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Review

Analytical approaches for the determination of pesticide residues in nutraceutical products and related matrices by chromatographic techniques coupled to mass spectrometry

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A B S T R A C T

A review of sample preparation and analytical techniques currently used to analyze pesticides in nutraceutical products is shown. Different sample treatments are commented, and the QuEChERS method is the most used (quick, easy, cheap, effective, rugged and safe). For the chromatographic determination, gas chromatography (GC) and liquid chromatography (LC) are evaluated. Different detection modes are discussed, and simple quadrupole mass spectrometry (Q-MS) and triple quadrupole tandem mass spectrometry (QqQ-MS/MS) are the most used. Finally, a review of the occurrence of pesticides (from the revised literature) in real samples is presented, evaluating several matrices, such as nutraceuticals, dietary supplements, medicinal plants, and fish oil. The occurrence of several pesticides was reported: γ-HCH (lindane), endosulfan, procymidone, azoxystrobin, p,p'-DDE, metalaxyl, quintozene, tolclofos-methyl, chlorpyrifos and hexachlorobenzene.

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1. Introduction

In the last years, a number of diseases are becoming increasingly prevalent in the industrialized countries. Arthritis, diabetes and heart diseases are becoming more common because of the increase of unhealthy habits in modern society. When pharmaceutical products are not completely effective against this problem, people usually self-medicate with other kind of products like nutraceuticals, considering that they will be more effective on preventing or treating diseases [1]. Many consumers believe that nutraceutical products will improve their health, and they also think that these "natural" remedies are both effective and free from the side effects that may occur with other medications [2]. The consumption of this type of products is increasing: the nutraceutical industry has grown since 2000, getting to $22 billion in the United States (US) [3] and reaching $12 billion on the Asiatic market [3]. The US National Institute of Standards and Technology (NIST) reported that approximately 75% of the US population takes dietary supplements, including vitamins and mineral supplements [2]. The explosive growth of the US and Japanese markets has created similar expectations for the European market. Thus, the nutraceutical products market in Europe is currently valued at $31.6 billion [3].

Nutraceuticals legislation is sometimes ambiguous because there is not a common definition for this kind of products. Several definitions can be found in bibliography, and Lockwood discusses them, defining "nutraceutical" as "a term used to describe a medicinal or nutritional component that includes a food, plant or naturally occurring material, which may have been purified or concentrated, and that is used for the improvement of health, by preventing or treating a disease" [1]. Unlike foods, dietary supplements are allowed to use "nutritional support statements", and they can be marketed without any study looking for substances that can bring along a risk for human health [1]. The Dietary Supplement Health and Education Act (DSHEA) is the US law that establishes regulations and limits label claims on dietary supplements [4]. DSHEA defines "nutraceutical" as "a dietary supplement that may contain an herb or other botanical, or a concentrate, metabolite, constituent, extract or combination of any ingredient from the other categories" [5]. In Europe, the legislation covers food supplements (Directive 2002/46/EC) [6] and herbal medicinal products (Directive 2004/27/EC) [7], but there is no formal legislation regulating nutraceutical products across the European Union (EU) [5].

Nutraceutical products can be divided into three categories: (i) dietary supplements (vitamins, minerals, co-enzime Q, carnitine, ginseng, Ginkgo Biloba, Saint John's Wort, Saw Palmetto), (ii) functional foods (oats, bran, psyllium, lignin, prebiotics, omega-3, canola oil, stanols), and (iii) medicinal foods (transgenic cows, lactoferrin, transgenic plants, health bars) [8]. These products represent a huge food market and their quality controls should not be different from conventional food. Bearing in mind that a nutraceutical product is a concentrated form of a food or plant, it is possible to find substances utilized in plant protection, such as pesticides. The quality guide for botanical food supplements released by the European Botanical Forum specify that for botanical extracts, contaminant controls should be performed on the processed extract, whenever it has been demonstrated that certain organic chemical contaminants can be concentrated during the extraction process [9]. Moreover, there are occasional reports of inaccurate labeling, adulteration, contamination (e.g. with pesticides, heavy metals, or toxic botanicals), and drug interactions for these products [2]. Pesticide maximum residue levels (MRLs) for every food and animal feed have been defined by different organizations across the world, like the World Health Organization (WHO), the Food and Agriculture Organization of the United Nations (FAO) [10], the US-Environmental Protection Agency (US-EPA) [11] or EU [12]. However, the Regulation EC 396/2005 [13], which includes MRLs, only concerns raw materials. Therefore, MRLs should be defined in these nutraceuticals, in order to assure the safety of this type of products.

In this sense, analytical methods that allow the detection and quantification of pesticides on these products are necessary. Because of the complexity of this type of matrices, the first step in the analytical methods used for the determination of pesticide residues in nutraceutical and related products is the extraction and/or clean-up of the target compounds from the matrix. Several extraction approaches such as QuEChERS (acronym of quick, easy, cheap, effective, rugged and safe), pressurized liquid extraction (PLE) and matrix solid phase dispersion (MSPD) could be applied [14]. Then, chromatographic techniques such as gas (GC) and liquid (LC) chromatography are coupled to several detectors such as electron capture detection (ECD) [15], or diode array [16] for the determination of pesticide residues in this type of matrices. However, they have being replaced by mass spectrometry (MS) detection [17,18], considering that reliable confirmation is achieved with this detection technique.

The objective of this paper is the review of sample preparation and analytical techniques currently used to determine pesticides in nutraceutical products; special attention will be paid on GC and LC coupled to classical and advanced detectors, such as MS.

2. Sample extraction

According to the revised bibliography, most of the studies are focused on medicinal plants (raw material), and only in some cases on dietary supplements (final product) that come from medicinal plants. In other particular cases, fish oils were also evaluated. All these products are considered nutraceuticals bearing in mind the aforementioned definitions. Therefore, the different extraction techniques applied for these particular products will be discussed in this section. Table 1 shows a summary of the main procedures.

2.1. Medicinal plants and herbal infusions

For this type of matrix, a variety of sample treatments can be used, such as QuEChERS [17,19–29], PLE [30], Soxhlet extraction [31–35], solid–liquid extraction (SLE) [36–38], MSPD [15,39], solid phase micro-extraction (SPME) [40,41] and solid-phase extraction (SPE) [16,42], as it can be observed in Table 1.

The QuEChERS method has been used for the extraction of a large variety of medicinal plants, and it is currently the preferred option for the determination of pesticides in plant-based products. This extraction procedure was originally developed by Anastassiades et al. in 2003 [43] for the analysis of pesticides in vegetables and fruits. Nowadays, it represents a simple, rapid, effective and inexpensive methodology to extract pesticide residues from different matrices. The QuEChERS method basically consists of an extraction with acetonitrile followed by a clean-up stage using the dispersive solid-phase extraction (dSPE) with primary-secondary amine (PSA) [44]. A high number of studies reported the application of this methodology using either the original QuEChERS or the modified versions. The subsequent study by Lehotay et al. [45] described a modification of the original method using a buffered solvent (also known as the American QuEChERS version): this method uses acetonitrile with 1% of acetic acid (v/v), magnesium sulfate and sodium acetate to determine multiple pesticides, obtaining recoveries between 68% and 96% and precision, expressed as relative standard deviation (RSD), between 15% and 33%. It was introduced to improve the recoveries of more acidic compounds (e.g. chlortalonil, captan). Chang [20,21] also utilized

Table 1
Sample treatment for pesticide determination on nutraceutical products.[a]

Analytes	Matrix	Extraction	Clean-up	Recovery (%)	Precision[b] (%)	Ref.
46 OCPs	Dietary supplement (Dandelion)	QuEChERS (acetonitrile, magnesium sulfate, sodium chloride, trisodium citrate dehydrate disodium hydrogen citrate sesquihydrate)	dSPE (magnesium sulfate, GBC, PSA)	46–131	N.A.	[44]
135 OCPs/OPPs	Green and black tea	QuEChERS (acetonitrile, magnesium sulfate, sodium chloride)	SPE (magnesium sulfate, C_{18}, PSA)	70–120	< 20	[19]
44 OCPs/OPPs	229 herbal crude materials	QuEChERS (acetonitrile, magnesium sulfate, sodium chloride)	–	53–113	10–14	[20,21]
236 OCPs/OPPs	Ginseng, saw palmetto, gingko biloba	QuEChERS (acetonitrile, magnesium sulfate, sodium chloride)	SPE (magnesium sulfate, C_{18}, PSA)	91–102	< 20	[22]
162 OCPs/OPPs	Medicinal plants	QuEChERS (acetonitrile, magnesium sulfate, sodium chloride)	SPE (PSA, magnesium sulfate)	N.A.	N.A.	[23]
41 OCPs/OPPs	Dietary supplement (*Scutellaria baicalensis* + *Acacia catechu*)	QuEChERS (acetonitrile, magnesium sulfate, sodium chloride)	SPE (neutral alumina)	80–120	5	[46]
116 OCPs/OPPs	Medicinal plants	QuEChERS (acetonitrile, magnesium sulfate, sodium chloride)	SPE (PSA, GBC, magnesium sulfate)	70–120	< 15	[24]
18 OCPs/OPPs	Ginseng	QuEChERS (acetonitrile, sodium chloride)	SPE (Florisil, sodium sulfate)	72–117	< 5	[25]
23 OCPs/OPPs Pyrethroids	Medicinal plants	QuEChERS (acetonitrile, sodium chloride, magnesium sulfate)	SPE (PSA, GBC, magnesium sulfate)	78–119	3–8	[26]
3 OPPs	*Cinnamomi cortex*	QuEChERS (acetonitrile, anhydrous sodium sulfate, sodium chloride)	SPE: (1) activated charcoal mini-column acetonitrile-toluene; (2) Florisil mini-column acetone-hexane	88–91	4–8	[27]
168 OCPs/OPPs	Ginseng root	QuEChERS (acetonitrile, magnesium sulfate, sodium chloride)	SPE: (1) C_{18} sorbent and magnesium sulfate; (2) PSA, GBC and magnesium sulfate	70–120	< 10	[17,28]
39 OCPs/OPPs Neonicotinoids	*Camellia sinensis*	QuEChERS (acetonitrile, acetic acid)	dSPE (magnesium sulfate, PSA, GBC)	70–120	< 10	[29]
100 OCPs/OPPs	Dietary supplements (ginseng, dandelion)	QuEChERS (acetonitrile, magnesium sulfate, sodium chloride)	dSPE (magnesium sulfate, PSA, C_{18} sorbent, GBC)	N.A.	N.A.	[18]
52 OCPs/OPPs	Medicinal plants	PLE (diatomaceous earth, cyclohexane, ethyl acetate)	GPC (cyclohexane, ethyl acetate)	62–127	2–27	[30]
6 OCPs	Ginseng root	Soxhlet procedure (sodium sulfate, dichloromethane and isooctane)	Sulfuric acid and acid washed cooper powder were used to remove interferences	N.A.	0.6–1.4	[31]
28 OCPs/OPPs Pyrethroids	Medicinal plants	Soxhlet procedure (hexane, acetonitrile)	SPE (Florisil, sodium sulfate)	90–93	N.A.	[32–35]
170 OCPs/OPPs	Ginseng root	SLE (ethyl acetate, cyclohexane)	GPC (ethyl acetate, cyclohexane) + SPE (GBC, PSA)	81–95	3–8	[36]
20 OCPs	Medicinal plants	SLE (ethyl acetate)	GPC (ethyl acetate)	N.A.	N.A.	[37]
8 OCPs	Plant extract (raspberry, maize, cranberry, rose, horsetail)	SLE (acetone, isooctane)	–	> 86	N.A.	[47]
> 600 Multi-class pesticides	Tea	SLE (acetonitrile, hexane)	SPE (acetonitrile, toluene)	60–120	< 20	[38]
10 OCPs	Fish oil	SLE (hexane, TCN)	SPE (Na_2SO_4, activated silica modified with sulfuric acid)	81–118	< 4	[48]
4 OCPs	Medicinal plants	MSPD (acetone, dichloromethane, Na_2SO_4)	SPE (Florisil, Na_2SO_4)	88–98	5–10	[15]
6 OCPs	*Cordia salicifolia*	MSPD (propylene column with silanized glass wool, Na_2SO_4, C_{18} sorbent). Cyclohexane and dichloromethane for elute	–	68–130	6–26	[39]
9 OCPs	Herbal infusions	SPME (NiTi–ZrO_2-PDMS activated with NaOH and HCl)	–	77–120	3–10	[40]
9 OCPs	Herbal infusions (*Mikania laevigata, Maytenus ilicifolia*)	SPME (polydimethylsiloxane fibers)	–	90–108	< 17	[41]
20 OCPs/OPPs	*Radix paeoniae*	SPE (acetone/dichloromethane, silica gel column prewashed with petroleum ether)	–	74–115	< 10	[42]
7 Pyrethroids	*Melissa officinalis*	SPE (C_{18}, acetonitrile, acetic acid)	SPE (same conditions)	36–57	9–18	[16]
13 OCPs	Fish oil	Diluted with dichlorometane + GPC (dichlorometane)	Two SPE: (i) silica gel modified with sulfuric acid, sodium sulfate; (ii) Florisil, sodium sulfate	30–102	N.A.	[49]
30 OCPs	Fish oil	Diluted with ethyl acetate, cyclohexane + GPC (ethyl acetate, cyclohexane)	GPC (same conditions)	64–122	1–25	[50]

[a] Abbreviations: GBC: Graphitic black carbon; GPC: Gel permeation chromatography; MSPD: Matrix solid-phase dispersion; N.A.: Data not available; NiTi–ZrO_2-PDMS: poly(dimethylsiloxane) fiber sol–gel coated onto NiTi alloy electrodeposited with zirconium oxide; OCPs: organochlorine pesticides; OPPs: organophosphorus pesticides; PLE: Pressurized liquid extraction; PSA: Primary secondary amine; RSD: Relative standard deviation; SLE: Solid–liquid extraction; SPE: Solid phase extraction; SPME: Solid phase micro-extraction; TCN: Tetrachloronaphtalene.

[b] Expressed as relative standard deviation (RSD).

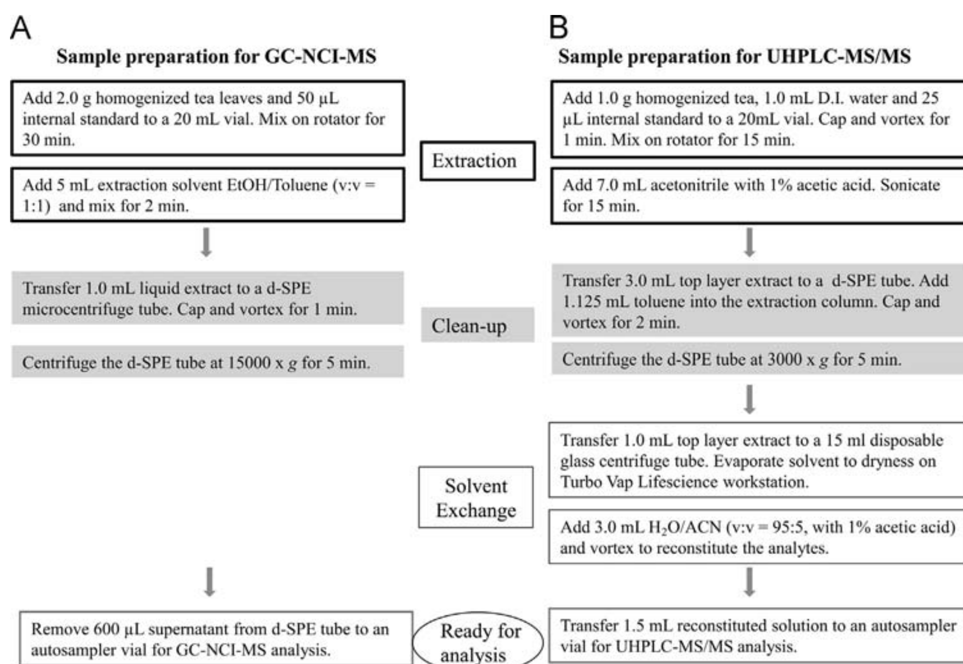

Fig. 1. Flowchart of sample preparation for AR analysis by GC–NCI-MS (A) and UHPLC–MS/MS (B).
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this approach to determine 44 pesticides, including organochlorine (OCPs) and organophosporus (OPPs), in 229 herbal crude materials. The recoveries obtained by this study ranged from 53% to 113% and precision, expressed as RSD, was lower than 15%. Harris et al. [23] also applied this procedure to determine 162 pesticides (OCPs and OPPs) on different medicinal plants (recoveries not reported).

The addition of a clean-up step by the dSPE has normally been useful to obtain better recovery and precision percentages (Table 1). Chen et al. [22] used the same QuEChERS procedure described by Chang, but with an additional clean-up step by dSPE, consisting of a mixture of octadecyl (C_{18}), PSA and anhydrous magnesium sulfate in order to determine 236 pesticides (OCPs and OPPs) on ginseng, saw palmetto and gingko biloba, obtaining better recovery values (91–102%) and similar precision ($<20\%$). The use of other clean-up sorbents can help to improve the performance of the QuEChERS method. Thus, Zhang et al. [29] applied a dSPE clean-up step with PSA, magnesium sulfate and graphitized black carbon (GBC) in order to determine 39 OCPs on the medicinal plant *Camellia Sinensis*, obtaining recoveries between 70% and 120% and RSD values below 10%. This study also described the addition of a solvent exchange step for the analysis by LC–MS (Fig. 1) since peak shape can be affected due to the injection of acetonitrile extracts. The injection of extracts with compositions close to the initial composition of the gradient improves peak shape for early eluting compounds [29]. Chen et al. [24] applied the same procedure in order to determine 116 pesticides (OCPs and OPPs) on different medicinal plants (including *Herba Lophatheri*, *Dogwood*, *Radix Ginseng*, *Semenpersicae* and *Flos Loncerae*), obtaining recoveries between 70% and 120% and RSD values below 15%. This study indicated that different edible parts of the plants could be chosen to make each dose in traditional chinese medicine formulation. Moreover, the plants used in these formulations normally vary in chemical constituents. Furthermore, dSPE and SPE performances were also compared in terms of recovery, obtaining better results when dSPE was used [24]. Ronghua et al. [26] also used this kind of procedure for the extraction of 23 OCPs, OPPs and pyrethroids from different medicinal plants (*Radix Gentianae*, *Cortex Phellodendri* and *Bulbus Fritillariae Ussuriensis*) obtaining suitable recoveries (78–119%) and precision (3–8%) values. Park et al. [25] modified the dSPE clean-up step, using Florisil and sodium sulfate to determine 18 pesticides (OCPs and OPPs) on ginseng, obtaining similar recoveries (74–115%) but improving the RSD values (below 5%). In this sense, Gang et al. [30] explained that in order to achieve effective one-step sample preparation, it is crucial to choose a suitable sorbent for pesticide residue analysis. For example, PSA can effectively remove saccharides, polar organic acids and lipids from food samples, whereas Florisil can preferentially absorb polar and low-fat components [30].

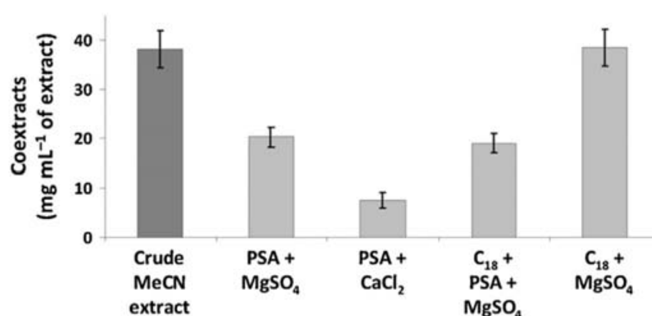

Fig. 2. Amount of matrix co-extracts determined gravimetrically in green tea extracts after purification employing various sorbents/desiccants for dispersive-SPE.
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Cajka et al. [19] evaluated different clean-up sorbents to determine 135 pesticides (OCPs and OPPs) in green and black tea, obtaining recoveries between 70% and 120% and RSD values lower than 20%. They explained that tea represents a complex matrix with high amounts of polyphenols, methyl xanthines (e.g. caffeine), purines and phenolic acids, which might be co-isolated to some extent during sample extraction. This co-extracted material can affect negatively the method performance in different ways. The non-volatile matrix components deposited at the inlet part of the system are typically responsible for the formation of new active sites. On the other hand, the abundant (semi)-volatile matrix components can cause distortion of peak shapes and

shifting of the retention time of target analytes. In addition, interfering ions (formed during the ionization of matrix co-extracts) with masses close to those of target residue and, even ion suppression, are the main factors that limit the achievement of low detection limits and reliable analyte identification. Therefore, clean-up steps were necessary, as it can be observed in Fig. 2, which shows how these matrix co-extracts are reduced by the use of different dSPE and liquid–liquid extraction (LLE) clean-up steps [19].

The use of more than one clean-up step in the QuEChERS method can improve the validation results. Thus, Tagami et al. [27] applied two clean-up steps by SPE using first an active charcoal mini-column, and afterwards, a Florisil mini-column in order to determine 3 OPPs on the medicinal plant, *Cinnamomi cortex*. The recoveries obtained by this procedure ranged from 88% to 91% and the RSD values were 4–8% [27]. Wong et al. [17,28] used two dSPE clean-up steps, one with a C_{18} sorbent+magnesium sulfate and the other one with PSA+GBC+magnesium sulfate, in order to

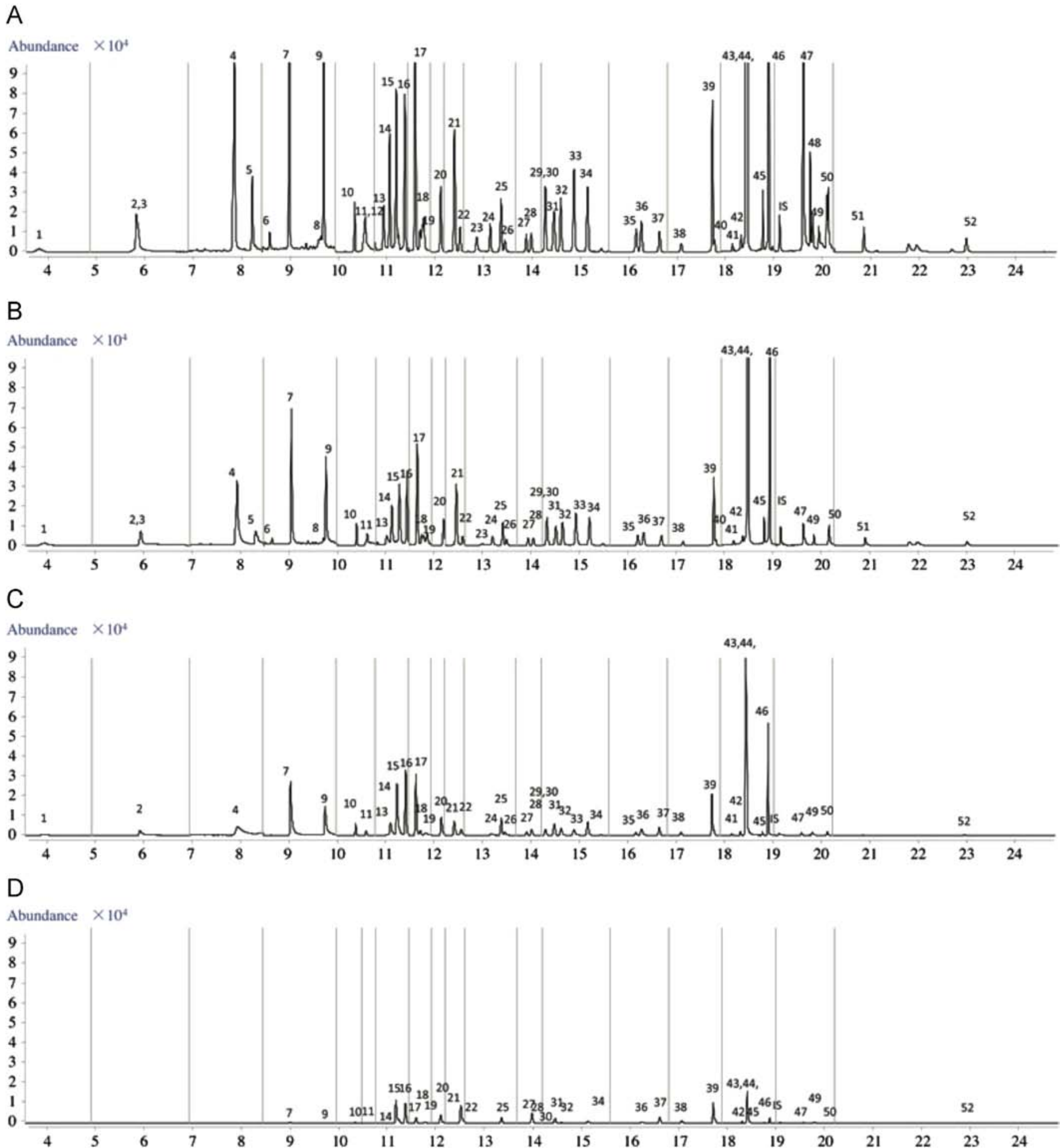

Fig. 3. The typical total ion chromatograms of 52 pesticide residues were acquired by multiple reactions monitoring mode. Spiked samples were performed by different methods including SPLE (A), PLE-GPC (B), SPE (C), and SPME (D).
Reprinted from [30], copyright 2012, with permission of John Wiley and Sons.

determine 168 OCPs and OPPs on ginseng root. The recoveries obtained by this procedure range from 70% to 120%, with RSD values lower than 10% [17,28].

PLE has also been used to determine pesticide residues in medicinal plants. Comparing with other extraction techniques such as Soxhlet extraction, PLE can simplify the extraction step and significantly reduce the extraction time and solvent consumption [30]. In the revised literature, when PLE was used, a clean-up step was necessary. Gang et al. [30] applied PLE using cyclohexane and ethyl acetate to extract 52 OCPs and OPPs from different medicinal plants. The clean-up step applied in this case was gel permeation chromatography (GPC) using a mixture of cyclohexane and ethyl acetate (1:1 v/v) as mobile phase [30]. The recoveries obtained by this procedure ranged from 62% to 127% and precision was between 2% and 27% [30]. They also evaluated different sample treatments such as PLE, SPE, SPME, observing that better results were obtained when PLE was used, as it can be observed in Fig. 3.

Soxhlet procedures were also applied for sample treatment. Chan et al. [31] applied a Soxhlet apparatus and sodium sulfate, dichloromethane and isooctane to extract 6 OCPs from the ginseng root. To remove interferences, sulfuric acid and acid washed copper powder were used and the precision obtained by this procedure was between 0.6% and 1.4% [31]. Different works performed by Kakkar et al. [32–34] used the same Soxhlet apparatus but with *n*-hexane and acetonitrile to extract 28 OCPs, OPPs and pyrethroids from different medicinal plants. A dSPE with Florisil and sodium sulfate was used as the clean-up step, obtaining recoveries between 90% and 93%. Mruthyumjaya et al. [35] also used the same procedure for the determination of pesticides in *Emblica officinalis*, *Terminalia chebula*, *Terminalia belerica* and *Withania somnifera*, obtaining similar recoveries.

SLE has been used for specific nutraceutical products and the use of a further clean-up step is recommendable to obtain suitable extracts. Hayward et al. [36] determined 170 OCPs and OPPs from the ginseng root using SLE with ethyl acetate and cyclohexane followed by two clean-up steps: one using GPC (ethyl acetate: cyclohexane, 70:30, v/v) and the other one by dSPE (GBC+PSA). The recoveries obtained by this procedure ranged from 81% to 95%, and precision values were lower than 8% [36]. Leung et al. [37] also applied this procedure to determine 20 OCPs in different medicinal plants but recoveries were not reported. Because this type of sample treatment is less specific, more coextracted material is found in the raw extracts and further clean-up steps are required. Besides, GPC is employed for the clean-up since this technique efficiently removes matrix material; however, it is highly time-consuming and requires increased solvent volume. Fang et al. [38] determined more than 600 multi-class pesticides using acetonitrile and *n*-hexane in the SLE procedure. The recoveries obtained by this method ranged from 60% to 120% with precision below 20%.

MSPD can be carried out simultaneously with sample homogenization, extraction, and clean-up and it only requires a small sample size and small amounts of solvent. It avoids some drawbacks generally associated with other techniques, such as the use of large solvent volumes, the occurrence of troublesome emulsions and the analysis time [39], but the number of pesticides analyzed by this technique is much lower and the optimization of the extraction conditions is more complex. Viana et al. [39] determined 6 OCPs on *Cordia salicifolia* applying MSPD, using a propylene column with silanized glass wool and sodium sulfate and C_{18} as a sorbent. Cyclohexane and dichloromethane were used to elute the compounds and the recoveries obtained by this procedure ranged from 68% to 130%, and precision values were between 6% and 26% (RSD) [39]. Abhilash et al. [15] used a MSPD extraction procedure to determine 4 OCPs on different medicinal plants with an acetone:dichloromethane (3:1, v/v) suspension and anhydrous sodium sulfate using Florisil as sorbent. The recovery values and precision using this sorbent were improved, achieving recoveries from 88% to 98% and precision lower than 10%.

The SPME technique is another alternative that has been utilized in nutraceutical analysis; it integrates extraction, preconcentration and clean-up in a single step, avoiding the use of organic solvents [40]. It can be suitable for the analysis of liquid matrices, such as herbal infusions. However, several well-known operation disadvantages hinder its applicability, such as the fragility of the support, which traditionally is a fused silica rod. Other materials have been used to overcome this drawback. Thus, Budziak et al. [40] applied SPME to determine 9 OCPs on herbal infusions using a poly(dimethylsiloxane) (PDMS) fiber sol–gel coated onto a NiTi alloy electrodeposited with zirconium oxide (NiTi–ZrO_2-PDMS) submerged in NaOH and HCl solutions. The recoveries obtained by this experiment were between 77% and 120% with precision values lower than 10% [40]. Furthermore, Rodrigues et al. [41] applied SPME in order to determine 9 OCPs on two herbal infusions, *Mikania laevigata* and *Maytenus ilicifolia*, using PDMS fibers with a SPME holder apparatus. They explain that bearing in mind OCPs are non-polar compounds, the PDMS fiber coating was selected for the SPME studies. The recoveries obtained by this procedure were between 90% and 108% and precision lower than 17% [41].

SPE is one of the most common extraction techniques in pesticide residues [14] and it has been applied for the clean-up of pesticide residues in medicinal plants after SLE. For instance, Qian et al. [42] used a silica gel column for the clean-up during the extraction of 20 pesticides (OCPs and OPPs) from *Radix paeoniae*, obtaining recoveries between 74% and 115% with precision below 10%. In this case, the elution solvent was a mixture of petroleum ether/acetone (40/60, v/v). Tuzimski [16] used C18 cartridges during the determination of 7 pyrethroids in *Melissa officinalis*, obtaining recoveries between 36% and 57% with precision between 9% and 18%. The elution solvent was tetrahydrofuran.

Comparing the discussed methodologies, the QuEChERS approach can be used for a high variety of matrices with adequate recoveries and precision values. PLE and SPME also offer similar recoveries but require special apparatus. Moreover, the number of reported pesticides analyzed is higher with the QuEChERS procedure (236) than with other extraction techniques. SLE and MSPD are very specific and would depend on the matrix that is being analyzed. Finally, SPE does not offer adequate recoveries although the precision is similar to the other methods. In general, the use of clean-up steps after the extraction procedure improved the recoveries and precision values of the applied extraction techniques.

2.2. Dietary supplements and other final products

Considering the revised literature, two types of processed materials or final products were found: dietary supplements that were obtained from medicinal plants (capsules, tablets) and fish oil.

For dietary supplements, the sample treatment is similar to those described for the raw material, applying QuEChERS [18,44,46] and SLE [47] procedures. Kowalski et al., [44] used the original QuEChERS method to determine 46 OCPs from a dietary supplement obtained from the dandelion root. A clean-up step based on dSPE was also applied, using magnesium sulfate, GBC and PSA. This mixture was effective to remove several common matrix compounds like fatty acids, sugars and other co-extractives [44]. The recoveries obtained by this procedure ranged from 46% to 131% [44]. Mastovska et al. [18] also used the same QuEChERS procedure followed by a dSPE with magnesium sulfate, PSA, C_{18} sorbent and GBC in order to determine 100 OCPs and OPPs in two dietary supplements from the ginseng and dandelion root but the

recovery values were not reported. Lee et al. [46] found that the best clean up sorbent in the dSPE is neutral alumina compared to PSA, C_{18} or Florisil. In this work, 41 pesticides (OCPs and OPPs) were determined from a dietary supplement that contained a mixture of *Scutellaria baicalensis* and *Acacia catechu*. The recoveries obtained by this procedure ranged from 80% to 120% with precision values lower than 6% [46].

SLE was applied by Tusa et al. [47] to determine 8 OCPs on different plant extracts (raspberry, maize, cranberry, rose and horsetail). This procedure used acetone and isooctane to extract the compounds and the recoveries obtained were higher than 86% [47]. Despite SLE provides similar results as the QuEChERS procedure, the latter is suitable for the extraction of a wider range of compounds.

For the extraction of pesticides from fish oil, SLE proved to be more effective [48–50], although the use of clean-up steps was necessary. Since many pesticides are lipophilic and tend to accumulate through the aquatic food chain, they can be found in the lipid compartments of fish and also in the products extracted thereby. Thus, it has been reported that fish oils contain relatively high levels of OCPs [48]. Berzas et al. [48] determined 10 OCPs applying SLE with *n*-hexane and tetrachloronaphtalene, followed by a SPE using activated silica modified with sulfuric acid and sodium sulfate. The recoveries obtained by this procedure ranged from 81% to 118%, and precision values were lower than 4% [48]. Rawn et al. [49] determined 13 OCPs by dissolving the matrix in dichloromethane and then applying a GPC using the same solvent as the mobile phase. After that, two SPEs were applied in order to concentrate the samples: one with silica gel modified with sulfuric acid and sodium sulfate, and another with Florisil and sodium sulfate, obtaining recoveries between 30% and 102% [49]. Hoh et al. [50] diluted the fish oil with a mixture of cyclohexane and ethyl acetate (1:1, v/v) and then, two stages of GPC were applied using this mixture as the mobile phase to determine 30 OCPs. The GPC steps were applied in order to eliminate interferences and to obtain a more sensitive analysis [50]. The recoveries obtained were 64–122%, and precision values ranged from 1% to 25% [50]. Nevertheless, the procedure was highly time-consuming.

In conclusion, for processed materials (tablets, capsules) the selected sample treatment is still the QuEChERS method, although in the specific case of fish oil, SLE proved to be more effective and a thorough clean up step is normally applied to remove lipidic material.

3. Chromatographic separation

3.1. Separation by GC

A variety of columns can be used in GC to determine pesticides. One of the most used is the typical fused silica column with stationary phase composed of 95% dimethyl-5% diphenylpolysiloxane (e.g. HP-5 ms, VF-5 ms, DB-5 ms, OV-5, etc.), and with dimensions such as 30 m × 0.25 mm × 0.25 μm film thickness [17,20,21,26,28,36,39–42,46–48]. This column was used to separate from 6 OCPs [39], (9 OCPs) [40] to 170 pesticides (OCPs and OPPs) [36]. Different dimensions have been additionally employed. Rawn et al. [49] used a 60-m column to separate 13 OCPs from fish oil; Gang et al. [30] used a shorter column with a lower internal diameter and film thickness (20 m × 0.18 mm × 0.18 μm) to separate 52 pesticides (OCPs and OPPs) from different Chinese medicinal plants; and Mastovska et al. [18] also utilized a short column (15 m × 0.25 mm × 0.25 μm) to separate 100 pesticides (OCPs and OPPs) from dietary supplements (dandelion and ginseng).

Other columns which have been used for pesticide analysis are HP-1707MS (more polar than typical DB-5 ms; 6 OCPs) [31]; Rxi-5Sil MS (low polarity phase; 1,4-bis(dimethylsiloxy)phenylene dimethylpolysiloxane; 46 OCPs) [44]; DB-1701 (more polar than DB-5 ms; 14% cyanopropylphenyl-86% dimethyl polysiloxane; 3 OPPs) [27]; and Elite 35 (midpolarity phase, 35% diphenyl-65% dimethylpolysiloxane; 4 OCPs) [15]. These specific columns have been used to separate a low number of pesticides comparing to the typically fused silica column composed of 95% dimethyl-5% diphenylpolysiloxane. Only a 14% cyanopropylphenyl-86% dimethyl polysiloxane column was used to separate 490 multi-class pesticides from different types of tea [38].

The use of coupled GC columns is also reported. Cajka et al. [19] described the use of a column system with a 95% dimethyl-5% diphenylpolysiloxane ultra inert column (15 m × 0.25 mm × 0.25 μm) coupled to a 95% dimethyl-5% diphenyl polysilarylene ultra inert column (0.50 m × 0.15 mm × 0.15 μm) in order to separate 135 pesticides (OCPs and OPPs) from green and black tea. The coupling operated with a pneumatics pressure control. The authors explained that for a suitable separation of the target compounds, coupling a 15-m narrow bore non-polar (5%-phenyl)-methylpolysiloxane GC column to a 0.5 m microbore column with the same stationary phase (required for backflush operation) was recommended. A total cycle run time of 29 min was used, including a pre-injection step of 1 min, a 21 min GC run, a 2 min post-run, post-column backflush and a 3 min cool-down of the GC oven and injection port [19].

Another interesting application of GC is the one denominated two-dimensional gas chromatography (GC × GC). Basically, this technique consists of two GC separations on fundamentally different separation mechanisms in order to create the so-called orthogonal separation conditions. The first separation occurs on a column containing a non-polar stationary phase, and the second separation in a column of a polar or shape-selective nature. The fast separation in the second dimension results in very narrow peaks [51]. Hoh et al. [50] applied this procedure with a Restek Rtx-5Sil-MS (1,4-bis(dimethylsiloxy)phenylene dimethyl polysiloxane, 15 m × 0.25 mm × 0.25 μm) as the first column, and a DB-17 MS (50% phenyl-50% dimethyl polysiloxane, 2 m × 0.18 mm × 0.18 μm) as the second column in order to separate 30 OCPs from fish oil. The authors explained that the greater selectivity of GC × GC affords cleaner mass spectra in complex extracts (fewer interferences), which helps in quantitation and qualitative identifications of targeted and nontargeted chemicals, providing greater separation and sensitivity than GC alone [50].

Regarding type of injection, majority of the studies used splitless mode. Normally, splitless injection is the preferred technique for trace analysis since it permits to achieve a high sensitivity according to the low concentrations found for pesticides in comparison to the matrix components. Nevertheless, some particular studies applied split injection, such as Hayward et al. [36], who used a split ratio of 10:1 (170 pesticides), Park et al. [25], who used a split ratio of 30:1 (18 pesticides) or Chang [20,21] (split ratio of 50:1). Hoh et al. [50] employed two different injection modes: split injection (split ratio, 50:1; 44 pesticides) and pulsed splitless injection (pulsed pressure, 35 psi and 2 min; 30 pesticides). The use of split injection for the analysis of pesticides is not common because this mode is more appropriate when the concentration of the analytes in the sample is high (e.g. mg kg^{-1}) but pulsed injection is an interesting alternative to normal splitless injection since it can help in preventing thermal degradation of some compounds by reducing the residence time in the injector and the interaction with active sites of the inlet. Alternatively, cold splitless injection and solvent vent programmed temperature vaporization (PTV) have also been applied [19].

Basically for GC, a variety of pesticides are separated with the fused silica column with stationary phase composed of 95% dimethyl-5% diphenyl polysiloxane, as discussed before, and it

Table 2
Analytical methods used for pesticides determination and quantification in nutraceutical products[a].

Analytes	Matrix	Technique	Separation remarks	Detection remarks	LOD	LOQ	Ref.
4 OCPs	Medicinal plants	GC-ECD	Elite-35 (30 m × 0.32 mm × 0.5 μm) Injection: Splitless	–	2–6 μg kg^{-1}	N.A.	[15]
9 OCPs	Herbal infusions	GC-ECD	OV-5 (30 m × 0.25 mm × 0.25 μm) Injection: Splitless	–	0.001–0.01 μg L^{-1}	0.004–0.05 μg L^{-1}	[40]
20 OCPs	Medicinal plants	GC-ECD	N.A.	–	N.A.	20 μg kg^{-1}	[37]
28 OCPs/OPPs Pyrethroids	Medicinal plants	GC-ECD	N.A.	–	0.1–0.5 μg kg^{-1}	N.A.	[32–35]
18 OCPs/OPPs	Ginseng	GC-ECD	HP-5 (30 m × 0.25 mm × 0.25 μm) Injection: Split (30:1)	–	3–50 μg kg^{-1}	10–200 μg kg^{-1}	[25]
20 OCPs/OPPs	*Radix paeoniae*	GC-ECD	HP-5 (30 m × 0.32 mm × 0.25 μm)	–	0.1–2.5 μg kg^{-1}	0.4–7.3 μg kg^{-1}	[42]
13 OCPs/OPPs	*Passiflora* L	GC-ECD/FPD	Mega 13 (50 × 0.25 mm, 0.15 μm)	–	1.0–14.3 μg L^{-1}	NA	[53]
108 OPPs	Gingseng root	GC-FPD	DB-5 (30 m × 0.55 mm × 1.5 μm) Injection: Splitless	–	0.025–0.05 mg kg^{-1}	NA	[54]
18 OPPs	Chinese medicine wine	GC-FPD	DB-5 (30 m × 0.25 mm i.d. with 0.25 μm) Injection: Splitless	–	1–15 μg L^{-1}	4–50 1–15 μg L^{-1}	[55]
6 OCPs	Ginseng root	GC-Q-MS	HP-1707 MS (30 m × 0.25 mm × 0.25 μm) Injection: Splitless	SIM	0.2–0.5 μg kg^{-1}	0.7–2.0 μg kg^{-1}	[31]
44 OCPs/OPPs	229 herbal crude materials	GC-Q-MS	DB-5 MS (30 m × 0.25 mm × 0.25 μm) Injection: Split (50:1)	Full scan	1–40 μg kg^{-1}	N.A.	[20,21]
41 OCPs/OPPs	Dietary supplement (*Scutellaria baicalensis*+*Acacia catechu*)	GC-Q-MS	DB-5 MS (30 m × 0.25 mm × 0.25 μm) Injection: Splitless	SIM	20–180 μg kg^{-1}	24–235 μg kg^{-1}	[46]
9 OCPs	Herbal infusions (*Mikania laevigata, Maytenus ilicifolia*)	GC-Q-MS	HP-5 MS (30 m × 0.25 mm × 0.25 μm) Injection: Splitless	SIM	0.060–4 μg kg^{-1}	0.2–12 μg kg^{-1}	[41]
23 OCPs/OPPs Pyrethroids	Medicinal plants	GC-Q-MS	HP-5 MS (30 m × 0.25 mm × 0.25 μm) Injection: Splitless	SIM	0.05–3 μg kg^{-1}	0.3–10.1 μg kg^{-1}	[26]
3 OPPs	*Cinnamomi cortex*	GC-Q-MS	DB-1701 (30 m × 0.25 mm × 0.25 μm) Injection: Splitless	SIM	$<$20 μg kg^{-1}	N.A.	[27]
6 OCPs	*Cordia salicifolia*	GC-Q-MS	DB-5 MS (30 m × 0.25 mm × 0.25 μm) Injection: Splitless	SIM	100–150 μg kg^{-1}	150–250 μg kg^{-1}	[39]
168 OCPs/OPPs	Ginseng root	GC-Q-MS	HP-5 MS (30 m × 0.25 mm × 0.25 μm). Injection: Splitless	SIM	25 μg kg^{-1}	50–5000 μg kg^{-1}	[17]
490 Multi-class pesticides	Variety of teas	GC-Q-MS	DB-1701 (30 m × 0.25 mm × 0.25 μm) Injection: Splitless	SIM	1–500 μg kg^{-1}	2–1000 μg kg^{-1}	[38]
13 OCPs	Fish oil	GC-IT	DB-5 (60 m × 0.25 mm × 0.25 μm) Injection: Splitless	–	0.002–0.009 μg kg^{-1}	N.A.	[49]
8 OCPs	Plants extract (raspberry, maize, cranberry, rose, horsetail)	GC-IT	DB-5 MS (30 m × 0.25 mm × 0.5 μm) Injection: Splitless	–	N.A.	2–41 μg kg^{-1}	[47]
135 OCPs/OPPs	Green and Black Tea	GC-QqQ–MS/MS	HP-5ms (15 m × 0.25 mm × 0.25 μm) coupled to DB-5ms (0.50 m × 0.15 mm × 0.15) Injection: Cold splitless and solvent vent PTV	SRM	0.2–2 μg L^{-1}	N.A.	[19]
52 OCPs/OPPs	Medicinal plants	GC-QqQ–MS/MS	HP-5 MS (20 m × 0.18 mm × 0.18 μm) Injection: Splitless	SRM	0.2–5 μg kg^{-1}	1–10 μg kg^{-1}	[30]
162 OCPs	Medicinal plants	GC-QqQ–MS/MS	N.A.	–	10–50 μg kg^{-1}	10–50 μg kg^{-1}	[23]
168 OCPs/OPPs	Ginseng powder	GC-QqQ–MS/MS	VF-5 (30 m × 0.25 mm × 0.25 μm) Injection: Splitless	SRM	N.A.	1–20 μg kg^{-1}	[28]
100 OCPs/OPPs	Dietary supplements (ginseng, dandelion)	GC-QqQ–MS/MS	HP-5 MS (15 m × 0.25 mm × 0.25 μm)	SRM	N.A.	N.A.	[18]
46 OCPs	Dietary supplement (Dandelion)	GC-QqTOF–MS	Rxi-5Sil (30 m × 0.25 mm × 0.25 μm) Injection: Splitless	Full scan	N.A.	N.A.	[44]
10 OCPs	Fish oil	GC-QqTOF–MS	HP-5 MS (30 m × 0.25 mm × 0.25 μm) Injection: Splitless	SIM	0.1–1.26 μg L^{-1}	0.33–4.20 μg L^{-1}	[48]
170 OCPs/OPPs	Ginseng root	GC-QqTOF–MS	HP-5 MS (30 m × 0.25 × 0.25) Injection: Split (10:1)	SIM	N.A.	$<$3 μg L^{-1}	[36]
30 OCPs	Fish oil	GC-QqTOF–MS.	Rtx-5Sil (15 m, 0.25 mm × 0.25 μm) Injection: Split (50:1)	Full scan	0.0004–0.0006 μg kg^{-1}	N.A.	[50]
7 Pyrethroids	*Melissa officinalis*	HPLC-DAD	C_{18} (150 mm × 4.6 mm, 5 μm) A: water ; B: acetonitrile	–	30–440 μg L^{-1}	100–1350 μg L^{-1}	[16]

236 OCPs/OPPs	Ginseng, saw palmetto, gingko biloba	UHPLC-QqQ–MS/MS	C_{18} (2.1 mm × 100 mm, 1.8 μm) A: 5 mM ammonium formate in water with 0.01% formic acid; B: 0.01% formic acid in acetonitrile	ESI SRM	2–4 μg kg^{-1}	7–13 μg kg^{-1}	[22]
116 OCPs/OPPs	Medicinal plants	UHPLC-QqQ–MS/MS	C_{18} (100 mm × 2.1 mm, 1.7 μm). A: 0.1% (v/v) formic acid in water; B: acetonitrile	ESI SRM	N.A.	< 10 μg kg^{-1}	[24]
39 OCPs/OPPs Neonicotinoids	*Camellia sinensis*	UHPLC-QqQ–MS/MS	BEH phenyl analytical column (2.1 × 50 mm, 1.7 μm) A: 5 mM ammonium formate in deionized water; B: 5 mM ammonium formate in methanol.	ESI SRM	0.51–20.4 μg kg^{-1}	1.53–61.2 μg kg^{-1}	[29]

[a] Abbreviations: DAD: Diode array detector; ECD: Electron capture detector; ESI: Electrospray ionization; FPD: Flame photometric detection; GC: Gas chromatography; HPLC: High performance liquid chromatography; IT: Ion trap; MS: Mass spectrometry; N.A.: Data not available; OCPs: Organochlorine pesticides; OPPs: Organophosphorus pesticides; PTV: programmed temperature vaporization; Q: Single quadrupole; QqQ: Triple quadrupole; SIM: Single ion monitoring; SRM: Selective reaction monitoring; TOF: Time of flight; UHPLC: Ultra high performance liquid chromatography.

represents a good option for the separation of pesticides from nutraceutical products.

3.2. Separation by LC

There are several works that use LC for pesticide determination on nutraceutical products. The use of LC has increased clearly in the last decade for the determination of pesticides in general. Many of the newly registered pesticides, which are replacing traditional OCPs and OPPs, are not amenable to the elevated temperatures used in the GC methods, and therefore, they are suited to LC. Consequently, LC is now commonly used to separate a variety of thermally labile or non-volatile compounds [21], but also including typical GC-amenable compounds. The correct choice of the column and mobile phase will be important to achieve a good separation of the target compounds. Although planar chromatography could be utilized [52], most of the applications have been based on the use of LC.

In relation to the type of column, the most used phase is the octadecyl carbon chain (C_{18})-bonded silica with different dimensions depending on the chromatographic system and number of pesticides to be analyzed. For typical LC systems, column size is typically in the range 150 mm to 250 mm, with particle size from 2.1 to 5 μm. This configuration is normally employed for the determination of a low number of compounds (e.g. < 50 compounds). In this sense, the use of ultra high performance liquid chromatography (UHPLC) [22,24,29] has increased significantly and it was the most used LC system in the revised literature. UHPLC employs columns with a lower particle size (< 2 μm), which increases the number of theoretical plates and, therefore, chromatographic resolution, but applying higher pressures in the LC. The studies monitoring a higher number of compounds per injection use the UHPLC systems [22,30,24].

As aforementioned, the correct choice of the mobile phase is critical, specially for applications determining a high number of compounds. Chen et al. [24] tested different mobile phases consisting of methanol, acetonitrile, and water with formic, acetic acid, ammonium acetate or ammonium formate at different concentrations. For most pesticides, wider peak shape was observed with methanol as organic solvent in the mobile phase instead of acetonitrile. Furthermore, acetonitrile is preferred for multi-residue analysis. On the other hand, the addition of formic acid provided better results than acetic acid and it was used to improve the ionization efficiency. Ammonium acetate and ammonium formate did not significantly improve the ionization efficiency than formic acid [24]. Bearing in mind this explanation, it can be seen that, for multi-residue analysis (116–236 compounds), acetonitrile with an aqueous solution of formic acid is selected as the mobile phase [22,24] and methanol with an aqueous solution of ammonium formate for the analysis of fewer pesticides (39 compounds) [29].

Bearing in mind the previous information, the use of UHPLC seems more appropriate for multi-residue analysis utilizing C_{18} columns and acetonitrile-formic acid mixtures as the mobile phase. For multi-residue analysis with a narrower scope (e.g. < 50 pesticides), HPLC can be applied using the same type of stationary phase (C_{18}) and a binary based composed of methanol and an aqueous solution of ammonium formate.

4. Detection of pesticide residues in nutraceuticals

From classical detectors, such as electron capture detector (ECD) in GC and diode array detector (DAD) in LC, to advance detectors, such as MS detectors and the different analyzers (e.g. triple quadrupole analyzer (QqQ)), there exist different choices in

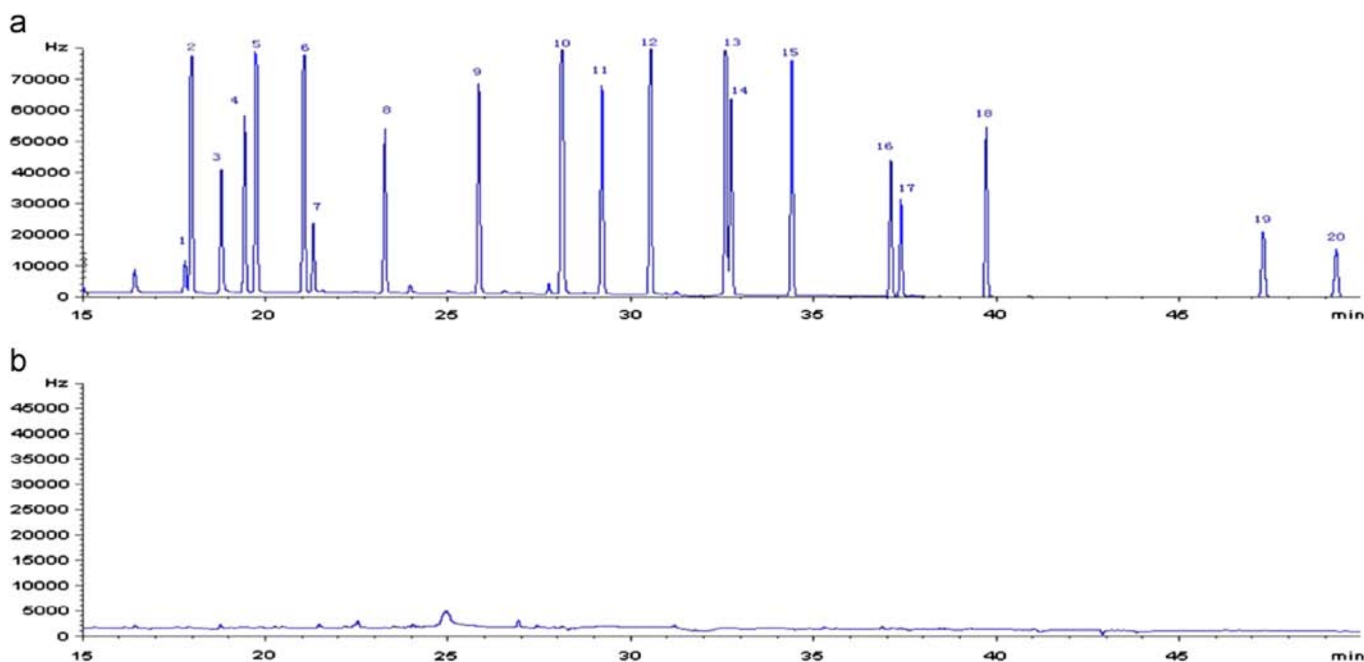

Fig. 4. Capillary GC-ECD chromatograms of 20 pesticides. (a) Mixture solution of 20 pesticides for separation of standards; (b) extract solution of Radix paeoniae Alba for the effect of metric; (1) phorate (3.0 mg/kg), (2) α-HCH (1.0 mg/kg), (3) dimethoate (1.0 mg/kg), (4) β-HCH (1.0 mg/kg), (5) γ-HCH (1.0 mg/kg), (6) δ-HCH (1.0 mg/kg), (7) chlorothalonil (1.0 mg/kg), (8) vinclozolin (1.0 mg/kg), (9) chlorpyrifos (2.0 mg/kg), (10) fipronil (2.0 mg/kg), (11) α-endosulfan (1.0 mg/kg), (12) pp′DDE (1.0 mg/kg), (13) pp′DDD (1.0 mg/kg), (14) op′DDT (1.0 mg/kg), (15) pp′DDT (1.0 mg/kg), (16) befenthrin (3.0 mg/kg), (17) fenpropathrin (2.0 mg/kg), (18) λ-cyhalothrin (3.0 mg/kg), (19) s-fenvalerate (3.0 mg/kg), (20) deltamethrin (3.0 mg/kg).
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order to accomplish the adequate detection and quantification of pesticides in nutraceutical products. In the revised bibliography, MS is widely preferred and the most used analyzers are the single quadrupole (Q) and QqQ. The use of other analyzers, such as the ion trap (IT) and the quadrupole time of flight (QqTOF) is less frequent. Table 2 shows a summary of the different detection systems used for the determination of pesticide residues in nutraceuticals.

4.1. Classical detection

For GC, ECD has been used in order to detect and quantify pesticides in nutraceutical products. Abhilash et al. [15] used ECD in order to determine 4 OCPs on different medicinal plants, obtaining limits of detection (LODs) lower than 6 μg kg^{-1}. Budziak et al. [40] also used ECD in order to determine 9 OCPs on different herbal infusions, obtaining LODs and limits of quantification (LOQs) between 0.001–0.01 μg L^{-1} and 0.004–0.05 μg L^{-1} respectively. Leung et al. [37] also used this detector to determine 20 OCPs from different medicinal plants, obtaining LOQs below 20 μg kg^{-1}.

In different works performed by Kakkar et al. [32–34], ECD was selected to determine 28 OCPs, OPPs and pyrethroids in different medicinal plants, obtaining LODs from 0.1 to 0.5 μg kg^{-1}. Mruthjumyaya et al. [35] used the same procedure, obtaining similar LODs. Park et al. [25] also used this detector to determine 18 OCPs and OPPs in ginseng, obtaining LODs and LOQs between 3–50 μg kg^{-1} and 10–200 μg kg^{-1} respectively. These authors observed that small peaks at retention times ranging from 10 to 20 min and 28 min appeared in the ECD chromatogram of the blank matrix, spiked ginseng and real samples but, after looking for each peak, it became clear that these peaks belong to the ginseng and did not correspond to any of the pesticides that were being tested. In general, good resolution of the pesticide mixtures was achieved in approximately 43 min [25]. Qian et al. [42] also used ECD in order to determine 20 OCPs and OPPs in the medicinal plant *Radix paeoniae*, obtaining LODs and LOQs from 0.1 to 2.5 μg kg^{-1} and from 0.4 to 7.3 μg kg^{-1} respectively, shown in Fig. 4 a representative chromatogram [42] where a standard solution of the 20 pesticides can be seen (Fig. 4a) and a *Radix paeoniae* blank (Fig. 4b).

Flame photometric detection (FPD) has been used for the determination of OPPs in several matrices. For instance, Zuin et al. [53] determined 7 OPPs and 6 OCPs in Brazil's medicinal plants, combining ECD and FPD. LODs lower than 14.3 μg L^{-1} were obtained in extracts of *Passiflora* L. Pesticide residues were determined by Wong et al. [54] in dried ground ginseng root, observing that LODs for most of pesticides were 0.025–0.05 μg g^{-1}. Moreover, 18 OPPs were also determined in the Chinese medicinal health wine [55], and LODs ranged from 1 to 15 μg L^{-1}.

Up to our knowledge and according to the revised bibliography, LC was only used coupled to DAD. Tuzimski [16] applied HPLC with DAD in order to determine 7 pyrethroids in the medicinal plant *Melissa officinalis*, obtaining LODs and LOQs between 30–440 μg L^{-1} and 100–1350 μg L^{-1} respectively. Tuzimski [16] explains that the analytes were identified on the basis of their retention times and by comparison between the UV spectrum of the reference compound in the library and the UV spectrum of the detected peak in the sample. Also, a match between both the spectra equal to or higher than 990 was regarded as confirmation of the identity for all the analytes, observing in the original work [16] the purity of the analytes.

4.2. Mass spectrometry

Different mass analyzers have been used in order to determine pesticides on nutraceutical products, such as Q [17,20,21,26,27,31,36,38,41,46,48], IT [47,49], QqQ [18,19,22–24,28–30] and QqTOF [36,44,48,50].

The most widely used analyzer for pesticide determination in nutraceutical products is Q, but basically on medicinal plants (raw

product). Chan et al. [31] explained that this technique offers the advantages of good sensitivity and selectivity, and it is largely employed in fit-for-purpose methods for most trace-level measurements. However, unavailability of certified reference materials for OCPs in plant matrices hindered evaluation of the overall accuracy and the traceability of the measurements [31]. From the different works studied, this analyzer has been used for the determination of 6 OCPs [39] to 490 pesticides (multi-class pesticides) [47] (Table 2) using GC. Moreover, the lower limits for this analyzer are between 0.060–500 μg kg^{-1} for the LODs and between 0.2–1000 μg kg^{-1} [38,46] for the LOQs (Table 2). Chang [20,21] also determined 44 pesticides (OCPs and OPPs) in 229 herbal crude materials employing GC-Q, and lower limits (only LODs were evaluated) ranged from 1 to 40 μg L^{-1}. Examples of chromatograms obtained by this technique are show in Fig. 5, where polar OPs, such as acephate, dichlorvos, methamidophos and menvinphos, and non-polar OPs, such as coumaphos, leptophos and temephos can be seen [17].

Some problems may appear with this technique. For instance, Lee et al. [46] explained that a challenge of his study was to overcome undesirable high recoveries for most pesticides when they were analyzed by the GC–MS. The high recoveries appear to be from a matrix-induced enhancement effect. Coextracted compounds from samples block the active sites in the GC inlet, resulting in higher signals from the analyte compared to the matrix-free solution. This matrix enhancement not only causes inaccurate quantitation, but also decreases the GC method ruggedness. Several solutions are known to minimize matrix interferences, such as the use of matrix-matched standards, isotope-labeled internal standards and analyte protectants [46]. Tagami et al. [27] had a similar problem, explaining that there are some methods to prevent matrix enhance effect like effective sample clean-up after extraction, improvement of the chromatographic system and the use of matrix-match standards. In this study, working standard solutions were diluted and the recovery rates were satisfactory, although the LODs obtained by this study were equal to or lower than 20 μg kg^{-1} [27]. Viana et al. [39] also observed the matrix enhancement effect. The problem was that the matrix components can cause variation in the detector response when pesticides are monitored. Therefore, the matrix effect was evaluated by comparing the detector response for pesticide standards prepared in dichloromethane and that obtained with standards prepared in herb extract. When standards were prepared by spiking blank herb samples with known amounts of pesticides, higher peak areas were obtained for the same pesticide concentrations. Consequently, the quantification of pesticide residues was carried out using the matrix-matched standards [39]. Wong et al. [17] agreed with these authors about the reason of the occurrence of the matrix effect. They attributed matrix induced enhancement to components in the matrix, which block active sites in the injection liner, and protect the analyte from thermal degradation. The OCPs showing matrix enhancement are usually polar or thermally labile, and these compounds prepared in toluene are more susceptible to thermal degradation than the standards prepared in the ginseng matrix. At higher pesticide concentrations, enhancement is minimized due to the presence of a larger number of analytes adsorbed onto the active sites, which compensate for any initial losses due to thermal degradation [17].

QqQ coupled to GC has been intensively used for the determination of organic pollutants. Typically, the selected reaction monitoring (SRM) mode gives the possibility of simultaneous confirmation and quantification of the target compounds with excellent selectivity and sensitivity [30] but the number of product ions screened is limited by the scan speed of the instrument [28]. Harris et al. [23] applied GC-QqQ–MS/MS to determine 162 OCPs in different medicinal plants, obtaining LODs and LOQs between 10 and 50 μg kg^{-1}. Mastovska et al. [18] also applied this technique to determine 100 pesticides (OCPs and OPPs) from different dietary supplements (ginseng and dandelion). Unfortunately, the LODs and LOQs were not reported.

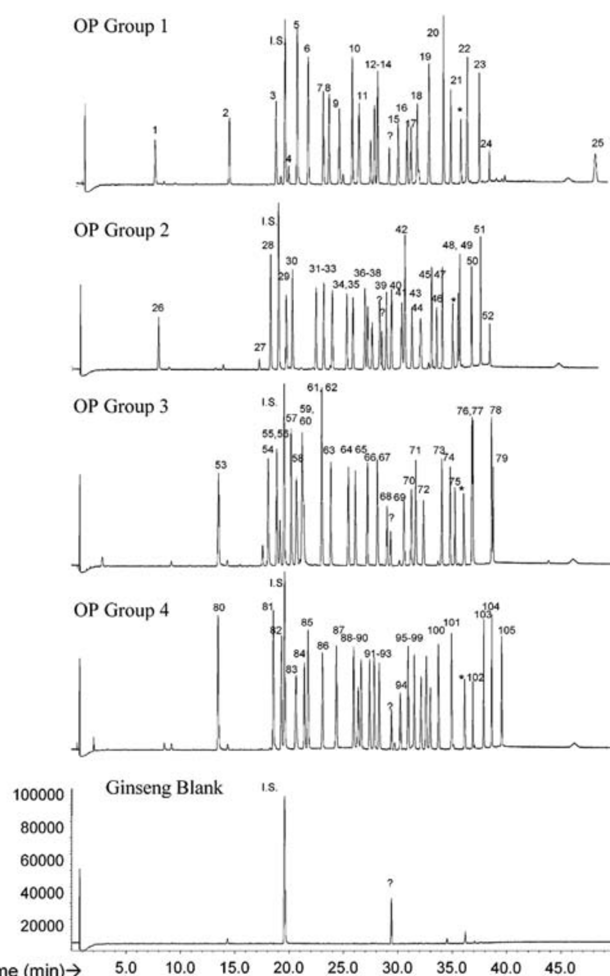

Fig. 5. Reconstructed GC–MS/SIM chromatograms from a ginseng extract fortified at 5 μg/mL.
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Repeated injections of non-volatile matrix components, which are gradually deposited in the GC inlet and/or front part of the GC column, can give rise to the successive formation of new active sites, which may be responsible for matrix induced GC signal diminishment. Gradual decrease in analyte responses associated with this phenomenon, together with distorted peak shapes (broadening and/or tailing) and shifting of the retention times towards higher values, negatively impact the ruggedness of the analytical method [19].

LC–MS/MS is now commonly used to detect a variety of thermally labile compounds because of its stability, sensitivity, and selectivity. Fig. 6 shows a total ion chromatogram obtained by the LC-QqQ–MS/MS, indicating that optimal separation of the 116 compounds was achieved using a gradient elution with acetonitrile and an aqueous solution of formic acid at 0.1% (v/v) within 15 min. Due to the high selectivity of SRM detection, it is not necessary to achieve the complete resolution between the pesticides [24]. Overall, with QqQ the number of pesticides analyzed was between 39 [29] and 236 [22] OCPs and OPPs, with LODs and LOQs between 0.2–50 μg kg^{-1} [23,30] and 1–61 μg kg^{-1} [28,29] respectively (Table 2).

When LC–MS/MS is used as a determination technique, it is well-known that the presence of interferences can affect the

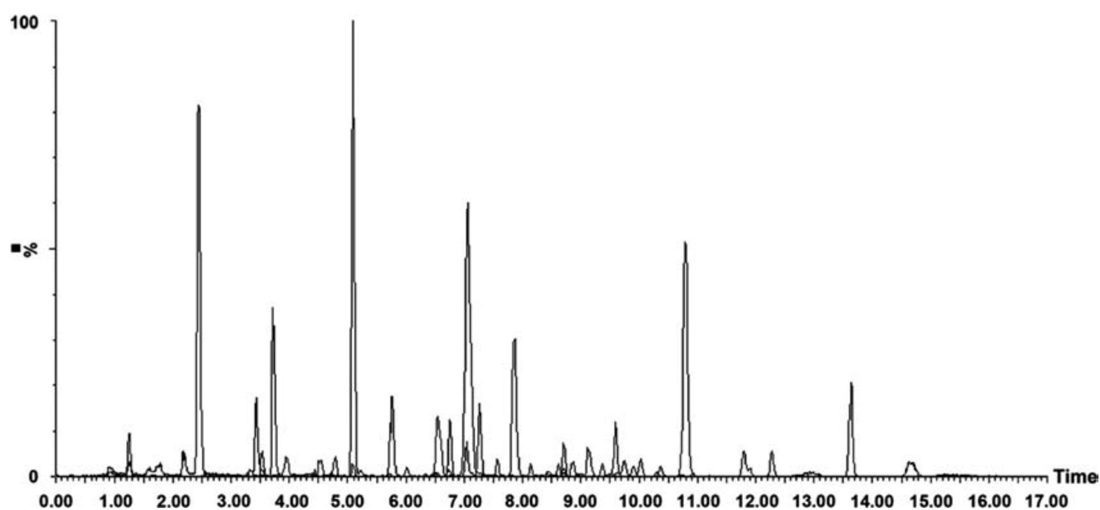

Fig. 6. Total ion chromatogram of ginseng fortified at 0.02 mg/kg.
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ionization process of the target compounds (competition process), reducing or enhancing the response in relation to the same concentration in the solvent. When pesticide residues are determined in nutraceuticals and related products, the high content of polyphenols or chlorophylls usually provoked a matrix suppression [22] and matrix matched calibration was usually used in order to counteract this matrix effect.

Other mass analyzers, as IT, offer a range of operating modes that allows a fast collection of data, confirming the identity of compounds and reducing the sample clean-up costs. When the GC-IT analyzer is used, the number of pesticides analyzed were between 8 and 13 OCPs with LODs and LOQs between 0.002–0.009 μg kg^{-1} [49] and 2–41 μg kg^{-1} [47], respectively.

On the other hand, QqTOF analyzers can have higher spectral acquisition rates than the Q or QqQ analyzers, which reduces spectral skewing [36] and is usually coupled to LC. This analyzer offers powerful data processing, providing fast acquisition rates and unbiased mass spectra. Moreover, it provides enough sensitivity (picogram level) in full mass range mode, which also allows the potential for finding nontarget pesticides or unknown peaks can also be identified [44]. Berzas et al. [48] explained that although MS, usually in the selective ion monitoring (SIM) mode, is the preferred method of choice for pesticide determination, confidence in the confirmation of identity may be reduced if one or more of the selected ions are affected by matrix interferences, which give poor spectral information. The obtained results can be improved by the use of TOF or QqTOF analyzers. This technique reduces the sample preparation step significantly, although it is either too expensive or too sophisticated to be implemented for routine analysis [48]. The number of pesticides analyzed by this procedure are between 10 [48] and 170 [36] pesticides (OCPs and OPPs), with LODs and LOQs between 0.0004–1.26 μg kg^{-1} [50] and 0.33–4.20 μg L^{-1} [48], respectively.

Comparing the different techniques mentioned before, Q or QqQ can analyze higher amount of pesticides. Although the QqTOF can quantify a similar or higher amount of pesticides, the analyzer is more expensive than the other two for routine analysis. Concerning the LODs and LOQs, it appears that IT can provide lower limits, but the number of pesticides analyzed is lower. Overall, QqQ and QqTOF obtain lower limits for multiresidue methods. In conclusion, QqQ or QqTOF can be suited for routine analysis of multiple pesticides, although GC or LC coupled to Q or QqQ are still widely applied in routine analysis as the preferred techniques.

5. Occurrence of pesticide residues in real samples

Some of the works discussed in this review applied the developed methods to real samples to detect and quantify the presence of pesticides in nutraceutical products. Most of the studies have been focused on medicinal plants and in some cases on dietary supplements that come from medicinal plants. In other cases, fish oils were also evaluated and Table 3 presents a summary of the analyzed pesticides and their concentrations found in real samples.

5.1. Medicinal plants

Budziak et al. [40] studied different herbal infusions in Brazil, finding 0.005 μg L^{-1} for *p,p′*-DDD and 0.067 μg L^{-1} for endosulfan II as the lowest and highest pesticide concentration respectively. Another pesticide detected in 5 different samples was aldrin (0.006–0.017 μg L^{-1}) [40]. The values found in the infusions investigated are below the MRL permitted by Regulation no.518 of the Brazilian Health Ministry [56]. In two works, Chang [20,21] investigated 44 OCPs and OPPs in 229 crude materials from China. From these samples, pesticides were detected in 18 samples, and 3 pesticides were detected at concentrations higher than their respective MRLs: BHC with 215 μg kg^{-1} (200 μg kg^{-1} MRL), procymidone with 572 μg kg^{-1} (100 μg kg^{-1} MRL) and endosulfan with 349 μg kg^{-1} (200 μg kg^{-1} MRL). Carbofuran at 34 μg kg^{-1} and procymidone at 579 μg kg^{-1} were the lowest and highest pesticide concentration respectively detected in the samples [20,21]. Gang et al. [30] determined 52 OCPs and OPPs on different Chinese medicinal plants. Ten pesticides were detected in the 20 tested samples, and four of them were forbidden, including monocrotophos, endrin, lindane, and *p,p′*-DDT. The lowest and highest pesticide concentration were 4 μg kg^{-1} for *p,p′*-DDT and 267 μg kg^{-1} for metalaxyl, respectively [30].

Harris et al. [23] evaluated 162 OCPs in different Chinese medicinal plants, detecting 42 pesticides in the 108 samples studied. From these pesticides, 21 were not registered for their use in the US [23]. The lowest and highest pesticide concentration was 11 μg kg^{-1} for terbufos sulfone and 650 μg kg^{-1} for chlorpyrifos, respectively [23]. Chen et al. [24] determined 116 OCPs and OPPs from different medicinal plants from China, indicating that in the 132 analyzed samples, 55 compounds were detected in 95 samples. The detected pesticides showed a concentration lower than their respective MRLs, and carbendazim, carbofuran,

Table 3
Summary of pesticides detected in real samples.[a]

Analytes	Matrix	Samples	Concentration[b]	Ref.
10 OCPs	Fish oil (salmon and cod liver)	3	0.11 (HCB) – 9.9 (γ-HCH) μg kg^{-1}	[48]
9 OCPs	Herbal infusions	5	0.005 (*p,p′*-DDD) – 0.067 (α-endosulfan) μg L^{-1}	[40]
135 OCPs/OPPs	Green and black tea	37	10 (triazophos) – 462 (bifenthrin) μg kg^{-1}	[19]
44 OCPs/OPPs	229 herbal crude materials	18[c]	34 (carbofuran) – 579 (procymidone) μg kg^{-1}	[20,21]
236 OCPs	Ginseng, saw palmetto, gingko biloba	4	2.7 (carbendazim) – 198 (azoxystrobin) μg L^{-1}	[22]
13 OCPs	Fish oil (mixed, salmon, shark, menhaden and seal)	8	0.024 (mirex) – 1810 (sum DDT) μg kg^{-1}	[49]
170 OCPs/OPPs	Ginseng root	4	1.6 (hexachlorobenzene) – 450 (*p,p′*-DDE) μg kg^{-1}	[36]
52 OCPs/OPPs	Medicinal plants	20	4 (*p,p′*-DDT) – 267 (metalaxyl) μg kg^{-1}	[30]
162 OCPs	Medicinal plants	108	11 (terbufos sulfone) – 650 (chlorpyrifos) μg kg^{-1}	[23]
30 OCPs	Fish oil (salmon and cod liver)	4	0.083 (heptachlor epoxide) – 57 (p,p-DDE) μg kg^{-1}	[50]
41 OCPs/OPPs	Dietary supplement (*Scutellaria baicalensis*+*Acacia catechu*)	52	1 (*p,p*-DDE) – 800 (endosulfan) μg kg^{-1}	[44]
20 OCPs	Medicinal plants	10	4.6 (lindane) – 2045 (quintozene) μg kg^{-1}	[37]
116 OCPs/OPPs	Medicinal plants	138	55 compounds lower than the MRLs	[24]
28 OCPs/OPPs Pyrethroids	Medicinal plants	30	0.4 (α-endosulfan) – 920 (γ-HCH) μg kg^{-1}	[32–34]
18 OCPs/OPPs	Ginseng	364	6–1600 (tolclofos-methyl) μg kg^{-1}	[25]
20 OCPs/OPPs	*Radix paeoniae*	5	3–26 (chlorpyrifos) μg kg^{-1}	[42]
23 OCPs/OPPs Pyrethroids	Medicinal plants	9	7.25 (bifenthrin) – 25.32 (hexachlorobenzene) μg kg^{-1}	[26]
8 OCPs	Plants extract (raspberry, maize, cranberry, rose, horsetail)	5	31 (metoxychlor) – 230 (lindane) μg kg^{-1}	[47]
168 OCPs/OPPs	Ginseng powder	12	< 1 (hexachlorobenzene) – 4000 (quintozene) μg kg^{-1}	[28]
39 OCPs/OPPs Neonicotinoids	*Camellia sinensis*	27	1–1684 μg kg^{-1} (Sum OCPs)	[29]

[a] Abbreviations: OCPs: organochlorine pesticides; OPPs: organophosphorus pesticides; MRL: Maximum residue level.
[b] Compounds showing the minimum and maximum values of the range are shown in parentheses.
[c] Samples contaminated.

propoxur, triazophos and acetamiprid were the most frequently detected [24]. Kakkar et al. [32–34] performed several studies in Indian herbs, and 9 out of 28 pesticides were detected. The pesticide concentrations were lower than their respective MRLs, determining 0.4 μg kg^{-1} for α-endosulfan and 920 μg kg^{-1} for γ-HCH [32–34].

Ronghua et al. [26] also investigated 23 OCPs and OPPs on different medicinal plants from China, finding 6 pesticides on 3 different matrices (below their respective MRLs). The lowest and highest pesticide concentrations were 7.25 μg kg^{-1} for bifenthrin and 25.32 μg kg^{-1} for hexachlorobenzene respectively [26]. Qian et al. [42] determined 20 OCPs and OPPs on the medicinal plant *Radix paeoniae*, finding that only chlorpyrifos was detected in the 5 samples studied, and the highest concentration was 26 μg kg^{-1} below the MRL (200 μg kg^{-1}) [42]. Tusa et al. [47] investigated 8 OCPs on different plant extracts (raspberry, maize, cranberry, rose and horsetail), finding lindane, DDT and metoxychlor on the studied samples, at concentrations ranging from 31 μg kg^{-1} (metoxychlor) and 230 μg kg^{-1} (lindane) [47].

Chen et al. [22] studied 236 OCPs on different Chinese medicinal plants (ginseng, saw palmetto, gingko biloba) detecting 13 pesticides at concentrations ranging from 2.7 μg kg^{-1} (carbendazim) to 198 μg kg^{-1} (azoxystrobin). Hayward et al. [36] evaluated 170 OCPs and OPPs on ginseng root, detecting 17 pesticides in the 4 different samples studied. Three replicates were used per matrix and the lowest and highest pesticide concentration quantified were 1.6 μg kg^{-1} for hexachlorobenzene and 450 μg kg^{-1} for *p,p′*-DDE respectively [36]. Leung et al. [37] also investigated 20 OCPs on different types of ginseng (10 samples per matrix), founding that hexachlorobenzene (480 μg kg^{-1}) and quintozene (2045 μg kg^{-1}) concentration exceeded their respective MRLs (100 and 1000 μg kg^{-1} respectively). These findings generally agreed with previous studies reporting quintozene and hexachlorobenzene as the most commonly detected OCPs in ginseng roots [57]. The lowest and highest pesticide concentration detected were 4.6 μg kg^{-1} for lindane and 2045 μg kg^{-1} for quintozene respectively [37]. It is important to highlight that the levels of contaminants present in local samples were found to be generally higher than those observed for the mainland counterparts. As a traditional common practice, most of the mainland imported herbs selling in Hong Kong would have had the cork or skin removed to improve the exterior appearance. Obviously this practice did not help to remove pesticide residues as the majority of these contaminants not only adhered to the surface but were already absorbed into the plant tissues [37]. Park et al. [25] studied 18 OCPs and OPPs in ginseng, finding that on the 364 samples, only tolclofos-methyl was above the MRL (250 μg kg^{-1}), and the highest concentration for this pesticide was 1600 μg kg^{-1} [35]. Wong et al. [28] also determined 168 OCPs and OPPs in ginseng powder, detecting 19 pesticides in the 12 different samples. Many of these samples contained OCPs, such as quintozene and its metabolites, DDT and its metabolites, benzene hexachloride (BHC) isomers, chlordane, procymidone, iprodione, chlorothalonil and dacthal. In addition, two OPPs, chlorpyrifos and diazinon, were present in the same ginseng sample [28]. The found concentrations ranged from < 1 μg kg^{-1} (hexachlorobenzene) and 4000 μg kg^{-1} (quintozene) [28]. Wong et al. [28] explain that the presence of banned OCPs, such as *p,p′*-DDT and its metabolites and BHC isomers, is probably due to the persistence of these pesticides in the environment where these ginseng roots were cultivated. Furthermore, Plaza et al. [58] explain that the presence of DDT metabolites on real samples is considered as an indicative of historical DDT residues, and thus, the contamination by DDT product is due to old applications.

Cajka et al. [19] evaluated 135 OCPs and OPPs on 37 samples of green and black tea, finding that 81% of the samples were positive (≥ 10 μg kg^{-1}) containing at least one pesticide residue. Cypermethrin (68%), endosulfan and related products (41%), propargite (38%), bifenthrin (38%), λ-cyhalothrin (24%) and buprofezin (24%) were the most frequently found pesticides [19]. It was also found that some samples contained residue concentrations close to the MRLs and in one sample, buprofezin (179 μg kg^{-1}) and triazophos (214 μg kg^{-1}), exceeded their corresponding MRLs (50 and 20 μg kg^{-1} respectively) [18]. Zhang et al. [29] also studied 39 pesticides (OCPs, OPPs and neonicotinoids) in *Camellia sinensis*, detecting 11 pesticides in 27 samples. The lowest and highest pesticide concentrations, reported as a sum of the different compounds found, ranged from 1 μg kg^{-1} to 1684 μg kg^{-1} respectively [29], and imidacloprid and acetamiprid were the most commonly detected pesticides, in 12 and 11 samples, respectively.

5.2. Processed material: tablets and capsules

On the revised bibliography, only one work evaluated the presence of pesticide residues in dietary supplements. Lee et al. [46] investigated 41 OCPs and OPPs on a dietary supplement containing *Scutellaria baicalensis* and *Acacia catechu*. Only 8 pesticides were detected in the 52 lots analyzed [46]. Pesticide concentrations found in this study did not surpass their respective US MRLs, and the lowest and highest detected concentrations were 1 μg kg^{-1} for *p,p′*-DDE and 800 μg kg^{-1} for endosulfan respectively [46].

On the particular cases of fish oil, Berzas et al. [48] investigated 10 OCPs from three different samples (two salmons and cod liver). In all the cases, the dominant detected compounds are polychlorinated biphenyls (PCBs), followed by DDTs. The prevalence of DDE, which is one of the metabolites of DDT degradation (DDT→DDD→DDE), could indicate that exposure to DDT of all samples was far behind in time [48]. The found concentrations ranged from 0.11 μg kg^{-1} (HCB) to 9.9 μg kg^{-1} (γ-HCH) [48]. Rawn et al. [49] studied 13 OCPs in 8 different oil samples (including mixed, salmon, vegetable, shark and seal). OCPs levels in the oil supplement samples varied widely between supplement types. Although individual contributors to the sum of HCH (for example, α-HCH, γ-HCH) and the sum of chlordane (for example, oxychlordane) were below LODs in a few samples, only 1 mixed fish oil sample, with no salmon content, was found to have non-detectable residues of HCH [49]. The supplements containing seal, shark and salmon oils had elevated levels of OCPs in comparison to those containing other fish and vegetable oils [49]. The lowest and highest pesticide concentrations were 0.024 μg kg^{-1} for mirex and 1810 μg kg^{-1} for the sum of DDT (*p,p′*-DDT and *p,p′*-DDE) respectively [49]. Hoh et al. [50] also studied 30 OCPs from 4 different oils (three cod livers and salmon). 10 pesticides were detected, and concentrations ranged from 0.083 μg kg^{-1} (heptachlor epoxide) to 57 μg kg^{-1} (*p,p′*-DDE) [50].

6. Conclusions

For pesticides determination in nutraceutical products, different sample treatments were discussed, and QuEChERS like methods are the most used. Other treatments include PLE, SLE, MSPD and SPE. The selection of the most suitable extraction procedure will depend on the matrix itself and how much simple, rapid, effective and costly the method can be. In general, the use of clean-up steps during the extraction step is recommended to obtain better recovery percentages and to minimize the matrix effect.

For chromatographic separation, GC and LC were applied, and GC is the most widely used. One or two columns can be used in order to obtain a good pesticide separation from the matrix. In relation to LC, the use of UHPLC can reduce running time and thus increase the number of compounds that can be simultaneously analyzed in one single run.

Different detection systems were evaluated for pesticides determination and quantification, including classical and advanced analyzers. Advanced analyzers were Q-MS, IT, QqQ-MS/MS and QqTOF, Q-MS and QqQ-MS/MS is the most used, although for a large quantity of pesticides the use of QqQ-MS/MS is recommended because of the possibility of simultaneous confirmation and quantification of the target compounds with excellent selectivity and sensitivity.

It is important to indicate that the studies analyzing capsules or tablets of nutraceuticals are really scarce. In this sense, considering that pesticide residues were found in such studies, further analysis to obtain data about the occurrence of pesticides (and other possible contaminants) should be necessary. Medicinal plants, dietary supplements and fish oils have been analyzed. Basically OCPs, OPPs, pyretroids and neonicotinoids were detected. The presence of these pesticides in the analyzed samples indicates that monitoring programs should be established in order to assure the safety of these products, as well as legislation should be updated in order to cover the current gap regarding the lack of MRLs for some types of nutraceutical products. Also, new studies should be focused on more polar pesticides, bearing in mind the lack of analysis in this field.

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