Pesticide Residues in Natural Products with Pharmaceutical Use: Occurrence, Analytical Advances and Perspectives

Andrés Pérez-Parada[1], Marcos Colazzo[1], Natalia Besil[1],
Eduardo Dellacassa[1], Verónica Cesio[1],
Horacio Heinzen[1] and Amadeo R. Fernández-Alba[2]
[1]Pharmacognosy and Natural Products, Facultad de Química
Universidad de la República, Montevideo
[2]Pesticide Residue Research Group, Universidad de Almería, Almería
[1]Uruguay
[2] Spain

1. Introduction

A pharmaceutical raw material is every active or inactive substance used in the manufacturing process of a pharmaceutical dosage form. According to their function in the medicament, they could be classified into two groups: (i) active ingredients with pharmacological activity; and (ii) excipients which allow the active ingredients dosification and makes suitable the route of administration (de la Rosa et al., 1995). In spite of the high number of synthetically produced pharmaceuticals and the progress in biotechnology and gene engineering, there are a number of raw materials of natural origin, which are still used for manufacturing active pharmaceutical products (Balandrin et al., 1985).

Quality is a mandatory requirement in the materials to accomplish the Pharmaceutical 'Good Manufacturing Practices'. Nowadays, the presence of pesticides in animal and vegetal commodities is a topic of public concern for the potential health hazards derived from them (WHO, 1998). The presence of pesticide residues in animal or vegetal raw materials can be originated in agricultural practices, environmental contamination or cross contamination. For example, oils rendered from whole fish and from fish offal represent an important dietary component in many areas of the world. In addition, fish oils are extensively used as ingredients in food and in dietary supplements for their therapeutic benefits in the treatment of cardiovascular, arthritic and dermatological diseases. Fish oils, however, are susceptible to contamination with lipophilic organic chemicals, in particular many organochlorine chemicals are now ubiquitous contaminants of marine ecosystems, but there is no specification on the pesticide residue allowed (Jacobs et al., 1997).

The number of trace pollutants to be monitored is continually growing and the levels at which these compounds need to be determined are becoming lower. Multi-residue methods (MRMs) are the preferred strategy over single analyte or class residue methods (SRMs). Analytical features of the MRMs allow to cover a large range of pesticides in a single analysis. Many MRMs employing different extraction and clean-up techniques and a variety

of detection methods, have been reported for the determination of pesticide residues in raw materials. The analytical method to be used depends mainly on the matrix composition, the physicochemical properties of the target analytes as well as the chemical nature of interferents from the analyzed matrices and/or the available instrumentation.

Currently, general methods to control pollutants in materials intended to be used in pharmaceutical preparations are described in the United States and European Pharmacopeias (USP 2007, EP 2008). Many herbs and spices that have been included in the Pharmacopeias, like Cinammon and Ginger, even when they were evaluated to detect low contaminant levels using the newest analytical methodologies and procedures available, their specifications remain unchanged. The different classes and wide range of pesticides and agricultural commodities containing them have fostered the development of new methods. However, the difficulty to cover all analytes in one single method and for all the possible matrices, has turned this approach into an almost impossible task, despite the advances in instrumentation in the last decade. Moreover, new pesticides have been introduced in the market which are more polar, non-chlorinated, less persistent or unstable leading to a change in the concept of the analytical and detection features required.

The actual trend in pesticides residue analysis focuses on the use of liquid chromatography coupled to mass spectrometry techniques (LC-MS) instead of gas chromatography (GC) with selective detection as electron capture (ECD), flame photometric (FPD), nitrogen-phosphorus (NPD) and thermal conductivity (TCD) detectors which represented the state-of-the-art at the end of the last century. They are intended for lipophillic (in many cases obsolete) pesticides like most organochlorine (i.e. p,p'-DDT, aldrin, dieldrin, lindane). Moreover, the targeted compounds monitored by such methods sometimes are neither pesticides of toxicological relevance or the most commonly found in a particular sample. Furthermore, MRMs could not be suitable and additional steps or SRMs are required. The wide scope of targeted analytes lies unchanged in the last editions of the most important Pharmacopeias as well as the methods for their anaylsis. The new instrumentation and sample preparation procedures, which allowed to diminish the limits of detection and consequently lowered the maximum residue levels (MRLs) in food commodities to 10 µg/kg for the great majority of pesticides in the European regulation, has not been yet included in the most important Pharmacopeias. The USP includes in its last Edition the detection of impurities in heparin using sophisticated high field NMR (nuclear magnetic resonance) techniques (USP, 2010). Nevertheless, the use of LC-MS/MS for pesticide residues and other contaminants is not mentioned at all. It is worth to consider that, these raw materials could be employed for pharmaceuticals which would be taken by people whose general health situation is jeopardized by their particular condition and therefore, the toxicological risk could be magnified. Therefore, strict regulations should rule the content of noxious substances like pesticides.

There is also a growing market for medicinal plants and their pharmaceuticals that is freely available in markets of Europe and USA: these Over The Counter (OTC) products are actually sold as "panaceas" for a well and long lasting living. There is a grey zone, from the regulatory point of view, with relation to pesticide residue allowable contents where all these kind of products are laid. The self medication and, therefore, uncontrolled use or misuse as well the lack of specific regulation in pesticide residue limits for OTC products deserves attention from the regulatory bodies. Nutraceuticals, natural cosmetics and phytopharmaceuticals share the same kind of regulatory alibi where the risk due to the

presence of pesticides residues has not been yet particularly considered. For many food products like oils or meals, processing factors have been estimated, but which is the processing factor for a specific pesticide in a Hammamelis tincture? Or in propolis tincture? Pesticides are concentrated or diluted? Undoubtely, there are more open questions than answers.

New trends in pesticides residue analysis have been focused on the miniaturization of the sample preparation methodology, moving to the development of straightforward, faster, cost-effective, and environmentally friendly procedures, adaptable for routine use in laboratories. Even with the advent of highly sensitive mass spectrometry (MS) and tandem mass spectrometry (MS/MS) detection techniques, the total knowledge of the contribution of pesticides in pharmaceutical products has not been completely developed yet, as for food and feed commodities or environmental samples. This sitution makes the development of new methods in organic contaminants residue analysis in pharmaceutical raw materials, a non closed and challenging issue.

This chapter addresses the main features of pesticide residue analysis in natural complex matrices frequently used in the pharmaceutical industry and folk medicine. An overview in the literature for methods and new improvements to determine pesticide residues in some critical pharmaceuticals raw materials is here presented. Expected pesticides, strategies in sample preparation procedures, recent applications in detection capabilities about these particular uses of the above mentioned pesticides are critically discussed. The authors are aware of reports in literature that were published in a variety of languages or in restricted access publication that were not included in this chapter. However, we tried to set priority on the most relevant, high impact and recent developments in the area.

2. Natural products of economical importance in the pharmaceutical industry

As above mentioned, some natural products are widely used in pharmacy. Table 1 shows an overview of selected commodities studied in this chapter and features for which are used. Particularly, traditional Chinese medicines are the most used, being more than 1.5 billion people all over the world which trust in their efficacy and safety (Li et al., 2010). The use of medicinal plants in both crude and prepared forms has increased greatly. The WHO has estimated that 80% of the global population relies on traditional medicine for health care. About 51% of all drug preparations in industrialized countries derive from plants, acting as sources of therapeutic agents or models for new synthetic compounds, or as raw material for semi-synthetic production of highly complex molecules (Zuin & Vilegas, 2000).

Aromatic plant drugs including essential oils and natural aromachemicals have been used extensively in the pharmaceutical industry as flavouring, and to mask the foul odour or taste of some pharmaceuticals as excipients.

However, in recent years, many aromatic plant drugs are used as active ingredients of botanicals. The simplest common traditional and modern use of aromatic plant drugs is as herbal tea. In many countries, pharmacies can freely dispense established tea formulations to patients for mild indications (Wichtl et al., 1994). Thus, essential oils could also make their way from the traditional into the modern medical domain (Bakkali et al., 2008).

Beeswax, instead, is constituted by saturated and unsaturated hydrocarbons, long chain saturated, mono and di-unsaturated esters and is being used as a lipid in pill coatings for drug delivery systems from tablets although current cosmetic uses are also known (Üner et al., 2005). Propolis is well known for therapeutic and dermal contact applications due to

its antibiotic and antiviral activities; more activities of interest were widely reported principally citotoxic, antioxidant and antifungal ones (Marcucci, 1995). Lanoline is a complex matrix composed principally by a mixture of esters involving different aliphatic acids combined with different alcohols. Lanolin is the by-product obtained after wool wax purification and it is widely used in cosmetic and pharmaceutical formulations because its unique surfactants properties. It also represents the world first source of cholesterol and lanosterol (Jover et al., 2002).

Commodity	Features/Properties	Pharmaceutical use
Beeswax	Compatibility with skin oils , UV inhibitor	Hand and body creams, ointments and pill coatings
Essential oils	Aromatic	Flavoring
Lanolin	Compatibility with skin oils, water absorbing, surfactant and emulsifying	Creams and ointments
Propolis	Antibiotic, anti-inflamatory, healing, antioxidant	Dermal contact, dietary supplement, cough candies
Medicinal Aromatic herbs	Wide variety of active constituents (i.e. antibiotic, anti-inflamatory)	Depending on active constituents. Dietary supplement

Table 1. Selected commodities and their uses in pharmacy.

3. Pesticide residues occurrence in natural products of economical importance

Since 1963 pesticide residues were detected in natural drug matrices although most monitoring reports have been published in the last 20 years. Organochlorines (OCs) such as DDT, aldrin, dieldrin, endrin, hexachlorocyclohexane (HCH) isomers and hexachlorobenzene (HCB) have been widely used as insecticides since the introduction of pesticides in management strategies for the protection of crops. Due to its well known environmental persistence, most OCs are currently banned worldwide. Synthetic pyrethroids and organophospates (OPs) insecticides were then introduced as replacements, being most of them currently commercially available in several countries. The occurrence of pesticides residues in phytopharmaceuticals and phytomedicines reviewed by Zuin and Vilegas (2000) state evidence that most reports were focused on the detection of OCs and OPs. On the other hand scarce reports have deal with the monitoring of other insecticide families such as carbamates and bridged diphenyls; triazine and urea herbicides; phtalamides and benzimidazole fungicides; a trend which is slowly overcome nowadays. Efforts in stricter regulation in MRLs settling for pesticides was stated by Codex Alimentarious, European and United States legislation for food commodities which has change the concept of contamination with pesticide residues. Therefore, more and more compounds are being targeted in raw materials although also banned OCs are still being found.

3.1 Pesticide residues in citrus essential oils
Depending on the extraction procedures, some oils could concentrate pesticide residues, as the case of citrus oils and extracts, being necessary to control the impact of the different

processing steps from fruit to the essential oil and the storage materials used. Citrus essential oils are by far the most studied essential oil commodity for pesticide residues. Di Bella and co-workers have been extensively reported the occurrence of variety of contaminants in Italian citrus essential oils. From the technological package applied in citrus crops, liphophilic pesticides are suggested to be preferably present in the oil phase (Dugo & Di Bella 2002). Residues of the bridged diphenyls (BDPs) tetradifon and dicofol along with the its metabolite, 4,4'- dichlorobenzophenone, were firstly reported in a five years monitoring campaign in lemon, mandarin, bergamot and orange essential oils (Saitta et al., 2000). On the other hand, Uruguayan lemon essential oils were found containing the OPs methidathion and chlorpyriphos (Dellacassa et al., 1999). More recently, a wide variety of OPs were reported in bergamot essential oil (Di Bella et al., 2004) and biological celementine, lemon, mandarin and orange essential oils (Di Bella et al., 2006).

Finally, a recent study with variety of samples from Argentina, Brazil, Italy, South Africa and Spain, revealed the introduction of more compounds to the target list since residues of the BDP bromopropilate, and OPs fenthion, malathion, phentoate and pyridafenthion were found (Di Bella et al., 2010). From this work, the authors conclude the wide occurrence of chlorpyriphos and dicofol residues in most samples, being Brazilian and Spanish ones the largest contaminated with individual levels up to 4.8 and 8.5 mg/L respectively.

3.2 Pesticide residues in bee's by-products: Propolis and beeswax

The contamination of bee's by-products with acaricides residues was extensively investigated by Bogdanov and co-workers. Important contamination with the most worldwide used synthetic acaricides to control Varroasis such as achrinathrine, amitraz, bromopropilate, coumahos, flumathrin and τ-fluvalinate was depicted in several studies (Bogdanov, 2003, Bogdanov, 2006, Bogdanov et al., 2003, Wallner, 1999). Even in a less extent, studies reporting the occurrence of several lipophilic pesticides from environmental pollution in beeswax were established. Not only acaricide contamination occurs, residues of the OC lindane, and the OCs metabolites p,p'-TDE, endosulfan sulphate and 3-phenoxybenzaldehyde were found in beeswax from Spanish beehives (Jiménez et al., 2005). A wide variety of residues of OPs, synthetic pyrethroids and dicarboximide fungicides (procymidone and vinclozolin) were also detected in beeswax from France (Chauzat & Faucon 2007). Recently, Spanish commercial beeswax were found containing chlorimefon, chlorfenvinphos, chlorpyriphos, endosulfan and malathion residues (Serra-Bonvehí & Orantes-Bermejo, 2010). Over 197 samples analysed, the authors reported residues of chlorfenvinphos residues in 96 % of samples with concentrations up to 10.6 mg/kg (pesticide not legally authorised for use in beekeeping) whereas τ-fluvalinate was detected in 93.6% of samples with concentrations up to 88.7 mg/kg (Serra-Bonvehí & Orantes-Bermejo 2010). Currently, a critical situation with pesticide residues in bee's by-products is being found worldwide. A recent study carried out in hive matrices of USA and Canada, showed the occurrence of 121 different pesticides and metabolites (a variety of acaricides but also insecticides, fungicides and herbicides introduced in the hive by the bees from pesticide containing crops) within 887 beeswax samples analyzed (Mullin et al., 2010). The authors found that almost 98 % of the North American beeswax samples were contaminated with up to 204 and 94 mg/kg residues of τ-fluvalinate and coumaphos respectively. With relevant incidence, lower amounts of amitraz degradates and the fungicide chlorothalonil were found while an average of 6 pesticide detection per sample and a high of 39 was stated (Mullin et al., 2010).

On the other hand, since the composition of raw propolis contains high amount of beeswax, pesticide residues occurrence in propolis are expected to be in the same line to beeswax contamination (Bogdanov 2006, Wallner 1999). Recently our group investigated the presence of coumaphos, the most used acaricide, in propolis tinctures from Uruguay. Moreover, minor residues of ethion and chlorpyriphos were also detected (Pérez-Parada et al., 2011).

3.3 Pesticide residues in lanolin

To prevent fleece damage by sheep ectoparasites and to protect wool during storage, OCs, OPs and pyrethroid insecticides were extensively used in sheep sanitary treatments. Because of their lipophilic nature, these pesticides tend to accumulate in wool wax. Nowadays ectoparasite control is achieved exclusively through the application of OP and pyrethnoid insecticides and chitin synthesis inhibitors such as diflubenzuron and triflumuron (Jones 1996). However, few efforts were carried out in the last decade to determine the occurrence of pesticide residues in wool wax and processed wool wax (lanolin). In a previous study, our group found residues of chlorpyriphos, cypermethin, diazinon and ethion in Uruguayan lanolin samples (Pérez et al., 2010). Furthermore, when recently searching for 40 OPs residues in raw sheep wool from Uruguay, the extracted wool wax was found only containing diazinon and ethion residues (Niell et al., 2011).

3.4 Pesticide residues in medicinal plant materials

The interest in pesticide residues in medicinal herbs have been mainly focused on method developments rather than in the publication of results for public concern. However, the main fact can be divided into (i) findings are still OCs and OPs insecticides and (ii) the occurrence of a growing amount of pesticides, mainly fungicides, contained in medicinal products which enlarge the target list of pesticides to search for. Most studies still revealed the high incidence of OCs pesticides in several herbal drugs worldwide (Abhilash & Singh, 2008, Jeon et al., 2007, Leung et al., 2005, Mishra et al., 2007, Sun et al., 2007, Zuin et al., 2003).

The occurrence of banned OCs is accentuated when determining pesticide occurrence in commodities based on roots principally due to the cultivation in contaminated soils with several decades of agricultural use (Hayward & Wong, 2009, Leung et al., 2005, Li et al., 2010, Wong et al., 2010, Wu et al., 2011). Additional interest lies on OCs residues in those commodities which are consumed with dietary supplement purpose such as Ginseng root powders (Wong et al., 2010). Other authors reported residues of carbendazim, cyazofamid, diethofencarb and pyrimethanil in Asian Ginseng (Choi et al., 2007).

A wide variety of pesticides were found in a study carried out with Korean herbs (Nguyen et al., 2010). Chlorfenapyr, chlorfluazuron, λ-cyhalotrin, metalaxyl, pyridalyl, fenvalerate, tebuconazole and tebufenozide residues were found suggesting new strategies in pest control for medicinal plants which include herbicides, fungicides and insecticides. However, residues of p,p'-DDE were also detected as main contaminants in several plants (Nguyen et al., 2010). Residues exceding MRLs were reported (Abou-Arab & Abou Donia, 2001).

Monitoring campaign in Chinese and Korean medicinal drugs showed main contamination with 5 pesticides: methoxychlor, DDT, γ-HCH (lindane), endosulfan and procymidone with residues concentration ranging from 0.044 to 0.501 mg/kg. Intensive monitoring campaigns were suggested since the detection rate of pesticides in 30 different types of drugs was determined as 3.1% from the 229 samples analyzed. On critical observation was the detected amount of procymidone (0.501 mg/kg) and methoxychlor (0.382 and 0.312 mg/kg) (Oh

2009). Other studies showed that, from the 8 detected pesticides (residues between 0.034–0.579 mg/kg), 4 of them were fungicides (captan, chinomethionate, procymidone and tolyfluanid) (Oh 2007). Moreover, major residues of chlorothalonil fungicide were also detected in Brazilian *Passiflora L.* leaves (Zuin et al., 2003).

4. Pesticide residue analysis in natural products

Scientific advances in the employment of natural products with pharmaceutical relevance have focused on the analysis of constituents with the purpose of standardizing the applicability of such commodities. Aware for recent interest in residue analysis is intended to ensure assessment for safety consumption. Main pitfall in the study of pesticide residues in such matrices is the amount and nature of different commodities of interest and pesticides applied. Residue testing in this field is a major challenge because of the wide range of target agrochemicals but also it is an unexplored field, i.e. most medicinal plants are still lacking on analytical studies. This has lead to the development of fit-for-purpose methods since official methodologies are often described for not to face real life conditions (i.e. laboratory infrastructure, variability between different commodities, variability between samples from different geographical origins, residue legislation of different countries and non-harmonized application of used pesticides or commodity consumption) (Zuin et al., 2003b). Therefore, method development for contaminants is currently of paramount interest to determine the quality of these materials as demonstrated for the increasing numbers of publications in the area. Effort has been carried out for the residue analysis of pesticides in medicinal derivatives in the last ten years by modifying sample preparation, the inclusion of unstudied pesticides along with the introduction of more reliable detection and quantitation techniques. In accordance to pesticide residue research in food, the need was directed to more accurate, faster and more sensitive analytical methods. Dynamic sample treatment methodologies are mandatory. Trends were intended for avoiding tedious; time consuming and expensive extraction and clean-up protocols. Generally, classical procedures (as those described in official methodologies) drawbacks were related to the use of liquid-liquid or solid-liquid extraction which uses high amount of hazardous organic solvents. Modern techniques are focused on miniaturization as well as rapid or cost-effective features of the selected sample preparation. This has promote methodologies based on the use of new sorbents (i.e. primary-secondary amine, PSA; graphitized carbon black, GCB) or tailored sorbents (molecular imprinted polymers, nanomaterials, immunosorbents); integrative methods (defined as those which perform simultaneously several steps, i.e. extraction and clean-up); automated or even non-automated methods which can be carried out without the need of specific equipments. Remarkable attention has been conducted since the development of versatile MRMs such as the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) (Anastassiades et al., 2003a) along with its modifications; or MSPD (Matrix Solid Phase-Dispersion) (Barker 2007) which are suitable for large scale residue analysis in a wide variety of matrices.

At the same time, numerous regulations have set MRLs for pesticides and their relevant metabolites in a broad range of commodities (principally for food, feed and environmental matrices). Nowadays, chromatography coupled to MS has emerged as the default tool for residue analysis because of its improved sensitivity and selectivity provided for quantitative and qualitative analysis in comparison to conventional detection systems (i.e. ECD and FPD). The successful coupling of LC-MS and MS/MS have become the most used

configuration for large scale residue analysis according to the offered capabilities such as simultaneous identification, confirmation and quantitative determination for residue analysis. However, natural products were comparatively neglected in such a trend.

4.1 Sample preparation: Extraction and clean-up techniques

Despite the increasing progress in detection, sample preparation still remains as the bottleneck in natural products residue analysis. Current methods involve the use of one or a combination of sample preparation steps. Table 2 shows an overview of reported methodologies in the latest ten years for the sample treatment and chromatographic determination of pesticide residues in pharmaceutical raw materials and medicinal plants. A valuable indicator of the advances in residue analysis scenario for natural products, can be found in the pesticide determination in medicinal plants reviewed by Zuin & Vilegas (2000) years ago and the current situation with the latest improvements concerning miniaturized sample preparation (Zhou et al., 2011) and hyphenated MS techniques (Wong et al., 2010).

4.1.1 Official methodologies and related techniques

Testing for pesticide residues in pharmaceutical raw materials is described in official monographs for pesticides in botanical origin commodities and lanolin (USP 2007, EP 2008). They are based in liquid phase extraction (LPE) and further size-exclusion chromatography (gel permeation chromatography, GPC) as clean-up step. Subsequent clean-up step using SPE (solid phase extraction) in alumina is used in EP prior to gas chromatography (GC) injection. By far, these procedures are rugged and slightly differ in the experimental conditions.

4.1.1.1 Solid-liquid / liquid–liquid extraction

Most sample preparation methods in phytopharmaceuticals are based on LPE procedures either liquid-liquid (L-L) or solid-liquid (S-L) methodologies. Maceration of coarsely powdered solid substances, both fresh and dry materials with acetone is currently employed for extraction of pesticide residues as described in both official documents (EP 2008, USP 2007). The obtained extract, is then purified using GPC and analyzed by GC with selective detectors. Earlier literature was based on this scheme (Lino et al., 1999, Lino et al., 1997, Zuin & Vilegas, 2000). After maceration, further L-L partitioning between an immiscible organic phase (commonly petroleum ether or hexane) was widely assayed as a variation of the official methods. Subsequent clean-up using sorbent based techniques such as column chromatography and SPE was required for GC determination. L-L partitioning between a polar phase such as methanol or acetonitrile (MeCN) and n-hexane was reported as a preliminary clean-up in the determination of variety of lipophilic pesticides from beeswax (Jiménez et al., 2005, Jiménez et al., 2004a, Jiménez et al., 2004b). Likewise, L-L was proposed as the first clean-up stage in order to remove most waxes for the residue determination of 17 OCs in raw propolis (Chen et al., 2009).

Recent literature still uses these procedures because of the offered versatility derived from independence from expensive analytical instrumentation which are limiting requirements for laboratories. Acidic digestion procedures of the obtained organic extracts employing H_2SO_4 were reported for the analysis of OCs pesticides in Ginseng root (Chan et al., 2007, Quan et al., 2004). Similarly, a selected group of OCs and OPs were determined in herbal essential oils by using this technique (Yoon et al., 1999). However this strategy is limited for

the analysis of a reduced group of non-hydrolysable compounds. Moreover, Soxhlet extraction was reported as enhanced extraction methodology for OCs determination in Ginseng root using EtAc (ethyl acetate):petroleum ether (7:3) (Chan et al., 2007) and acetone:petroleum ether (3:1) solvent mixtures (Quan et al., 2008). A selected group of carbamates (metalocarb, isoprocarb, fenobucarb, carbofuran, pirimicarb and carbaryl were extracted with Soxhlet based extraction in CH_2Cl_2 from three different Chinese herbal medicines (Wu et al., 2005). Major pitfall of this strategy is its restriction for thermally stable compounds.

4.1.1.2 Gel permeation chromatography

GPC is a well established method for the fractionation and clean-up of non-fatty and fatty matrices in both plant and animal origin samples. It is generally recommended for purifying extracts obtained from complex samples. GPC is widely extended technique in routine laboratories for the analysis of pesticide residues after a preliminary LPE step and usually followed by GC analysis with different detectors.These conventional methodologies for pesticide residue analysis are often displayed as costly, time consuming and environmentally unfriendly because of the requirement of large amount of samples and greater volumes of hazardous solvents. The classical approach used in described official methodologies merely focus on GC based analysis with selective detection (i.e. ECD, TCD) or MS for confirmatory purposes. Although the inherent ruggedness, such methods cannot be applied to different conditions (target pesticides and different matrices) with the same analytical performance. Consequently several modifications were applied as evidenced for GPC based sample preparation of lanolin (Jones 1996, Jover & Bayona, 2002). The development of more straightforward, cost-effective and environmentally aware procedures while also providing the same analytical performance and fulfilling the requirements of regulatory issues is often suggested to substitute this kind of procedures (Pérez et al., 2010, Zuin et al., 2003a, Zuin et al., 2003b).

4.1.2 Recent advances, trends and achievements for pesticide residue analysis in natural products

Many modern multiresidue procedures employing different clean-up techniques and a variety of detection methods have been reported for the determination of pesticide residues in natural products with pharmaceutical application. Despite advances in the sensitivity of the analytical instrumentation for the end-point determination of analytes a pre-treatment is compulsory required to extract and isolate the target pesticides from the matrix, thus facilitating their determination (Gilbert-López et al., 2009). The sample treatment applied depends heavily in the complexity of the matrix. Modern sample treatment strategies for food and feed use to classify matrices depending on the water, sugar, acidic or fatty content matrices (DG SANCO 2009). Similarly, different natural product matrices can be grouped depending on its water content (low or high, i.e. dry and fresh herbs), waxy content (lanolin, beeswax and raw propolis) and polyphenolic content (propolis). This categorization is helpful because extraction strategies and involved inteferents are of similar nature which is relevant for the development of the sample preparation step. Moreover, the selection of sample preparation methodology is directly related to the detection method available. The more sensitive and specific detection method is used, the less stages of sample treatment will be required (Gilbert-López et al., 2009). In general, selective capability of the detector would compromise the followed sample treatment methodology (i.e. TCD, ECD detectors in GC and diode array detection (DAD) in

LC) require exhaustive and dedicated clean-up stages. In all cases, it is often necessary further clean-up to remove non desired interferents of the matrix such as pigments (i.e. chorlophylls, polyphenols, carontenoids) and waxes for increased sensitivity and reliability of the results along with maintenance of the instrumentation.

It must be highlighted that most recent reports in such matrices focus on the sample preparation instead of the use of modern detection. The advent of advanced MS detectors equipped with high selectivity, specificity and sensitivity capabilities, was recently introduced in the field of pesticide residue analysis of pharmaceuticals raw materials. Another key issue is the nature and number of targeted analytes included in the method. There is a wide range of agrochemicals with different physicochemical properties. For this reason, large scale methods use generic or less specific sample treatment at the expense of the sensitivity and selectivity provided by the MS determination.

4.1.2.1 Solid phase extraction

Nowadays, SPE is by far the most popular technique in the extraction and clean-up of complex extracts. Distinctive purposes can be accomplished depending on the used strategy: to retain target compounds and exclude interferents from the solid-phase or either retain interferents while pesticides are selectively eluted. Several advantages of SPE over liquid partitioning procedures are highlighted in higher sample throughput and lower solvent consumption. Moreover, the formation of emulsions is avoided with concomitant increased repeatability of the sample preparation. The flexibility to work with different commercially available solid phases offer increased features in the application of difficult to handle samples with inexpensive equipment (Gilbert-López et al., 2009). The main issue in method development for SPE lies on the selection of the sorbent material and the elution protocol. As commented above widely reported is the combination of preliminary L-L partitioning clean-up prior to SPE in order to avoid saturation of the material capacity. Methods using combined clean-up steps based on GPC and SPE (Hayward & Wong, 2009, Jones 1996) or sequential SPE (Chen et al., 2009, Jiménez et al., 2004b) are often suggested for better GC maintenance and improved sensitivity. Early methods were focused on the use of silica gel, alumina and Florisil columns but currently there are a wide variety of polymeric phases, carbon based, basic and acidic sorbents or even a combination of phases for particular necessities. A validated multiresidue method was developed for the analysis of a selected group of LC and GC amenable pesticides in lemon essential oils using SPE on Florisil cartridges. The vast majority of terpenic oil is selectively removed in a rinsing step using pentane while elution of the pesticides is performed with CH_2Cl_2 (Barrek et al., 2003). Several applications were developed for the residue analysis of lipophilic insecticides and acaricides in beeswax. In this case, the preferred strategy is based on the retention of waxes in the sorbent whereas pesticides are eluted. Methodologies using Florisil (Adamczyk et al., 2007, Frison et al., 1999, Tsigouri et al., 2000, Tsigouri et al., 2004), C_{18} (Chauzat & Faucon, 2007, Jiménez et al., 2004a, Kamel & Al-Ghamdi, 2006) also combined with HLB (Jiménez et al. 2004b) were reported. Selective eluting step is carried out using non-polar mixtures (n-hexane:diethylether mixtures) to polar ones (based on MeOH and MeCN), respectively. A group of 17 OCs pesticides was determined in raw propolis after L-L extraction in n-hexane:acetone (1:1) mixture with further double column series SPE on graphitized carbon and Florisil with 6mL elution of EtAc:n-hexane (2:8) mixture and GC-ECD (Chen et al., 2009). Perez-Parada et al., (2011) proposed the use of final SiO_2 removal of polyphenolic interferents for increased maintenance of the equipment in OPs routine analysis.

Most relevant works in this matter addresses the use SPE for the clean-up of medicinal plants extracts. OCs residues were determined in three different leafy plants harvested in Brazil using a mixed cartridge containing Florisil and silica-gel and performing elution with n-hexane:CH$_2$Cl$_2$ mixture (Rodrigues et al., 2007). Combined salting-out MeCN extraction and SPE based multiresidue determination of pesticides in Ginseng root was firstly introduced for the analysis of 18 insecticides and fungicides by GC-NPD/ECD determination (Park et al., 2007). Compulsory for selective detection was the Florisil based clean-up in which the elution was performed by hexane:acetone (8:2) solvent.

Large scale methods with MS detection are currently the top choice for advanced residue surveys. Nevertheless, these multiresidue methods with increased scope of analytes need to be able to selectively eliminate most in interferents while quantities of pesticides are obtained in the final extract. Initially, 102 multiclass residue determination of medicinal plant extracts was stated using GPC clean-up with further Envicarb based SPE. Residues were determined by GC-MS after elution from the cartridge with acetone:EtAc:hexane (1:2:1) solvent (Huang et al., 2007). In addition, a 170 multiclass method containing PSA-GCB based SPE column was developed for final purification of GPC extracts. The combined capacity of GPC for co-extractives removal is complemented with this further clean-up. Quantitative elution of most pesticides was accomplished using a EtAc:toluene (3:1) mixture (Hayward & Wong, 2009).

4.1.2.2 Matrix solid-phase dispersion

Matrix solid phase dispersion (MSPD) is a procedure usually applied for sample treatment in a variety of matrices. This procedure often referred as a modified SPE, is based on the mechanical disruption of the sample in a proper sorbent material with a mortar and a pestle. Sample homogenization, extraction and clean-up can be accomplished simultaneously by using relatively small sample size, low solvent consumption and minimum amount of sorbent phase. After blending, the sorbent material is packed into a column and the analytes are eluted using a suitable eluting solvent. However, the selection of the experimental conditions is critical in the selective extraction and purification of the sample extract (Barker 2007).

Many MSPD procedures uses co-columns, which is the use of packed sorbent with complementary features of the disrupting phase at the bottom of the column in order to obtain exhaustive removal of interferents. Hence, the selection of proper dispersant phase plus elution volume is mandatory to achieve enhanced extraction of the matrix while giving purified extracts for quantitative analysis of pesticides. The use of co-columns has improved the applicability of MSPD by increasing the versatility of the purification step.

MSPD can also be used for extracting analytes from both solid and liquid samples. The potential of this strategy in tricky matrices is relatively new in the literature. A procedure for the determination of buprofezin, tetradifon, vinclozolin and bifenthrin in raw propolis was reported using MSPD over silica gel and Florsil co-colum cleanup by elution with CH$_2$Cl$_2$:EtAc (9:1) mixture and GC-MS determination (Santana dos Santos et al., 2008). Analysis of propolis extracts is a challenging issue because of the high polyphenolic content of this matrix. The appropriate selection of conditions for enhanced extraction of polyphenols in propolis tinctures was recently stated by Pérez-Parada et al., (2011.) Popolis tinctures were dispersed on anhydrous Al$_2$(SO$_4$)$_3$ using a MSPD approach with Florisil co-colum clean-up by performing elution with CH$_2$Cl$_2$:EtAc (9:1) solvent for the determination of coumaphos, ethion and chlorpyriphos.

On the other hand, various authors have used MSPD for the analysis of pesticides residues in medicinal plants. Firstly, Zuin et al., (2003b) reported the determination of 7 OCs and 4 OPs in *Passiflora L.* leaves using a MSPD on Florisil with a Na_2SO_4 co-column to remove wet by eluting with a n-hexane:EtAc (7:1) mixture and GC-ECD determination. Method performance was evaluated and compared to that described in EP. Moreover, matalaxyl, triadimefon, paclobutrazol, vinclozolin, tebuconazole and fenatimol residues were determined by GC-NPD in *Isatis indoctica* and *Paeonia lactifora* herbs using MSPD on SiO_2 and Na_2SO_4 co-column by eluting with acetone (Tang et al., 2006). Similarly, HCH isomers were determined in *Withania somnifera* and *Ocimum sanctum* by GC-ECD using using Florisil MSPD and $MgSO_4$-NaCl co-column by eluting with n-hexane:EtAC (7:3) mixture (Abhilash et al., 2007, Abhilash et al., 2009, Abhilash & Singh, 2008). Analytical features were in the same line to those official methodologies but with more flexibility to work. Other authors have determined acephate, chlorpropham, pyrimicarb, bifenthrin, tetradifon and phosalone residues in *Cordia salicifolia* leaves by using neutral alumina and peat as dispersant phases plus Na_2SO_4 and C_{18} co-columns and eluting with cyclohexane:CH_2Cl_2 and GC-MS determination (de Carvalho et al., 2009a, de Carvalho et al., 2010). In addition, Qi (2010) proposed sonicated MSPD approach on Florisil sorbent and EtAC:hexane (7:3) elution to determine pentachloronitrobencene, pentachloroaniline, methylpentachlorophenyl sulphide and procymidone in Ginseng extracts by GC-ECD. On the other hand, Pérez et al., (2010) reported the determination of a variety of OCs, OPs and pyrethoids in lanolin using MSPD on C_{18} plus alumina co-column with elution performed by MeCN:n-hexane saturated and further GC-ECD/FPD determination. Method performance was also evaluated to that attainable in USP and EP without significant loss of reliability.

Finally, the applicability of modern MSPD sorbents was assayed for the quantitative residue determination of bifenthrin, tetradifon and phosalone in *Cordia salicifolia* leaves using the two dimensional DPA (di-2-pyr-idylamine) coordination polymer (∞[Gd(DPA)(HDPA)]). Purified extracts were obtained after elution with acetone:petroleoum ether (5:3) while residues were determined by GC-MS (de Carvalho et al., 2009b)

4.1.2.3 Ultrasonic and microwave assisted extraction

A straightforward approach on LPE based extractions is the employment of waves for rapid and enhanced extraction of analytes from solid matrices. Ultrasonic wave assisted extraction (UAE) was reported for the pesticide residue analysis in different medicinal plants materials. Determination of 18 different OPs from *Flos lonicera* herbal material was performed by using UAE extraction and further SPE and GC-FPD analysis (Xiang et al., 2006). Furthermore, UAE in acetone:petroleum ether (5:3) solvent mixture was proposed for rapid extraction of 15 fungicides from *Isatis indigotica* herb and granule formulation. Subsequent clean-up to obtain proper extracts was performed by using L-L extraction in n-hexane (Tang et al., 2005). GC-ECD residue analysis of 20 multiclass pesticides (OCs, OPs, pyrethoids and fungicides) in five different traditional Chinese medicines using UAE in acetone:CH_2Cl_2 (2:1) mixture with subsequent SiO_2 column chromatography clean-up was recently reported (Qian et al. 2010). Most relevant improvement of this strategy was recently reported by Wang et al. (2011) for large scale determination of 195 multiclass pesticides in different traditional Chinese herbs using UAE in acetone with further GPC clean-up and GC-MS determination.

Microwave assisted extraction (MAE) was also employed for extracting pesticides in medicinal plants. Water was used as extracting solvent for MAE of 7 OCs in Chinese herbs

as reported Ho & Hsieh, (2001) with further SPME (solid phase microextraction) and GC-ECD determination. Moreover, a rapid strategy was proposed employing MAE in ethanol for the extraction of 16 OPs in 4 different Chinese herbs with posterior dispersive SPE based PSA clean-up and GC-FPD determination (Wan et al., 2010).

4.1.2.4 Supercritical fluid extraction

Quantitative extraction of pesticides using supercritical fluid extraction (SFE) was less reported in the last years principally due to the need of high cost instrumentation and the difficulties in method development concerning the optimization of the extraction conditions. This methodology is based on the modification of a fluid extractability at the supercritical state by performing variation cycles of temperature and pressure. Main advantages are the use of non-toxic, non-flammable and inexpensive fluids, principally CO_2 (Gilbert-López et al., 2009). Firstly, SFE with CO_2 was assayed to extract a variety of OCs, OPs and pyrethroids from raw wool wax by GC-ECD/TCD determination. Extraction and clean-up was performed simultaneously with good recoveries and repeatability (85-108 %, RSD <8 %) (Jones, 1997). Fist attempts in the extraction and clean up in phytopharmaceuticals by SFE, were made for OCs and OPs pesticide residues in camomile using CO_2 as supercritical fluid and GC-ECD/FPD determination and GC-MS confirmation of residues (Carisano & Rovida, 1995). Other authors reported SFE for residue extraction of a variety of OCs and OPs pesticides in *Passiflora L.* and *Angelica sinensis* leaves by GC-ECD and GC-FPD (Zhao et al., 2002, Zuin et al., 2003a)

4.1.2.5 Accelerated solvent extraction

Pressurized liquid extraction (PLE), so-called accelerated solvent extraction (ASE) is a solvent based methodology working under elevated temperatures (50–200 ºC) and pressure (500–3000 psi) conditions for short time periods (5–10min). Tipically, a solid sample is packed into the extraction cell and analytes are extracted from the matrix with conventional low-boiling solvents or solvent mixtures at elevated temperatures up to 200 ◦C and pressure (30–200 atm) to maintain the solvent in the liquid state. A very interesting feature of this technique is the possibility of full automation and many samples can be extracted sequentially with good repeatability (Gilbert-López et al., 2009). However, the extraction efficiency of ASE is dramatically influenced by the extraction pressure and temperature conditions as well as the nature of the sample and its water content. Therefore, the extraction behaviour of ASE is not plain and the optimization of operating conditions is laborious. Fifteen OPs pesticide residues were extracted from Ginkgo leaves by using ASE in MeCN. Extracts for proper GC-FPD determination were cleaned-up by SPE cartridges (Yi & Lu, 2005). Moreover, also reported was ASE for routine extraction of 74 multiclass pesticides in six traditional Chinese herbs by LC-MS (Mao et al., 2010).

4.1.2.6 Solid phase microextraction and stir bar sorptive extraction

Solid phase microextraction (SPME) and stir bar sorptive extraction (SBSE) are solvent free methodologies applied for GC-amenable compounds in liquid samples. Interesting capabilities of these techniques derives from the integration of extraction, pre-concentration and injection of the analytes in the GC inlet by thermal desorption in one single step (Gilbert-López et al., 2009). With this aim, a suspended coated fiber either directly immersed in the liquid sample (normal SPME) or in the headspace (HS-SPME) is introduced in a vial. SPME was successfully assayed in a variety of infusions and extracts. Polydimethylsiloxane (PDMS) coated fibers were reported in the determination of a variety of herbal infusions and

formulations (Hwang & Lee, 2000). HS-SPME was applied for the residue analysis of five pesticides (3OCs and 2OPs) using a PDMS-polyvinylalcohol (PVA) fiber in *Passiflora L.* infusions by GC-ECD determination (Zuin et al., 2004).

SBSE is based on the sorption of the targeted analytes onto a coated stir bar. Solid phase normally used is typically also PDMS material. The analytes are extracted by stirring a magnetic the bar immersed in the aqueous sample and recovered by desorbing the stir bar thermally in the GC inlet. SBSE has successfully been applied to the analysis of 11 different OCs and OPs residues in *Passiflora L.* herbal infusions by GC-ECD/FPD (Bicchi et al., 2003).

4.1.2.7 Dispersive Liquid-Liquid Micro Extraction (DLLME)

Dispersive liquid–liquid microextraction (DLLME) has become a very popular environmentally benign sample-preparation technique, because it is fast, inexpensive, easy to operate with a high enrichment factor and consumes low volume of organic solvent. DLLME is a modified solvent extraction method in which acceptor-to-donor phase ratio is greatly reduced compared with other methods. Its combination with different analytical techniques such GC, HPLC, inductively coupled plasma-optical emission spectrometry (ICP-OES) and electrothermal atomic absorption spectrometry (ETAAS) makes DLLME an intersting tool for contaminant analysis (Rezaee et al., 2010, Fariña et al., 2007).

4.1.2.8 QuEChERS and other means

Since the introduction of the QuEChERS method for fruits and vegetables (Anastassiades et al., 2003a) it has been widely expanded to other matrices principally due to the versatility of a miniaturized solvent based extraction and easy applicability for most laboratories. This fit-for-purpose procedure start with a liquid partitioning or salting-out of MeCN:water followed by a dispersive SPE (dSPE) clean-up step in dedicated sorbents (i.e. primary secondary amine (PSA), graphitized carbon black (GCB)) which is applied depending on the nature of the matrix and requirements for removal of interferents. Main advantages are the low solvent consumption and cost-effectiveness. Several modifications of the original method has been successfully introduced ranging from the modification of the salts, used extraction solvents and dSPE sorbents depending on the nature of the matrix and the targeted species. Few reports were recently stated for the QuEChERS method in these matrices. Firstly, a procedure for American and Asian dried Ginseng residue analysis was described (Wong et al., 2007).

Wu et al., (2011) has proposed QuEChERS sample preparation for the residue analysis of 15 OCs pesticides in American Ginseng root by GC-ECD determination. Excellent extraction rates were obtained with recoveries ranged from 81.4-95.2 % and RSD < 8 %. Major improvement was reported for the high throughput residue analysis in Ginkgo leaves of 81 multiclass pesticides in agreement to those widely accepted method performance requirements stated by DG SANCO (2009) guidelines. Of special interest was the recent proposal of Wong et al., (2010) for the residue analysis of 168 pesticides in Ginseng powder using MeCN o acetone based extraction. The inclusion of C_8 based dSPE step prior to the GCB+PSA dispersive clean-up, showed to be straightforward, relatively inexpensive and fast. Moreover, analytical features were similar to those attained in GPC based ones (Hayward & Wong, 2009). More recently, other miniaturized large scale analytical method based in the same scheme was reported by Nguyen et al., (2010) for the determination of 234 pesticides in Korean herbs, while Mullin et al., (2010) reported the determination of 121 pesticides residues in beeswax using a modified QuEChERS strategy for partitioning of the

wax material between of MeCN:H$_2$O. Widespread use of QuEChERS based methods for pesticide multiresidue analysis in these matrices is expected in a short term.

4.2 Separation sciences and detection techniques
4.2.1 Gas chromatography

In accordance to official methodologies, capillary GC is the most used separation technique in residue analysis of non-polar and semi-polar pesticides for this kind of matrices. Major attention has paid target determination of OCs, OPs and pyrethroids GC-amenable compounds. As discussed previously, most reports focuses on selective detection of pesticides using ECD, NPD, TCD and FPD detectors. The increased selectivity of FPD allowed direct injection of diluted citrus essential oil with proper internal standard as previously assayed for the determination of OPs residues without the use of clean-up steps (Dellacassa et al., 1999, Di Bella et al., 2004, Saitta et al., 1997). ECD is described as more sensitive than the rest of selective detectors although the lack of specificity needs to be companied with exhaustive clean-up steps. Evidence when performing determination of OCs in citrus essential oil by GC–ECD, was the required silica gel column chromatography for the removal of polyphenolic interferents (Di Bella et al., 2004, Saitta et al., 2000).

Most recent application of ECD still relies on the sensitive detection of OCs. ECD was recently described for the successful determination of variety of OCs, and halogenated OPs, pyrethroids or fungicides using different clean-up steps in several matrices.

Using hydrolysis and derivatization with heptafluorobutyric anhydride (HFBA), total amitraz residues were determined in beeswax with LODs < 0.08 mg/kg by GC–ECD (Jiménez et al., 2004a).

On the other hand, SPE is by far the most reported clean-up strategy prior to GC–ECD analysis. Fluvalinate residues were determined in beeswax by ECD after Florisil SPE (Tsigouri et al., 2000). Regarding medicinal plants, GC–ECD determination was widely used for the determination of HCH isomers with LODs at the ppb level (Abhilash et al., 2007, Abhilash et al., 2009, Abhilash & Singh, 2008). Twenty multiclass pesticides were also determined in variety of medicinal plants using this detection technique (Qian et al., 2010). Pentachloronitrobence, pentachloroaniline, methylpentachlorophenyl sulphide and procymidone residues were determined in Ginseng extracts by μECD with LODs at sub ppb level. Also QuEChERS sample treatment with GC–ECD analysis was used for the determination of 15 OCs in Ginseng (Wu at al., 2011).

Adequate selectivity attained with FPD operated in the phosphorous mode is often referred for proper determination of OPs pesticides. Most relevant report was described by Wong et al., (2007) for the determination of 108 OPs in Ginseng root; similar quantitative performance plus lower LODs for troublesome compounds than when applying GC–MS determination were obtained. Advances in carbamate determination was stated for metalocarb, isoporocarb, fenobucarb, carbofuran, pirimicarb and carbaryl analysis in traditional Chinese herbs by GC–NPD obtaining LOQs ≤ 0.05 mg/kg(Wu et al. ,2005). Moreover, NPD was described for the multiclass determination of metalaxyl, triadimefon, paclobutrazol, vinclozolin, tebuconazole and fenatimol (Tang et al,. 2006).

Dual determination of residues using ECD/FPD was applied to medicinal plants (Tang et al., 2005, Zuin et al., 2003a), lanolin (Pérez et al., 2010) and propolis tinctures (Pérez-Parada et al,. 2011) whereas ECD/NPD was also reported for Ginseng root (Park et al., 2007) and beeswax (Kamel & Al-Ghamdi, 2006).

The use of sample preparation should be considered together with the accessible detection technique. Generally, the most sensitive and selective detection is used; the more generic sample preparation would be required. However the sample features are of major importance independently of the determination technique as exposed for highly polyphenolic content matrices. As an example, exhaustive clean-up using SPE series was mandatory for the determination of OCs pesticides in raw propolis by GC–ECD (Chen et al., 2009). Selective features of ECD for the determination of OCs are not efficiently replaced when using other MS based detectors; therefore it is widely being used in laboratories dealing with the determination of OCs in Ginseng root as reported for proficiency testing results in Asia Pacific region (Chan et al., 2010).

In spite of the increased sensitivity of ECD for the determination of organohalogen pesticides, or increased selectivity of FPD and NPD, the use of MS is being compulsory introduced in order to obtain reliable identification and confirmation of residues. Commonly used in routine analysis, is to combine selective detection with MS confirmation. In this sense, our group has used combined ECD and FPD for the determination of OCs, OPs and pyrethroids in lanolin whereas confirmation of residues was carried out by GC–MS (Pérez et al. 2010). After selective detection, confirmation of residues by GC–MS was also described in citrus essential oils (Saitta et al., 2000), medicinal plants (Quan et al., 2004) and beeswax (Kamel & Al-Ghamdi, 2006).

Concerning GC–MS, fingerprinting features of EI (electron impact) mass spectra coupled to commercially available mass spectral libraries is widely used for identification purposes of small organic molecules. Nevertheless, this approach is insufficient when assessing residues due to the loss of ion specificity from the co-eluted matrix. The approach of enhanced selectivity of the selected ion monitoring (SIM) mode of single quadrupole (Q-MS) mass spectrometer results in improved sensitivity and is widely used in the determination of residues as seen in Table 2. In the same line, EI has been used as ionization technique in almost all of the studies described in these matrices although few reports based on chemical ionization (CI) were reported. Increased selectivity and enhanced detectability is achieved when using negative chemical ionization (NCI) for electron-capturing compounds (mainly organohalogens) because the co-eluted matrix does not efficiently ionize. Consequently, few ions with high abundance are usually observed in the relevant mass spectrum resulting in improved sensitivity. NCI was recently used for the analysis of 56 multiclass pesticides (Tagami et al., 2008) as well as in 10 pyrethoids residues (Tagami et al., 2006) and 10 OCs (Guo et al., 2010) in herbal medicines. The advent of MS has opened new perspectives in order to increase the scope of targeted analytes and reliability of the findings. EI-GC–(Q)MS working in the SIM mode was employed in the determination of 12 GC-amenable pesticide residues in lemon essential oil (Barrek et al. 2003).

Regarding bee's byproducts, Jiménez et al. (2005) and Santana dos Santos et al. (2008) have determined by GC–MS pesticide residues in beeswax and raw propolis, respectively. Significant attention in large scale methods using GC–(SIM)MS was recently described for the target trace analysis of 54 (Miao el al., 2010), 81 (Zhou et al., 2011), 102 (Huang et al., 2007), 108 (Wong et al., 2007), 168 (Wong et al., 2010), 170 (Hayward & Wong, 2009), 195 (Wang et al., 2011) and 234 (Nguyen et al., 2010) pesticides in plant matrices with medicinal use.

Less described in the literature is the use of advanced GC–MS techniques although some improvements were made in the last years. Major advantages of modern MS configurations, both full scan MS or MS/MS over conventional MS, are the increased specificity and

sensitivity to quantify residues principally when dealing with concentrations approaching the detection limit. Furthermore, performance in terms of selectivity with confirmation capability is improved remarkably when MS/MS is used. Multiple reaction monitoring mode of triple quadruple (QqQ) MS/MS is currently the more appropriate determination technique for target analysis since it achieves lower LODs but also offers high dynamic ranges (almost four orders of magnitude). Selected qualifiers and quantifiers transitions ions are focused on quantitative and qualitative purposes respectively. Firstly, GC–(QqQ)MS/MS was employed for the determination of 18 pesticides (acaricides, insecticides and fungicides) in beeswax (Chauzat & Faucon, 2007). Other authors, have recently introduced comparative studies of multiresidue determination performance by GC–MS working in SIM mode and GC–(QqQ)MS/MS in Ginseng powders (Wong et al., 2010). Improved reliability in confirmation and sensitivity was stated which even allowed dilution of the extracts for better maintenance of the instrument but also decreasing the matrix effect and improving accuracy of results. On the other hand, pentachloronitrobenzene and its metabolites (pentachloroaniline and pentachloroanisole) were determined by GC–quadrupole-ion trap (QIT)MS/MS configuration (Li et al., 2009).

Target analysis is frequently referred to nominal Q-MS and QqQMS/MS (less often to ion traps) in which the need to select ions (SIM) or transitions limits the number of compounds that can be analyzed. Time-of-flight (TOF) analyzers have overcome these limitations thanks to the ability of accurate mass measurements of the ions by offering resolution powers at full width al half medium (FWHM) in the 10,000-20,000 range. These instruments are typically mentioned as high resolution mass spectrometers (HRMS) which have recently found application in this topic. Mass accuracy with mass deviation errors < 5 ppm, permits the elucidation of the elemental composition of the ions by the coupling to an adequate software tool. Identification and confirmation capability of the compounds via parent and fragment ions recognition is highly improved. A comparative study was carried out using two different GC–MS instruments such as classical Q-MS operated in the SIM mode and TOF-MS for the analysis of 170 organohalogen and OPs pesticides (Hayward & Wong, 2009). In this case, although no significant differences were found in quantitative results, identification of incurred troublesome pesticides was only unambiguous when using accurate mass measurements provided by TOF-MS.

Compounds	Matrix	Sample treatment and clean-up step	Determination	Recovery rates (%) (RSD (%))	Analytical features	Reference
9 OPa, 10 OCs, 4 Pys	Lanolin	MSPD on C_{18} plus neutral alumina co-column. Elution MeCN saturated in n-hexane	GC-ECD/FPD GC-MS confirmation	83-118 % (<20 %)	LOQs OPs ≤ 0.070 mg kg^{-1} LOQs OCs & Py ≤0.087 mg kg^{-1}	(Pérez et al., 2010)
Simazine and cypermethrin	Orange essential oil	Extraction in methanolic phosphate buffer for simazine. Cypermethrin was extracted in hexane-acetonitrile partitioning followed by silica SPE	ELISA	-	LOQs simazine ≤ 0.04 mg kg^{-1} LOQs cypermethrin ≤ 0.5 mg kg^{-1}	(Nichkova et al., 2009)

Compounds	Matrix	Sample treatment and clean-up step	Determination	Recovery rates (%) (RSD (%))	Analytical features	Reference
22 Multi-class (12 GC amenable; 10 LC amenable) pesticides	Lemon essential oil	SPE (C$_{18}$ and Florisil). Pentane rinsing, elution with CH$_2$Cl$_2$	LC-(Q)MS and GC-MS	57-114.6 % (<6.6 %)	LC amenables LODs ≤ 0.06 mg L^{-1} GC amenables LODs ≤ 0.4 mg L^{-1}	(Barrek et al., 2003)
70 Multi-class pesticides	Lavandin essential oil	10 fold dilution and direct injection	LC-(QTRAP)MS/MS	-	LOQs: ≤1 µg/L for 9 pesticides, ≤5 µg/L for 44, ≤10 µg/L for 9, and ≤20 µg/L for 5	(Fillâtre et al., 2011)
Synthetic acaricides (fluvalinate, coumaphos, bromopropylate and its metabolite 4,4'-dibromobenzophenone)	Beeswax	Dissolution in isooctane. SPE (Florisil) clean-up with elution by MeOH	LC-DAD	70-110 % (<15 %)	LODs ≤ 0.2 mg kg^{-1}	(Adamczyk et al., 2007)
16 insecticides and acaricides 2 fungicides	Beeswax	SPE C$_{18}$, elution with MeCN	GC-(QqQ)MS/MS	N.D.	LODs ≤ 0.05 mg kg^{-1}	(Chauzat & Faucon, 2007)
Total amitraz (amitraz and its degradation products)	Beeswax	Dissolution in MeOH and n-hexane L-L extraction. Hydrolysis to 2,4-dimethylaniline (DMA) and derivatization with Heptafluorobutyric anhydride (HFBA)	GC-ECD	~80 % (<15 %)	LOD = 0.08 mg kg^{-1}	(Jiménez et al., 2004a)
Chlorfenvinphos, fluvalinate, amitraz, bromopropylate, acrinathrin, flumethrin, coumaphos, chlorpyrifos, chlordime-form, endosulfan andmalathion	Beeswax	Dissolution in n-hexane plus heating : freezing cycles. Further SPE (Florisil) clean-up with elution by acetone:hexane (1:1)	GC-µECD/NPD/MS	86-108%	LODs ≤ 0.3 mg kg^{-1} (GC-µECD/NPD) and ≤ 0.085 (GC-MS)	(Serra-Bonvehí & Orantes-Bermejo, 2010)
Total amitraz	Beeswax	Hydrolysis in acidic buffer and HS-SPME	GC-ITD	-	LODs = 0.01 mg kg^{-1}	(Leníček et al., 2006)
Up to 15 lipophilic pesticides (including acaricides, PCBs, OPs, Pys and OCs)	Beeswax	A) Dissolution in n-hexane and L-L extraction with MeCN. B) Subsequent SPE in HLB cartridges and additional SPE of C$_{18}$.	A) GC-MS (SIM) B) GC-ECD	A) 93-108 % (<16 %) B) 94-107 % (<20 %)	A) LOQs ≤ 0.065 mg kg^{-1} B) LOQs ≤ 0.12 mg kg^{-1}	(Jiménez et al., 2005, Jiménez et al., 2004b)

Compounds	Matrix	Sample treatment and clean-up step	Determination	Recovery rates (%) (RSD (%))	Analytical features	Reference
121 pesticides	Beeswax	Modified QuEChERS: addition of MeCN:H$_2$O, NaAc, MgSO$_4$. Organic phase is clean-up by dSPE using PSA, C18 and MgSO$_4$. For GC further SPE in GCB+PSA was used eluting with acetone:toluene (7:3).	LC- (Q)MS/MS and GC-MS (SIM)	-	LOD ≤ 0.03 mg kg^{-1}	(Mullin et al., 2010)
Flumethrin, tau-fluvalinate, coumaphos, and amitraz	Beeswax	Dissolution in MeCN and SPE of C$_{18}$.	GC-NPD/ECD GC-MS	90-102 %	B) LODs ≤ 0.05 mg kg^{-1}	(Kamel & Al-Ghamdi, 2006)
Fluvalinate	Beeswax	Dissolution in n-hexane, and the solutions is sonicated and transferred to Extrelut columns. The fluvalinate was extracted with MeCN, and a portion of the extract was cleaned-up on a Florisil cartridge. Elution with diethyl ether-n-hexane (1:1)	GC-ECD	77.4 to 87.3 (<8.31 %)	LOQs = 0.1 mg kg^{-1}	(Tsigouri et al., 2000)
Coumaphos, ethion and chlorpyriphos	Propolis tinctures	MSPD on Al$_2$(SO$_4$)$_3$ and Florisil co-column. Elution with CH$_2$Cl$_2$:EtAc (9:1). Subsequent silica gel clean-up eluting with. CH$_2$Cl$_2$	GC-FPD and GC-MS confirmation	87.4 -115.0 % (<12.8 %)	LODs ≤ 0.0143 mg kg^{-1}	(Pérez-Parada et al., 2011)
17 OCs	Raw propolis	L-L extraction n-hexane-acetone (1:1) plus tandem SPE graphitized carbon and Florisil cartridge clean-up with elution by EtAc and hexane (2:8)	GC-ECD	62.6-109.6 (<9.4 %)	LODs ≤ 0.038 mg kg^{-1}	(Chen et al., 2009)
Buprofezin, tetradifon, vinclozolin, and bifenthrin	Raw propolis	MSPD on SiO$_2$ and Florisil clean-up. Elution with CH$_2$Cl$_2$:EtAc (9:1).	GC-MS (SIM)	67-175 % (<12.1 %)	LOQs ≤ 0.25 mg kg^{-1}	(Santana dos Santos et al., 2008)
7 OCs	Radix et Rhizoma Rhei	SiO$_2$ hollow fiber sorptive microextraction	GC-MS (SIM)	63-115 %- (<10 %)	-	(Li et al., 2010)
16 OPs	4 Chinese herbs	MAE in ethanol and dSPE in PSA	GC-FPD	73.8–123% (<15.2 %)	LODs ≤ 0.09 mg kg^{-1}	(Wan et al., 2010)

Compounds	Matrix	Sample treatment and clean-up step	Determination	Recovery rates (%) (RSD (%))	Analytical features	Reference
20 OCs	Herba epimedii	Maceration in EtAc and further SPE in Florisil+Na_2SO_4 clean-up. Elution with diethylether: hexane (15:85).	GC-MS (SIM)	75.4-90.7 % (<18.3%)	LODs ≤ 0.055 mg kg^{-1}	(Guo et al., 2010)
7 OCs 4 OPs	Passiflora L. leaves	MSPD on Florisil. Co-column of neutral alumina and Na_2SO_4. Elution with n-hexane:EtAc (7:1)	GC-ECD	75.2-110.6 (<17.4 %)	LODs ≤ 0.038 mg kg^{-1}	(Zuin et al., 2003b)
A) 7 OCs & 6 OPs B) 12 OCs	A) Passiflora L. leaves B) Angelica sinensis leaves	SFE using CO_2 as mobile phase	A) GC-ECD/FPD B) GC-ECD	A) 69.8-107.1 (<14.7 %) B) 83.3-98.1 (<6.1 %)	A) LODs ≤ 0.014 mg kg^{-1} B) LODs ≤ 0.010 mg kg^{-1}	A) (Zuin et al., 2003a) B) (Zhao et al., 2002)
3 OCs 2OPs	Passiflora L. infusions	Headspace solid-phase microextraction (HS-SPME) with polydimethylsiloxane- poly(vinyl alcohol) (PDMS/PVA) fiber	GC-ECD	78.7-91.5 (< 14.2 %)	LODs ≤ 0.015 mg L^{-1}	(Zuin et al., 2004)
102 multi-class pesticides	Traditional Chinese herbal infusions	L-L extraction acetone:EtAc:hexane (1:2:1) plus GPC and SPE (Envicarb) elution with the same solvent mixture	GC-MS (SIM)	59.7-120.9 ((< 20.8 %)	LOQs ≤ 2.5 mg L^{-1}	(Huang et al., 2007)
15 OPs	Ginkgo leaves	ASE (accelerated solvent extraction)in MeCN and further SPE (Envicarb) eluting with MeCN:toluene (3:1)	GC-FPD	95.2 % (< 4.6 %)	LODs ≤ 0.044 mg kg^{-1}	(Yi & Lu, 2005)
18 OPs	Flos lonicerae	Ultrasonic wave assistant extraction (UAE) in acetone and further SPE clean-up.	GC-FPD	83.6-88.7 % (< 6.0 %)	LODs ≤ 0.018 mg kg^{-1}	(Xiang et al., 2006)
6 Carbamates (metolcarb, isoprocarb, fenobucarb, carbofuran, pirimicarb, and carbaryl)	Traditional Chinese herbs	Soxhlet extraction with CH_2Cl_2	GC-NPD (SIM)	80.8-154.6	LOQs ≤ 0.05 mg kg^{-1}	(Wu et al., 2005)
Multi-class 195 pesticides	Traditional Chinese herbs	UAE in acetone and further GPC clean-up.	GC-MS (SIM)	Normally 80-120 %	LOQs ≤ 0.05 mg kg^{-1}	(Wang et al., 2011)

Compounds	Matrix	Sample treatment and clean-up step	Determination	Recovery rates (%) (RSD (%))	Analytical features	Reference
Acephate, chlorpropham, pirimicarb, bifenthrin, tetradifon, and phosalone	Cordia salicifolia	MSPD using the 2 dimensional coordination polymer (∞[Gd(DPA)(HDPA)])	GC-MS (SIM)	20-107.7 % (< 29.1 %)	LOQs ≤ 0.25 mg kg^{-1}	(de Carvalho et al., 2009b)
Multi-class 15 pesticides (OCs, OPs, Pys and fungicides)	Isatis indigotica raw material, granule formuation and infusion	Twice UAE extraction in acetone: petroleum ether (5:3) mixture and L-L clean-up with hexane for raw material or granule. Infusion L-L extraction with petroleum ether.	GC-ECD/FPD	70.2-119.5% for raw material, 73.2-105.1% for granule formulation, and 72.8-113.3% for infusion formulation	LODs ≤ 0.035 mg kg^{-1}	(Tang et al., 2005)
6 multi-class (metalaxyl, triadimefon, and paclobutrazol, vinclozolin, tebuconazole, fenatimol)	Isatis indigotica Fort and Paeonia lactiflora	MSPD on silica gel. Packing on a column with Na$_2$SO$_4$ co-column. Elution with acetone.	GC-NPD	80.6-106.1% (< 17.7 %)	LOQs ≤ 0.05 mg kg^{-1}	(Tang et al., 2006)
A) 9 OCs B) 19 OCs	A) Plant infusions B) Chinese herbal formulations	SPME with PDMS coated fiber	GC-MS (SIM)	A) 90-108 % (< 17.0 %) B) – (< 31.0 %)	A) LOQs ≤ 0.012 mg kg^{-1} B) LODs ≤ 0.001 mg kg^{-1}	A) (Rodrigues et al., 2005) B) (Hwang & Lee, 2000)
7 OCs	Atractylodis rhizoma, Glycyrrhizae radix and poria	MAE in water and SPME with PDMS coated fiber	GC-ECD	-	LODs ≤ 0.00013 mg kg^{-1}	(Ho & Hsieh, 2001)
11 pesticides (hexachlorobenzene, lindane, chlorothalonil, parathion methyl, parathion ethyl, fenitrothion, malathion, dieldrin, α- and β-endosulfan, and tetradifon)	Passiflora L.	SBSE with PDMS coated fiber	GC-ECD/FPD	30-90 %	B) LOQs ≤ 0.117 mg kg^{-1}	Bicchi et al., 2003
9 OCs	Mikania laevigata, Maytenus ilicifolia and Cordia	Solid-liquid extraction (SLE) with n-hexane: CH$_2$Cl$_2$ (4:1), followed by clean-up in solid phase mixed	GC-MS (SIM)	70-124 % (< 7.3%)	LOQs ≤ 0.03 mg kg^{-1}	(Rodrigues et al., 2007)

Compounds	Matrix	Sample treatment and clean-up step	Determination	Recovery rates (%) (RSD (%))	Analytical features	Reference
	verbe-nacea	cartridge (Florisil and silica-gel) eluting with n-hexane- CH$_2$Cl$_2$ (3:2)				
A) 9 OCs B) 18 multi-class (fungicides, insecticides)	Ginseng root	A) Soxhlet extraction with acetone: petroleum ether (3:1) and cleanup with 5 mL H$_2$SO$_4$. The supernatant is separated. B) Chopped into small portions, add MeCN and NaCl and centrifugation for L-L extraction. The organic phase is separated, concentrated and directly injected for GC-NPD. Further clean-up is carried out using SPE (Florisil) elution with hexane:acetone (8:2) for GC-ECD.	A)GC-ECD and GC-MS confirmation B) GC-ECD/NPD	A) - B) 72.3-117.2 % (< 7.3%)	A) - B) LOQs ≤ 0.2 mg kg^{-1}	A) (Quan et al., 2004) B) (Park et al., 2007)
5 OCs (hexachlorobenzene and hexachlorocyclohexanes (α-, β-, δ-, and γ-isomers))	Ginseng root	Soxhlet with EtAc: petroleum ether (7:3) mixture. H2SO4 cc. digestion and the supernatant analyzed	Isotope dilution GC-MS (SIM)	-	Repeatability ≤ 1.4 % Expanded relative uncertainty ranging from 4.0-6.5 %	(Chan et al., 2007)
170 multi-class (OPs and OCs)	Ginseng root	S-L extraction with EtAc and combined clean-up by GPC plus (PSA)/graphitized carbon black (GCB) SPE column eluting with EtAc:toluene (3:1).	A) GC-(Q)MS (SIM) B) GC-TOF MS (HRMS)	A) Mean = 83; 79; 75 for 25, 100, and 500 μg kg^{-1} levels (< 5.0%) B) Mean = 93; 85; 81 for 25, 100, and 500 μg kg^{-1} levels (< 8.0%)	A) Geometric mean LOQ = 0.004 mg kg^{-1} B) Geometric mean LOQ = 0.003 mg kg^{-1}	(Hayward & Wong, 2009)
168 multi-class (OPs and OCs) pesticides	Ginseng root	S-L extraction with A) MeCN or B) acetone/cyclohexane/EtAc (2:1:1) mixture. Further SPE (C8 + GCB/PSA) sorbents clean up and toluene elution.	1) GC-MS (SIM) 2) GC-MS/MS (QqQ)	86-88 % (< 14.0%)	Geometric mean LOQ A1)=0.053; A2)=0.006; B1)=0.048; B2)=0.007 mg kg^{-1}	(Wong et al., 2010)

Compounds	Matrix	Sample treatment and clean-up step	Determination	Recovery rates (%) (RSD (%))	Analytical features	Reference
20 multi-class (OCs, OPs, Pys and fungicides)	Radix paeoniae, Isatis indigotica Fort, Pltycodon grandiflorum, Cotex mouta and Poria cocos	Powdered and UAE in acetone/ CH_2Cl_2(2:1) with subsequent clen-up on silica gel column chromatography eluting with ether:acetone (40:60).	GC-ECD	72.5-113.5 % (< 14.0 %)	LOQs ≤ 0.082 mg kg^{-1}	(Qian et al., 2010)
Pentachloronitrobenzene, pentachloroaniline, methylpentachlorophenylsulphide and procymidone	Ginseng extract	Sonicated MSPD. Dispersion on Florisil and extracted twice in EtAc-hexane (70:30)	GC-µECD	83.5-97.4 % (< 10.0 %)	LODs ≤ 0.004 mg kg^{-1}	(Qi, 2010)
54 multi-class pesticides	6 traditional Chinese herbs	Extraction with MeCN and clean-up on SPE (C18/Envicarb/ PSA).	GC-MS (SIM)	70-120 % (< 20.0 %)	LODs ≤ 0.01 mg kg^{-1}	(Miao et al., 2010)
74 multi-class pesticides	6 traditional Chinese herbs	ASE and combined purification on GPC and SPE.	LC-(QqQ)MS/MS	70-110 % (< 15.0 %)	LODs ≤ 0.01 mg kg^{-1}	(Mao et al., 2010)
Acephate, chlorpropham, pyrimicarb, bifenthrin, tetradifon, and phosalone	Cordia salicifolia leaves	MSPD on A) neutral alumina or B) peat, using Na_2SO_4 and C_{18} co-column.and eluting with cyclohexane:CH_2Cl_2 (3:1).	GC-MS (SIM)	67.7-129.9 % (< 15.0 %) 64-118.0% (< 26.4 %)	LOQs ≤ 0.25 mg kg^{-1}	A) (de Carvalho et al., 2009a) B) (de Carvalho et al., 2010)
4OCs: hexachlorocyclohexanes (α-, β-, δ-, and γ- isomers))	Withania somnifera and Ocimum sanctum	MSPD with Florisil and blended with MgSO4 and NaCl and eluting with f n-hexane:EtAc mixture (70:30).	GC-ECD	93-103 % (< 10%)	LODs ≤ 0.005 mg kg^{-1}	(Abhilash et al., 2007, Abhilash et al., 2009)
Aldrin, endrin, dieldrin and HCH isomers	Withania somniferea, Ocimum sanctum L. and Achyranthes aspera.	Modified EP method: maceration in acetone: CH_2Cl_2 (3:1). Further MSPD (Florisil) eluting with the same solvent mixture.	GC-ECD	88-98 % (< 9.85%)	LODs ≤ 0.001 mg kg^{-1}	(Abhilash & Singh, 2008)
108 OPs pesticides	Ginseng root	QuEChERS based MeCN:water extraction. dSPE with PSA:GCB	1) GC-MS (SIM) 2) GC-FPD	> 90 % for most compounds (< 37 %)	1) LODs ≤ 0.50 mg kg^{-1} 2) LODs ≤ 0.05 mg kg^{-1}	(Wong et al., 2007)

Compounds	Matrix	Sample treatment and clean-up step	Determination	Recovery rates (%) (RSD (%))	Analytical features	Reference
15 OCs	American Ginseng	QuEChERS based MeCN:water. dSPE with PSA:GCB	GC-ECD	81.4-95.2 (<8%)	-	(Wu et al., 2011)
234 pesticides	Korean herbs	QuEChERS based MeCN(HAc):water extraction. dSPE with PSA:GCB	GC-MS (SIM)	62-119 % (<21%)	1) LODs ≤ 0.40 mg kg⁻¹	(Nguyen et al., 2010)
81 multiclass pesticides	Ginkgo leaves	QuEChERS based MeCN:water. dSPE with PSA:GCB	GC-MS (SIM)	70-110 % for most compounds (<20%)	LOQs ≤ 0.058 mg kg⁻¹	(Zhou et al., 2011)

Table 2. Analytical features of most recent reports in pesticide residue analysis in pharmaceutical and medicinal plants.

4.2.2 Liquid chromatography

The trend, in which these matrices were not excluded, was the introduction of non-persistent and biodegradable pesticides which have lead to the introduction of more polar (and less volatile) agrochemicals. In agreement to food and environmental matrices, such compounds have prompted the use of LC–MS, which at the moment is widely accepted technique for monitoring purposes of polar and most semi-polar pesticides as well as for regulatory issues. Concerning this matter, few attempts were made in pharmaceutical matrices although major advances are expected in a short term.

LC-DAD was used in beeswax (Adamczyk et al., 2007) and medicinal plants (Choi et al., 2007, Peng et al., 2007, Tuzimski 2011) to determine pesticide residues. Main pitfall is the attained selectivity and sensitivity for proper and unambiguous trace determination.

Due to its versatility for wide variety of organic molecules, electrospray ionization (ESI) operating in positive mode is the preferred interface for most studies that uses LC-MS. In general, ESI can be applied for polar, ionized, and semi-polar analytes if adequate mobile phases are selected. Depending on the polarity, ionization process in ESI usually gives protonated or deprotonated molecules ([M+H]⁺, [M-H]⁻) although adducts can also be obtained (i.e. [M+Na]⁺, [M+NH₄]⁺).

Residues of methamidophos, imidacloprid, benomyl, thiophanate-methyl, bendiocarb, diflubenzuron, chlorpyrifos, flufenoxuron, carbosulfan and bifenthrin were determined in citrus essential oils by using LC–ESI(+)–(Q)MS with LODs ≤0.05 mg/kg (Barrek et al. 2003). The authors noted high matrix effect for the analyzed extracts which cannot be overcome by simple LC−MS but also suggest the use of MS/MS experiments in further studies. Unfortunately, LC−MS/MS has not been reported until recently in the literature for the determination of pesticides residues in this topic. Nevertheless, advances in the application of LC−MS/MS for testing pesticides in pharmaceutical matrices are expected in a short term since the use of ESI sources and conventional QqQ analyzer is currently the most reported technique for target screening of pesticide residues in food and environmental samples.

Several analytical features are provided when using LC−MS/MS ranging from excellent performance for quantitative analysis when working in the selected reaction monitoring (SRM) mode; allowing the selection of two specific SRM transitions with subsequent confirmation of the analyte in the sample. A combined QqQ scanning functionality with a

sensitive linear ion trap (LIT) is offered in the QLIT (quadrupole-linear ion trap) system. Working in the LIT mode, the hybrid QLIT provides improved performance, higher versatility as well as enhanced sensitivity, both in full scan MS (EMS) and product ion (enhanced product ion; EPI) scan modes. Improvements in this hybrid system are also related to software developments as seen in the sSRM (schedule SRM mode) or the combination of SRM and EPI scans by the built-in information dependant acquisition (IDA) software tools used for confirmation purposes of the residues. Direct injection analysis of 10 fold diluted lavandin essential oil was recently reported for the determination of 70 multiclass pesticides using sSRM acquisition mode in a new generation LC−(ESI)−QLIT MS/MS instrument in both positive and negative ionization (Fillâtre et al., 2011). Obtained LODs were all below 20 µg/L. Major technological improvements in QLIT were focused on enhanced sensitivity. Nowadays the impressively low instrumental detection limits (IDLs) are offering new workflows and providing independence from time consuming sample preparation steps. Last but not least, LC−HRMS is expected to find application in large scale screening of contaminants and reliable conformation of residues using accurate mass measurements. An interesting example was stated by Schürmann and co-workers in the correct identification of false positive sebuthylazine residues in tarragon. The situation lies on the fact that nominal LC−MS/MS confirmation is accomplished by the use of 2 specific transitions plus retention time matching with standards. However, sometimes this approach is not possible to apply for troublesome analytes which even could lead to inadequate report of residues. However, it was found that false positive findings can be discarded by the use of resolving power provided by TOF-MS for the identification of the endogenous matrix compounds (Schürmann et al., 2009).

4.2.3 Matrix effect

Matrix effect is considered to be a suppression or enhancement of the analyte response due to the influence of the matrix. However, when dealing with MS determination, either in GC and LC, matrix effect is normally referred to ion suppression/enhancement. Compensation of the matrix effect is then compulsory for accurate quantitation. Several strategies were employed to reduce the matrix effect such as the use of external calibration, matrix matched calibration, isotopically labeled surrogate compounds, analyte protectants (APs) or internal standard addition. Matrix effect can be estimated by comparing the response obtained from the standard solution and that from the spiked sample extracts. Matrix effect may be partially solved before the detection technique for instance by exhaustive clean-up through the reduction of co-extractives, improved separation (comprehensive two dimensional gas chromatography (GCxGC) or ultra high pressure liquid chromatography (UPLC) by using sub 2µm particle size), sample dilution if enough sensitivity is obtained or reduction of the injected sample (Gilbert-López et al., 2009).

In GC this effect was widely investigated for food matrices principally when GC–(SIM)MS was used (Anastassiades et al. 2003b, Poole 2007). On the other hand, in LC this effect is typically faced at the expense of the sensitivity of MS/MS applying sample dilution (Gilbert-López et al. 2009). Among others, in GC, matrix-induced effect is mainly related to the silanol active sites present in the liner as well as in the chromatographic column which might interact with the analyte, resulting in analyte loses and distorted peak shapes (Anastassiades et al. 2003b). Chan and co-workers introduced the use of isotope dilution (ID) for the determination of HCB and HCH isomers by GC–MS in Ginseng root. ID-GC–MS showed to be a good quality assurance approach since inaccuracy and uncertainty were

significantly reduced in a difficult matrix (Chan et al., 2007). However, this strategy is generally expensive, time consuming for routine analysis and is intended for MS only.

The use of matrix matched calibration (MMC) or APs is being increasingly used since most reports still face determination of semi-volatile compounds by GC with different detection techniques. Moreover, note that when using selective and non specific detection such as ECD, matrix effect could involve co-eluted compounds which can increase the noise while negatively affect the peak area and reducing the quantitative performance. Jiménez et al., (2004b) reported MMC for the multiresidue analysis of pesticide residues in beeswax by GC–ECD. Moreover, Pérez et al., (2010) used MMC for the quantitative determination of pesticides in lanolin by GC–ECD/FPD when using MSPD as sample treatment. On the other hand, several authors used MMC in plant origin samples with medicinal purposes by GC–ECD (Zuin et al. 2003), GC–MS (Hayward & Wong 2009, Wong et al. 2007) and even when more sophisticated GC–(QIT)MS/MS (Li et al., 2009) and GC–(QqQ)MS/MS (Wong et al., 2010) were used. It should be highlighted that MMC is not referred in USP and EP.

Since the possibility of not getting reference matrices or representative materials to perform MMC, the use of alternatives such as APs is of great concern. Ideally, APs overcome these limitations by interacting with the active sites and conducting reliability in the response of the analyte. Dealing with real samples the compensation of the matrix effect cannot be solely related to new method developments, since it could be easily applied to official methodologies to obtain more accurate results. Recently, in accordance to food residue analysis, eight different APs were evaluated for GC–MS determination of an extensive group of 195 pesticides in medicinal plants (Wang et al., 2011). Troublesome and early eluting pesticides such as acephate and omethoate were succesfully determined using d-ribonic acid-γ-lactone (2 mg/mL) whereas sorbitol showed the best compensation effect for late eluted compounds such as fenitrothion and methidation. From the results obtained, the authors concluded a mixture of d-ribonic acid-γ-lactone and d-sorbitol for the reliable determination of most pesticides by GC–MS although there is neither an ideal AP nor mixture of APs that can completely resemble these complex matrices in order to compensate the matrix-induced enhancement for such an amount of pesticides (Wang et al. 2011).

The need for sample treatment step in LC–MS determination encompasses the undesired effects of interferents on analytical performance to requested LODs for proper trace analysis of pesticides. In LC–MS instead, matrix effect is usually referred to co-elutants during the ionization process when using atmospheric pressure interfaces (such as ESI) which are prone to ion suppression and enhancement effects. The approach of MMC and sample dilution is widely assayed in LC–MS. A combined sample dilution and MMC strategy was also employed in QLIT-MS/MS determination of 70 pesticide residues in lavandin essential oil. The authors reported that using this approach, weak matrix effect (≤20%) for 70 % of the compounds was obtained (Fillâtre et al. 2011).

5. Conclusions

This chapter has outlined latest improvements concerning pesticide residues in natural products commodities employed as raw materials used in the pharmaceutical industry or for medicinal purposes. Occurrence of pesticide residues is demonstrated to be moving from classical compounds, in which official methodologies still focus their interest, to more specific compounds and crop treatments. As demonstrated, environmental pollution plays an important role in the occurrence of unexpected pesticides, principally for roots materials

or lipophilic matrices such as beeswax. Current occurrence of previously unstudied pesticides in these matrices corresponds to trends in the use of different pesticide classes and biological target actions (fungicides were also integrated to the sanitary packages) to more environmentally-friendly, more polar, less toxic but also troublesome to analyze. Notably, monitoring trends and exposure monitoring for risk assesment are reavealing lack of legislation as well as dedicated studies in these matrices.

Scientists and regulators should recognize the current pesticide reality about residues occurrence in matrices with dermal contact or direct ingestion applications.

Last but not least, pest control is nowadays a matter of critical concern for bee's by-products which shows the higher contamination levels with pesticides from the studied matrices.

Advances in pesticide residue analysis were stated and discussed. Nowadays, it is being carried out an update in techniques for residue analysis as experimented for foodstuffs and environmental samples years ago. Both newer sample preparation and determination techniques based on chromatography coupled to mass spectrometric detection, are offering improved knowledge and reliability for residue analysis. However, since the magnitude of this problem, several strategies should be necessary to employ in order to cover more target analytes and matrices of interest.

Perspectives in this field are expected to be focused on the implementation of miniaturized, high throughput sample preparation methodologies and widespread use of advanced mass spectrometric techniques. This will help for the comprehensive assessment on pesticide occurrence in matrices particularly not yet well studied and difficult to handle. An update on residue methodologies is a necessary step in further Pharmacopeias along with urgent specific regulations for pesticide residues which are currently being found. Such a lack of information will encourage investigation in this topic in the years ahead.

6. Acknowledgments

7. References

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