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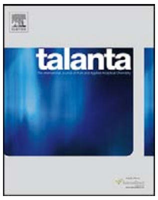

Review

Analytical methods for estimation of organophosphorus pesticide residues in fruits and vegetables: A review

Dipakshi Sharma [a], Avinash Nagpal [a], Yogesh B. Pakade [b], Jatinder Kaur Katnoria [a,*]

[a] *Department of Botanical and Environmental Sciences, Guru Nanak Dev University, Amritsar 143005, Punjab, India*
[b] *Hill Area Tea Science Division, Institute of Himalayan Bioresources and Technology (CSIR-IHBT), Palampur 176061, Himachal Pradesh, India*

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ABSTRACT

Use of pesticides has turned out to be an obligatory input to agriculture and public health. Versatile use of pesticides had resulted in contamination of all basic necessities of life, i.e. air, water and food. Among various pesticides, organophosphorus pesticides (OPPs), derivative of phosphoric acid, are the most extensively used insecticides or acaricides in many crops. Due to low persistency and high killing efficiency of OPPs, many agriculturalists regularly use this group of pesticides for various vegetables and fruits crops. The continuous use of pesticides has caused the deleterious effects to ecosystem. In response to this, a number of methods have been developed by several regulatory agencies and private laboratories and are applied routinely for the quantification and monitoring of multi pesticide residues in vegetables and crops. The present review pertains to various extraction and quantification procedures used world wide to analyze OPPs residues in various vegetables and fruits.

Contents

1. Introduction

Fresh vegetables, fruits and pulses are the important part of a healthy diet because of the presence of significant amount of nutrients and minerals in them. However, at the same time, they can also turn out to be source of toxic substances such as pesticides [1]. The use of pesticides in agriculture has increased after World War II in order to increase the world food production. Since then there had been marked development of different types of pesticides belonging to various groups. The use of pesticides and additional environmental pollution due to industrial emissions during the production of pesticides have resulted in occurrence of residues of these chemicals and their metabolites in every component of environment, i.e. air, water and soil along with that in the crops, vegetables and fruits [2].

Among various pesticide classes, organophosphorus pesticide (OPPs) group is the most widely used class of agricultural pesticides [2–8]. In recent years, many studies have proved OPPs to be mutagenic, carcinogenic [5,8–12], cytotoxic [13,14], genotoxic [15–17], teratogenic [18] and immunotoxic [19,20]. Some of the

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* Corresponding author. Tel.: +91 9815741922; fax: +91 0183 2258820.
E-mail address: jkat08@yahoo.com (J.K. Katnoria).

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pesticides belonging to OPPs group are also known for their interferences in reproduction especially in males [21]. There are reports on the testicular toxic effects of parathion (OPP) in males and mouse [22,23] and chromosomal aberrations in peripheral lymphocytes of mouse [15]. Few studies reported that the exposure of OPPs had posed a direct potential risk to human health by inhibiting acetyl cholinesterase and modifying cholinergic signaling [5,8]. OPPs have tendency to bind to the enzyme acetyl cholinesterase and to disrupt nerve functioning which further result in paralysis and death [2,24–37].

In different fruits and vegetables, for better yield and quality, pesticides are repeatedly applied during the entire period of growth and sometimes even at the fruiting stage. These are absorbed by the vegetables and turned out to be noxious when consumed by human beings [38]. It is anticipated that over 1000 compounds are applied to agricultural crops in order to control undesirable weeds, moulds, insects and pests [39]. Many scientists have estimated the pesticide residues (PRs) in various fruits including banana, mango, apple, peach, watermelon, melon, grape, orange, lemon, pear, pineapple, strawberry, raspberry, kiwi fruit, beet, papaya and litchi, etc. [1,10,39–47], vegetables like tomato, cabbage, green beans, pepper, cucumber, pea, eggplant, spinach, marrow, onion, potato, carrot, cauliflower, lettuce, aubergine, chard, sprout, leek, sweet pepper, green salad, brinjal, okra, green chilly, mint, radish, ginger and smooth gourd [32,48–57] and reported the occurrence of pesticide residues to be even more than maximum residue level (MRL) values recommended by european union (EU), world health organization (WHO) and food and agricultural organization (FAO). The content of pesticides in various fruits and crops does not only depend on the sprayed amount over them but also on the content present in soil or water used for irrigation. Therefore, some works have also been carried out to know the content of various pesticide residues in soil [58–60] and water [59,61–69] directly.

Different extraction and quantification methods are used by various scientists for estimation of multi class pesticide residues in several vegetables and fruits. The main criteria for opting any methodology is that analytical method should be fast, easy, inexpensive and applicable to different matrices. In recent years, gas chromatography (GC), gas chromatography-mass spectrometry (GC–MS), gas chromatography–ion trap mass spectrometry (GC-ITMS) [11] and gas chromatography–tandem mass spectrometry (GC–MS/MS) [70] had made a legendary progress in field of monitoring pesticides because of the high separation power, selectivity and identification capabilities of MS [10,55,71–73]. The variety of sensitive detectors coupled with GC such as electron capture detector (ECD) [74–77], nitrogen phosphorus detector (NPD) [7,38,45,74–76,78,79], flame ionization detector (FID) [80], pulsed flame photometric detector (GC-PFPD) [55], flame photometric detector (FPD) [2,74,81] improved the detection and quantification procedures of pesticide residues monitoring in different matrices. Besides GC–MS methods, there are other traditional quantification methods like high performance liquid chromatography (HPLC) [61,80], liquid chromatography–mass spectrometry (LC–MS) [82–84], liquid chromatography–tandem mass spectrometry (LC–MS/MS) [53] and low-pressure gas chromatography–mass spectrometry (LP–GC/MS) [54,71,85].

The most frequently used extraction solvents in pesticide residue studies are acetonitrile, acetone, dichloromethane, hexane, ethyl acetate and methanol which provide high recoveries of pesticides over a wide range of polarity [1,7–10,38,53,55,56,78–80,86]. The increasing public concern about the possible health risk of pesticide residues in the diet has profoundly modified crop production strategies with emphasis on food quality and safety. Apart from this, the wide spread concern for health of society has led to the strict regulation of MRL of pesticide residues in food [10]. Seeing the current need to address the awareness for long term moderate exposure of pesticides, the following review focuses on occurrence of the different types of the OPPs and organochlorine pesticides (OCPs) in various fruits and vegetables along with methodology adopted by various scientists to analyze their residues (Table 1).

2. Analysis of pesticide residues in fruits and vegetables

The literature survey has revealed that gas chromatography has been the most widely used technique for analysis of organophosphorus pesticides (OOPs).

2.1. Gas chromatography (GC)

Different scientists all over the world have adopted different extraction procedures for estimation of OPPs using GC. Accordingly, the following section of review focuses on extraction procedures adopted by various workers for pesticide residues using GC technique.

Gonzalez-Rodriguez et al. [48] estimated the residue of methamidophos in vegetables of Southern Spain (Almeria) by using GC. The samples of chopped tomato and green bean were mixed with 100 g anhydrous sodium sulfate and homogenized in a blender with 100 ml of dichloromethane for 2 min. The homogenate was transferred into a Kuderna Danish tube through a glass wool filter and was concentrated to approximately 0.5 ml. The concentrated filtrate was exposed to the slight stream of nitrogen till dryness. The residue was dissolved in acetone to prepare 4 ml solution. The final solution was subjected to gas chromatography-flame photometric detector (GC-FPD) for analysis. The recovery rates for both vegetable samples were more than 89% whereas that diminution rate of methamidaphos was higher in tomato than in green beans.

Patel et al. [87] developed a method of resistive heating gas chromatography (RH-GC) with FPD for the screening of OPPs (dichlorvos, methamidophos, acephate, fonofos, dimethoate, parathion methyl, ethion, phosmet, chlorpyrifos methyl, chlorpyrifos, diazinon, pyrazophos and etrimfos) in fruits and vegetables (peach, grapes, lettuce and sweet pepper). Organically produced fruits and vegetables were extracted in ethyl acetate solution and analyzed on RH-GC-FPD. 30 g of homogenized sample was mixed with 60 ml ethyl acetate, 30–40 g anhydrous sodium sulfate and 5–6 g sodium hydrogen carbonate. The sample was placed in a water bath at 30 °C for at least 20 min and then was homogenized for 30 s. The filtered organic layer was evaporated to dryness under a stream of oxygen-free nitrogen. The resultant extract was reconstituted with ethyl acetate and then injected into RH-GC-FPD for analysis. It took only 4.3 min for the separation of 20 pesticides. Average recoveries obtained were 70% and 116% for pesticides spiked at 0.01 mg/kg and 0.1 mg/kg, respectively, with associated relative standard deviation (RSD) values to be less than 20%.

Parveen et al. [80] monitored number of pesticide residues, *viz.*, endosulfan, chloropyrifos, dimethoate, ethion, methyl parathion, monocrotophos, cypermethrin, deltamethrin, profenfos, carbendazim, metalaxyl, TBZ and acetamiprid belonging to different pesticide classes like OPPs, OCPs, carbamates and pyrethroids in 206 vegetable samples including 27 different types like carrot, garlic, ginger, onion, potato, radish, sugarbeet, brassica leaves, cucumber, mint, methi, chilli, french beans, tomato, pumpkin, cabbage and cauliflower from Karachi, Pakistan. 30 g of sample was extracted with 75 ml of extraction mixture containing tolulene, n-hexane and ethyl acetate in the ratio of 3:1:1. The extracts were analyzed using HPLC and GC-FID. 63% samples were contaminated with one or another pesticide and 46% of contaminated samples had pesticide residues more than MRL values as given by FAO/WHO [88].

Table 1
Summary of analytical procedures for estimation of pesticide residues (PRs) in various fruits and vegetables.

S. no.	Study region/Country	Fruits/Vegetables	Chemical class	% Recovery	LOD[a]/LOQ[b]	Analytical technique	Reference
1.	Croatian cities, China	Apple, orange, peach, pear, grapefruits, lettuce, tomato, cabbage, potato, onion, leek.	OPPs[c], OCPs[d]	–	–	GC[e]–MS[f]	Knezevic and Serdar [1]
2.	Shaanxi area, China	Cereals, vegetable and fruit samples	OPPs	88–107 (for 0.1 mg/kg spiked level), 89–108 (for 0.5 mg/kg)	0.002–0.006 mg/kg[a]	GC-FPD[g]	Bai et al. [2]
3.	Nanjing, China	Shanghai green, Chinese cabbage, carrot, spinach	OPPs	61.8–107	0.05–0.2 g[a] (appropriate dosage range)	GC-NPD[h]	Wang et al. [7]
4.	Beijing, China	Apple juice	OPPs	85–98 89–104	0.005 mg/l[a] –methamidaphos. 0.002 mg/l[a] – chlorpyrifos	GC-FPD	Chen et al. [8]
5.	Kanpur, India	Apples, bananas, mangoes, litchi, papaya, etc., spinach, carrot, cabbage, cucumber, cluster bean, etc.	OPPs, OCPs	>90	–	GC-ECD[i]	Sanghi and Tewari [9]
6.	Slovak Republic	Apples	OPPs, OCPs, pyrethroids, carbamates, aromatic hydrocarbons, phenol, OSPs	–	0.10–6.50 ng/ml[a]	GC–MS	Huskova et al. [10]
7.	Beijing, China	Cucumber, cabbage, cole, capsicum	OPPs, OCPs, carbamates, pyrethroids	>70	0.02–0.1 mg/kg[a]	GC-ion trap mass spectrometry	Tao et al. [11]
8.	Hisar, India	Brinjal, okra, cauliflower, cabbage, pea, potato, cucumber, smooth gourd, summer squash, knol-knol	OPPs, OCPs, pyrethroids, carbamates	83–125 (OPPs), 80–111 (OCP), 73–95 (SP), 82–104 (carbamates)	10–50 ng,[a] 5–20 pg,[a] 50–100 pg,[a] 25–50 ng[a]	GLC[j]-ECD/NPD	Kumari et al. [38]
9.	Geneva, Switzerland	Apple, grapes, strawberry, nectarine, tomato, carrot, green salad, cucumber, eggplant, spinach, potato, pepper, lemon	Carbamates, neonicotinoids, OPPs, pyrethroids	–	0.01 mg/kg[b]	LC-ESI[k]–MS/MS	Ortelli et al. [39]
10.	Almeria, Spain	Orange, green pepper, tomato, cucumber	OPPs, OCPs, carbamates, triazins, phenylureas, neonicitinoids	–	0.04–150 µg/kg[a]	LC[l]–TOF[m]-MS	Ferrer and Thurman [40]
11.	Estonia	Orange, raspberries, bananas, apple, grapes, grapefruit, pear, peach, tomato, sweet pepper, cucumber, lemon, leek, red currant	OPPs, carbamates, benimidazoles	–	–	LC-MSD[n]	Kruve et al. [41]
12.	Amarante Portugal	Grapes, musts and wines	OPPs, OCPs, pyrethroids, dicarboxamide, carbamates	3–21	0.02–5.0 µg/g[a]	LP[o]-GC/MS	Cunha et al. [43]
13.	Canary Island, Spain	Bananas	OPPs	67–118	0.01–0.14 mg/kg[b]	GC-NPD	Hernandez-Borges et al. [45]
14.	Almeria, Spain	Tomato, green beans	OPPs	>89 (for vegetables), >90 (air)	–	GC-FPD	Gonzalez et al. [48]
15.	Czech Republic	Orange, Cabbage, Wheat grains	OCPs, OPPs, pyrethroids, fungicides, herbicides	62 – pyrethroid, 91 – cypermethrin, 95 – fenvalerate, 92–103 – others	–	GC–MS-ECD/NPD	Hajslova et al. [49]
16.	Poland	Cabbage	OPPs	80.6–113.0	0.01–0.10 ppm[a]	GC-NPD	Szymczyk and Malczewska [50]
17.	Kuwait	Apple, bananas, dates, water melon, mangoes, orange, broad beans, cucumber, green salad, tomato. 12 Grain items, milk products, 2 fats and oils, 7 beverages, etc.	OPPs	73–124	0.0036 ppm[a] (omethoate), 0.052 ppm[a] (chloropyrifos methyl)	GPC, GC-ECD	Sawaya et al. [51]

Table 1 (*Continued*)

S. no.	Study region/Country	Fruits/Vegetables	Chemical class	% Recovery	LOD[a]/LOQ[b]	Analytical technique	Reference
18.	Spain	Tomato, lemon, raisin, avocado	OPPs, N-methyl carbamates, fenoxiacids, benzomidazole, neonicotinoids, macrocyclic lactones, etc.	70–110	0.01 mg/kg[b]	GC-NPD	Hernandez et al. [53]
19.	Poland	Tomato, onion	OPPs, OCPs, pyrethroids, carbamates, triazine	84.0–124 (GC–MS), 92.7–110.3 (LP-GC)	–	LP-GC–MS	Walorczyk and Gnusowski [54]
20.	Almeria, Spain	Green beans, cucumber, pepper, tomato, eggplant, etc.	OPPs	73–110	<0.01 mg/kg[a]	GC–MS–MS GC-PFPD[p]	Salvador et al. [55]
21.	Accra, Kumasi, Tamale, Ghana	Cabbage, spring onion, lettuce	OCPs, OPPs, pyrethroids	–	–	GC-ECD/FID[q]	Amoah et al. [56]
22.	Almeria, Spain	Watermelon, melon, marrow, green beans, cucumber, pepper, tomato, eggplant	OPPs, OCPs, carbamates, pyrethroids	71–119	<1 ng/g,[b] <1 ng/g[b]	GC–MS–MS	Vidal et al. [70]
23.	El Ejido, Almeria South Spain	Tomato, cucumber, pepper	OPPs, OCPs, carbamates, organothiophosphate, fungicides, synthetic pyrethroids	70.3–126.9	0.1–10.0 μg/kg,[a] 0.2–22.0 μg/kg[b]	LP-GC–MS–MS	Arrebola et al. [71]
24.	Belgium	Vegetables, fruits, baby food	OPPs, fungicides	94–100 (water), 46–72 (methanol), 43–75 (SBSE)	–	SBSE[r]-thermal desorption-RTL[s]-GC–MS	Sandra et al. [73]
25.	Sao Paulo City, Brazil	Beet, apple, grape, guava, mangoes, melon, orange, peach papaya, strawberry, persimmon, carrot, cucumber, tomato, summer squash, onion, spice	OPPs, OCPs, carbamates, pyrethroids	70–120	0.01 mg/kg[b]	GC-ECD-NPD-FPD	Gebara et al. [74]
26.	Cameron Highlands, Malaysia	Soil samples	OPPs, OCPs	60–120	0.01 ng/ml[a]	GC–MS/ECD	Ngan et al. [75]
27	Greece	Tomato, courgette	OPPs	5–500 μg/kg (linearity)	<MRL value (10 μg/kg)	GC-ECD/NPD	Amvrazi and Tsiropoulos [76]
28.	Agra, India	Cauliflower, cucumber, brinjal, cabbage, spinach, etc.	OPPs	–	–	GC-ECD	Bhanti and Taneja [77]
29.	Ludhiana, Punjab, India	Cucumber	OPPs	>80	0.01 mg/kg[a]	GC-ECD, GLC-NPD	Singh et al. [78]
30.	Venezuela	Potato, tomato, onion, red pepper, green onion, lettuce	OPPs	72–110	0.0027–0.0081 mg/kg[a]	GC-NPD	Quintero et al. [79]
31.	Karachi, Pakistan	Carrot, garlic, ginger, onion, potato, radish, sugarbeet, cucumber, brassica leaves, mint, etc.	OPPs, neonicotinoids, pyrethroids, OCPs	–	–	HPLC[t], GC-NPD/FID	Parveen et al. [80]
32.	Kumasi, Ghana	Tomato, eggplant, pepper	OPPs	88–102 – tomato, 90–105 – eggplant, 91–98 – pepper	0.001–0.015 mg/kg[a]	GC-FPD	Darko and Akoto [81]
33.	Netherlands	Grapes, cabbage.	Polar OPPs	80–101	0.001–0.004 mg/kg,[a] 0.01 mg/kg[b]	LC–MS/MS–atmospheric pressure chemical ionization	Mol et al. [82]
34.	Beijing, China	Apples, tomato, carrot, cabbage	OPPs, carbamates	70–110	0.5–10 ng/ml[a]	LC–MS	Liu et al. [83]
35.	Almeria, Spain	Avocado	OPPs, OCPs, pyrethroids	70–110	0.01–2.50 μg/kg,[a] 0.04–8.33 μg/kg[b]	LP-GC–MS/MS	Moreno et al. [85]
36.	Hisar, India	Cabbage, cauliflower, pea grain, brinjal, tomato, potato, green chilly	OCPs, SP[u], OPPs, carbamates	83–125 (OPPs), 80–111 (OCP), 73–95 (SP), 82–104 (carbamates)	10–50 ng,[a] 5–20 pg,[a] 50–100 pg,[a] 25–50 ng[a]	GC-ECD/NPD	Kumari et al. [86]
37.	UK	Peach, grapes, lettuce, sweet pepper	OPPs	70–116	0.01 μg/ml[a]	Resistive Heating GC–FPD	Patel et al. [87]
38.	Slovenia	Apple, orange, green beans,spinach, carrot, etc.	OPPs, OCPs, carbamtes, pyrethroids	>80	0.01 mg/kg[b]	GC–MS	Stajnbaher and Zupancic-Kralj [93]
39.	El Ejido, Almeria South Spain	Green bean, pepper, cucumber, pea, eggplant, tomato, marrow	OPPs, OCPs, carbamates, organothiophosphate, fungicides, herbicides	73–108	–	GC–MS–MS	Arrebola et al. [94]

Table 1 (*Continued*)

S. no.	Study region/Country	Fruits/Vegetables	Chemical class	% Recovery	LOD[a]/LOQ[b]	Analytical technique	Reference
40.	Spain	Cucumber	OPPs	61.09–88.0	–	GC-PFPD, RTL GC–MS	Munoz et al. [95]
41.	Slovenia	Vegetable samples	OPPs, OCPs, carbamtes, pyrethroids, neonicotinoids	70–110	0.01 mg/kg[a]	GC–MS	Stajnbaher and Zupancic-Kralj [96]
42.	Ourense, Spain	Lettuce, swiss chard, spinach	OPPs, carbamates, pyrethroids, triazole, dicarboximide, fungicides	80–110	<0.010 mg/kg[a]	GC-PolarisQ Ion trap–MS	Gonzalez-Rodriguez et al. [97]
43.	Spain	Water samples. Potato, pepper, tomato. Grain samples	OPPs	95–98	0.01 ng/ml[a]	HPLC, Flurometric detection	Perez-Ruiz et al. [105]
44	Uppsala, Sweden	Grapefruits, lemon, oranges, apple, pears, strawberries, mangoes, kiwi fruit, pineapple, etc., carrot, sweet potato, onion, cucumber, cabbage, cauliflower, Brussels, sprouts, lettuce, spinach, celery, leeks	OPPs, carbamates, Nmethylcarbamates, benzimidazoles, neonicotinoids	70–100	–	LC–MS/MS	Jansson et al. [108]
45	Hefei, Anhui province, China	Leafy vegetables	OPPs, carbamates, triazine, neonicotinoids		>83 <1.4 g/ kg[b]	LC–MS/MS	Pan et al. [112]

[a] Limit of detection.
[b] Limit of quantification.
[c] Organophosphorus pesticides.
[d] Organochlorine pesticides.
[e] Gas chromatography.
[f] Mass spectrometry.
[g] Flame photometric detector.
[h] Nitrogen phosphorus detector.
[i] Electron capture detector.
[j] Gas liquid chromatography.
[k] Electrospray ionization.
[l] Liquid chromatography.
[m] Time of flight.
[n] Mass selective detector.
[o] Low pressure.
[p] Pulsed flame photometric detector.
[q] Flame ionization detector.
[r] Stir bar sorptive extraction.
[s] Retention time locked.
[t] High pressure liquid chromatography.
[u] Synthetic pyrethroids.

Pesticide residues of OPPs, OCPs, carbamates and pyrethroids groups (acephate, captan, chlorpyrifos, diazinon, dieldrin, endosufan, HCH, ethion, iprodione and quintozene) in fruits and vegetables (beet, carrot, cucumber, tomato, summer squash, onion, spice, apple, grape, guava, mango, melon, orange, peach, papaya, strawberry and persimmon) were monitored in Sao Paulo City, Brazil by Gebara et al. [74]. Total 2223 samples comprising 700 vegetables and 1523 fruits collected from general stores and wholesale shops were analyzed for 100 pesticide residues of insecticides and fungicides with GC equipped with different detectors (ECD, NPD and FPD). The extracts were prepared by using acetone, water and dichloromethane. Among all samples, 67.4% samples contained the residues below the detectable limit. Fruit samples (34.6%) had more detectable pesticide residues than in vegetable samples (29.0%). 5.8% of vegetables and 11.4% of fruit samples contained multiple residues. 3.0% of vegetable and 0.9% of fruit samples violated the MRL values. Among all the vegetables, green bean and tomato had the maximum multiple residues whereas among fruits, peach and strawberry had the maximum multiple residues. The recovery rates ranged from 70% to 120% with limit of quantification (LOQ) as 0.01 mg/kg.

Different organophosphorus pesticide residues (dichlorvos, demeton, diazinon, dimethoate, parathion methyl, pirimiphos-methyl, sumithion and parathion) in market food samples (cereals, vegetables and fruits) in Shaanxi area, China were estimated by Bai et al. [2] using GC-FPD. Out of 200 samples, 18 samples had five OPPs, *viz.*, dichlorvos, dimethoate, parathion methyl, pirimiphos-methyl and parathion in concentrations ranging from 0.004 mg/kg to 0.257 mg/kg. The most common OPPs residues found in vegetable and fruit samples of Shaanxi area were dichlorvos, dimethoate and parathion. The mean levels of dimethoate in fruits and parathion in vegetables exceeded the MRL values given by the Ministry of Health of China whereas demeton, diazinon and sumithion were absent in all samples. At different spiking levels, recoveries were 88–107% (at 0.1 mg/kg) and 89–108% (at 0.5 mg/kg). The limit of detection (LOD) value of the method ranged from 0.002 mg/kg to 0.006 mg/kg.

A total of 180 vegetable samples including lettuce, cabbage and spring onion, collected from 9 major markets and 12 specialized individual vegetable and fruit sellers of Accra, Kumasi and Tamale were analyzed for chlorpyrifos, lindane, endosulfan, DDT and lambda-cyhalothrin (OPPs, OCPs and pyrethroid, respectively) by Amoah et al. [56]. The extraction was done using Soxhlet extractor and sample was cleaned, concentrated and eluted with hexane in a solid phase extraction (SPE) column. Resultant solution was injected to the GC columns connected to various detectors (ECD and FID). 78% of lettuce vegetable samples were found to contain chlopyrifos, 31% lindane, 36% endosulfan, 11% lambda-cyhalothrin and 33% DDT.

Wang et al. [7] analyzed 8 OPPs residues in fresh vegetables retailed from agricultural product markets of Nanjing, China. The activated carbon was used for pigment removal and SPE for reduction of matrix interference and contamination. The vegetables (shanghai green, chinese cabbage, carrot and spinach) collected from Lanyuan, Suojingcum and Fuzimiao were extracted in ethyl acetate and were analyzed for different pesticides like fonofos, methyl parathion, malathion, chlorpyrifos, fenthion, phorate, chlorfenvinfos and dimethoate using GC-NPD. All the pesticides except fonofos and fenthion were found to be present in collected vegetable samples. The LOD for spiked samples were between 0.05 g and 0.2 g whereas 61.8–107% average recovery rate was achieved for all pesticides.

Quintero et al. [79] monitored OPPs residues like methamidophos, diazinon, chlorpyrifos, and malathion in vegetables, *viz.*, potato, tomato, onion, red pepper, green onion and lettuce from Venezuela. The samples were chopped fine in food processor and 10 ml of ethyl acetate/acetone (90:10, v/v) was used to extract pesticides. 5 g sodium sulfate was added to the mixture and left for 10 min until organic matter got separated out from solvents. The solution was concentrated to 2 ml. 1 ml solution was taken in a vial and spiked with internal standard (triphenyl phosphate) and injected into GC-NPD for quantification. 48.0% samples were found to be contaminated by one or other pesticides studied. Methamidophos was found in all analyzed vegetables, *viz.*, tomato (62.5%), red pepper (37.5%), lettuce (37.5%), potato (31.3%), onion (12.5%) and green onion (6.3%). Diazinon was detected only in onion (12.5%), chorpyriphos in tomato (12.5%), malathion in red pepper (31.3%), tetrachlorvinphos in red pepper (18.8%) and in lettuce (25%). The concentrations of dimethoate and parathion methyl were less than the detectable limits in all samples whereas among all tested samples, 16.7% of the samples had residues higher than the MRL values. LOD was 0.0027–0.0081 mg/kg. The recovery rate values of method were in range of 72–110%.

Darko and Akoto [81] reported dietary intake of OPPs residues (methyl-chlorpyrifos, ethyl-chlorpyrifos, dichlorvos, dimethoate, malathion, monocrotophos, omethioate, methyl parathion and ethyl parathion) through vegetables (tomatoes, eggplant and pepper) from Kumasi, Ghana. 2 g of each homogenized vegetable sample was extracted in 5 ml of acetonitrile on rotatory shaker for 15 min. The solvent layer was removed and was centrifuged for 2 min at 3000 rpm. The supernatant layer was shifted into a vial. The extract was concentrated to about 2 ml using a rotary evaporator at 30 °C. The internal standard was added to the extract and was made up to 2 ml with acetonitrile and injected into GC-FPD. The residue of ethyl-chlorpyrifos was at an average level of 0.211 ± 0.010 mg/kg in 42% tomato, 0.096 ± 0.035 mg/kg in 10% eggplant and 0.021 ± 0.013 mg/kg in 16% pepper whereas dichlorvos pesticide was present frequently in all samples analyzed. The exceeded levels of malathion residues beyond MRL values (0.1 mg/kg) in tomatoes (0.120 ± 0.101 mg/kg) and pepper (0.143 ± 0.042 mg/kg) were related to the health risks of the population of Ghana. The recoveries achieved for tomato, eggplant and pepper were 88–102%, 90–105% and 91–98%, respectively, whereas LOD of the method ranged from 0.001 mg/kg to 0.015 mg/kg.

Knezevic and Serdar [1] analyzed 240 samples, *viz.*, 106 fruit samples and 134 vegetable samples collected from supermarkets and markets of four Croatian cities: Zagreb, Osijek, Rijeka and Split, China, for presence of pesticide residues. Fruit samples included orange, apple, peach, pear and grapefruit whereas vegetable samples included lettuce, tomato, cabbage, potato, onion and leek. The samples were extracted in water, acetone and cyclohexane/ethyl acetate. Equal amount of water followed by 100 ml acetone was added to 50 g of homogenized samples. The solution was homogenized for 2 min. Sodium chloride (17.5 g) and 50 ml mixture of cyclohexane/ethyl acetate (1:1, v/v) were added and homogenized again for 1 min. The upper organic phase (100 ml) was filtered through glass wool plug layered with 50 g sodium sulfate in funnel. The filtrate along with 40 ml mixture of cyclohexane/ethyl acetate (1:1, v/v) (collected from four times washing of funnel) was concentrated with using rotary evaporator at 40 °C. The dried residue was redissolved in 7.5 ml ethyl acetate. Approximately 2.5 g salt mixture (Na_2SO_4 anhydrous + NaCl, 1:1, w/w) and 7.5 ml cyclohexane were added to it. The solution was filtered through a membrane filter (0.45 μm pore size, 25 mm diameter) and was analyzed for 100 different types of fungicides, insecticides, nematicides, plant growth regulators and acaricides by gas chromatography with mass selected detector. The pesticide residues were absent in 66.7% samples while in 25.8% samples, pesticide residues level was less than MRL values. The most frequently found pesticides were imazalil and chlorpyrifos which were found in 35 and 24 samples, respectively.

Hernandez-Borges et al. [45] monitored four pesticides, *viz.*, ethoprofos, malaoxon, malathion and chlorpyrifos in bananas purchased from local markets of Canary Island, Spain. The aliquot mixture was subjected to extraction with acetonitrile and disodium hydrogen citrate solvents using Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) method. The analysis was carried out using GC-NPD. It was observed that 50 samples contained chlorpyrifos (highly occurred pesticide in samples) while 5 contained malathion, 4 fenitrothion and 1 buprofezin. All those values were found less than MRL values except for 2 samples which contained fenitrothion. The distribution of chlorpyrifos pesticide in the pulp and peel of banana was also investigated and observed that most of the pesticides remained in the peel with a concentration of 0.87 mg/kg as compared to pulp with concentration ranging from 0.07 mg/kg to 0.12 mg/kg. The different pesticides were recovered at rate of 67–118% with a good LOQ value ranging from 0.01 mg/kg to 0.14 mg/kg.

Some studies on estimation of pesticide residues in fresh vegetables and fruits have also been carried out in India. Being an agricultural country, India is the third largest consumer of pesticides in the world and holds its position at the top among different South Asian countries. Due to the subtropical climatic conditions, proliferation of insects is very high in India and therefore, pesticides became an inevitable tool to control the pests of various fruits and vegetable crops [9,38,77,78,86]. Sanghi and Tewari [9] monitored pesticide residues in summer fruits and vegetables from Kanpur, India. They monitored vegetables like spinach, carrot, cabbage, cucumber, and cluster beans and fruits like apple (supplied from Kashmir), banana (from Bhusaval), mangoes (from Lucknow region), litchi (from Nainital belt) and papaya (from Kanpur). These vegetables and fruits were sampled for detection of OPPs (methyl parathion, malthion and ethion) and OCPs (DDT, DDE, BHC and endosulfan, etc.). 20 g of each sample was extracted for 4 h at the rate of 4–5 cycles per hour in 150 ml n-hexane in a Soxhlet extractor. The obtained extract was concentrated using vacuum rotary evaporator. The organic part was extracted in n-hexane with the help of separating funnels. To this mixture, a pinch of sodium sulfate was added. Filtered solution was concentrated and then reconstituted in 5 ml n-hexane. The resultant solution was injected to GC-ECD. The average recovery rates for all pesticides by Soxhlet extraction were found to be more than 90%. However, OCPs (94%) had shown high recoveries than OPPs (91%). Malathion was the most abundantly present insecticide both in vegetables and fruits. However, its concentration was higher in vegetables as compared to fruits. Apart from malathion, few samples also contained DDT and its metabolite, DDE. Tropical fruit, mangoes showed high concentration of malathion as compared to other fruits grown in colder region.

Kumari et al. [86] determined the pesticidal contamination in 80 winter vegetable samples (cabbage, cauliflower, pea grains, brinjal, tomato, potato, and green chilly) from Hisar, Haryana. The samples were extracted in ethyl acetate and detected with the help of GC-ECD and GC-NPD systems. All the samples were contaminated with measurable amount of pesticide residues. Among four pesticide groups tested, residue level of organophosphorous insecticides was the highest. 32% samples had the residue level higher than the MRL values. In another study, 84 farmgate seasonal vegetable samples, *viz.*, brinjal, okra, cauliflower, cabbage, knolknol, summer squash, smooth gourd, cucumber, pea and potato from Hisar were analyzed for monocrotophos, endosulfan, cypermethrin, malathion, methyl parathion, chloropyrifos, aldicarb and quinalphos by Kumari et al. [38]. 25 g of fresh sample was macerated in 5–10 g anhydrous sodium sulfate to make a fine paste. It was further extracted with 100 ml acetone on mechanical shaker for 1 h. Extract was concentrated to 40 ml and subjected to liquid–liquid partitioning with ethyl acetate (50, 30, 20 ml) after diluting 4–5 times with 10% aqueous NaCl solution. It was concentrated to 10 ml on rotary evaporator and was used for estimating OCPs, OPPs, synthetic pyrethroids and carbamates. The residues of monocrotophos and chloropyrifos were more than the MRL values whereas the residues of OCPs like HCH, DDT and endosulfan did not exceed the MRL values.

Bhanti and Taneja [77] evaluated the residue levels of selected OPPs (methyl parathion, malathion and chlorpyrifos) in different seasonal vegetables, *viz.*, spinach, cucumber, brinjal, bottle gourd, ridge gourd, cauliflower and cabbage collected from the fields of 15 blocks of Agra district, India. They also studied the related health risk of these pesticides in the same region. Vegetables grown in different seasons were cut into small pieces and mixed with acetonitrile in a blender. The resultant mixture was then poured into separatory funnel and shaken with 400 ml of 3:2 mixture of *n*-hexane and dichloromethane for 1 h. After shaking, funnel was left in same position for about 30 min to have distinct layer. n-Hexane layer was collected and dried using rotary evaporator. The solution was then subjected to cartridge packed with florisil and activated charcoal (5:1, w/w) and a layer of anhydrous Na_2SO_4 was added in between two layers. Extract was eluted with 125 ml mixture of acetate:hexane (3:7, v/v). Eluted extract was dried, redissolved in 2 ml of n-hexane and injected to GC-ECD. They found that concentration of malathion was maximum in all seasons while concentration of methyl parathion was minimum in summer and rainy seasons. They also found that out of three season vegetables, winter vegetables were highly contaminated. From risk associated studies on pesticides in all three seasons, methyl parathion was found to be causing maximum health risk in population of the region.

Single drop micro-extraction (SDME) followed by GC-FPD was successfully applied by Zhao et al. [89] for estimation of OPPs in a highly complex orange juice matrix. The orange juice was centrifuged and diluted with water and analyzed by GC-FPD. The relative mean recoveries for all pesticides were in the range 76.2–108.0%. The LOD of method was below 5 μg/l and the repeatability varied between 4.6% and 14.1% ($n = 5$) for all target pesticides. The results indicated that SDME method can be employed for the determination of majority of organic contaminants normally found in orange derived food samples.

Amvrazi and Tsiropoulos [76] studied the SDME technique coupled with GC-NPD-ECD for the determination of OPPs chemical class pyriproxyfen in vegetables collected from food stores. Their results showed good linearity, LOD at the sub-μg/kg level and high precision. Among all pesticides, the recovery of methamidophos (108.6%) was maximum while that of malathion (98.5%) was second highest. Therefore, the method was applicable in detection of a number of pesticides in vegetable samples.

Lopez-Perez et al. [90] studied the dynamics of commercial formulations of two insecticides, a nematicides, a herbicide and combination of two fungicides. The concentrations of the active ingredients were monitored throughout the growing season of the potatoes and in the 0–1 cm and 1–15 cm soil layers (where potatoes were grown). The technique used for pesticide determination was gas chromatography with mass selective detection. The only pesticide detected in potato tubers was metalaxyl and the concentration of it was less than half the maximum residual limit.

Other than fruits and vegetables, some scientists have also estimated the pesticide residues in honey [91,92]. Fernandez-Muino et al. [92] analyzed 101 honey samples for determination of acaricides such as amitraz, bromopropylate, coumaphos and fluvalinate, by using GC-ECD/FID. 16 samples contained bromopropylate residues while fluvalinate residues were found in 11 honey samples whereas no residues of amitraz and coumaphos were detected in the collected samples.

2.2. Gas chromatography–mass spectrometry (GC–MS)

Many scientists used GC–MS for estimation of pesticide residues because of its high sensitivity, selectivity and quantification potential to wide range polarities of pesticides.

Hajslova et al. [49] estimated 37 common pesticides (OPPs, OCPs, pyrethroid and carbamates) like parathion, ethion, diazinon, dichlorvos, parathion methyl, methamidophos, metalaxyl, fenitrothion, endosulfan sulfate, I, II-endosulfan, chlorfenvinphos, chlorpyrifos, iprodione, lindane and permethrin in wheat, orange, cabbage and other fruits/vegetables obtained from local retail markets of Czech Republic by using GC–MS-ECD/NPD. 50 g of each sample was blended for 5 min with 250 ml ethyl acetate and 100 g anhydrous sodium sulfate. The suspension was filtered through a layer of sodium sulfate and extract was concentrated by using rotary evaporator to 50 ml. The sample volume was adjusted to 100 ml by adding cyclohexane. For extract clean up, 2 ml aliquot of crude was loaded on high performance gel permeation chromatography (HPGPC) column. The flow rate of mobile phase (cyclohexane/ethyl acetate, 1:1, v/v) was 1 ml/min with dump time of 16 min and collection time of 14 min. The eluate was further evaporated under rotary evaporator and gentle stream of nitrogen. The residue was then reconstituted in 1 ml of toluene and injected into GC–MS-ECD/NPD for analysis of various pesticide residues. The percentage recovery rates for pyrethroid, cypermethrin, fenvalerate and others were found to be 62%, 91%, 95% and 92–103%, respectively.

Vidal et al. [70] used GC–MS/MS to analyze 31 multi class pesticides in 8000 fresh fruit and vegetable samples. Green bean, cucumber, pepper, tomato, eggplant, watermelon, melon, and marrow were extracted with 105 ml dichloromethane. To the mixture, 80 g anhydrous sodium sulfate was added and allowed to rest for 2 min. Extract was filtered through 12 cm Buchner funnel and filtered through a paper filter with anhydrous sodium sulfate into the spherical flask. The solvent was evaporated to dryness using rotary evaporator at 40 °C. The residue was reconstituted in 5 ml of cyclohexane and injected into GC–MS–MS for analysis of different pesticides like methamidophos, acephate, ethoprophos, malation, etc. from OPPs and lindane, chlorthaonil, vinclozoline, triadimefon from OCPs class. The average recoveries obtained for each pesticide were 71% and 119% at two different fortification levels, i.e. 7 ng/g and 300 ng/g, respectively, with RSD less than 19% for all compounds tested. The calculated LOD and LOQ were within the MRL level decided by European Legislation. The obtained average recovery rate was between 71% and 119% and the LOD of the method was observed to be less than 1 ng/g.

Stajnbaher and Zupancic-Kralj [93] developed a selective multi residue method for the quantitative determination of 90 pesticides using GC–MS. The method involved a rapid extraction with acetone followed by SPE using glass styrene–divinylbenzene copolymer (LiChrolut EN). The extraction column was washed with 6 ml of ethyl acetate and conditioned by passing 6 ml of methanol followed by 8 ml of deionized water through the column. The sample was loaded and column was dried by vacuum aspiration under increased vacuum until the sorbent changed its color from brown to orange. The pesticides were eluted with 2 ml of ethyl acetate with 1% triethylamine and thrice with 2 ml of ethyl acetate-acetone (90:10, v/v). The eluted extract was evaporated to 1 ml under nitrogen stream, reconstituted in 2 ml of acetone and injected to GC–MS. After completing whole process, the final solution was analyzed for multi pesticide residues like acephate, captan, chlorpyrifos ethyl, chlorpyrifos methyl, deltamethrin, diazinon, dichlorvos, ethion, dicofol, fenitrothion and malathion belonging to different pesticide classes, *viz.*, OPPs, carbamates and OCPs. Almost all the recoveries were found to be more than 80% with few exceptions, i.e. polar pesticides like methamidophos, acephate and omethoate. LOQ value of method was found to be 0.01 mg/kg.

Arrebola et al. [71] developed a GC–MS method comprising high analysis speed with reduced analysis time. Analyte vegetables (tomato, cucumber and pepper) collected from El Ejido (Almeria, Spain) were chopped and homogenized. An aliquot of 15 g was mixed with 50 ml of dichloromethane for 2 min. 50 g anhydrous sodium sulfate was added to the mixture and allowed to rest for 2 min. They filtered the extract firstly through 9 cm Buchner funnel and then through paper filter with anhydrous sodium sulfate. The content was evaporated to dryness using rotary evaporator. Dried residue was redissolved with 5 ml of cyclohexane. The final solution was injected in LP-GC–MS/MS for the analysis of 72 pesticides (OPPs and OCPs) like dichlorvos, acephate, ethoprophos, dimethoate, disulfan, lindane, chlorpyrifos methyl, parathion methyl, metalaxyl, malathion, fenthion, dicofol, tetraconazole, chlorpyrifos and ethion, etc. By following this methodology, the total time required to determine pesticide residues could be reduced to less than half of the time used in other methods. It was also inferred that the differences between conventional capillary GC–MS/MS and advanced LP-GC–MS/MS techniques were less than 0.01 mg/kg. The recovery rate obtained was between 70.3 and 126.9 with LOD value ranging from 0.1 μg/kg to 10.0 μg/kg. The LOQ of method was found to be 0.2–22.0 μg/kg.

Fresh foodstuffs of El Ejido, Almeria, Spain were collected from September 2001 to July 2002 to monitor 81 multiclass pesticides (OPPs, OCPs and pyrimidine) in approximately 4000 real vegetable samples (green bean, pepper, cucumber, pea, eggplant, tomato and marrow) by Arrebola et al. [94]. The analysis was carried out through single injection using gas chromatography–chemical ionization and electron ionization tandem mass spectrometry. The vegetable samples were extracted in dichloromethane for multi pesticide residues of dichlorvos, methamidophos, mevinphos, acephate, omethoate, lindane, diazinon, disulfoto, parathion methyl, chlorpyrifos, malthion, fenthion, dicofol, ethion, endosulfan-I, II, permethrin, cyfluthrin, deltamethrin and azoxystrobin and were analyzed with GC–MS–MS having recovery range between 73% and 108% for all pesticides. The LOD and LOQ were lower than the MRL values established by EU.

Munoz et al. [95] analyzed OPPs residues in cucumber using a double gas chromatographic system, i.e. gas chromatography-pulsed flame photometry (GC-PFP) and retention time locking gas chromatography–mass spectrometry (RTL-GC/MS). 15 g of cucumber sample was mixed with 20 g of anhydrous sodium sulfate and 50 ml of ethyl acetate. The mixture was then homogenized with a polytron for 1 min and centrifuged for 5 min at 4500 rpm. Supernatant was filtered through a layer of 5 g anhydrous sodium sulfate and remaining solid was further treated with additional 30 ml ethyl acetate. Under vacuum rotary evaporator, extract was dried at 45 °C. The residue was redissolved with 5 ml of acetone and injected into GC-PFPD for analysis of carbophenothion, chlorpyrifos, diazinon, dichlorvos, ethion, fenthion, parathion, omethoate, fenamiphos, etrimfos, fenitrothion, chlorpyrifos methyl, parathion methyl, heptenophos and triazophos. The presence of these compounds in vegetable and fruit samples by using gas chromatography with mass spectrometric detection (GC–MSD) in the electron impact (EI) mode with full scan acquisition was also confirmed. The percentage recovery rates for all pesticides ranged from 61.9% to 88.0%. The LOD was found to be less than the MRL values.

Sandra et al. [73] used stir bar sorptive extraction (SBSE) with thermal desorption capillary GC–MS for screening of pesticides (OPPs and OCPs) in vegetables, fruits and baby food. 30 ml of methanol was added in 15 g of sample which was finely homogenized by using an Ultra Turrax mixer for 5 min and the flask was placed in an ultrasonic bath for 15 min. The supernatant was transferred into 20 ml vial and centrifuged for 5 min at 5000 rpm. 1 ml extract was placed in a 20 ml vial and 10 ml of HPLC grade water was added. A SBSE stir bar, 10 mm long coated with 0.5 mm PDMS layer was added and the mixture was stirred for 60 min at 1000 rpm. After sampling, stir bar was removed with tweezers, dipped briefly in bi-distilled water, placed on lint free tissue to remove residual droplets and finally placed in a liner of thermal desorption unit (TDU). For quantification, the extract was inserted on GC–MS. The recoveries for spiked samples ranged between 94% and 100% for water samples, 46–72% for methanol and 43–75% for SBSE. Residues of various pesticides (fenthion, dichlofenthion, fonofos, dodemorphI, pentachloroaniline, teccnazene and methoxychlor) were present in different crops. The association of SBSE-RTL-GC–MS operated in scan mode could monitor simultaneously about 300 pesticides present in vegetables, fruits and baby food. The SBSE in combination with thermal desorption-RTL-GC–MS could detect residues from mg/kg to the sub-μg/kg level.

Salvador et al. [55] monitored 24 OPPs (chlorpyriphos, disulfoton, parathion methyl, malathion) in 20 samples of different vegetables (green bean, cucumber, pepper, tomato and eggplant) from Spain. 15 g of finely chopped and homogenized sample was mixed with 50 ml dichloromethane for 2 min at 19,000 rpm. 50 g anhydrous sodium sulfate was added to mixture and was kept for 2 min and filtered through 9 cm Buchner funnel. Solvent was evaporated to dryness on rotary evaporator. The final solution was diluted with cyclohexane and injected into GC-PFPD detector. It was found that the recovery rates were between 73% and 110% and precision was better than 15%. Its LOD was less than 0.01 mg/kg much lower than the MRL values stipulated by EU.

Stajnbaher and Zupancic-Kralj [96] determined pesticide residues in fruits and vegetables using GC–MS. 24 pesticides (methamidophos, dichlorvos, lindane, fonofos, chlorpyriphos-methyl, malathion, fenthion, quinalphos, methidathion, endosulfan-I, II, imazalil, deltamethrin, azoxystrobin, permethrin and dicofol) representing different chemical classes (OPPs, OCPs, carbamates and pyrethroids) were chosen for optimization of large volume injection (LVI) parameters. The vegetable and fruit samples collected from local markets were homogenized and extracted by adding 18 ml acetone. The sample was thoroughly mixed on vortex for 2 min and the extract was centrifuged for 10 min at 4000 rpm. Supernatant was transferred to a volumetric tube and acetone:water (2:1, v/v) mixture was added. After shaking, a 2.5 ml aliquot corresponding to 1 g of sample was transferred to a 15 ml reservoir of a funnel shaped SPE column filled with 150 mg of LiChrolut EN sorbent which was washed with 4 ml of ethyl acetate and preconditioned with 4 ml of methanol followed by 5 ml of deionized water. After addition of aqueous acetone extract, the reservoir was filled with deionized water. The diluted extract was passed through the sorbent with a flow rate 5–10 ml/min. The sorbent was dried by passing air through the sorbent bed until color of the sorbent changed from brown to orange (20 min). 1 ml SPE column containing 100 mg of DEA sorbent topped with approximately 1 cm of anhydrous sodium sulfate and washed with 3 ml of ethyl acetate. The pesticides retained on the LiChrolut EN phase were then eluted with 4 ml of ethyl acetate. The eluate was concentrated under a gentle stream of nitrogen to approximately 780 μl. Afterwards, 20 μl of PChB solution was added prior to GC–MS analysis. Recoveries were found to be between 70% and 110% for most of the pesticides except imazalil, fenthion, phorate and dicofol. It was reported that the miniaturized SPE method in connection with Programmed temperature vaporizing (PTV)-based large volume injection was faster in sample preparation and economically beneficial as it saved costs of SPE material and other solvents. It was also observed that the large volume injection of 10 μl ethyl acetate extract in to an empty multi baffled or CarboFrit packed liner using PTV injectors and GC–MS analysis enabled the detection and quantification of 124 pesticides in fruit and vegetable samples at the 0.01 mg/kg level.

Gonzalez-Rodriguez et al. [97] determined residues of 12 different insecticides (acrinathrin, bifenthrin, carbofuran, cyfluthrin, chlorfenvinphos, deltamethrin, fenamiphos and methiocarb) and 11 fungicides (cyprodinil, fludioxonil, iprodione, metalaxyl, vinclozolin and procymidone) in leafy vegetables (lettuce, swiss chard and spinach) purchased from food stores and sellers in Ourense, NW Spain, with recovery rates ranging from 80% to 110% and LOD value less than 0.010 mg/kg.10 g sample was mixed with 30 ml of acetonitrile and vigorously homogenized on ultrasound bath for 10 min. 3 g NaCl and 12 g anhydrous $MgSO_4$ were added. The solution was vigorously shaken for 5 min and left for salting out. The extract was filtered and dried under rotary evaporator at 40 °C, transferred to SPE cartridge filled with graphitized carbon black/primary secondary amine (GCB/PSA) and eluted out by using a mixture of acetonitrile:toluene (3:1, v/v). Extract was evaporated to dryness and reconstituted in 0.5 ml of acetone before injecting it into GC-PolarisQ-ion trap-mass spectrometer. 84% (63 from 75 samples) of the total samples were contaminated with pesticide residues and among all samples, 18 samples were reported to violate MRL value.

Huskova et al. [10] proposed a GC–MS method for the detection of multiple pesticide residues in apples grown with and without the treatment of dichlorvos, methamidophos, diphenyl, acephate, *o*-phenylphenol, chlorpropham, captan, pyridaben, lambda-cyhalothrin, monocrotophos, diazinon, dimethoate, malathion, methiocarb, chloropyrifos and mirex belonging to pesticide classes OPPs, OCPs, pyrethroids, carbamates, aromatic hydrocarbon, phenol and OSs. Apple samples with peel were homogenized in a blender and by using QuEChERS procedure with acetonitrile reagent, the extraction was completed. GC–MS analysis was performed with PTV, an autoinjector and a quadrupole bench-top MS detector, for about 61 pesticides of different classes. 5 pesticides were present in apple samples and concentration ranged from 1.00 μg/kg to 21.47 μg/kg with RSD of 20%. It was observed that the LOD value of the method ranged between 0.10 ng/ml and 6.50 ng/ml.

Tao et al. [11] used an analytical method for determination of 39 different pesticides belonging to OPPs, OCPs, pyrethroids and carbamates groups in vegetables like cucumber, cole, capsicum and cabbage purchased from supermarket in Beijing, China. 50 g aliquot was well homogenized and mixed with 15 g sodium chloride, acetone (200 ml) and dichloromethane (150 ml). The mixture was vigorously shaken on mechanical shaker for 40 min. The extract was filtered through 12 cm Buchner funnel and residue was again treated with additional acetone (30 ml) and dichloromethane (30 ml). Through salting out process, the aqueous lower layer was discarded in separating funnel. To the resultant solvent layer, 10 g anhydrous sodium sulfate was added and was shaken for 30 s. Extract was filtered through coarse filter paper and evaporated to dryness at 45 °C by using rotary evaporator. The residue was dissolved in a 5 ml (v/v, 1/1) mixture of acetone and ethyl acetate and 2 ml for further cleanup. Extraction and purification was performed employing cartridges filled with 500 mg different sorbents (florisil, graphitized carbon black, silicon dioxide, or graphitized carbon black in combination with acidic aluminum oxide). On top of cartridge, 2 cm anhydrous sodium sulfate layer was added and then preconditioned with 2 ml n-hexane and loaded with acetone/ethyl acetate (2 ml). The elute was discarded. Analytes were eluted with 30 ml acetone/ethyl acetate (1:1, v/v) and was concentrated in a rotary evaporator and completely dried under nitrogen purge. The resultant residue was reconstituted in ethyl acetate for GC-IT/MS analysis. Among different vegetables, the percentage recovery of multiple pesticide residues varied from 34.2% to 125%. In cucumber, the maximum recovery was reported for DDT (109%), whereas in cabbage, cole, capsicum, the recovery of pesticides varied from 39.8% to 125.7%, 34.2% to 129% and 41.3% to 114.7%, respectively. For all 39 pesticides, LOD was between 0.02 mg/kg and 0.1 mg/kg.

Zhang et al. [98] developed new approaches for determination of nine OCPs in vegetables by SDME coupled with GC–MS in EI and SIM mode. The result showed a good linearity, precision and recoveries, and LOD in the range of 0.05–0.2 ng/ml. The optimized parameters, 1.00 μl mixed organic solvent (8:2, v/v) of p-xylene and acetone, stirring rate of 400 rpm and extraction time of 30 min were employed for the analytical determination of all target compounds. This study was found to be useful for monitoring of pesticide residue in food which is a major concern in the present state of affairs.

The dissipation rates of cyprodinil, fludioxonil, procymidone and vinclozoline during storage of grape juice were estimated by Pose-Juan et al. [99]. A method based on liquid–liquid extraction (LLE) and gas chromatographic separation, followed by mass spectrometric detection (GC–MSD) was applied for the analysis. Fortified white grape juice samples were taken three times a week from the oven and the degradation rates of the fungicides were monitored for 56 days. The half-life periods of vinclozoline and procymidone at 40 °C were found to be 11 and 20 days, respectively. For fungicides like fludioxonil and cyprodinil half-life periods were slightly high (33 and 44 days, respectively). From first-order rate constants, the fungicide dissipation rate was found to be in the following sequence: vinclozoline (0.062 d^{-1}) > procymidone (0.035 d^{-1}) > fludioxonil (0.021 d^{-1}) > cyprodinil (0.016 d^{-1}).

Gonzalez-Rodriguez et al. [100] used GC-IT/MS for the estimation of 23 fungicide and insecticide residues in 75 green and leafy vegetables (Swiss chards, spinach and lettuce) collected from Ourense, Spain. Samples were extracted with acetonitrile and cleaned by using SPE. The solution was concentrated under PTV and then analyzed on GC-IT/MS. Pesticide residues were found to be above the MRL values in 15 of the 75 analyzed samples, with total of 18 violations from MRL values. The maximum concentration (12 mg/kg) of fungicides (procymidone) was found in lettuce while maximum concentration (6 mg/kg) of insecticides (cypermethrin) was found in Swiss chard.

Fernandez-Gonzalez et al. [101] determined the residues of 10 fungicides (cyprodinil, fludioxonil, pyrimethanil, etc.) in white grapes used for winemaking by GC–MS detection. The study also assessed the induced matrix effects during the estimation of pesticide residues. The method has good precision (relative standard deviation of <10%) and detection limits (ranging between 1 μg/kg and 18 μg/kg) that are lower than the MRL values set by the 76/895/European Economic Community (EEC) and 90/642/EEC Directives. The concentrations of the fungicides identified in grapes were lower than the MRLs established by the European legislation.

2.2.1. Low-pressure gas-chromatography–mass spectrometry (LP-GC–MS)

The literature survey has revealed some of the studies which report the use of LP-GC–MS for pesticide residues [54,85].

The use of pesticides in vegetable products which contain high contents of fats involves an additional risk to human health because of the solubility of non-polar pesticides in the lipidic material and consequent concentration effects. The extraction process of pesticides in these types of matrices requires an additional clean up steps so that high percentage of lipid material co-extracts with target analytes could be reduced, which otherwise could cause interferences [54,102–104]. Moreno et al. [85] developed a multi residue method for the evaluation of different sample treatments to determine 65 pesticide residues (OPPs, OCPs and pyrethroids) in fatty vegetable matrices like Avocado by LP-GC–MS/MS. They compared conventional organic solvent extraction assisted by a high speed homogenizer to pressurized liquid extraction (PLE). For extraction assisted by high speed homogenizer, the fresh vegetable sample was chopped and homogenized. 50 ml of acetate–cyclohexane mixture (1:1, v/v) and 10 g anhydrous Na_2SO_4 were added and thoroughly mixed using high speed homogenizer for 2 min. The extract was filtered through a porous plate funnel. For PLE extraction process, 5 g of fresh sample was mixed and homogenized. The sample was transferred to the cell and the extraction process was performed automatically with the same solvents used for extraction assisted by high speed homogenizer. Before injecting the final solution to GC–MS/MS, these samples were cleaned up by gel permeation chromatography (GPC). This method has shown the applicability of LP-GC–MS/MS for fatty vegetable matrices. This study showed that the recovery rates were in the range 70–110% with RSD values lower than 19% at 12–50 μg/kg spiking levels. The LOQ (0.04–8.33 μg/kg) and LOD (0.01–2.50 μg/kg) were with in a range below the MRL values set by EU.

Walorczyk and Gnusowski [54] developed a method for the determination of pesticide residues (OPPs, OCPs and pyrethroids, etc.) in vegetables (tomato and onion) using low-pressure gas chromatography with triple quadrupole mass spectrometry (LP-GC–MS). 20 g vegetable sample was homogenized with 40 ml ethyl acetate for 2 min in a blender. 20 g of anhydrous Na_2SO_4 was added to it and homogenized for 1 min. The sample was centrifuged for 10 min at 2000 rpm and 10 ml of extract was transferred to a test tube. 0.25 g NH_2 adsorbent and 105 g anhydrous $MgSO_4$ were added to the tube containing extract. It was shaken vigorously for 1 min. The extract was recentrifuged at 2000 rpm for 10 min and 1 ml aliquot was transferred to an auto sampler vial for the determination of 78 different pesticides like methamidophos, dichlorvos, acephate, α,β-HCH, lindane, chlorpyrifos, parathion ethyl, malathion, parathion methyl, metalxyl, quinalfos, cypermethrin, fenarimmol and deltamethrin by using LP-GC–MS. Out of 78 pesticides, 12 pesticides were tested for validation experiments so that a comparison of performance of LP-GC and GC–MS operated in scan (selected ion monitoring), SIM and MS/MS mode could be drawn. The study indicated that the rate of false negative results (β error) was higher in case of conventional GC–MS analysis. Moreover, LP-GC–MS technique was able to reliably identify pesticides at the lower spiking level as resulting peaks were improved in both shape and size. In the present method, the linearity of calibration curves at concentrations between 0.01 μg/ml and 0.5 μg/ml showed a practical way to overcome the matrix enhanced effects. The coefficients of determination (r^2) of LP-GC–MS were 0.999 whereas, for conventional GC analysis it was 0.973–0.999. Finally this study demonstrated the applicability of this method for analysis of pesticide residues using tomato samples.

2.3. Liquid chromatography (LC)

The multi-residues determination of pesticides in vegetables and fruits is generally carried out by GC–MS due to its excellent characteristics of efficient chromatographic separation, sensitivity and confirmation power based on electron impact ionization mass spectra. However, LC–MS allows the rapid and efficient determination of many compounds that have been scarcely investigated in food or determined with difficulties by using laborious and time consuming GC or conventional LC procedures [105]. In recent years, a modified LC–MS/MS has become a powerful tool for pesticide residue analysis (PRA) in a variety of complex matrices [53,82,106–109].

Perez-Ruiz et al. [105] analyzed OPPs in potatoes, peppers and tomatoes purchased from local markets. They used HPLC assay for determination of pesticide residues. Three to four units of each fresh vegetable was macerated individually to homogeneity in an electric food mixture. 25 g of each sample was weighed into round bottom flasks and spiked with 5 ml solution of OPPs containing 0.125 μg and 12.5 μg of each and was allowed to stand for at least 1 day at room temperature. 25 ml of methanol was added and stirred for 30 min on a shaker. The extract was removed and residue was re-extracted with equal volume of methanol to ensure complete extraction. The combined extracts were filtered through *Whatman* filter paper. The filtrate was evaporated to near dryness in vacuum evaporator at 40 °C. The residue was dissolved in 1 ml of ultrapure water and filtered through 0.45 μm membrane filter prior to LC analysis. They observed that there was 95–98% recovery of spiked pesticides in all the three vegetable samples. They further used photolysis of the organocompounds by irradiation with a low-pressure mercury lamp in presence of peroxydisufate. The fluorescence intensity of thiochrome was measured at 440 nm with excitation at 375 nm. The detection system was used to determine phosphate, acephate, and methamidophos, which were separated on ODS column by isocratic reversed phase chromatography with acetonitrile–water as the mobile phase. They also found that relationship between peak area and analyte concentration was within the range 0.016–7.0 μg/ml with correlation coefficient greater than 0.9995 and detection limits between 4 ng/ml and 12 ng/ml. In the analysis of vegetables and grains the detection limit was about 1 μg/kg.

Rial-Otero et al. [110] developed a new multi pesticide residues determination method for fourteen fungicides in white grapes. The proposed method was based on LLE and SPE followed by liquid chromatography and diode array detection (HPLC-DAD). Dichloromethane–acetone (75:25, v/v) was found to be the most appropriate solvent mix for extracting fungicides in white grapes. Similarly, silica cartridges were found to be most appropriate for extract purification purposes. Quality parameters such as recovery rates (ca. 85% for almost all target compounds) and precision (between 1.5% and 16%) were found to be good. The detection limits was lower than maxima residual limits set by the 76/895/ECC and 90/642/ECC Directive. Five different white grape samples used for vinification, produced in Rias Baixas area in Galicia (NW Spain) were analyzed and it was found that the concentrations for those identified fungicides in grape samples were lower than those established by European legislation.

2.3.1. Liquid chromatography–mass spectrometry (LP-MS)

Ferrer and Thurman [40] adopted LC–time of flight mass spectrometry (LC-TOF-MS) method for multi residue analysis of 101 pesticides (OPPs, OCPs, carbamates, triazins, phenylureas and neonicotinoid, etc.) in food and water samples. Different fruit and vegetable samples like green pepper, tomato, cucumber and orange were prepared by using QuEchERS method. 15 g of homogenized sample was taken in a centrifuged tube, mixed with 15 ml of acetonitrile and vigorously shaken for 1 min. 1.5 g of NaCl and 4 g of $MgSO_4$ were added to the mixture and was again shaken for 1 min. The solution was centrifuged at 3700 rpm for 1 min. 5 ml aliquot supernatant was taken in another centrifuged tube containing 250 mg of PSA and 750 mg of $MgSO_4$ and was again shaken for 20 s. The extract was centrifuged for 1 min at 3700 rpm. The extract was evaporated and reconstituted up to 1 ml and injected on LC–TOF-MS and HPLC for the analysis of pesticides like dimethoate, malathion, chlorpyrifos methyl, captan, carbaryl, alachlor, aldicarb, acetamiprid, imazali, metalaxyl, nicosulfuron, trifluralin, propanil, thiosultap and simazine, etc. The LOD in green pepper samples ranged from 0.04 μg/kg to150 μg/kg. About 82% (a total of 20) of the samples analyzed had shown presence of at least two pesticides while only 15% of samples had shown high MRL values.

A LC–MS/MS method was developed by Mol et al. [82] for determination of less GC-amenable OPPs like acephate, metamidaphos, monocrotophos, omethoate, oxydemeton methyl and vamidothion in cabbage and grape samples collected from local shops of Netherlands. They took cabbage as reference vegetable which had relatively high wax content and grapes as reference fruit matrix which had high sugar content. 25 g finely homogenized sample was extracted with 50 ml of ethyl acetate using ultra turrax for 2 min. In case of ethyl acetate, 25 g of sodium sulfate was added. If no clear liquid phase was obtained after settling then perform centrifugation. Supernatant was transferred to a vial and evaporated to dryness at 35 °C under a gentle flow of nitrogen gas. The residue was redissolved in a solution of 0.1% acetic acid in water with the aid of vortex or ultrasonic bath and filtered through PTFE filter. The solution was inserted for analytical quantification and detection by using LC-MS/MS. In the present study several solvents were evaluated with respect to the possibility of direct injection, matrix induced suppression or enhancement of response and extraction efficiency. It was found that ethyl acetate was the most favorable solvent for extraction. Pesticides were ionized using atmospheric pressure chemical ionization (APCI) on a tandem mass spectrometer in multiple reactions monitoring mode. In this study, the final extraction was done with ethyl acetate and solvent of 0.1% acetic acid/water. The method was found to be validated at 0.01 mg/kg and 0.5 mg/kg level, for both cabbage and grapes having recoveries between 80% and 101%, respectively, with quantification limit 0.01 mg/kg.

Jansson et al. [108] determined pesticide residues of benzimidazole, carbamates and OPPs (oxidisable sulfide group) in number of vegetables and fruits (grapefruits, lemon, orange, apple, pear, apricot, grapes, banana, mango, carrot, onion, sweet potato, cucumber, pepper, tomato, cabbage, brussel, lettuce, spinach, celery and leek). The samples were extracted in ethyl acetate in presence of sodium sulfate. Sodium hydroxide was added to extracts having pH lower than 4.5. The extract was filtered and evaporated to dryness [111]. The resultant residue was dissolved in methanol and injected into LC–MS/MS. The method was valid for 57 different pesticides (imazalil, imidacloprid, linuron, methiocarb, methomyl, oxamyl, oxamyl-oxime, disulfoton, disulfoton sulfone, ethiofencarb sulfoxide and phorate, etc.) and their metabolites. The recovery rates were 70–100%. The proposed method was quick for the analysis of pesticides in single determination step at 0.01 mg/kg without requiring any extra clean up step [108].

Liu et al. [83] determined carbamates and OPPs in 25 samples including root vegetables (carrot and potato), leafy vegetables (lettuce, cabbage and spinach), bulb vegetables (onion, pumpkin-squash and eggplant), fruit vegetables (cucumber and tomato), bean vegetables (kidney bean-legume) and prome fruits (apple, melon and peach) by using LC-MS. All the samples were collected from local markets and supermarkets of China. 10 g of each sample was dissolved in 10 ml acetonitrile and homogenized on vortex for 1 min. 4 g of anhydrous $MgSO_4$ and 1 g NaCl were added to it and remixed on vortex for 1 min. The extract was further centrifuged at 3000 rpm for 10 min. 1 ml supernatant in acetonitrile was transferred into micro-centrifuge tube containing 50 mg primary secondary amines (PSA) sorbent and 100 mg anhydrous $MgSO_4$. It was vortexed for 1 min and centrifuged for another 5 min at 5000 rpm. The sample was then transferred into sample vial and subjected for analysis by using LC–MS. They reported that more than 30% of samples contained multi-residues of one or other pesticides except carbaryl, baycarb, methicarb, pyraclofos and etrimfos. Azinphos methyl and malathion were widely reported in almost all types of fruits and vegetables. Among all samples, alone potato samples were found to contain 10 different pesticides within the MRL values (Japan and EU). The precision for spiked samples was 0.05 mg/kg. The recovery rates were in range of 70–110% with RSD less than 8%.

Hernandez et al. [53] compared LC–MS/MS with the GC–MS for estimation of pesticide residues in vegetables and fruits. 52 non-GC-amenable pesticides and metabolites belonging to different chemical classes like *N*-methyl carbamates, sulfonylureas, phenylureas, strobilurines, fenoxiacids, organophosphorus, benzomidazoles, neonicotinoids, macrocyclic lactones and others, in four types of food commodities like lemon (high acidity); raisin (high sugar content), tomato (high water content) and avocado (high lipid content) from an Organic Farming Grocery by using LC–MS/MS were determined in this study. Samples were cut into small pieces and macerated without any pre washing or removing their peels. 20 g of homogenized sample was mixed in 60 ml of methanol:water ($MeOH:H_2O$) (80:20, v/v) 0.1% HCOOH. The mixture was extracted for 2 min with high speed blender at 8000 rpm and then filtered through a filter paper. The filtrate was diluted with $MeOH:H_2O$ (80:20, v/v) 0.1% HCOOH to make a final volume of 100 ml. Then, the extract was diluted eight times with LC-grade water. 2.5 ml aliquot was taken and diluted to 20 ml. 5 ml of diluted extract was passed through a SPE cartridge, preconditioned with 5 ml of MeOH, 5 ml of methanol:methyl *tert* buthyl ether (MeOH:MTBE) (10:90, v/v) 0.1% HCOOH, 5 ml of MeOH 0.1% HCOOH and finally with 5 ml of acidified water. The cartridge was dried for 1 h before loading of sample and eluted with 5 ml MeOH:MTBE (10:90, v/v) 0.1% HCOOH. 0.5 ml of water was added and the extract was evaporated to 0.5 ml under nitrogen stream at 40 °C. The extract was reconstituted with a volume of $MeOH:H_2O$ (10:90, v/v) and then injected into LC–MS/MS for analysis. 43 pesticides (belonging to different chemical families of insecticides, acaricides, fungicides, herbicides and plant growth regulators and 9 pesticides metabolites) were present in all samples. The recovery rate ranged from 70% to 110% with satisfactory precision (CV $\leq$ 15%) that could be used for the accurate determination of 52 pesticides and metabolites in one single determination step at the 0.01 mg/kg level.

Pan et al. [112] analyzed leafy vegetables like spinach received from the local supermarkets in Hefei (Anhui province), China for multi pesticide residues of OPPs, carbendazim, carbaryl, simazine, neonicotinoid, carbamate and triazine by using ultrasonic solvent extraction (USE) and liquid chromatography-tandem mass spectrometry (LC–MS/MS). The extraction was completed with two techniques, one was with USE method which provided high contact between sample matrix and solvent and the second was simple homogenized extraction which was done with ethyl acetate. The homogenized vegetables were mixed with 45 ml of ethyl acetate for 2 min. 8 g anhydrous sulfate was added and further homogenized for more 2 min. The liquid layer was filtered and additional 20 ml ethyl acetate was added to wash the residue. The extract was again mixed for about 2 min and then filtered. Under gentle stream of nitrogen the solution was evaporated to dryness. Resultant extract was redissolved in 1 ml methanol:water (40:60, v/v) solution and injected into LC–MS/MS. The extracts thus obtained were analyzed with electron microscope to see the effects of USE method. Pan et al. [112] found that the proposed procedure allowed the extraction of six pesticide residues in a single step with 40 ml of ethyl acetate for 35 min sonication, providing recovery over 83% and LOQ less than 1.4 μg/kg. In this study, the optimized USE method was a simple, low cost and an effective preparation method for determination of pesticide multi-residues at trace levels in leafy vegetables in comparison with homogenized extraction method.

2.4. Gas liquid chromatography (GLC)

Kumari et al. [38] used Hewlett Packard 5890A gas liquid chromatograph (GLC) coupled with capillary columns using ECD and NPD for analysis of various pesticides. 26% samples contained pesticide residues more than MRL values. Among all pesticides, monocrotophos residue was higher than MRL in 3 samples of brinjal and 1 sample of okra, cauliflower and smooth gourd each. Chlorpyriphos was present in 3 samples of cauliflower and 8 samples of cabbage. The LOD of the method was found to be 10–50 ng for OCPs, 5–20 pg for synthetic pyrethroid, 50–100 pg for OPPs and 25–50 ng for carbamtes. In spiked samples, the recoveries for different classes of pesticides were 83–125% for OPPs, 80–111% for OCPs, 73–95% for SP and 82–104% for carbamates.

2.5. Chemiluminescence method (CL)

Many scientists determined the pesticide residues by using GC, thin layer chromatography (TCL) and HPLC, etc. Since the pesticides especially OPPs are known to be cholinesterase inhibitors, a few scientists utilized the inhibitory effects of these compounds on acetylcholinesterase or other enzymes to develop many biosensors for the determination of these pesticides [29]. The chemiluminescence (CL) method has become an attractive analytical tool in pesticide residue determination in recent years as the GC and LC are comparatively costly [32].

Xiao-Zhou et al. [32] used SPE-CL method for determination of methamidaphos in vegetables like cabbage, cole and spinage from China. The method was based on enhancing effects of methamidaphos on the CL reaction of luminol with hydrogen peroxide in alkaline solution. Under the optimal conditions, the CL intensity was linear to the methamidaphos mass concentration and ranged between 0.2 μg/ml and 1.3 μg/ml. The recovery ranged from 90% to 109% for cabbage, cole and spinage with RSDs 1.8%, 2.5% and 3.7%, respectively.

3. Conclusion

Pesticides have been widely used in agriculture to fight against insects, pests, weeds and moulds for many years, thereby, increasing crop productivity. Besides their positive effects, they also have been posing various health risks to consumers. Therefore, the concentration of pesticide residues must be monitored not only in various food commodities including fruits, vegetables, pulses and cereals but also in all the three components of environment, *viz*., soil, air and water in order to check the current status. The present review is an attempt to summarize various extraction procedures followed by different quantification techniques used to estimate the pesticide residues in fruits and vegetables.

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