethylphosphonic acid) in cereals after derivatization by isotope dilution and UPLC-MS/MS. Food Anal Methods 2012;5:1177−85.

[6] Alechaga É, Moyano E, Galceran MT. Simultaneous analysis of kasugamycin and streptomycin in vegetables by liquid

chromatography-tandem mass spectrometry. Anal Methods 2015;7:3600−7.

[7] Chamkasem N. Rapid determination of polar pesticides and plant growth regulators in fruits and vegetables by liquid chromatography/tandem mass spectrometry. J Environ Sci Health, Part B 2018;53:622−31.

[8] Botero-Coy AM, Ibáñez M, Sancho JV, Hernandez F. Direct liquid chromatography−tandem mass spectrometry determination of underivatized glyphosate in rice, maize and soybean. J Chromatogr A 2013;1313:157−65.

[9] Gasparini M, Angelone B, Ferretti E. Glyphosate and other highly polar pesticides in fruit, vegetables and honey using ion chromatography coupled with high resolution mass spectrometry: method validation and its applicability in an official laboratory. J Mass Spectrom 2020;55:e4624.

[10] Vass A, Robles-Molina J, Pérez-Ortega P, Gilbert-López B, Dernovics M, Molina-Díaz A, et al. Study of different HILIC, mixed-mode, and other aqueous normal-phase approaches for the liquid chromatography/mass spectrometry-based determination of challenging polar pesticides. Anal Bioanal Chem 2016;408:4857−69.

[11] Hidalgo-Ruiz JL, Romero-González R, Vidal JLM, Frenich AG. Monitoring of polar pesticides and contaminants in edible oils and nuts by liquid chromatography-tandem mass spectrometry. Food Chem 2021;343:128495.

[12] Gormez E, Golge O, Kabak B. Quantification of fosetyl-aluminium/phosphonic acid and other highly polar residues in pomegranates using Quick Polar Pesticides method involving liquid chromatography-tandem mass spectrometry measurement. J Chromatogr A 2021;1642:462038.

[13] Kaczyński P. Clean-up and matrix effect in LC-MS/MS analysis of food of plant origin for high polar herbicides. Food Chem 2017;230:524−31.

[14] López SH, Scholten J, Kiedrowska B, de Kok A. Method validation and application of a selective multiresidue analysis of highly polar pesticides in food matrices using hydrophilic interaction liquid chromatography and mass spectrometry. J Chromatogr A 2019;1594:93−104.

[15] Adams S, Guest J, Dickinson M, Fussell RJ, Beck J, Schoutsen F. Development and validation of ion chromatography−tandem mass spectrometry-based method for the multiresidue determination of polar ionic pesticides in food. J Agric Food Chem 2017;65:7294−304.

[16] Cutillas V, Fernández-Alba AR. Analysis by LC-MS/MS of polar pesticides in fruits and vegetables using new hybrid stationary phase. MethodsX 2021;8:101306.

[17] Ł Rajski, Díaz Galiano FJ, Cutillas V, Fernández-Alba AR. Coupling ion chromatography to Q-orbitrap for the fast and robust analysis of anionic pesticides in fruits and vegetables. J AOAC Int 2018;101:352−9.

[18] Anastassiades M, Lehotay SJ, Štajnbaher D, Schenck FJ. Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. J AOAC Int 2003;86:412−31.

[19] Han Y, Song L, Zhao P, Li Y, Zou N, Qin Y, et al. Residue determination of glufosinate in plant origin foods using modified Quick Polar Pesticides (QuPPe) method and liquid chromatography coupled with tandem mass spectrometry. Food Chem 2016;197:730−6.

[20] Savini S, Bandini M, Sannino A. An improved, rapid, and sensitive ultra-high-performance liquid chromatography-high-resolution orbitrap mass spectrometry analysis for the determination of highly polar pesticides and contaminants in processed fruits and vegetables. J Agric Food Chem 2019;67:2716−22.

[21] Lara FJ, Chan D, Dickinson M, Lloyd AS, Adams SJ. Evaluation of direct analysis in real time for the determination of highly polar pesticides in lettuce and celery using modified Quick Polar Pesticides Extraction method. J Chromatogr A 2017;1496:37−44.

[22] Tóth E, Tölgyesi Á, Bálint M, Ma X, Sharma VK. Separation of fosetyl and phosphonic acid in food matrices with mixed-mode HPLC column coupled with tandem mass spectrometric detection and method application to other highly polar pesticides. J Chromatogr B 2022;1189:123083.

[23] Sekiyama A, Toriumi E, Yamada Y. Single-and multiple-laboratory validation of LC-MS/MS method for simultaneous determination of fosetyl-Al and phosphonic acid in cereal grains and analysis of rice, wheat, and barley. J AOAC Int 2021;104:1298−307.

[24] Ciasca B, Pecorelli I, Lepore L, Paoloni A, Catucci L, Pascale M, et al. Rapid and reliable detection of glyphosate in pome fruits, berries, pulses and cereals by flow injection−Mass spectrometry. Food Chem 2020;310:125813.

[25] Domingos Alves R, Romero-González R, López-Ruiz R, Jiménez-Medina M, Garrido Frenich A. Fast determination of four polar contaminants in soy nutraceutical products by liquid chromatography coupled to tandem mass spectrometry. Anal Bioanal Chem 2016;408:8089−98.

[26] Aramendía MA, Borau V, Lafont F, Marinas A, Marinas JM, Moreno JM, et al. Determination of diquat and paraquat in olive oil by ion-pair liquid chromatography−electrospray ionization mass spectrometry (MRM). Food Chem 2006;97:181−8.

[27] Bo L, Xiaojun D, Dehua G, Shuping J. Determination of glyphosate and aminomethylphosphonic acid residues in foods using high performance liquid chromatography-mass spectrometry/mass spectrometry. Chin J Chromatogr 2007;25:486−90.

[28] Polyakova Y, Ho Row K. HPLC of some polar compounds on a porous graphitized carbon HypercarbTM column. J Liq Chrom Relat Tech 2005;28:3157−68.

[29] Li H, Jiang Z, Cao X, Su H, Shao H, Jin F, et al. Simultaneous determination of three pesticide adjuvant residues in plant-derived agro-products using liquid chromatography-tandem mass spectrometry. J Chromatogr A 2017;1528:53−60.

[30] Wang L, Fei T, Qi D, Sha Y, Wu D, Liu B. Development of microwave-assisted extraction and liquid chromatography-tandem mass spectrometry for determination of maleic hydrazide residues in tobacco. Anal Methods 2015;7:5103−7.

[31] Sabatino L, Scordino M, Caruso R, Chiappara E, Traulo P, Belligno A, et al. LC/MS/MS detection of short-chain aliphatic amines in glazing agents for fruit coating. Eur Food Res Tech 2012;235:177−84.

[32] Bauer K-H, Knepper TP, Maes A, Schatz V, Voihsel M. Analysis of polar organic micropollutants in water with ion chromatography−electrospray mass spectrometry. J Chromatogr A 1999;837:117−28.

[33] Bauer A, Luetjohann J, Rohn S, Kuballa J, Jantzen E. Ion chromatography tandem mass spectrometry (IC-MS/MS) multimethod for the determination of highly polar pesticides in plant-derived commodities. Food Control 2018;86:71−6.

[34] Melton LM, Taylor MJ, Flynn EE. The utilisation of ion chromatography and tandem mass spectrometry (IC-MS/MS) for the multi-residue simultaneous determination of highly polar anionic pesticides in fruit and vegetables. Food Chem 2019;298:125028.

[35] Lesellier E, West C, Lemasson E, Hennig P, Bertin S. Mixed-mode chromatography−a review. 2017.

[36] Lopez SH, Dias J, Mol H, de Kok A. Selective multiresidue determination of highly polar anionic pesticides in plant-based milk, wine and beer using hydrophilic interaction liquid chromatography combined with tandem mass spectrometry. J Chromatogr A 2020;1625:461226.

[37] Blondel A, Krings B, Ducat N, Pigeon O. Validation of an analytical method for 1, 2, 4-triazole in soil using liquid chromatography coupled to electrospray tandem mass spectrometry and monitoring of propiconazole degradation in a batch study. J Chromatogr A 2018;1562:123−7.

[38] Wang P-C, Lee R-J, Chen C-Y, Chou C-C, Lee M-R. Determination of cyromazine and melamine in chicken eggs using quick, easy, cheap, effective, rugged and safe (QuEChERS) extraction coupled with liquid chromatography−tandem mass spectrometry. Anal Chim Acta 2012;752:78−86.

[39] Beyer J, Peters FT, Kraemer T, Maurer HH. Detection and validated quantification of toxic alkaloids in human blood plasma—comparison of LCAPCIMS with LCESIMS/MS. J Mass Spectrom 2007;42:621—33.

[40] Thräne C, Isemer C, Engelhardt UH. Determination of nicotine in tea (Camellia sinensis) by LC—ESI—MS/MS using a modified QuEChERS method. Eur Food Res Tech 2015;241: 227—32.

[41] Mol HG, van Dam RC, Vreeken RJ, Steijger OM. Determination of daminozide in apples and apple leaves by liquid chromatography—mass spectrometry. J Chromatogr A 1999; 833:53—60.

[42] Jong T-T, Lee M-R, Chiang Y-C, Chiang S-T. Using LC/MS/MS to determine matrine, oxymatrine, ferulic acid, mangiferin, and glycyrrhizin in the Chinese medicinal preparations Shiau-feng-saan and Dang-guei-nian-tong-tang. J Pharmaceut Biomed Anal 2006;40:472—7.

[43] Berton T, Mayhoub F, Chardon K, Duca R-C, Lestremau F, Bach V, et al. Development of an analytical strategy based on LC—MS/MS for the measurement of different classes of pesticides and theirs metabolites in meconium: application and characterisation of foetal exposure in France. Environ Res 2014;132:311—20.

[44] Constantinou P, Louca-Christodoulou D, Agapiou A. LC-ESI-MS/MS determination of oxyhalides (chlorate, perchlorate and bromate) in food and water samples, and chlorate on household water treatment devices along with perchlorate in plants. Chemosphere 2019;235:757—66.

[45] Golge O. Validation of quick polar pesticides (QuPPe) method for determination of eight polar pesticides in cherries by LC-MS/MS. Food Anal Methods 2021;14:1432—7.

[46] Padilla-Sánchez JA, Plaza-Bolaños P, Romero-González R, Grande-Martínez Á, Thurman EM, Garrido-Frenich A. Innovative determination of polar organophosphonate pesticides based on high-resolution Orbitrap mass spectrometry. J Mass Spectrom 2012;47:1458—65.

[47] Manzano-Sánchez L, Martínez-Martínez JA, Domínguez I, Martínez Vidal JL, Frenich AG, Romero-González R. Development and application of a novel pluri-residue method to determine polar pesticides in fruits and vegetables through liquid chromatography high resolution mass spectrometry. Foods 2020;9:553.

[48] Jasak J, Blanc YL, Speer K, Billian P, Schoening RM. Analysis of triazole-based metabolites in plant materials using differential mobility spectrometry to improve LC/MS/MS selectivity. J AOAC Int 2012;95:1768—76.

[49] Rahman MM, Abd El-Aty A, Choi J-H, Kim S-W, Shin SC, Shim J-H. Consequences of the matrix effect on recovery of dinotefuran and its metabolites in green tea during tandem mass spectrometry analysis. Food Chem 2015;168:445—53.

[50] Abdelwahed MH, Khorshed MA, Elmarsafy AM, Elshabrawy MS, Souaya ER. Polar reversed-phase liquid chromatography coupled with triple quadrupole mass spectrometer method for simple and rapid determination of maleic hydrazide residues in some fruits and vegetables. Food Anal Methods 2021;14:172—85.