Journal of Applied Pharmaceutical Research
Volume 8, Issue 4, Year of Publication 2020, Page 01 – 09
DOI: 10.18231/j.joapr.2020.v.8.i.4.1.9

Review Article

JOURNAL OF APPLIED PHARMACEUTICAL RESEARCH | *JOAPR*
www.japtronline.com *ISSN: 2348 – 0335*

INVESTIGATING THE PRESENCE OF PESTICIDES IN FRUITS AND VEGETABLES BY THE CHROMATOGRAPHIC TECHNIQUES: A REVIEW

Shweta Borkar*, Jessica Gonsalves

Article Information
Received: 17th January 2020
Revised: 21st September 2020
Accepted: 16th October 2020

Keywords
Pesticides, Extraction, Analytical Method, Spinach, Chromatography

ABSTRACT

The intensive development of agriculture means more and more toxic and inorganic compounds are entering the environment. Because of their widespread use, stability, selective toxicity, and bioaccumulation, pesticides are among the most toxic substances contaminating the environment. They are particularly dangerous in fruits and vegetables, by which people are exposed to them. It is therefore crucial to monitor pesticide residues in fruits and vegetables using various analytical methods. This article reviews various stages in the determination of pesticide residues in fruits and vegetables. Here the target analyte has been isolated and then enriched before final determination which may be carried out either by Gas Chromatography-Mass Spectrometer (GC-MS) method or Liquid Chromatography-Mass Spectrometer (LC-MS) method. This review explores the analysis of multiple pesticide residues in spinach samples and walks through the step-by-step process of developing the analytical method, from sample preparation to analysis, best suited to the data requirements

INTRODUCTION

Fruits and Vegetables are an important part of a balanced diet. We are told to eat fruits and vegetables every day, but are they safe to eat? What about the Chemicals the farmers use to keep their crops free from pests? Do these Chemicals stay on the food we eat? The answer to all the above questions explains to us what are pesticides? What health impacts it can have on humans, animals, and the environment. Further how these chemical hazards can be detected using various techniques. This review article focuses on the various techniques of extraction of pesticides further detecting this using sophisticated instruments like GC-MS, LC-MS. A cpesticide is any substance or mixture of drugs intended for preventing, destroying or controlling any pest, including vectors of human or disease,

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*Department of Pharmaceutical Analysis, Goa College of Pharmacy, 18th June Road, Panaji–Goa, India

***For Correspondence:** swe_bha26@rediffmail.com

unwanted species of plants or animals causing harm during or otherwise interfering with the assembly, processing, storage, transport or marketing of food, agricultural commodities, wood and wood products or animal feedstuffs, or substances which can be administered to animals for the control of insects or other pests in or on their bodies [1]. The term includes substances intended to be used as a plant phytohormone, defoliant, desiccant, or agent for thinning fruit or preventing the premature fall of fruit. It is also used as substances applied to crops either before or after harvest to protect the commodity from deterioration during storage and transport [2].

Pesticides v/s Health:

Pesticides must be toxic or poisonous to be effective against the pests they're intended to regulate. Because pesticides are toxic, they are also potentially hazardous to humans and animals. Toxicity may be a measure of the capacity of a pesticide to cause injury; it's a property of the chemical itself. The toxicity of a specific pesticide is decided by subjecting test animals (usually rats, mice, rabbits, and dogs) to different dosages of the active ingredient and every of its formulated products. Toxicity can be acute or chronic [1].

Acute Toxicity and Acute Effects

The acute toxicity of a pesticide refers to the power of the chemical to cause injury to an individual or animal from one exposure, generally of short duration. Acute toxicity is determined by at least three methods: (1) dermal toxicity is determined by exposing the skin to the chemical; (2) inhalation toxicity is determined by permitting test animals to breathe vapors of the chemical; and (3) oral toxicity is decided by feeding the chemical to check animals. The harmful effects that occur from one exposure by any route of entry (dermal, inhalation, oral) are termed acute effects. Besides, the effect of the chemical as an irritant to the eyes and skin is examined under laboratory conditions [1].

Chronic Toxicity and Chronic Effects

Chronic toxicity is decided by subjecting test animals to long-term exposure to a pesticide. The harmful effects that occur from small doses repeated throughout for your time, usually years, are termed chronic effects. Some of the chronic effects found in test animals exposed to certain pesticides include birth defects (teratogenesis); toxicity to a fetus (fetotoxic effects); production of tumors (oncogenesis), either benign (noncancerous) or malignant (cancerous/carcinogenesis); genetic changes (mutagenesis); blood disorders (hemotoxic effects); nerve disorders (neurotoxic effects); endocrine disruption; and reproductive effects. The chronic toxicity of a pesticide is more difficult to determine through laboratory analysis than the acute toxicity [2].

Determination of pesticides in fruits and vegetable samples [3]

For determination, the target analyte must be isolated from the matrices and then be enriched before the final determination can be undertaken. Figure 1 summarizes the stages involved

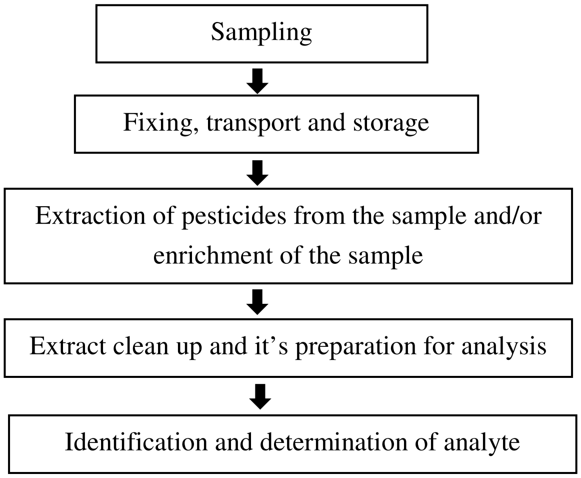


Figure 1: Main stages in the analytical procedure for determining pesticides in the sample of fruits and vegetables

Preparation of samples for analysis

This is an important stage as this stage can affect the final results. The sample of material for analysis must be homogenous and representative. A representative sample has a chemical composition that resembles as closely as possible to the average composition of the analyte material. Sample preparation consists of a number of steps such as removal of surface contaminants by washing the samples in distilled water. The sample is dried at elevated temperature or with the aid of a desiccant. The sample is broken up and crushed, or ground in a mill or with a pestle and motor, after which the sample is homogenized [3].

Isolation of pesticides from samples [4]

This stage is essential, as in many cases the available analytical method is not sufficiently sensitive to carry out a final determination of the trace constituent directly from the sample. Isolation and/or preconcentration mean the transfer of the analytes from the primary matrix to a secondary one with the simultaneous removal of interferents and increase in target-analyte concentrations to levels above the limit of determination of the analytical technique applied. With fruits and vegetables, the solid matrix often has to be replaced by a liquid. This is done using a suitable extraction method as given in figure 2

Extraction of pesticides from the matrix and/ or sample enrichment

- Liquid - Liquid Extraction (LLE)
- Ultrasound - Assisted Extraction (UE)
- Soxhet - Soxtec Extraction
- Microwave - Assisted Soxhlet (MAE)
- Supercritical Fluid Extraction (SFE)
- Accelerated Solvent Extraction
- Membrane Extraction

Figure 2: Techniques for extracting of pesticides from samples of fruits and vegetables

Extract clean up

The isolation of analytes from biological samples involves a certain clean up effect, and, like every clean-up process ensures a certain degree of isolation. Extraction yield not only the target analyte but also interferents (e.g. sugar, fat, and chlorophyll), which may distort the result of the analysis. Hence, extract clean-up is essential and should always precede the analysis of the extract [4]. The usual technique s for cleaning up fruits and vegetable extract is:

1) Solid-phase extraction (SPE)
2) Solid-phase microextraction (SPME)
3) Matrix solid-phase dispersion extraction (MSPDE)
4) Stir-bar sorption extraction (SBSE)
5) Adsorption chromatography
6) Gel-permeation chromatography (GPC)

SPE is the most popular clean up technique. As the sample passes through a column of sorbent, the target analytes are adsorbed on the sorbent particles. The compounds retained are then liberated with a solvent and analyzed. A salt, usually NaCl or Na_2SO_4 is often added to the solution to increase its ionic strength, and that increases the proportion of analytes extracted to the sorbent. The sorbent used for SPE includes C_{18} polymers, graphitized non-porous carbon, and ion-exchangers [5].

The $B_{15}C_5$ sorbent is the best one for determining organophosphorous pesticides (OPPs). The method is simple and can be readily automated. However, against that the solvent bed has to be conditioned before use and analyte recovery is small.

In SPME, analyte is adsorbed on a fiber coated with a suitable solid phase that is pushed out from a microsyringe. The analyte is then thermally desorbed and transferred to the GC injector. The benefits of this method are that the solvent can be eliminated and that it is impossible to overload the column because of the limited volume of adsorbent. Depending on where the fiber is placed about to with concerning the sample, SPME can be divided into

1) Direct immersion (DI-SPME)
2) Headspace (HS-SPME)

GPC is just as frequently used as a clean-up technique. It enables micro molecular pesticides to be separated from macromolecular substances present in the matrix. While the long lifetime of the columns is an advantage, the poorer resolution compared to adsorption techniques, especially when gradient-elution techniques are used, is a disadvantage [6].

Approaches are being sought to develop pesticide determination techniques that are quick, easy, cheap, effective, rugged and, safe (QuEChERS) which is a combination of liquid-liquid extraction (LLE) and SPE [7]. It is based on the number of stages as shown in figure 3.

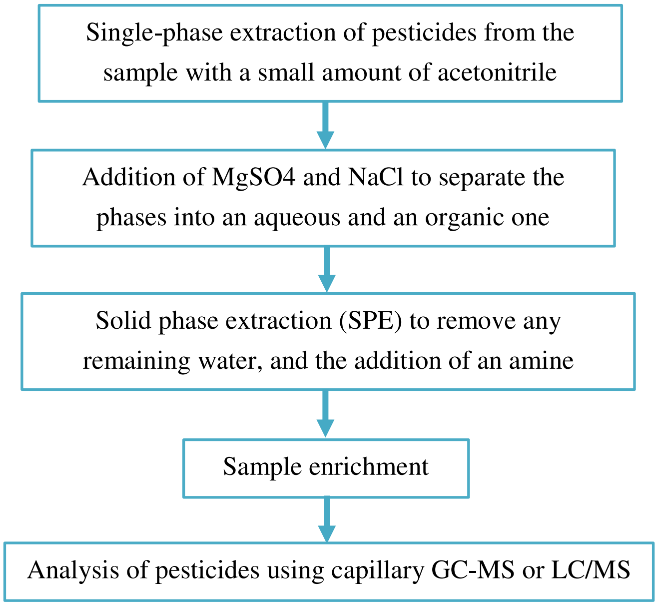


Figure 3: Stages in the determination of pesticides using Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) method

In the QuEChERS method the consumption of samples and toxic solvents is minimal. By applying QuEChERS in the pesticide determination in fruits and vegetables, elimination of matrix effects and high recoveries of the target analytes are possible.

The method can be modified depending on the type of sample and the target analytes. To improve the extraction of polar organophosporous pesticides, the method is modified by the addition of acetic acid. When samples of citrus fruits are under investigation, a protective wax coating can be removed by freezing the sample for at least 1 h. For the analysis of citrus fruits; blackcurrants and raspberries, it is recommended to add aqueous NaOH to reach pH=5 and to improve the analysis. The last stage in the QuEChERS is the final determination of analytes by GC or LC [7].

Identification and determination of analytes [5, 6]

The last stage in the analytical procedure is the identification of compounds and their quantitative determination of compounds and their quantitative determination using appropriate instrumentation. The choice of final determination technique depends above all on the properties of the analytes. The usual techniques for final determination of pesticides include capillary gas chromatography (GC) and high-performance liquid chromatography (HPLC) (usually in reversed-phase mode) for determining pesticides that are unsuitable for determination by GC. Pesticides to be determined by GC should be volatile and thermally stable. For determining OPPs, GC equipped with a suitable column and detector is used. GC can be used to determine the residues of all classes of pesticides.

The choice of a chromatographic column is extremely important for separating analytes and for their qualitative and quantitative determination. The chromatographic column should be highly efficient and resistant to changes in the parameters of the separation process. The solid (stationary) phase should be thermally stable and highly selective concerning for to the constituents of the mixture being analyzed. The multi-residue determination of pesticides in fruits and vegetables is generally carried out by GC-MS, due to its excellent characterization of efficient chromatographic separation, sensitivity and confirmation power based on electron-impact ionization mass spectra. However LC-MS allows rapid, efficient determination of many compounds that have rarely been investigated in food or determined with difficulty by using laborious, time-consuming GC or conventional LC procedures.

The following techniques can also be employed to determine OPPs and organonitrogen pesticides (ONPs) in fruits and vegetables.

- MS (mass spectrometry)- for the determination of pesticides of various classes
- ECD (electron-capture detector)-highly sensitive about to with concerning compounds containing electronegative atoms and generally used for quantification of organochlorine pesticides (OCPs)
- FPD (flame-photometric detector)- applied in the determination of OPPs
- NPD (nitrogen- phosphorus detector)- for the simultaneous determination of ONPs and OPPs
- TSD (thermionic specific detector)- for the determination of compounds containing nitrogen or phosphorus

CASE STUDY

Analysis of Multiresidue Pesticides from Food Using the QuEChERS Sample Preparation Approach, LC–MS–MS and GC–MS Analysis [13-15]:

Spinach was chosen as the sample matrix because it is representative of highly pigmented fruits and vegetables, which are notoriously difficult samples to analyze. Because of spinach's dark green pigmentation, consisting principally of chlorophyll and carotenes, sample preparation was critical. Before beginning method development, the overall scope and goal of the analysis are clearly defined. In this particular analysis, four requirements were established:

(1) Multiple classes of pesticides must be extracted from the spinach samples using a single sample preparation procedure;

(2) Chlorophyll, carotenes and other interferences in the matrix must be removed before analysis

(3) The chromatographic conditions must be able to separate and accurately quantify each of the pesticides

(4) The resulting method must be validated in terms of recovery and repeatability (RSD) for the 16 pesticides planned to study.

QuEChERS is an abbreviation for Quick, Easy, Cheap, Effective, Rugged, and Safe. The approach was developed to provide a highly flexible sample preparation method that could extract multiple classes of compounds from the food of plant origin while eliminating or minimizing interferences such as organic acids, pigments, and fats. Since the development of the technique, two independent and inter-laboratory validated methods have been established: AOAC International and the European Committee for Standardization. The two methods are similar; however, they rely on different salt combinations to aid in the extraction process.

Table 1: Pesticides and analytical methods used to analyze them [8-13]

S. No	Types of pesticides	Examples	Analytical methods
1.	Chlorinated Pesticides	Endosulfan-α, Endosulfan-β and Endosulfan Sulphate, α-hexachlorocyclohexane, β-hexachlorocyclohexane, γhexachlorocyclohexane, δ-hexachlorocyclohexane, Aldrin, Dieldrin, Chlordane, Heptachlor, Heptachlor Epoxide	GC
2.	Organophosphorus Pesticides	Acephate, Chlorpyriphos, Chlorpyriphos-methyl, Demeton O, Diazinon, Dimethoate, Ethion, Fenitrothion, Malaoxon, Malathion, Methamidophos, Monocrotophos, Omethoate Paraoxon, Paraoxonmethyl, Parathion, parathion-methyl Phosalone	GC
3.	Synthetic Pyrethroid	bifenthrin, fenopropathrin, cyhalothrin, permethrin, cypermethrin, fluvalinate, fenvalerate, and deltamethrin	GLC
4.	N-Methylcarbamate Insecticides, Pendimethalin	carbaryl and carbofuron	GLC
5.	Fumigants	ethylene dibromide, carbon tetrachloride and methyl bromide	GC
6.	Fungicides and Herbicides	Acetamiprid, Atrazine, Cymoxanil, Imidacloprid, Isoprothiolane, Metalaxyl, Propiconazole, Simazine, Thiamethoxam, Thiodicarb, Triadimefon, Triadimenol	LC-MS/MS
7.	Dithiocarbamates	Ferbam, Ziram, Thiram, Maneb, Zineb, Mancozeb and Nabam	UV
8.	Ethylene Thiourea And Ethylene Urea	acetamiprid, azoxystrobin, cyprodinil, fenhexamid, fludioxonil, folpet, iprodione, metalaxyl, primicarb, tolyfluanid	HPLC
9.	Diquat and Paraquat, Carbamate, Dithiocarbamate (DTC) Pesticides	carbaryl, bendiocarb, carbofuran, methiocarb, promecarb and propoxur	UV
10.	OP Pesticides;	malathion and dimethoate	UV
11.	Benomyl, tebuthiuron, simazine, atrazine, profenofos, diuron, ametryn, triazophos, chlorpyrifos	--------------------	HPLC, UV
12.	Cyanazine, Simazine, Atrazine, and Promethazine	---------------------	UPLC-QTOF-TMS
13.	Isofenphos-Methyl	---------------------	LC-MS/ MS
14.	Dimethenamid, Saflufenacil	---------------------	LC-MS
15.	Pydiflumetofen	---------------------	UPLC-MS/MS

GC – Gas Chromatography; GLC – Gas-Liquid Chromatography; LC – Liquid Chromatography, MS – Mass spectroscopy, UV – UV-Vis spectrophotometer; HPLC – High Performance Liquid chromatography ; UPLC-QTOF-TMS – Ultra high performance liquid chromatography–quadrupole time of flight tandem mass spectrometry

QUEChERS is comprised of two steps — extraction followed by Dispersive Solid-Phase Extraction (dSPE). The first step, extraction, relies on the use of organic solvents and salts that, when mixed with a food sample; cause the target analytes to partition into the organic layer (similar to a liquid-liquid extraction procedure). Once the initial extraction is performed, potential matrix interferences are removed from the Organic layer using dSPE. The dSPE uses SPE sorbents to specifically remove undesired matrix components. For example, a C18 sorbent can remove hydrophobic interferences such as fats and lipids while a primary-secondary amine (PSA) ion-exchange sorbent removes acids, sugars, and anthocyanin pigments that might act as instrumental interferences. The dSPE step can also use Graphitized Carbon Black (GCB), which is effective in removing a variety of planar pigments and sterols from the sample. Several different combinations of dSPE sorbents can be employed; choosing the most suitable dSPE sorbents is dependent on the characteristics of the commodity type (or food type) that is being analyzed (i.e. general, fats and waxes, pigmented, highly pigmented, pigmented and fats).

When developing the QuEChERS method for pesticide-spiked spinach samples, the AOAC Official Method 2007.01 was followed (figure 4). This method uses magnesium sulphate ($MgSO_4$) and sodium acetate (NaOAc) in the extraction step. The $MgSO_4$ induces liquid-liquid partitioning between water from the sample matrix and the organic solvent (acetonitrile). The NaOAc stabilizes the pH and buffers the sample during extraction, which is particularly important when analyzing pH-sensitive pesticides. A QuEChERS extraction kit (roQ AOAC Method 2007.01, Part no. KS0-8911, Phenomenex) was used for the extraction. Flat-bottomed, 50 ml centrifuge tubes were used, which stand upright without the use of a glass beaker, thus simplifying sample weighing. The tubes have been shown to produce very low extractable, thus eliminating interferences that could be introduced from the tubes. Prepacked salt packets were used to avoid manually having to weigh and add salts.

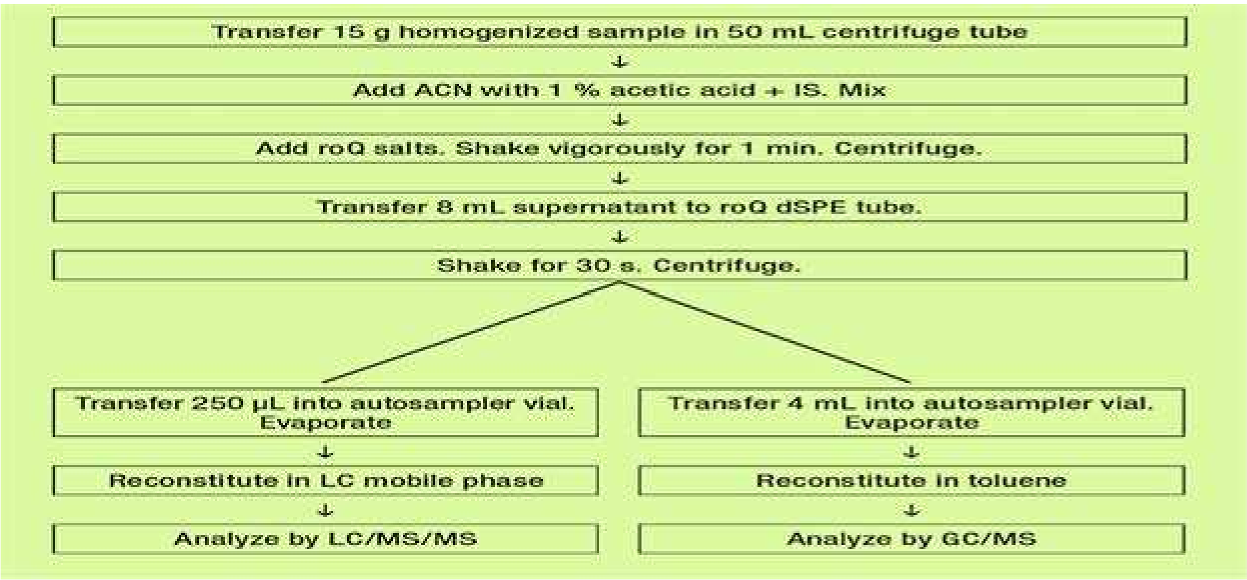
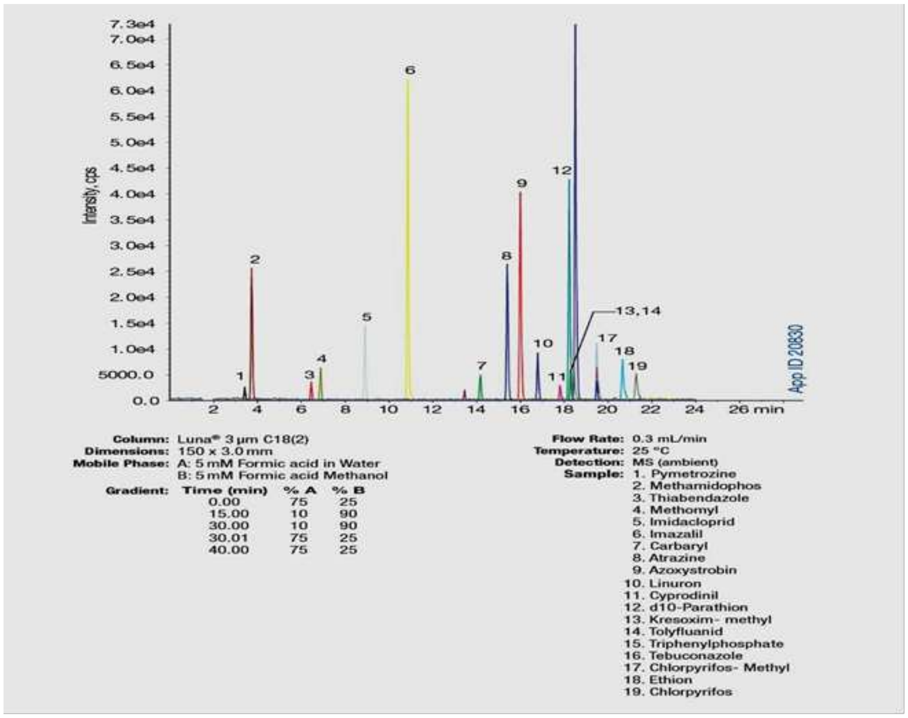


Figure 4: Flow chart summary for AOAC 2007.01 QuEChERS method.

After the extraction step, dSPE sorbent combination was chosen using pre-packed dSPE centrifuge tubes (roQ AOAC 2007.01 dSPE Kit for pigmented samples, Part no. KS0-8927, Phenomenex).The low-extractable 15 ml centrifuge tubes were prepacked with $MgSO_4$, primary-secondary amine (PSA) and graphitized carbon black (GCB). The PSA was able to remove organic acids, fatty acids, sugars and anthocyanine pigments while the GCB effectively removed planar molecules such as pigments and phytosterols (figure 5).

After clean-up of the extract by QuEChERS, the extract was split into two analytical portions, one to be analysed by LC–MS–MS and the other to be analyzed by GC–MS. Each portion was evaporated and reconstituted in a solvent suitable for this analysis: 5 mm formic acid in methanol for LC–MS–MS and toluene for GC–MS [16]. In terms of recovery and repeatability (RSD), some analytes gave good results in both LC–MS–MS and GC–MS analyses, while quite a few displayed acceptable results in either one or the other. Of the 16 representative pesticides analyzed, seven were studied by LC–MS–MS. The analysis was performed using a 150 x 3.0 mm HPLC column (Luna 3 µm C18(2), Phenomenex) coupled to an LC system (Agilent 1200, Agilent Technologies, Palo Alto, California, USA) and an API 4000 triple quadrupole mass spectrometer (AB Sciex, Framingham, Massachusetts, USA) [13, 14]. LC–MS–MS running conditions are listed in Figure 6.

Figure 5: Spinach extracts after dSPE clean-up. GCB removed a majority of the pigment from the sample matrix and the extracts were clear with a light green tint.



Column: Luna® 3 µm C18(2)
Dimensions: 150 x 3.0 mm
Mobile Phase: A: 5 mM Formic acid in Water
B: 5 mM Formic acid Methanol

Gradient:

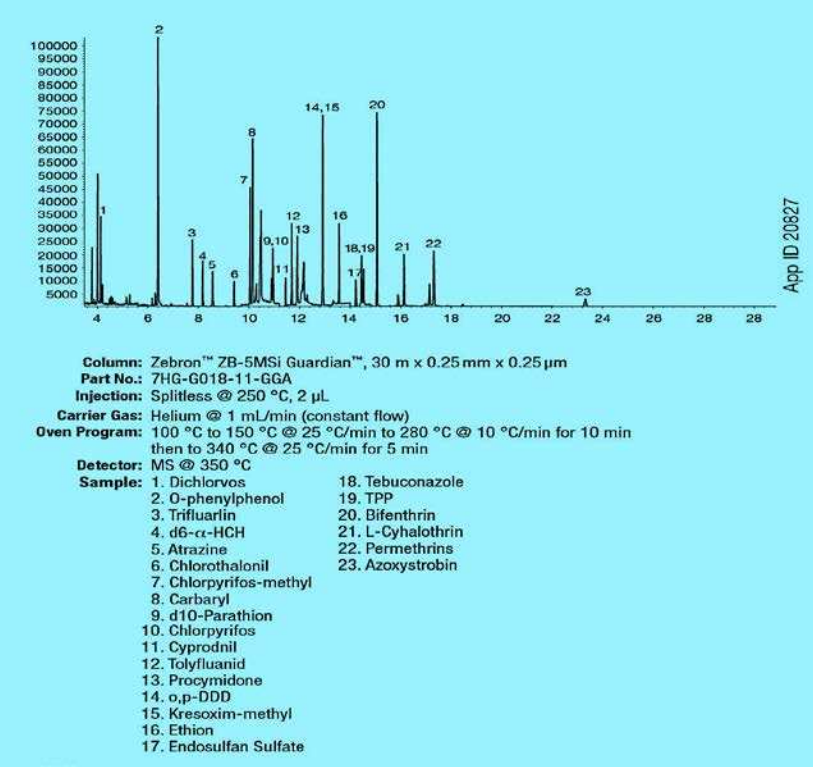
Time (min)	% A	% B
0.00	75	25
15.00	10	90
30.00	10	90
30.01	75	25
40.00	75	25

Flow Rate: 0.3 mL/min
Temperature: 25 °C
Detection: MS (ambient)
Sample:
1. Pymetrozine
2. Methamidophos
3. Thiabendazole
4. Methomyl
5. Imidacloprid
6. Imazalil
7. Carbaryl
8. Atrazine
9. Azoxystrobin
10. Linuron
11. Cyprodinil
12. d10-Parathion
13. Kresoxim- methyl
14. Tolyfluanid
15. Triphenylphosphate
16. Tebuconazole
17. Chlorpyrifos- Methyl
18. Ethion
19. Chlorpyrifos

Figure 6: LC–MS–MS chromatogram of spinach extract spiked at 200ng/g [13].

Table 2: Absolute recoveries of pesticides in two sets of five duplicated samples, fortified at 80 ng/g and 200 ng/g [14].

ANALYTE	**80 ng/g**				**200 ng/g**			
	LC/MS/MS Recovery (%)	**RSD (%)**	**GC/MS Recovery (%)**	**RSD (%)**	**LC/MS/MS Recovery (%)**	**RSD (%)**	**GC/MS Recovery (%)**	**RSD (%)**
Atrazine	76	3	89	3	72	6	88	3
Azoxystrobin	n/a	-	111	6	n/a	-	118	10
Bifenthrine	n/a	-	87	2	n/a	-	93	5
Carbaryl	n/a	-	105	8	n/a	-	94	17
Chlorothalonil	n/a	-	30	7	n/a	-	24	43
Chlorpyrifos	n/a	-	75	6	n/a	-	71	9
Enclosulfan Sulfate	n/a	-	111	6	n/a	-	109	12
Ethion	n/a	-	100	3	n/a	-	102	6
Imazalil	70	5	n/a	-	75	2	n/a	-
Imidacloprid	93	7	n/a	-	90	2	n/a	-
Kresoxim-methyl	82	2	95	4	87	6	96	7
L-Cyhalothrin	n/a	-	110	10	n/a	-	105	17
Linuron	77	4	n/a	-	78	10	n/a	-
o.p-DDD	n/a	-	98	3	n/a	-	97	6
o-phenylphenol	n/a	-	92	5	n/a	-	75	15
Permethrins	n/a	-	87	3	n/a	-	92	7
Tebuconazole	80	3	88	2	76	7	91	4
Thiabendazole	10	18	n/a	-	10	36	n/a	-



Column: Zebron™ ZB-5MSi Guardian™, 30 m x 0.25 mm x 0.25 µm
Part No.: 7HG-G018-11-GGA
Injection: Splitless @ 250 °C, 2 µL
Carrier Gas: Helium @ 1 mL/min (constant flow)
Oven Program: 100 °C to 150 °C @ 25 °C/min to 280 °C @ 10 °C/min for 10 min then to 340 °C @ 25 °C/min for 5 min
Detector: MS @ 350 °C
Sample:
1. Dichlorvos
2. O-phenylphenol
3. Trifluarlin
4. d6-α-HCH
5. Atrazine
6. Chlorothalonil
7. Chlorpyrifos-methyl
8. Carbaryl
9. d10-Parathion
10. Chlorpyrifos
11. Cyprodnil
12. Tolyfluanid
13. Procymidone
14. o,p-DDD
15. Kresoxim-methyl
16. Ethion
17. Endosulfan Sulfate
18. Tebuconazole
19. TPP
20. Bifenthrin
21. L-Cyhalothrin
22. Permethrins
23. Azoxystrobin

Figure 7: GC–MS chromatogram of spinach spiked at 200 ng/g [13]

Excellent recoveries and repeatability (RSD) of the pesticides analyzed by LC–MS–MS were obtained at two different spiked concentrations levels (Table 1) except for thiabendazole, which displayed low recoveries. This is most likely because, thiabendazole is a planar molecule and may have been absorbed by the GCB in the dSPE procedure [17]. GC–MS analysis was performed using a 30 m x 0.25 mm x 0.25 µm GC column (Zebron ZB-5MSi Guardian, Phenomenex) on a gas chromatograph (Agilent 6890N) with a network mass spectrometer (Agilent 5973, Agilent Technologies, Palo Alto, California, USA) [16]. GC–MS running conditions are listed in Figure 7. Recoveries for all pesticides analyzed by GC–MS were higher than 70 percent except for the planar molecule chlorothalonil, which is absorbed strongly by GCB. The QuEChERS sample preparation method was determined to be a quick and easy way to extract the many pesticides we wished to analyze from a difficult spinach matrix. Because the spinach sample is heavily pigmented, the use of a QuEChERS kit that contained GCB helped to remove a majority of the pigmentation before LC–MS–MS and GC–MS analysis. Food analysis, particularly of multi residues that have many different chemical properties, is a very difficult process that requires the careful choice of analytical methods for every sample matrix and compound. There is no one single solution that can be employed with all food matrices and target analytes. For this reason, careful consideration was given to the clean-up technique- and downstream analysis for multi residue pesticides from spinach samples. Although our QuEChERS method resulted in low recoveries of a few pesticides, overall the clean-up technique proved to be effective because it was nonselective. This technique extracted multi residues of various compound classes. It was able to remove interferences such as organic acids and pigments. Downstream analysis of pesticides by both LC–MS–MS and GC–MS proved to be vital because some pesticides in our screen were best analyzed by LC–MS–MS while others were more easily analyzed by GC–MS [17, 18]

CONCLUSION

Chemicals hazards due to pesticides in fruits and vegetables can be detected using various techniques. This review highlights various techniques of extracting pesticides; further detecting these using sophisticated instruments like GC-MS, LC-MS. Considering the ill effects in relation to health, pesticides in fruits and vegetables must be got to a standstill. Hence it is the role of the analyst to perform an enormous number if studies to bring analysis best suited to data requirement.

FINANCIAL ASSISTANCE

Nil

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

Jessica Gonsalves contributed to the compilation of the data, the study of the techniques used for designing the article related to the work as mentioned in the topic. Shweta Borkar analyzed, interpreted the compiled data and contributed to the drafting of the manuscript, and subsequently revised it. Jessica Gonsalves also approved the submitted version. Both the authors read and approved the final manuscript. Jessica Gonsalves and Shweta Borkar, both were accountable for the contributions and questions related to the accuracy or integrity of any part of the work

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