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Review

Pesticide residue analyses in plant material by chromatographic methods: clean-up procedures and selective detectors

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Abstract

This paper deals with the analysis of pesticide residues by chromatographic methods in samples of plant origin. Emphasis is put on the isolation and clean-up steps of the sample preparation for GC or LC determination. The problems of the extraction solvent selection and clean-up procedures using different types of adsorption column chromatography or gel permeation chromatography are discussed. Attention is also given to alternative techniques such as supercritical fluid extraction (SFE), matrix solid-phase dispersion (extraction) (MSPD) and sweep co-distillation that are used for sample processing prior to GC and LC analysis. Currently, pesticide residue analyses are typically multi-residue procedures with highly sensitive methods. Consumption of costly and toxic solvents is being minimized and fully automated analytical procedures can be expected in the future.

Keywords: Reviews; Extraction methods; Sample preparation; Detection, LC; Detection, GC; Environmental analysis; Food analysis; Fruits; Vegetables; Pesticides

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1. Introduction

The present state in the analysis of pesticide residues is characterized by the modern instrumental techniques of gas–liquid- and high-performance liquid chromatography. Capillary gas chromatography (GC) and high-performance liquid chromatography (HPLC) with selective detectors are dominating in the analyses of pesticide residues in samples of plant origin. Planar chromatography (TLC), including its modern alternatives [HPTLC, over-pressure layer chromatography (OPLC)], is still occasionally used for screening purposes, especially in combination with selective biochemical detection methods [1–3], but these methods are quickly loosing importance. The essential requirements for analytical methods for pesticide residues can be summarized in the following four points: multiresidual character, high recovery (min. 70%) and low limit of detection (LOD) and limit of quantitation (LOQ), high reproducibility and ruggedness of the method.

An analytical method for the determination of pesticide residues in a complex matrix such as fruit, vegetables, feeds or food is characterized by certain general features. Chromatographic methods used for the final determination require extraction of the residues from the matrix and a subsequent clean-up procedure. This article deals with different techniques for the isolation of pesticide residues from the plant matrix and with the cleaning of the extracts to make them suitable for chromatographic determination.

2. Classification of plant commodities under the consideration of the *Codex Alimentarius* Commission

The separation of pesticide residues from the plant prior to subsequent steps in the analytical procedure (usually gas–liquid chromatography) is a problem to which much effort has been applied in the area of method development. The determination of trace levels of pesticide residues (herbicides, insecticides, fungicides, etc.) in plant or food samples requires the use of sample preparation.

It involves sampling and sample handling, for which recommended approaches are described [4–6], extraction and clean-up procedures. The individual steps of the analytical procedures are chosen according to the chemical structure of the analysed compounds and according to the character of the matrix.

Classification and examples of the commodities (vegetables, fruits) under consideration by the *Codex Alimentarius* Commission (Table 1) are developed on the basis of the recommendations of the IUPAC Commission on Agrochemicals.

3. Extraction and clean-up procedures

The *Codex Alimentarius* classification of crops (Table 1) is based on the botanical types of the plants. For analytical purposes, plant samples have been divided by Ambrus et al. [7] into six main sample groups (Table 2), according to their chemical composition. The grouping is optimal for simple and quick orientation. The analysis of plant material for pesticide residues usually consists of the following steps; extraction of the residues, liquid–liquid partitioning (LLP), cleaning and determination.

Leoni et al. [8] described a multi-residue method for the quantification of organophosphorus pesticides in vegetable and animal foods. The foods are divided into four groups (Table 3) according to the extraction process (acetone and acetone–water for samples with moisture content <45%) and clean-up (on active carbon–Celite, on disposable mini-columns of Kieselguhr-type material and on disposable mini-columns of bonded-phase silica, according to fat and pigment content). Further fractionation on a silica gel micro-column can be included in the procedure.

The differences in the plant material and the texture of the samples necessitates different procedures for the extraction and especially for the clean-up of the concentrated extracts. Ambrus and Thier [9] classify samples for extraction into three groups; samples of medium and high water content, dry samples and fatty samples. Within the first group of samples, having a sugar content of 5 to 15% or 15 to 30% can be included.

Table 1
Classification and examples of commodities according to *Codex Alimentarius* Commission [5]

Number	Group	Commodities
1	Root and tuber vegetables	Beets, carrots, celeriac, chicory, horseradish, parsnips, potatoes, radishes, rutabagas, sugar beets, sweet potatoes, turnips
2	Bulb vegetables	Garlic, leeks, onions
3	Leafy vegetables	Beet leaves, chicory leaves, corn salad, endive, lettuce, parsley, radish leaves, spinach, sugar beet leaves, swiss chard
4	*Brassica* (cole) leafy vegetables	Broccoli, brussels sprouts, cabbages, cauliflower, collards, Kales, kohlrabi, mustard greens
5	Stem vegetables	Artichoke, asparagus, celery, rhubarb, witloof chicory
6	Legume vegetables	Brand bean, kidney beans, lima beans, runner beans, soybeans, peas, cow peas, lentil, sugar peas
7	Fruiting vegetables–edible peels	Cucumbers, egg plants, gherkin, okra, peppers, summer squash, tomato
8	Fruiting vegetables–inedible peels	Melons, pumpkin, squash, watermelon, sweet corn
9	Citrus fruits	Lemons, mandarins, orange, sweet
10	Pome fruits	Apples, pears, quince
11	Stone fruits	Apricots, cherries, sour cherries, sweet cherries, nectarines, peaches, plums
12	Small fruits and berries	Blueberries, cranberries, currants (black, red, white), dewberries, gooseberries, grapes, raspberries, strawberries
13	Assorted fruits–edible peel	Dates, figs, olives
14	Assorted fruits–inedible peel	Avocados, bananas, guavas, kiwi fruit, mangos, papayas, passion fruits, persimmons, pineapples, pomegranates
15	Cereal grains	Barley, maize, millet, oats, popcorn, rice, rye, sorghum, wheat
16	Fodder and straw	Barley fodder and straw, grasses, fodder, maize fodder and straw, mint fodder, oat fodder and straw, rice fodder and straw, rye fodder and straw, sorghum fodder, wheat fodder and straw, peanuts
17	Legume oilseed	Peanuts
18	Legume animal feeds	Alfalfa fodder, bean fodder, clover fodder, peanut fodder, pea fodder, soybean fodder
19	Tree nuts	Almonds, chestnuts, filberts, macadamia nuts, pecans, walnuts
20	Oilseed	Cottonseed, linseed, poppyseed, rapeseed, safflower seed, sunflower seed
21	Tropica seed	Cacao beans, coffee beans
22	Herbs	
23	Spices	Ginger-root, mustard-seed
24	Teas	Tea, dried, black, green

Table 2
Grouping of plant samples into six main sample groups [7]

Group	Plant sample
I	Root and bulb vegetables (e.g., carrot, parsley (root), onion, garlic)
II	Fruit and vegetables of low chlorophyll and oil content (e.g. pome fruits, stone fruits, citrus fruits, fruiting vegetables, berries, bananas, beets, radish)
III	Plants and crops of high chlorophyll content; commodities of high oil content are excluded (e.g., leafy and legume vegetables, *brassicas*, plant leaves, tobacco)
IV	Dried fruits of high sugar content (e.g., dates, figs, raisins, prunes)
V	Dry crops of low fat (oil) content that can be ground to powder (e.g., cereal grains, flours, maize, dried fodders, dried tea)
VI	Crops of high oil content (e.g., oil seeds, peanut, cacao beans, coffee beans, soybeans, nuts, dried red pepper)

Table 3
Grouping of food samples for extraction, shortened from reference [8]

Group	Water (%)	Fat (%)	Food type	Extraction
I	>45	<2	Vegetables, fresh fruits	Acetone
II	>45	>2	Whole milk, green cheese	Acetone
II a	>45	>2	Eggs, meat	Acetone
III	<45	>2	Cheese, oil, dried legumes	Acetone–water[a]
IV	<45	<2	Wheat meal, pasta, rice, bread	Acetone–water

[a] Except oil.

3.1. Liquid–liquid partitioning

In the case of multiresidue methods, the extracting solvent has to be suitable for the extraction of compounds within a wide polarity range from a variety of matrices containing different amounts of water, fat, sugar and other substances. The usual way for extracting pesticide residues from the sample is by thorough disintegration of the matrix in a high speed homogeniser (e.g., Ultra Turrax or equivalent) in the presence of the solvent or solvent mixture. In the multi-residue method (MRM), the most widely used solvents are acetone and acetonitrile. Both are miscible with water and consequently, the actual extracting agent is their mixture with the water derived from the sample. The merit of acetonitrile (MeCN) is that much lipophilic plant material, such as fats and waxes, is not extracted. The extract therefore contains only a minor load of co-extractives. The disadvantages of MeCN are its high price and toxicity. Acetone, which is widely used for different types of pesticide compounds, is chosen as the extracting solvent because of its advantages over MeCN, methanol (MeOH) or ethyl acetate (EtOAc) (non-toxic, easy to purify and evaporate, and it is inexpensive). In contrast, acetone has a low boiling point (56.5°C) which can cause evaporation problems and subsequent errors in quantitation. Acetone extraction is often followed by partition into dichloromethane.

Ambrus et al. [7] recommend acetone for the extraction of pesticide residues from plant materials belonging to groups I–III (Table 2), a water–acetone mixture for plant material samples from group IV and dichloromethane for the samples from group V. Liquid–liquid extraction (LLE) is not optimal for oily crops from group VI as the sample clean-up requires additional steps (e.g., gel permeation chromatography or liquid–liquid partitioning, usually between water and MeCN).

3.2. Solid-phase extraction

Besides the use of LLE for the isolation and preconcentration of a particular component from the sample, the use of extraction in the liquid–solid phase system is growing, mainly for matrices with a high content of water. In literature it is frequently denoted as solid-phase extraction (SPE).

SPE is a simple preparation technique based on the separation of liquid chromatography, where the solubility and functional group interactions of sample, solvent and sorbent are optimized to effect the retention and elution. Moderately polar to polar analytes are extracted from non-polar solutions onto polar sorbents. Sorbents for normal phase are modified with cyano-, diol- or amino groups. Non-polar-to-moderately polar analytes are extracted from polar solutions onto non-polar sorbents. Sorbents for reversed-phase are modified with octadecyl-, octyl-, cyclohexyl- or phenyl groups. SPE [10–13] may be carried out with several modifications, e.g., on columns (cartridges) or using membrane extraction discs.

Most MRMs include a clean-up step using adsorption columns, in particular Florisil, aluminium oxide (alumina) and silica gel. Most adsorbent columns provide good clean-up only when they are eluted with solvent mixtures of low polarity, eluting less polar residues and leaving more polar co-extractives in the column. The more the eluting solvent polarity is increased, the greater will be the portion of interfering substances eluted and the less effective the clean-up will be. Other details are provided in reference [9].

Florisil has gained the greatest attention of all the

sorbents used in residue analysis. As Florisil retains some lipids preferentially (25 g Florisil with 3% water retains 1 g of fat), it is particularly well suited for the clean-up of fatty foods. When a Florisil column is eluted with solvent mixtures of low polarity, non-polar residues are recovered almost quantitatively. The eluates are very clean for GC–electrochemical detection (ECD), nitrogen–phosphorus detection (NPD), flame photometric detection (FPD) as well as for TLC with selective detection using the Hill reaction inhibition technique [22]. A major disadvantage is, however, that activity may vary from one batch to another. For the analysis of plant material, Florisil has been recommended often in the framework of multi-residue procedures for fruits and vegetables.

Florisil columns have been used for the clean-up of plant extracts by many authors [16,23–31]. In many cases, Florisil can be replaced by alumina, particularly for the analysis of fatty foods. Basic alumina decomposes some organophosphates, and some more polar pesticides are not (or not quantitatively) eluted from neutral or acidic alumina columns. Alumina columns were used for clean-up by Kováč et al. [1], Ambrus et al. [7], Pestemer and Mann [30] and by Tekel' et al. [32]. A silver-loaded alumina column [23,33] can be used for elimination of sulphur-containing compounds from kale, onions, etc., prior to GC–ECD determination. However, for the GC–ECD determination of non-polar pesticides, silver-loaded alumina is preferable, as the clean-up efficiency is better for most products except for celery, parsnip, cabbage and cauliflower. Another advantage is that more pesticides are recovered [33]. In this case, alumina acts mainly as a support for reactive silver nitrate. Miniaturized methods are also available.

In general, silica gel is less efficient than alumina and does not adequately separate pesticides from plant co-extractives [9]. Its importance in the analysis of plant material resides in the fractionation of certain residues according to their polarity without appreciable losses. Many authors [16,21,27–30,34] have used silica gel columns or mini-columns for the clean-up of sample extract. Lores et al. [35] used an improved silica gel clean-up method for organophosphorus pesticides. The method used a 3.5-g silica column pre-loaded with 1% acetic acid. The recoveries ranged from 92 to 101% for the eleven organophosphorus compounds tested.

Mixed adsorbents have been used many attempts to combine the different properties of hydrophilic adsorbents and lipophilic carbon. Some of them have gained some importance, such as the silica–carbon column or mixtures of carbon with MgO and Celite. Petersen and Jensen [23] used column chromatography on activated charcoal–MgO–Celite for GC determination of organophosphate pesticide residues in black tea after their extraction with EtOAc.

Di Muccio et al. [36] used on-column partition clean-up of fatty extracts (olive oil, wheat, maize, barley, rice, peanuts, soybeans, beans, chick peas and lentils) for the determination of organophosphate pesticide residues. A fast, single step, efficient partition between *n*-hexane and MeCN on ready-to-use, disposable mini-columns of Kieselguhr-type material was developed for the cleaning of fatty extracts for determination by GC–FPD. The main feature of this clean-up system compared with the above-quoted methods are the good clean-up and recoveries of between 80 and 107%.

Di Muccio et al. [37] used single-step solid matrix clean-up of vegetable extracts from different crops (lettuce, onion, strawberry, apple, yellow pepper, peach, tomato, broccoli, cauliflower and radish) for the determination of organophosphorus pesticide residues. An aqueous acetone extract of the sample (15 ml aliquot of the extract equivalent to ca. 5 g of the crop) was transferred onto the top of an Extrelut-20 column filled with a macroporous Kieselguhr-type material. The column was eluted with four portions of light petroleum (b.p. 40–60°C), followed by four portions of dichloromethane–light petroleum (1:3, v/v) to elute dimethoate. The recoveries were between 75 and 110% (0.1 to 1.4 mg/kg).

In comparison with instrumental clean-up techniques (size-exclusion chromatography, sweep co-distillation) the described method is very simple, rapid, inexpensive and does not require the preparation or maintenance of costly apparatus or a skilled operator.

Increased attention is devoted to carbon sorbents [12,13], besides the ones with a SiO_2 matrix. Graphitized carbon black (GCB) is such a sorbent, being non-specific and generally of hydrophobic nature. Contrary to sorbents based on SiO_2, these

may be used without the pH of the treated solutions being taken into account. The GCB columns are extensively used in the analysis of pesticide residues in water.

3.3. Matrix solid-phase dispersion extraction

Extraction in a chromatographic column was also found to be very efficient for a wide range of compounds and samples with medium and high water contents. The sample of fruit or vegetable is homogenized with the addition of a small amount of water, if required. The sample is mixed with adsorbent (e.g., silica gel, Florisil). The free flowing dry mixture of sample pulp is transferred onto a column over a 5 mm layer of anhydrous sodium sulphate. The pesticide residues are extracted in a glass column with organic solvent, either individually or in a mixture. An advantage of this procedure is that no emulsion is formed.

Kadenczki et al. [14] used activated Florisil for MSPD extraction of pesticide residues from samples of plant origin. For the elution of residues (carbamate, organochlorine, organophosphate, synthetic pyrethroid, triazine and phenyl urea pesticides) EtOAc or dichloromethane–acetone (9:1, v/v) were used. The recovery was generally >80% and was independent of the sample material. The weight of sample pulp (W_p) taken for extraction and the amount of Florisil (W_f) depend on the volume of water (V) added to the sample. They can be calculated, by the following formula:

$$W_p = (200 + V)/40 \text{ and } W_f = 1.6 \times W_p$$

Lingh and Huang [15] used Florisil-based MSPD extraction and a GC–ECD method for the determination of six synthetic pyrethroids in vegetables (West Indian gherkin, eggplant, pak-choi, cabbage and garden peas). Schenk and Wagner [16] described a rapid MSPD extraction technique for five organochlorine and five organophosphorus pesticide residues from milk. Milk (5 ml) is blended with 2 g of C_{18} (octadecylsilyl-derivatized silica) and 1.5 ml of MeCN in a syringe barrel. The pesticide residues are eluted from the C_{18}–milk matrix with MeCN which is then eluted through a Florisil SPE column. The extract is directly analysed by GC–FPD. After further clean-up of the extract on a mini-Florisil column, the organochlorine pesticide residues are determined by GC–ECD.

3.4. Sweep co-distillation

Sweep co-distillation [17] is based upon the low vapor tension of analysed compounds in a stream of inert gas (e.g., nitrogen). Evaporated extracts of a sample in organic solvent is distilled in a glass tube filled with silanized glass wool or glass beads. Less volatile components are trapped by the filling material. After freezing, the distillate is evaporated again and analyzed by GC. It is an efficient purification for organochlorine and organophosphate pesticide residues, but its use is now being replaced by the more universal, gel permeation chromatography (GPC). Sweep co-distillation gained most popularity in Australia for the analysis of meats and diary products [18–21]. Results of sweep co-distillation clean-up are equivalent to those of GPC and adsorption chromatography on Florisil column clean-up [21].

3.5. Supercritical fluid extraction

A general trend in the isolation of pesticide residues is to decrease the consumption of expensive and/or toxic organic solvents and to increase the availability of a broad range of analytes and matrices. A possible solution is to use supercritical fluid extraction (SFE).

King et al. [38] and Poustka et al. [39] have published an application of SFE with carbon dioxide for the selective isolation of organophosphates from contaminated cereals. Resulting extracts were cleaned up by GPC and GC–FPD was used for quantitation. Comparison of the classic LLE–GPC method with the SFE method of sample preparation [39] demonstrates the power of the SFE method (reduction of the extraction time, lower organic solvent consumption, good quality of final extracts) for the analysis of organophosphates in cereals, rice, grain, flour, etc. Skopec et al. [40] used carbon dioxide modified with 5% (v/v) methanol for the isolation of organophosphates from rice. Extracts were analyzed without further clean-up by GC–atomic emission detection (AED). Extraction of methamidophos residues [41] from vegetables (pep-

per, cucumber and tomato samples) with SFE was developed. Vegetable samples were mixed with anhydrous magnesium sulphate (vegetable+ magnesium sulphate 5:7, w/w) and extracted with supercritical carbon dioxide with methanol as the static modifier and EtOAc as the trapping system. SFE was used for isolation of the residues of fluazifop-P in onions [42]. SFE coupled with supercritical fluid chromatography (SFC) was employed for the separation of sulfometuron-methyl residues [43] from several wheat matrices (grain, flour and straw).

The use of supercritical carbon dioxide with and without MeOH as a modifier to extract ^{14}C-labelled pesticide residues (deltamethrin, pirimiphos-methyl, fonofos, dieldrin a and atrazine) from soil plants (beans, onion radishes and canola) and wheat samples has been described [44]. Optimal SFE conditions were obtained for each pesticide by varying the temperature, pressure and the amount of modifier. Supercritical MeOH was found to be less efficient than supercritical carbon dioxide or methanol-modified supercritical carbon dioxide for the extraction of bound pesticide residues. The SFE method did not result in thermal degradation of residues. The SFE procedure employed required small samples, it was quick and it was not labor- and solvent-intensive.

3.6. Gel permeation chromatography

The most universally applicable clean-up is GPC. Separation is generally performed by using divinylbenzene-linked polystyrene gels, mostly Bio-Beads SX-3 (200–400 mesh, Bio-Rad, USA). It is suitable for organochlorine, organophosphorus and nearly all other types of pesticides and does not involve any losses by adsorption. The GPC column, which consists of porous polymer beads, retains molecules that are small enough to enter the pores. Lipid molecules that are too large to enter these pores are unretained and are therefore eluted from the column first. Most synthetic pesticides have molecular mass of between 200 and 400, whereas those of most lipids range from 600 to 1500. For the elution of pesticides, several solvent mixtures have been recommended (Table 4). The mixture cyclohexane–EtOAc (1:1, v/v) has proved to be suitable for the clean-up of pesticides and metabolites [45–47]. The mixture cyclohexane–methylene chloride (1:1, v/v) is useful for the clean-up of more than 120 pesticides [33,48]. Under the conditions used for plant extracts, GPC on Bio Beads SX-3 can be applied to the analysis of fats and oils, effectively removing lipid before the analysis of organochlorine and less polar organophosphates. Another valuable feature is that GPC can be carried out in an automatically controlled device. GPC using Bio Beads SX-3 and cyclohexane–EtOAc (1:1, v/v) as an elution mixture is well known for its capability of removing more than 95% of high molecular mass co-extractives and lipid from crude sample extracts [49].

Clean-up by GPC is adequate for GC and/or LC in most cases. However, some overlap of the large lipid chromatographic band with the pesticide fraction typically occurs and additional clean-up by adsorption chromatography on mini-columns is necessary in some cases. An alumina mini-column was used for pyrethroids [50] for further cleaning up. Some authors [33,46,49] used the mini-silica column. Chaput [48] used GPC with on-line Nuchar–Celite clean-up for crops with high chlorophyll and/or carotene content (e.g., cabbage and broccoli) for determining carbamates in fruits and vegetables.

A great advantage of GPC is the life time of the GPC column. In general, it could be used for several months without any effect on the retention volumes or the clean-up capacity [33].

4. Present trends in clean-up procedures for the analysis of pesticide residues in plant samples

As already mentioned, any analysis of residues by chromatographic methods requires cleaning of the extracts. The general trend is to minimize the consumption of organic solvents and to make it as universal as possible. SFE offers a suitable solution to both of these problems. As extractant here serves supercritical (liquid state) carbon dioxide which enables the rapid isolation of an analyte due to the high diffusion coefficient of the extraction fluid. It uses ecologically non-toxic extracting media such as CO_2 or N_2O. The extraction rates are high, because the surface tension of supercritical fluid is very low,

Table 4
Clean-up of plant samples by GPC on Bio Beads SX-3

Pesticide	Commodities	Extraction and partitioning	Elution mixture	Method	Reference
Benzoyl ureas	Apples	Acetone H_2O–CH_2Cl_2	Cyclohexane–$CHCl_3$ (3:2, v/v)	LC–DAD	[51]
Carbamate herbicides	Potatoes	MeCN Light petroleum–CH_2Cl_2	Cyclohexane–EtOAc (1:1, v/v)	LC–DAD	[45]
Carbamate insecticides	Fruits, vegetables	Methanol H_2O–CH_2Cl_2	Cyclohexane–CH_2Cl_2 (1:1, v/v)	LC–FD	[48]
Organochlorines, carbamates, dinitroaniline, phenyl ureas	Fruits, vegetables, cereals	Acetone *n*-hexane–CH_2Cl_2	Cyclohexane–CH_2Cl_2 (85:15, v/v)	GC–NPD GC–ECD	[52]
Organochlorines, organophosphates, carbamate insecticide	Fruits, vegetables	Acetone *n*-hexane–CH_2Cl_2	Cyclohexane–CH_2Cl_2 (1:1, v/v)	GC–ECD GC–FPD	[33]
Organochlorines, organophosphates, insect growth regulators	Wheat, rice	Acetone–methanol (1:1, v/v)	*n*-Hexane–CH_2Cl_2 (3:7, v/v)	GC–ECD GC–FPD LC–UV	[50]
Herbicidal phenoxy acids	Fruits, vegetables, cereals	Acetone H_2O–CH_2Cl_2	Cyclohexane–EtOAc (1:1, v/v)	GC–ECD	[53]
More than 400 pesticides and their metabolites	Foods and feeds of vegetables	Acetone–water (2:1, v/v)	Cyclohexane–EtOAc (1:1, v/v)	GC–NPD GC–FPD	[46]
23 N-containing pesticides	Agricultural products	Acetone water–*n*-hexane	Cyclohexane–acetone (1:1, v/v)	GC–MSD	[54]
100 pesticides	Olive oil, palm oil, sunflower oil	CH_2Cl_2	Cyclohexane–acetone (3:1, v/v) Light petrol–acetone (1:1, v/v)	GC	[55]
400 pesticides and metabolites	Plant foodstuffs	Acetone H_2O–CH_2Cl_2	Cyclohexane–EtOAc (1:1, v/v)	GC–ECD GC–NPD GC–AED GC–MSD	[47]
Ametryn and its metabolites	Tropical root crops	EtOAc–toluene (3:1, v/v)	EtOAc–toluene (3:1, v/v)	GC–NPD GC–FPD	[56]

which supports its penetration into the micropores of the sample. An important parameter in SFE is the usage of a modifier that can be added to extracting fluid to enhance its dissolving power. Water or methanol are usually used. Advantages are the saving of organic solvents and the speed of the process; a disadvantage is the need for carbon dioxide of ultrahigh purity. Difficulties may arise when analysing samples with a high content of water.

GPC is a particular case of the size exclusion technique, where the fractionation proceeds in non-aqueous medium. Respecting the nature of dominant pesticide residues with $M_r < 400$ in practice, there are gels based on styrene and divinylbenzene copolymers. The most used gel is Bio Beads SX-3. Mixtures of organic solvents are used as the mobile phase. Very often mixture of cyclohexane–EtOAc (1:1, v/v) or cyclohexane–dichloromethane (1:1, v/v) are used. GPC is suitable for routine analysis as an

isolation technique. It can be automated and it gives good results. It is usable for the purification of complicated matrices with high lipid contents.

The sweep co-distillation technique combines a rapid procedure and low capital cost for the sweep co-distillation apparatus, UNITREX (universal trace residue extractor), with the important advantage of greatly reduced solvent use [21]. In principle, it is suitable for a broad range of pesticides (e.g., organochlorines and organophosphates) and substrates (fat and plant material).

Column adsorption chromatography is a simple, proven technique but requires considerable organic solvent and has a low potential for automation. However, it is a rapid and reliable clean-up technique which is well suited for confirmatory analyses. Mini-columns (silica gel, Florisil) are often used for further clean-up of eluates after GPC.

Comparison of the universality, advantages and disadvantages of selected isolation and purification techniques (clean-up) is given in Table 5.

4.1. Use of selective detectors for GC and LC methods

An ideal selective detector for the analysis of pesticide residues would respond only to the target pesticides, while other co-extracted compounds remain transparent. Pesticides almost always contain heteroatoms and often have several in a single molecule. The most frequently encountered heteroatoms are O, P, S, N, Cl, Br and F. The use of different detectors in GC and LC analysis of pesticide residues in plant material is shown in Table 6. Therefore, most GC methods employ element-selec-

Table 5
Isolation and clean-up techniques in the analysis of pesticide residues

Technique	Isolation	Clean-up	Main commodities, limitation
Liquid–liquid extraction	+	+	Water, soil, foods, crops material High consumption of organic solvents, risk of emulsion creation, universal
LSE and SPE -cartridge -membrane extraction disc -solid-phase micro-extraction -graphitized carbon black	+	(+)	Water No emulsions, low consumption of organic solvents, universal Plant material
Matrix solid-phase dispersion extraction	+	+	Plant material, foods, feeds No emulsions, direct on-line clean-up, universal
Sweep co-distillation	+	+	Foods, crops material. Used usually for organophosphates
Column adsorption chromatography -Florisil column -Alumina column -Silica gel column	(+) (+) (+)	+ + +	Foods, crops material, universal
Supercritical fluid extraction	+	+	Soils, sediments, cereals, plant materials Using ecologically non-toxic extracting fluid (CO_2) Optimisation of modifier
Gel permeation chromatography	(+)	+	Samples with high content of fat, oil Universal

+ = Main effect, (+) = next or secondary effect.

Table 6
The use of different detectors in GC and LC analysis of pesticide residues in plant samples

Pesticide group	Method	Reference	Note
Organophosphates	GC–NPD; AFID	[23,27,29,35,57,58,64]	
	GC–FPD	[8,14,16,20,23,27,33,37,39,41] [46,50,57–59,62–65]	
	GC–ECD	[19,58,59,63,64]	
	GC–AED	[47,57]	
	GC–ELCD	[57,63]	
	GC–MS	[64]	
Organochlorines	GC–ECD	[14,16,21,23,27,33,46,50,57,58], [59,63]	
	GC–AED	[47,57]	
	GC–ELCD	[57,63]	
Carbamates	GC–NPD	[24,30,46,60,62,67]	
	GC–MS	[54]	
	GC–AED	[47]	
	GC–MSD	[60]	Derivatization with acetanhydride
	GC–FTD	[27]	
	GC–TID	[68]	
	LC–DAD	[45]	
	LC–FD	[10,11,48,66,98,99]	Derivatization with OPA–MERC
	LC–TSP–MS	[96]	
Synthetic pyrethroids	GC–ECD	[15,27,31,50,59]	
Bipyridyl cations	GC–NPD	[69]	Hydrogenation with sodium borohydride–nickel (II) chloride
	LC–UV; DAD	[70,71]	
Phenyl ureas and their anilines	GC–NPD	[61,72]	Derivatization with HFBA
	GC–ECD	[52,61,72]	Derivatization with HFBA
	GC–MSD	[60]	Derivization with acetanhydride
	LC–UV; DAD	[13,34,75,84]	
	LC–PCD	[76,84]	
	LC–FD	[74]	Derivatization with dansyl chloride
	LC–FD	[83]	Derivatization with OPA–MERC
	LC–TSP–MS	[96]	
Sulfonyl ureas	GC–ECD	[77]	Derivatization with PFBB
	LC–UV	[78]	
	LC–PCD	[79–82]	
Benzoyl ureas	LC–UV; DAD	[51,85]	
Triazines and their metabolites	GC–NPD; AFID	[14,24,30,46,56,59,67,88,89]	
	GC–FPD	[56,62]	
	GC–MS	[87]	
	LC–UV	[12]	
Diazines (uracils)	GC–NPD; AFID	[26,30,62,90,91]	
	GC–ECD	[30,59]	
	GC–MS	[54]	
	LC–UV	[73]	
Aryloxophenoxypropanoic acids (esters)	GC–NPD	[86,92]	Corresponding acids analyzed after conversion to methyl esters
	GC–ECD	[92]	
	GC–MS	[54]	
	GC–ELCD	[25]	
	LC–AD	[93]	
Phenoxyalcanoic acids (esters)	GC–ECD	[53]	Methylation with MeOH–H_2SO_4
Detto + chlorophenols	GC–ECD	[94]	Derivatization with PFBB
3,6-Dichloropicolinic acid	GC–ECD	[95]	Derivatization with diazomethane
	LC–UV	[73]	

tive detectors. Most commonly used are ECD and electrolytic conductivity detection (ELCD) for halogenated compounds, NPD for nitrogen- and phosphorus-containing pesticides, and FPD for sulphur or phosphorus compounds [57].

The analysis of pesticide residues in food and/or plant material is mainly performed by MRMs. Some vegetables with a complex matrix, such as onions and leeks, give rise to many problems in GC–ECD analysis. Two dimensional cGC using the techniques of heart-cutting and back-flush makes it possible to transfer small fractions or even single peaks to a second column where all relevant pesticides can be separated from their overlapping matrix compounds. Nitrogen- and phosphorus-containing pesticides in onion and leek samples are identified by selective detectors (NPD, FPD) without any problems [58]. The technique of two-dimensional GC is described in detail by Stan and Heil [59].

Linkerhänger and Stan [47] compared the customary detection by ECD–NPD with a new AED (detector that combines plasma excitation with optical emission spectroscopy) for the analysis of pesticide residues in plant foodstuffs. The screening analysis was performed with the combination GC–ECD–NPD as well as with the combination GC–AED in parallel. According to authors, the GC–ECD–NPD system is still a very reliable tool in screening analysis. Considering the original and the operating costs of a GC–AED system, which are comparable with those of a GC–MS system, the conventional GC–ECD–NPD system must be judged as a good choice for screening analysis in plant foodstuffs with regard to cost/profit calculations. Screening analysis of pesticide residues can be carried out reliably with the majority of foodstuffs with cGC and parallel ECD–NPD detection. Capillary GC–AED is the method of choice for screening so-called "problem foodstuffs", e.g., leek, garlic, onion and cabbage. The reliability of quantitative results at low concentration levels is notable. Ting and Kho [97] used GC–MIP–AED for six experiments including sensitivity and linearity studies for the elements S, P, Cl and N, a study of instrument response to Cl concentration in pesticide molecules, recoveries of organophosphates, carbamates and finally for an investigation of metallic pesticides (plictran and vendex). All positive results in screening analyses, however, have to be confirmed by GC–MS.

GC–MS was used for the determination of carbamate pesticides and some urea pesticides after derivatization with acetic anhydride [60]. Determination of thermolabile urea pesticides after derivatization with heptafluorobutyric acid (HFBA) using GC–ECD or GC–NPD and confirmation by GC–MS was described [61].

GC with AED was compared [57] to GC with other element-selective detectors for the analysis of ten pesticide residues in twelve agricultural products.

Table 7
Interferences caused by co-extractives in GC analysis of ten pesticides in crude plant extract (ethafluralin, dimethoate, diazinon, chlorothalonil, chlorpyrifos, parathion, chlorthal-di-methyl, folpet, dieldrin and azinphos-methyl) [57]

Plant	Detector type					
	FPD (S)	FPD (P)	NPD	ECD	ELCD (X)	AED
Alfalfa	x	x	– –	– –	–	x
Almonds	xx	x	x	–	–	xx
Broccoli	–	– –	–	x	x	xx
Carrot	xx	xx	x	x	xx	xx
Cauliflower	x	–	–	x	xx	xx
Green onion	– –	– –	x	– –	x	xx
Iceberg lettuce	xx	xx	x	x	xx	xx
Orange	xx	xx	x	–	–	xx
Romaine lettuce	xx	x	x	–	x	xx
Strawberry	xx	x	x	– –	–	xx
Sweet onion	– –	– –	x	– –	xx	xx
Zucchini	xx	x	x	x	xx	xx

Note: xx (best), x (good), – (unsuitable), – – (worst).

To analyze a broad range of volatile pesticides using conventional element-selective detectors, several different GC configurations are needed. As illustrated (Table 7), interferences from many commodities makes analysis of crude extracts virtually impossible; as a result, a clean-up step is required.

LC with fluorescence detection (LC–FD) was used for the determination of carbamate pesticides in vegetables and fruits after derivatization with *ortho*-pthalaldehyde–2-mercaptoethanol (OPA–MERC) [10,11,48,66,98,99]. LC–fluorescence detection (FD) was employed for the analysis of thermolabile phenylureas and their break-down products, anilines, after post-column derivatization with dansyl chloride [74] and OPA–MERC [83]. LC–TSP–MS is a powerful method for the qualitative confirmation of pesticide residues [96].

5. List of abbreviations

AD	Amperometric detection
AED	Atomic emission detection
AFID	Alkali flame detection
DAD	Diode array detection
ECD	Electron-capture detection
ELCD (X)	Electrolytic conductivity detection (halogen mode)
EtOAc	Ethyl acetate
FD	Fluorescence detection
FPD (P)	Flame photometric detection (phosphorus mode)
FPD (S)	Flame photometric detection (sulphur mode)
FTD	Flame thermoionic detection
GC (cGC)	Gas chromatography (capillary)
GCB	Graphitized carbon black
GC–MIP–AED	Gas chromatography–microwave-induced plasma atomic emission detection
GPC	Gel permeation chromatography
HFBA	Heptafluorobutyric acid (anhydride)
HPLC	High-performance liquid chromatography
HPTLC	High-performance thin-layer chromatography
IUPAC	International Union of Pure and Applied Chemistry
LC	Liquid chromatography
LC–TSP–MS	Liquid chromatography–thermospray mass spectrometric detection
LLE	Liquid–liquid extraction
LLP	Liquid–liquid partitioning
LOD	Limit of detection
LOQ	Limit of quantitation
LSE	Liquid–solid extraction
MeCN	Acetonitrile
MeOH	Methanol
MRL	Maximal residue limit
MRM	Multi-residue method (procedure)
MS	Mass spectrometry
MSPD	Matrix solid-phase dispersion (extraction)
NPD	Nitrogen–phosphorus selective detector
OPA–MERC	*ortho*-Phthalaldehyde–2-mercaptoethanol
OPLC	Over-pressure layer chromatography
PCD	Photoconductivity detection
PFBB	Pentafluorobenzyl bromide
SFC	Supercritical fluid chromatography
SFE	Supercritical fluid extraction
TID	Thermoionic detection
TLC	Thin-layer chromatography
UV	Ultra-violet (detection)

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