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Review on Current Analytical Methods with Chromatographic and Nonchromatographic Techniques for New Generation Insecticide Neonicotinoids

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1. Introduction

Neonicotinoid insecticides are a major group and the newest group among insecticides. They permeate the entire body of a plant and show excellent effects for the control of diseases and harmful insects. The history of neonicotinoid insecticides from development to their market release dates back to the late 1800s. Nicotine (Fig. 1), an alkaloid in tobacco leaves, is an early neonicotinoid insecticide that has been used as a natural insecticide, but it is extremely toxic to mammals ($LD_{50} = 50\text{--}60\text{ mg/kg}$; Tomizawa & Casida, 2005). Therefore, studies using nicotine as a model compound have been conducted actively to develop new pesticides with highly selective toxicity. In the 1970s, Shell developed nithiazine (Fig. 1), which showed strong insecticide activity (Soloway et al., 1978, 1979), although the compound was unstable in its application and remained commercially unavailable. Subsequently, the development of nithiazine derivatives was continued based on the relation between the chemical structure of nicotine compounds and insecticide activity. Eventually, Nihon Tokushu Noyaku Seizo (currently Bayer Crop Science) developed imidacloprid (Fig. 1) (Shiokawa et al., 1994). Subsequent to imidacloprid, acetamiprid (Nippon Soda Co. Ltd.), nitenpyram (Takeda Chemical Industries, currently Sumitomo Chemical Takeda Agro Co.), thiamethoxam (Ciba, currently Syngenta), thiacloprid (Bayer Crop Science), dinotefuran (Mitsui Chemicals Inc.) and clothianidin (Takeda Chemical Industries, currently Sumitomo Chemical Takeda Agro Co.) have been released on the market (Tomlin, 2003) with the subsequent new and recent development of imidaclothiz in China (Nantong Jiangshan Agrochemical and Chemical Co. Ltd.) (Fig. 1).

Neonicotinoid insecticides express insecticide activity by acting on the nicotinic acetylcholine receptor, nAChR, which is present on the postsynaptic membrane of the insect nerve. Excellent insecticide effects are expressed on hemipteran pest species including aphids, whitefly, and planthoppers by this mechanism of action. Although nAChR is present in both insects and mammals, neonicotinoid insecticides that act on them are highly selectively toxic to insects because the recognition site of insect nAChR is lipid-soluble, whereas mammalian nAChR must be ionized to a high degree. It is considered that the

selectivity results from the fact that neonicotinoid insecticides are not, unlike nicotine, fully ionic compounds. Therefore, they are transferred easily in lipophilic insect body fluids to reach nAChR to express the action, although affinity to nAChR is low in mammals (Kagabu, 1996; Shiokawa et al., 1994; Tomizawa, 1994; Tomizawa & Casida, 2003, 2005).

Neonicotinoid insecticides have come into worldwide use because, as described above, they have highly selective toxicity. Their toxicity to mammals, fish, and birds is low. They show a superior effect to control diseases and harmful insects that are resistant to insecticides including organophosphorus insecticides, carbamate insecticides, and synthetic pyrethroid insecticides. Recently however, it has been suggested that neonicotinoid insecticides are a possible cause of colony collapse disorder (CCD), i.e., sudden disappearance of bees that are pollinators of vegetables and fruits in modern agriculture (Decourtye & Devillers, 2010; El Hassani et al., 2008; Girolami et al., 2009; Iwasa et al., 2004; Mommaerts et al., 2010; Nauen et al., 2001). Studies have been undertaken to elucidate the relation between CCD and neonicotinoid insecticides, but the cause of CCD remains unclear.

As described above, neonicotinoid insecticides have come into use as insecticides of the next generation to replace classical insecticides such as organophosphorus insecticides for the stable supply of various crops. However, the influence on the ecosystem related to useful insects such as bees has been noted. Further studies must be undertaken from various viewpoints such as food safety including crops, environment and ecological influence, i.e., for risk management and risk assessment.

In this chapter, the author reports analytical methods that constitute the underlying technology that is indispensable for studies of risk management and risk assessment of neonicotinoid insecticides in the form of review to systemize cases reported to date, as well as to organize the trend, current situation, and future directions observed in the overview of respective analytical methods.

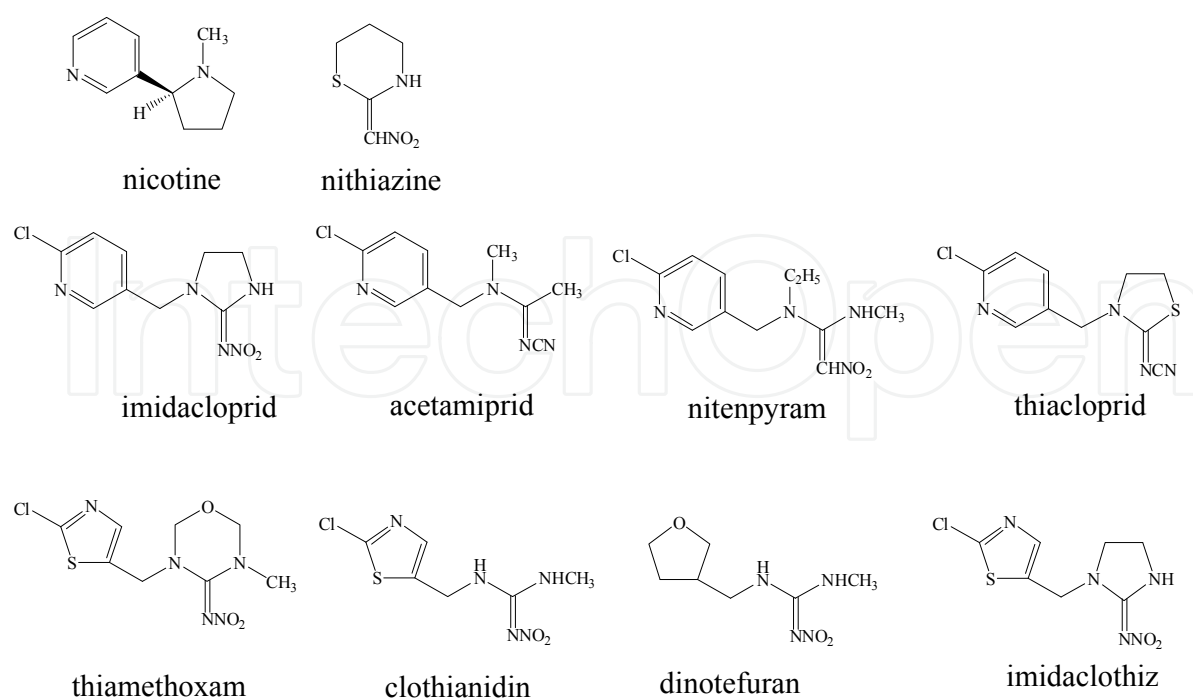


Fig. 1. Chemical structures of nicotine and neonicotinoid insecticides having nitromethylene moiety ($C=CHNO_2$), nitroguanidine moiety ($C=NNO_2$) and cyanoamidine moiety ($C=NCN$).

2. Analytical methods based on chromatographic techniques

Chromatography is a powerful tool for the determination of pesticides that might remain in widely various matrices such as food including crops, living bodies, and the environment. It also is used as an important method for the analysis of neonicotinoid insecticides, which were introduced into the environment about twenty years ago as the successor of organophosphorus, carbamate, and synthetic pyrethroid insecticides. This section refers to sample pre-treatments including extraction and clean-up needed before chromatographic determination and gives an exhaustive summary of the trend related to the development of residue analysis of neonicotinoid insecticides by chromatography.

2.1 Sample pre-treatment procedures prior to chromatographic determination

To analyze minute amounts of residual pesticides in complex matrices accurately, sample pre-treatment procedures must be conducted before chromatographic determination. It is no exaggeration to say that the results of the procedures have a decisive influence on the reliability of the data measured. This also applies to the analysis of neonicotinoid insecticides. In pesticide residue analyses conducted in the mid-1990s, when imidacloprid became available on the market, gas chromatograph (GC) equipped with so-called element-selective detectors, for example, electron capture ionization detector (ECD) for detection of pesticides with halogen atoms such as organochlorine insecticides and synthetic pyrethroid insecticides or flame photometric detector (FPD) for detection of pesticides with phosphorus atoms or sulfur atoms such as organophosphorus insecticides was mainly used. On the other hand, high-performance liquid chromatograph (HPLC) equipped with UV detectors or diode array detectors (DAD) were used for detection of pesticides that were unstable to heat. It is important in all measurement methods to conduct sample pre-treatment procedures to obtain accurate measurement data.

Table 1 shows that sample pre-treatment procedures roughly consist of (1) extraction of the target pesticide from the sample and (2) separation of the target pesticide from the extract and clean-up.

For (1) extraction of neonicotinoid insecticides, shaking extraction with organic solvents such as acetone, acetonitrile, or methanol (Baskaran et al., 1997; de Erenchun et al., 1997; Mohan et al., 2010; Tokieda et al., 1997b, 1998; Watanabe et al., 2007), blending extraction with a homogenizer (Agüera et al., 2004; Blasco et al., 2002a, 2002b; Di Muccio et al., 2006; Fernandez-Alba et al., 1996, 2000; Ferrer et al., 2005; Hengel & Miller, 2008; Hernández et al., 2006; Ishii et al., 1994; Jansson et al., 2004; Kamel et al., 2010; Mateu-Sánchez et al., 2003; Obana et al., 2002, 2003; Sannino et al., 2004; Ting et al., 2004; Tokieda et al., 1997a, 1997b; Venkateswarlu et al., 2007), and ultrasonic extraction (Bourgin et al., 2009; García et al., 2007; Ishii et al., 1994; Liu et al., 2005, 2010; Mayer-Helm, 2009; Rancan et al., 2006a, 2006b; Zhang et al., 2010) are commonly used. In addition to these, Bourgin et al. (2009) extracted five insecticides including imidacloprid from seeds coated with acetonitrile and Xiao et al. (2011) extracted seven neonicotinoid insecticides from bovine tissues with water by accelerated solvent extraction (ASE) to give quantitative extraction efficiency. For extraction from liquid samples such as water, milk and wine, solid-phase extraction (SPE) packed with e.g. C₁₈ or diatomaceous earth (Baskaran et al., 1997; Economou et al., 2009; Ferrer & Thurman, 2007; Pirard et al., 2007; Seccia et al., 2005, 2008; Zhou et al., 2006) and liquid-liquid extraction (Galera et al., 1998; Vilchez et al., 1996, 2001) are used. Moreover, matrix solid-phase dispersion (MSPD) was first reported in 1989. A small amount of solid or semisolid sample

was blended with adsorbents such as C_{18} to be packed in a disposable plastic column. It was then extracted with a suitable organic solvent (Barker et al., 1989). The method has been applied to the extraction of pesticides, pharmaceuticals, and antibiotics since then (Barker, 2000a, 2000b). MSPD is also applied to the extraction of neonicotinoid insecticides from crop, honey, and fruit juice samples (Blasco et al., 2002a; Pous et al., 2001; Radišić et al., 2009; Totti et al., 2006).

In any event, the first obstacle in the development of analytical methods for pesticide residues is to secure extraction efficiency from measurement samples. It is probably important to select extraction conditions that are suitable for the physicochemical features of the target pesticide and for the characteristics of the measurement sample.

In the process of (2) clean-up, classical liquid-liquid partitioning and column chromatography have been used. However, because of concern over the health impact of the use of organic solvents in large quantities on analytical staff as well as the environmental load, the strong need exists for the reduction of organic solvents used in sample pre-treatment procedures (Wan & Wong, 1996). SPE packed with widely various adsorbents such as silica gel, Florisil, C_{18} , polymeric materials, graphitized carbon black (GCB), and ion exchange resin has come into common use these days, making a great contribution to reduction of the use of organic solvents (Fritz & Macka, 2000).

In the 2000s, marked technical innovation in analytical instruments has taken place: LC-MS and LC-MS/MS have been brought into use for the determination of neonicotinoid insecticides. These methods have higher measurement sensitivity than classical HPLC-UV and HPLC-DAD. Moreover, in many cases, sample pre-treatment procedures have reportedly included only the extraction and dilution of the extract, with subsequent measurement, without even the need for additional clean-up (Table 1). The most common extraction procedure is the following: a measurement sample is homogenized; then it is dehydrated and extracted with ethyl acetate and anhydrous sodium sulfate with subsequent solvent evaporation and then LC-MS determination (Blasco et al., 2002a, 2002b; Fernández-Alba et al., 2000) or LC-MS/MS determination (Agüera et al., 2004; Jansson et al., 2004; Venkateswarlu et al., 2007).

In 2003, Anastassiades et al. (2003) reported fast, simple and easy sample pre-treatment procedures by extraction with acetonitrile and dispersive SPE. The method that was presented is known as a quick, easy, cheap, effective, robust, and safe (QuEChERS) method, which is a breakthrough in which the sample pre-treatment is completed via acetonitrile extraction, dehydration, and salting-out with anhydrous magnesium sulfate and sodium chloride. Thereafter, dehydration and clean-up of the extract are done using dispersive SPE with anhydrous magnesium sulfate and primary secondary amine (PSA), which is a weak anion exchange adsorbent. Reportedly dispersive SPE using PSA is extremely effective for the removal of organic acids, polar dye components, and saccharides (Anastassiades et al., 2003). QuEChERS is very different from general sample pre-treatment procedures by organic solvent extraction, re-extraction with the organic solvent phase, and clean-up with SPE in that no process of concentration (evaporation of solvents) is needed. The concentration process, a procedure that must be conducted after processes such as extraction and clean-up, unexpectedly accounts for a large percentage of the sample pre-treatment time. Therefore, QuEChERS is a technology that contributes much to speeding up and simplification of sample pre-treatment procedures. In addition, the amounts of organic solvents used are extremely as small: about 10 mL of acetonitrile per sample. For those reasons, it can be concluded that the technology has met all of the requirements described above (Wan & Wong, 1996).

GC						
Analyte(s)	Sample(s)	Extraction	Clean-up	Additional procedure prior to GC analysis	Determination	Ref.
Imidacloprid	Water	Mechanical shaking with chloroform	None	Hydrolysis in basic medium by application of heat	GC-MS	Vilchez et al., 1996
	Soil	Ultrasonic extraction with water and mechanical shaking with chloroform				
Imidacloprid	Tomato, cucumber, pepper and green bean	Ultrasonic extraction with water and mechanical shaking with chloroform	None	Hydrolysis in basic medium by application of heat	GC-MS	Navalón et al., 1997
Acetamiprid and 4 metabolites	Cabbage, green pepper, eggplant, potato, apple, orange, grape, strawberry, cucumber and radish (root, leaf)	Homogenization with methanol	Liquid-liquid partition and silica gel column chromatography	Hydrolysis in basic medium by application of heat, oxidation with KMnO ₄ by application of heat and esterification with CH ₂ N ₂	GC-ECD	Tokieda et al., 1997a
Acetamiprid	Cabbage, potato, radish (leaf, root), grape, orange, apple, strawberry, green pepper and eggplant	Homogenization and mechanical shaking with methanol	Liquid-liquid partition, Florisil column chromatography and C ₁₈ SPE (optional)	None	GC-ECD	Tokieda et al., 1997b
	Green tea (powder)	Mechanical shaking with methanol				
	Green tea (leachate)	Liquid-liquid extraction with methanol after soak in Boiling water				
3 pesticides including imidacloprid	White pine	Maceration with methanol/0.04% H ₂ SO ₄ (70:30, v/v)	C ₁₈ SPE and self-prepared Florisil minicolumn	Heptafluorobutryl derivative	GC-MS	MacDonald & Meyer, 1998
Acetamiprid	Vegetables	Homogenization with ethyl acetate	None	None	GC-MS/MS	Mateu-Sánchez et

al., 2003					
HPLC equipped with detectors except for MS or tandem MS					
Analyte(s)	Sample(s)	Extraction	Clean-up	Determination	Ref.
Imidacloprid	Japanese pear, apple, peach (pulp, peel), grape, radish (root, leaf), cucumber, eggplant, rice grain, rice green, rice straw and potato	Homogenization with acetonitrile/water (80:20, v/v)	Liquid-liquid partition and silica gel column chromatography	HPLC-UV	Ishii et al., 1994
	Soil	Ultrasonic extraction with acetonitrile/water (80:20, v/v)			
Imidacloprid	Pepper, tomato and cucumber	Homogenization with acetone	Liquid-liquid partition and C ₁₈ SPE	HPLC-DAD	Fernandez-Alba et al., 1996
Imidacloprid	Water	Extraction with methanol from C ₁₈ SPE	None	HPLC-UV	Baskaran et al., 1997
	Soil	Mechanical shaking with acetonitrile/water (80:20, v/v)	None		
Imidacloprid and 6-chloronicotinic acid	Soil	Mechanical shaking with acetonitrile/methanol/water (3:2:2, v/v)	None	HPLC-pulsed amperometric detector	de Erenchun et al., 1997
Imidacloprid and 6-chloronicotinic acid	Groundwater	Liquid-liquid extraction with dichloromethane	None	HPLC-DAD	Galera et al., 1998
Acetamiprid and 2 metabolites	Soil	Mechanical shaking with methanol/0.1 M NH ₄ Cl (8:2, v/v)	Liquid-liquid partition and Extrelut SPE packed with diatomaceous earth material	HPLC-UV	Tokieda et al., 1998
6-chloronicotinic acid		Mechanical shaking with methanol/0.1 M NH ₄ Cl (8:2, v/v) and methanol/0.5 M NaOH (8:2, v/v)	C ₁₈ SPE and liquid-liquid partition		
Imidacloprid and 6-chloronicotinic acid	Greenhouse air	Trap with Amberlite XAD-2 and desorption with acetonitrile and phosphate buffer	None	HPLC-DAD	Frenich et al., 2000
Acetamiprid, imidacloprid and nitenpyram	Cucumber, potato, tomato, eggplant, Japanese radish and grape	Homogenization with acetonitrile	PSA SPE and silica gel SPE	HPLC-DAD	Obana et al., 2002
3 pesticides including imidacloprid and thiamethoxam	Cabbage, tomato, chili, pepper and potato	Microwave-assisted extraction with acetone	Liquid-liquid partition	HPLC-UV	Singh et al., 2004

Imidacloprid	Grape, cantaloupe, strawberry, cucumber, tomatillo, lettuce and bell pepper	Homogenization with ethyl acetate and anhydrous Na ₂ SO ₄	NH ₂ and Florisil SPE	HPLC-nitrogen chemiluminescent detector and DAD	Ting et al., 2004
Imidacloprid	Tobacco leaf	Ultrasonic extraction with ethyl acetate	Liquid-liquid partition	HPLC-UV	Liu et al., 2005
Thiamethoxam	Honeybee	Ultrasonic extraction with acetone	None	HPLC-ECD	Rancan et al., 2006a
Imidacloprid and 2 metabolites	Bee and filter paper Maize leaf	Ultrasonic extraction with acetone	None Hydromatrix SPE packed with diatomaceous earth material	HPLC-ECD	Rancan et al., 2006b
Acetamiprid, imidacloprid and thiamethoxam	Water	Extraction with methanol from SPE packed with multiwalled carbon nanotubes	None	HPLC-UV	Zhou et al., 2006
Imidacloprid and 6-chloronicotinic acid	Honeybee	Ultrasonic extraction with acetone	Liquid-liquid partition	HPLC-FLD	García et al., 2007
Acetamiprid, clothianidin, dinotefuran, imidacloprid, nitenpyram, thiacloprid and thiamethoxam	Apple, carrot, cucumber, grape, peach, sweet pepper, spinach and tomato	Mechanical shaking with acetone	Chem Elut SPE packed with diatomaceous earth material and GCB/NH ₂ SPE	HPLC-DAD	Watanabe et al., 2007
Acetamiprid, imidacloprid, thiacloprid and thiamethoxam	Bovine milk	Extraction with dichloromethane from Chem Elut SPE packed with diatomaceous earth material	None	HPLC-DAD	Seccia et al., 2008
5 pesticides including imidacloprid	Coated seeds of wheat and corn	Ultrasonic extraction with acetonitrile ASE with acetonitrile	None	HPLC-UV	Bourgin et al., 2009
Imidacloprid	Orange juice, apple juice and mixture of pineapple and pear juice	None	Dilution of 0.05 M sodium dodecyl sulfate	HPLC-DAD	Chin-Chen et al., 2009
Acetamiprid, imidacloprid and thiacloprid	Cotton seed	Mechanical shaking with acetone/water (80:20, v/v)	C ₁₈ SPE	HPLC-UV	Mohan et al., 2010
HPLC equipped with MS or tandem MS					
Analyte(s)	Sample(s)	Extraction	Clean-up	Determination	Ref.
5 pesticides including imidacloprid	Pear and tomato	Homogenization with ethyl acetate and anhydrous Na ₂ SO ₄	None	LC-MS	Fernández-Alba et al., 2000
5 pesticides including imidacloprid	Strawberry, orange, potato, pear and melon	Extraction with dichloromethane from MSPD with C ₈	None	LC-MS	Pous et al., 2001
10 pesticides	Orange	Extraction with	None	LC-MS	Blasco et al.,

including imidacloprid		dichloromethane from MSPD with C ₈ Stir bar sorptive extraction after homogenization with methanol and water Homogenization with ethyl acetate and anhydrous Na ₂ SO ₄			2002a
4 pesticides including imidacloprid	Peach and nectarine	Homogenization with ethyl acetate and anhydrous Na ₂ SO ₄	None	LC-MS	Blasco et al., 2002b
Acetamiprid, imidacloprid, nitenpyram, thiacloprid and thiamethoxam	Bell pepper, cucumber, eggplant, grape, grapefruit, Japanese radish (root, leaf), peach, pear, potato, rice and tomato	Homogenization with methanol	GCB SPE	LC-MS	Obana et al., 2003
Imidacloprid	Soil	Mixing with methanol/0.05% NH ₄ OH (3:1, v/v)	None	LC-MS/MS	Bonmatin et al., 2003
	Maize, rape, wheat and sunflower	Grinding with methanol/0.05% H ₂ SO ₄ (4:1, v/v)	C ₁₈ SPE		
	Pollen	Mixing with ethanol/water (75:25, v/v)	None		
57 pesticides including imidacloprid and acetamiprid	Fruits and vegetables	Homogenization with ethyl acetate and anhydrous Na ₂ SO ₄	None	LC-MS/MS	Jansson et al., 2004
24 pesticides including imidacloprid	Apple puree, concentrated lemon juice and tomato puree	Homogenization with acetone and ethyl acetate/cyclohexane (50:50, v/v)	None	LC-MS/MS	Sannino et al., 2004
17 pesticides including acetamiprid, imidacloprid and thiacloprid	Pepper, lettuce and eggplant	Homogenization with ethyl acetate and anhydrous Na ₂ SO ₄ under alkaline	None	LC-MS/MS	Agüera et al., 2004
Acetamiprid, imidacloprid, thiacloprid and thiamethoxam	Honey	Extraction with dichloromethane from Extrelut SPE packed with diatomaceous earth material	None	LC-MS	Fidente et al., 2005
Acetamiprid, imidacloprid, thiacloprid and thiamethoxam	Drinking water	Extraction with ethyl acetate/methanol (50:50, v/v) from poly(styrene-divinylbenzene) SPE	None	LC-MS	Seccia et al., 2005
Acetamiprid, imidacloprid and thiacloprid	Cucumber, tomato, lettuce and pepper	Homogenization with ethyl acetate	None	LC-TOF-MS	Ferrer et al., 2005
146 pesticides including acetamiprid,	Lettuce and orange	QuEChERS method Shaking by hand with acetonitrile	Dispersive PSA SPE	LC-MS/MS	Lehotay et al., 2005

imidacloprid, thiacloprid and thiamethoxam					
Acetamiprid, imidacloprid, thiacloprid and thiamethoxam	Apricot, celery, courgette, peach and pear	Homogenization with acetone and extraction with dichloromethane from Extrelut SPE packed with diatomaceous earth material	None	LC-MS	Di Muccio et al., 2006
52 pesticides including acetamiprid, imidacloprid and thiacloprid	Lemon, raisin, tomato and avocado	Homogenization with methanol/ water (80:20, v/v) containing 0.1% formic acid	HLB (hydrophilic-lipophilic balanced copolymer) SPE	LC-MS/MS	Hernández et al., 2006
6 pesticides and metabolites including imidacloprid	Honeybee	Extraction with dichloromethane/ methanol (85:15, v/v) from MSPD with C ₁₈	None	LC-MS	Totti et al., 2006
52 pesticides including acetamiprid, imidacloprid and thiacloprid	Potato, orange and cereal-based baby food	Acetate buffered QuEChERS method Shaking with acetonitrile containing 1% acetic acid	Dispersive PSA SPE	UPLC-MS/MS	Leandro et al., 2007
19 pesticides and metabolites including imidacloprid	Honey	Extraction with ethyl acetate from Chem Elut SPE packed with diatomaceous earth material	None	LC-MS/MS	Pirard et al., 2007
101 pesticides and metabolites including acetamiprid, imidacloprid, nitenpyram and thiacloprid	Green pepper, tomato, cucumber and orange Water	QuEChERS method Shaking with acetonitrile Extraction with ethyl acetate from C ₁₈ SPE	Dispersive PSA SPE None	LC-TOF-MS	Ferrer & Thurman, 2007
42 pesticides including acetamiprid, clothianidin, dinotefuran, imidacloprid, nitenpyram, thiacloprid and thiamethoxam	Raisin, red grape and red wine Orange and wheat flour	QuEChERS method Shaking by hand with acetonitrile Shaking by hand with acetonitrile	Dispersive PSA SPE None	LC-MS/MS	Payá et al., 2007
10 pesticides including imidacloprid and thiamethoxam	Grape	Homogenization with ethyl acetate and anhydrous Na ₂ SO ₄	None	LC-MS/MS	Venkateswarlu et al., 2007
160 pesticides including acetamiprid, dinotefuran, imidacloprid, nitenpyram, thiacloprid and thiamethoxam	Tomato, pear and orange	QuEChERS method Shaking by hand with acetonitrile	Dispersive PSA SPE	LC-MS/MS	Kmellár et al., 2008
11 pesticides	Dried hop	Homogenization with	Polymeric styrene-	LC-MS/MS	Hengel &

including imidacloprid		acetonitrile	divinylbenzene methacrylate SPE and NH ₂ SPE		Miller, 2008
53 pesticides including acetamiprid, imidacloprid, nitenpyram, thiacloprid and thiamethoxam	Orange, strawberry and cucumber	Buffered QuEChERS method		UPLC-MS/MS	Frenich et al., 2008
		Vortex mixing with acetonitrile containing 1% acetic acid	None		
	Olive	Vortex mixing with acetonitrile containing 1% acetic acid	Florisil SPE		
46 pesticides including acetamiprid and imidacloprid	White and red wines	Extraction with methanol from HLB SPE	None	LC-MS/MS	Economou et al., 2009
52 pesticides including acetamiprid, imidacloprid and thiamethoxam	Tobacco	Ultrasonic extraction and shaking with methanol after soak in 1% acetic acid	Chem Elut SPE packed with diatomaceous earth material and Florisil SPE	LC-MS/MS	Mayer-Helm, 2009
12 pesticides including acetamiprid	Apple juice, peach juice, orange juice and raspberry juice	Extraction with dichloromethane from MSPD with diatomaceous earth material	None	LC-MS/MS	Radišić et al., 2009
7 pesticides and metabolites including imidacloprid and thiamethoxam	Honey and pollen	Extraction with methanol from Florisil SPE	None	LC-MS/MS	García-Chao et al., 2010
42 pesticides including acetamiprid, clothianidin, imidacloprid, thiacloprid and thiamethoxam	Tea (made tea, tea infusion and spent leaf)	Vortex mixing and homogenization with water, ethyl acetate/cyclohexane (9:1, v/v) and sodium chloride	Dispersive SPE with PSA, GCB and Florisil	LC-MS/MS	Kanrar et al., 2010
Acetamiprid, clothianidin, dinotefuran, imidacloprid, nitenpyram, thiacloprid and thiamethoxam	Rice and tea	Ultrasonic extraction with acetonitrile		UPLC-MS/MS	Liu et al., 2010
	Apple, cabbage, potato, chicken, pork, milk and egg	Vortex shaking with acetonitrile			
Clothianidin, dinotefuran, imidacloprid, thiamethoxam and 8 metabolites	Bee, bee pollen and bee honey	Modified QuEChERS method		LC-MS/MS	Kamel, 2010
		Homogenization with water and acetonitrile containing 2% triethylamine	C ₁₈ SPE		
36 pesticides including acetamiprid and imidacloprid	Tea	Ultrasonic extraction with acetonitrile containing 1% acetic acid	Dispersive SPE with PSA and GCB	UPLC-MS/MS	Zhang et al., 2010
22 pesticides	Milk, orange,	Homogenization with	Salting out	UPLC-MS/MS	Kamel et al.,

and metabolites including acetamiprid, clothianidin, dinotefuran, thiacloprid, thiamethoxam	spinach, apple, plum, watermelon, green bean, zucchini, broccoli, strawberry, grape and tomato	acetonitrile containing 1% triethylamine	(optional), C ₁₈ SPE and Florisil SPE		2010
93 pesticides and mycotoxins including acetamiprid, imidacloprid, thiacloprid and thiamethoxam	Wheat, cucumber and red wine	Acetate buffered QuEChERS method Vortex mixing with acetonitrile containing 1% acetic acid		None	UPLC-MS/MS Romero-González et al., 2011
150 pesticides including acetamiprid, imidacloprid, nitenpyram, thiacloprid and thiamethoxam	Tomato, pear and orange	Acetate buffered QuEChERS method Shaking by hand with acetonitrile containing 1% acetic acid		Dispersive PSA SPE	LC-MS/MS Kmellár et al., 2011
70 pesticides including imidacloprid, thiacloprid and thiamethoxam	Water	None	None	LC-MS/MS	Pareja et al., 2011
Acetamiprid, clothianidin, dinotefuran, imidacloprid, nitenpyram, thiacloprid and thiamethoxam	Bovine muscle and liver	ASE with water	HLB SPE	LC-MS/MS	Xiao et al., 2011
Acetamiprid, clothianidin, dinotefuran, imidacloprid, thiacloprid and thiamethoxam	Chestnut, shallot, ginger and tea	Vortex mixing with acetonitrile	Activated carbon SPE and HLB SPE	LC-MS/MS	Xie et al., 2011
Other chromatographic technique					
Analyte(s)	Sample(s)	Extraction	Clean-up	Determination	Ref.
Imidacloprid and 6-chloronicotinic acid	Greenhouse air	Trap with Amberlite XAD-2 and desorption with water	None	Capillary electrophoresis (micellar electrokinetic chromatography)-DAD	Carretero et al., 2003

Table 1. Overview of chromatographic techniques for determination of neonicotinoid insecticides.

With the presentation of QuEChERS as a turning point, many reports have described multiresidue analysis of various pesticides including neonicotinoid insecticides (Ferrer & Thurman, 2007; Frenich et al., 2008; Kamel, 2010; Kmellár et al., 2008, 2011; Leandro et al., 2007; Lehotay et al., 2005; Payá et al., 2007; Romero-González et al., 2011). Additionally,

reagents and adsorbents for QuEChERS are marketed as kits, implying that the method is extremely practical as multiresidue analysis for pesticide residues.

2.2 Determination of neonicotinoid insecticides based on chromatographic methods

Chromatographic determination of neonicotinoid insecticides are classifiable into two groups that use HPLC or GC (Table 1). However, because neonicotinoid insecticides are generally degraded by heat, additional processes such as derivatization are necessary for GC determination, making sample pre-treatment procedures more complicated than HPLC determination. Vilchez et al. (1996) and Navalón et al. (1997) used a hydrolyzed compound of imidacloprid [1-(6-chloro-3-pyridylmethyl)imidazolidin-2-one] formed by heat treatment of a measurement sample under alkaline conditions for GC-MS determination of imidacloprid in soil, water, and crops. MacDonald and Meyer (1998) extracted imidacloprid from white pine with water-containing methanol under acidic conditions with diluted sulfuric acid, cleaned up with C₁₈ SPE and a self-prepared Florisil minicolumn, heptafluorobutyl-derivatized, and then subjected to GC-MS determination.

As presented above, it is presumed that utilization of HPLC rather than GC is more advantageous for the determination of neonicotinoid insecticides from the viewpoints of speedup and simplification of sample pre-treatment procedures. It also is readily apparent that most cases reported to date used HPLC for determination (Table 1). In determination by HPLC, both HPLC-UV and HPLC-DAD contributed greatly to analyses of neonicotinoid insecticides until the 2000s, when MS and MS/MS began to become popularly used. Since the report by Ishii et al. (1994), HPLC-UV and HPLC-DAD have been applied to the determination of neonicotinoid insecticides in various matrices (Table 1). In addition to UV and DAD, methods were developed in which imidacloprid and thiamethoxam were separated using a column and then converted into electrochemically active compounds by ultraviolet irradiation to be detected by an electrochemical detector (ECD) (Rancan et al., 2006a, 2006b), or converted into fluorescent substances to be detected using a fluorescence detector (FLD) (García et al., 2007). ECD and FLD are generally more sensitive than UV or DAD. They are applied to residue analysis in the bodies of bees, where determination at low concentrations must be done.

In fact, HPLC-UV and DAD have less measurement sensitivity and selectivity than either LC-MS or MS/MS, which are the most widely used methods today. Therefore, thorough clean-up is indispensable for the pre-treatment procedures of samples consisting of complicated matrices such as crops. Liquid-liquid partition has been used since the initial phase of the market release of imidacloprid (Fernandez-Alba et al., 1996; García et al., 2007; Ishii et al., 1994; Liu et al., 2005; Singh et al., 2004). However, that technique presents problems: e.g. a large amount of organic solvent is used; and an emulsion is formed at the liquid-liquid interface depending on the extraction sample that is used. For that reason, it has been increasingly replaced by clean-up mainly by SPE (Mohan et al., 2010; Obana et al., 2002; Ting et al., 2004; Watanabe et al., 2007). Watanabe et al. (2007) used re-extraction with diatomaceous earth SPE and clean-up with GCB/NH₂ SPE in the development of simultaneous analysis by HPLC-DAD of seven neonicotinoid insecticides released on the market. However, the recovery of nitenpyram was not satisfactory (not more than 40%). According to their discussion, matrix components in the sample affected nitenpyram in some way, leading to the factor of reduced recovery.

In the 2000s, a dramatic increase occurred in the number of cases reported on the residue pesticide analysis by HPLC equipped with quadrupole MS, ion-trap MS, tandem MS (MS/MS), and time-of-flight MS (TOF-MS). Utilization of MS enabled not only the detection of trace pesticide residues in various matrices with high accuracy but also the elucidation of their respective chemical structures.

Obana et al. (2003) reported a method of extracting five neonicotinoid insecticides from 12 crop samples using methanol, clean-up with GCB SPE, and determination by LC-MS. They suggested that the analytical method that was developed was effective as a regular monitoring method. In determination methods including neonicotinoid insecticides by LC-MS/MS, good recovery is obtained in most reported cases. In one important case, quantification was achieved by direct injection of the filtered water sample in the LC-MS/MS determination of 70 pesticides including imidacloprid, thiacloprid, and thiamethoxam in paddy water samples (Pareja et al., 2011). It is exactly the benefit of LC-MS/MS, with which highly sensitive determination is possible.

Ultra-performance liquid chromatography (UPLC) was developed only a few years ago in which the mobile phase can be flown at a high pressure (about 15,000 psi) using a short column of about 50 mm packed with C₁₈ of particle size of not more than 2 µm. Its application to pesticide residue analysis has been examined because the utilization of UPLC enables not only the achievement of highly sensitive determination, but also improved high throughput attributable to reduced measurement time as well as substantially smaller amounts of organic solvents (mobile phase) used than in conventional HPLC (Frenich et al., 2008; Kamel et al., 2010; Leandro et al., 2007; Liu et al., 2010; Romero-González et al., 2011; Zhang et al., 2010). Liu et al. (2010) used UPLC-MS/MS to construct simultaneous analyses of seven neonicotinoid insecticides in crops and livestock products, obtaining good recovery except for the lower recovery of nitenpyram (not more than 70%) in some of samples including potatoes and cabbages. Romero-González et al. (2011) constructed highly sensitive ultra-rapid analysis of more than 90 pesticides including acetamiprid, imidacloprid, thiacloprid, and thiamethoxam as well as mycotoxins by the combination of sample pre-treatment by QuEChERS and UPLC-MS/MS.

As described above, the trend in the development of residue analysis of various pesticides including neonicotinoid insecticides by LC-MS and LC-MS/MS can be summarized via the overview of several cases. LC-MS and LC-MS/MS are suitable for highly sensitive determination of only slightly volatile and heat-unstable pesticides. They enable quantification only with extremely simple and rapid sample pre-treatment compared to those of HPLC-UV, HPLC-DAD, and GC with an element-selective detector, which requires complicated sample pre-treatment procedures. Particularly MS/MS can be characterized as an effective method for the structural analysis of the target pesticide and its confirmation because much chemical information can be acquired by obtaining product ions from the precursor ion. Moreover, little interference occurs by matrix components because ions can be selected at will, thereby enabling highly sensitive determination. Recently, newly marketed insecticides tend to be included in the subjects for HPLC determination. Therefore, increasing need is expected for LC-MS and LC-MS/MS in the future.

Although many advantages of LC-MS and LC-MS/MS are described above, the matrix effect must also be noted, which is a problem in the chromatographic determination of pesticide residues that might be present in various samples.

It has been pointed out that, as in GC-ECD and GC-FPD determination, quantitative determination is not possible because of the matrix effect when the sample clean-up is insufficient. Lee and Wylie (1991) have reported interesting observations by which the susceptibility (to the matrix effect) of several GC detectors is examined for individual crop samples. The matrix effect is a phenomenon that is also observed in LC-MS and LC-MS/MS determination; it was shown earlier that when the target pesticide is eluted together with matrix components in the sample, ion suppression or ion enhancement occurs during the ionization process, engendering error in the determination result (Niessen et al., 2006). The matrix effect can be avoided using a matrix-matched standard method or using isotope dilution with isotopically labeled internal standards (Niessen et al., 2006), in addition to thorough sample clean-up. In the matrix-matched standard method, the matrix effect is evaluated through the comparison of the responses of the standard solution and the target pesticide prepared in the measurement sample solution free of the target pesticide; this method is commonly used as a means to correct the matrix effect (Di Muccio et al., 2006; Economou et al., 2009; Ferrer et al., 2005; Ferrer & Thurman, 2007; Fidente et al., 2005; Frenich et al., 2008; Hernández et al., 2006; Kamel, 2010; Kamel et al., 2010; Kanrar et al., 2010; Kmellár et al., 2008; Leanro et al., 2007; Mayer-Helm, 2009; Payá et al., 2007; Pirard et al., 2007; Radišić et al., 2009; Romero-González et al., 2011; Totti et al., 2006; Venkateswarlu et al., 2007; Xiao et al., 2011; Xie et al., 2011).

Xie et al. (2011) reported observation of ion enhancement in dinotefuran, imidacloprid, and thiacloprid, as well as ion suppression in acetamiprid, clothianidin, and thiamethoxam by the matrix effect, and that acetamiprid and thiamethoxam among these were significantly affected. However, because the recovery was improved by correction using the matrix-matched standard method, they emphasized the effectiveness of the method in the avoidance of the matrix effect.

In any event, the matrix effect should be regarded as a common problem of pesticide residue analysis by HPLC and GC; it goes without saying that it is most important to evaluate the matrix effect that might be derived from the subject sample when the development of a new analytical method is attempted or when an established, existing analytical method is applied.

3. Analytical methods based on nonchromatographic techniques

Section 2 summarized the trends up to now in the development of the determination of neonicotinoid insecticides by chromatography. This section refers to analytical methods based on nonchromatographic techniques. The list in Table 2 shows that major nonchromatographic methods include flow injection analysis (FIA), direct MS analysis, and enzyme-linked immunosorbent assay (ELISA), which is an immunochemical determination using an antigen-antibody reaction with high specificity or selectivity. This section summarizes the trend and the current situation of the development of analytical methods for neonicotinoid insecticides using these.

3.1 FIA or MS analysis for direct determination of neonicotinoid insecticides

FIA is a method by which a predetermined amount of a sample solution is injected into carrier solution that flows continuously in a tube, and the target substance is detected or quantified using a detector, as might be done after a chemical reaction. Instruments used in

FIA are generally inexpensive. Furthermore, the method is known to be capable of rapid, easy, and highly sensitive detection of trace substances (Lara et al., 2010; Llorent-Martínez et al., 2011).

Reports on the determination of neonicotinoid insecticides by FIA have targeted imidacloprid to date. In all such studies, methods are constructed by which a measurement sample is irradiated with ultraviolet light for conversion into a fluorescent substance [1-(6-chloro-3-pyridylmethyl)-2-(hydroxyimino)-3,4-didehydroimidazolidene] which is detected using a spectrofluorometer (Flores et al., 2007; Vílchez et al., 2001). Alternatively, nitrite is detached from imidacloprid to be reduced into nitric oxide by iodide, which is detected by chemiluminescence detection with ozone (Lagalante & Greenbacker, 2007). All FIA methods show measurement sensitivity that is equal to or better than the detection limit of HPLC or GC. For the determination of liquid samples such as water, direct injection is possible with filtration only (Flores et al., 2007; Lagalante & Greenbacker, 2007). Actually, FIA is regarded as making a great contribution to fast, simple, and easy determination of pesticide residues, especially in liquid samples.

García-Reyes et al. (2009) reported rapid in situ qualitative and quantitative analysis of 16 pesticides including nitenpyram and thiacloprid by desorption electrospray ionization MS (DESI-MS) and MS/MS (DESI-MS/MS). In their determination, crop samples were pre-treated according to QuEChERS and the resulting sample solution was applied on the PTFE surface, while the skin of fruit and vegetable samples were fixed on glass slides, and electrospray was applied directly to the sample to ionize target pesticides in the sample. The measurement sensitivity was extremely high: on the order of $\mu\text{g/kg}$, and the measurement accuracy was comparable to LC-MS. Although such an analytical method remains under development at present, it can be anticipated as a new, highly sensitive, and rapid screening method.

Analyte(s)	Sample(s)	Extraction	Clean-up	Determination	Ref.
Imidacloprid	Water	Liquid-liquid extraction with dichloromethane	None	FIA with photochemically induced fluorescence detection	Vílchez et al., 2001
Imidacloprid	Water, hemlock xylem fluid and grape	Only filtration	None	FIA with chemiluminescence detection	Lagalante & Greenbacker, 2007
	Honey	Only dilution			
Imidacloprid	Water	Only filtration	None	FIA with photochemically induced fluorescence detection	Flores et al., 2007
16 pesticides and metabolite including nitenpyram and thiacloprid	Orange, lemon, apple, green pepper, persimmon, grapefruit, tomato, pear and grape	QuEChERS method		Desorption ESI-MS and MS/MS	García-Reyes et al., 2009
		Shaking with acetonitrile	Dispersive PSA SPE		
	Fruir and vegetable skin (peel)	None	None		

Table 2. Overview of nonchromatographic techniques with FIA and MS for determination of neonicotinoid insecticides.

3.2 ELISA analysis for neonicotinoid insecticides as a rapid and simple preliminary screening method

Since Yalow and Berson applied radioimmunoassay (RIA) to the determination of insulin in the 1950s (Yalow & Berson, 1959, 1960), RIA has been widely used mainly in the field of clinical laboratory tests. Although immunoassay for pesticide determination was not reported in two decades. Subsequently, antibodies selective to DDT and malathion were developed (Centeno et al., 1970). In addition, RIA for parathion determination was developed (Ercegovich et al., 1981). After Engvall and Perlmann (1972) proposed enzyme immunoassay (EIA, ELISA) using enzyme-labeled antigens instead of radioisotope-labeled antigens, a marked increase existed in the development of ELISA for various pesticides including organophosphorus and synthetic pyrethroid insecticides (Hennion et al., 1998; Meulenberg et al., 1995; Nunes et al., 1998; Shan et al., 2002).

The immunogenicity of small molecules such as pesticides themselves is extremely low. Therefore, it is necessary for the development of antigens to these compounds to design and synthesize hapten molecules that imitate the chemical structure of the target substances. It is known that the measurement sensitivity and selectivity of the resulting antibody is strongly dependent on the chemical structure of the designed hapten molecule (Shan et al., 2002; Szurdoki et al., 1995). However, the importance of hapten design is not explained in this chapter.

ELISA, based on an antigen-antibody reaction, is a method used to detect residual pesticides, etc., in various samples consisting of complex matrices such as food samples including crops. Therefore, fast analysis can be achieved because significant laborsaving is possible in complicated sample pre-treatment procedures before chromatographic determination. Moreover, ELISA is regarded as an economical, straightforward, and easy analytical method because only small amounts of organic solvents are used, instruments requiring expertise are not needed, and multisample treatment is possible using 96-well microplates (Ellis, 1996). In contrast, ELISA is disadvantageous compared to chromatographic determination in that it is limited to the determination of a single pesticide, it is incapable of identification, and it might produce a false positive result when it cross-reacts to a compound with similar chemical structure because it is a selective analytical method.

As shown in Table 3, ELISA for the determination of neonicotinoid insecticides was first developed in 2000 using polyclonal antibody (PoAb) specific to imidacloprid (Li & Li, 2000). It was followed by a report by Lee et al. (2001) in which the measurement sensitivity was improved to be approximately twice that obtained using another hapten. Watanabe et al. (2001) and Kim et al. (2004) developed monoclonal antibody (MoAb) specific to imidacloprid, and constructed ELISA that is 5-fold to 20-fold more sensitive than that obtained using PoAb. In addition to imidacloprid, ELISA using MoAb specific to acetamiprid (Watanabe et al., 2001) and thiamethoxam (Kim et al., 2003, 2006) were also developed.

As described above, ELISA uses highly specific antigen-antibody reaction. Theoretically, it responds sensitively only to the trace pesticide in matrices. In fact, however, ELISA is susceptible to the matrix effect described in Section 2.2, and it is important to evaluate the matrix effect for individual measurement samples (Jourdan et al., 1996; Nunes et al., 1998; Skerrett & Rani, 1996). While the matrix effect can be avoided or reduced by SPE, etc., such measures would eliminate advantages of ELISA shown above. Table 3 shows that the easiest method to avoid the matrix effect is dilution of the sample extract (mainly methanol extract)

with water or phosphate buffer. The recovery from various measurement samples were generally good in all reports (Byrne et al., 2005; Eisenback et al., 2009; Kim et al., 2006; Ma et al., 2009; Watanabe et al., 2001; Watanabe et al., 2004a, 2004b, 2006, 2007, 2011; Xu et al., 2010). The measurement sensitivity of ELISA is apt to be affected by the concentration of extraction solvent (mainly methanol) coexisting in the sample solution during measurement (Nunes et al., 1998). The sample extraction is diluted not only to avoid the matrix effect effectively but also to reduce the influence of the organic solvent.

Recently, kit-based ELISA for neonicotinoid insecticides was developed and marketed by Horiba Ltd. (Kyoto, Japan) and Enviroligix Inc. (Portland, ME). Kit-based ELISA package reagents needed for determination (96-well microplate pre-coated with antibody, washing solution, substrate solution, and stopping solution, etc.) can be used easily for monitoring tests of a specific neonicotinoid insecticide. However, it is important in the use of kit-based ELISA to remember that the matrix effect should be evaluated in advance (Byrne et al., 2005; Watanabe et al., 2004a, 2004b, 2006, 2007, 2011).

In any event, when ELISA is applied to a sample, it might be affected by the matrix effect. Therefore, it is important to examine in advance if the matrix effect is present, and to examine methods to avoid it. At least, ELISA can be applied sufficiently to routine analysis, especially as a screening method, by solving this problem.

Analyte(s)	Sample(s)	Extraction	Following sample preparation	Antibody	Assay format	IC ₅₀	Ref.
Imidacloprid	Water	None	None	PoAb	Indirect competitive ELISA	35 ppb	Li & Li, 2000
	Coffee cherry and coffee bean	Homogenization with methanol/1% sulfuric acid (3:1, v/v)	Evaporation, extraction with ethyl acetate and reconstitution with buffer				
Imidacloprid	Apple	Homogenization with methanol	Evaporation and reconstitution with buffer	PoAb	Indirect competitive ELISA	17.3 ng/mL	Lee et al., 2001
	Water	None	Dilution with buffer				
Acetamiprid	Cucumber, green pepper, tomato and apple	Extraction with ethyl acetate from C ₁₈ SPE	Evaporation and reconstitution with buffer	MoAb	Direct competitive ELISA	1.0 ng/mL	Watanabe et al., 2001
Imidacloprid		Shaking with methanol	Centrifugation and dilution with methanol/buffer (9:1, v/v)	MoAb		3.3 ng/mL	
Thiamethoxam	Water	None	Diluted with buffer	PoAb	Direct competitive ELISA	9.0 ng/mL	Kim et al., 2003
Imidacloprid	Apple	Shaking by hand with methanol	Filtration and dilution with water	MoAb	Direct competitive ELISA kit	8 ng/g	Watanabe et al., 2004a
Imidacloprid	Cucumber, eggplant, lettuce, green pepper and spinach	Shaking by hand with methanol	Filtration and dilution with water	MoAb	Direct competitive ELISA kit	-	Watanabe et al., 2004b
		Ultrasonic extraction with methanol					

Imidacloprid	<div>Water</div> <div>Cucumber</div>	None	<div>Dilution with buffer</div> <div>Centrifugation, filtration and dilution with buffer</div>	MoAb	Indirect competitive ELISA	0.8 µg/L	Kim et al., 2004
Imidacloprid	Avocado leaf	Homogenization with water	Dilution with water	-	Direct competitive ELISA kit	-	Byrne et al., 2005
Imidacloprid	Wiliwili leaf	Ultrasonic extraction with methanol/0.04% sulfuric acid (4:1, v/v)	Filtration, evaporation, centrifugation, liquid-liquid partition, reconstitution and dilution with water	MoAb	Indirect competitive ELISA	6.82 ppb	Xu et al., 2006
Thiamethoxam	<div>Water</div> <div>Potato, cucumber and apple</div>	None	<div>Filtration and dilution with buffer</div> <div>Centrifugation, filtration and dilution with buffer</div>	MoAb	Flow fluorescent immunoassay	30 pg/mL	Kim et al., 2006
Acetamiprid	Peach, apple, strawberry, cucumber, eggplant and tomato	Vortex mixing with methanol	Filtration and dilution with water	MoAb	Direct competitive ELISA kit	0.6 ng/g	Watanabe et al., 2006
Imidacloprid	Apple juice, grape juice and orange juice	None	Dilution with buffer	MoAb	Direct competitive ELISA kit	3.9 µg/L	Watanabe et al., 2007
Imidacloprid	Hemlock wood and needle tissues	Shaking by hand with water	Centrifugation and dilution with water	-	Direct competitive ELISA kit	-	Eisenback et al., 2009
Imidacloprid	Honey	None	Dilution with buffer	MoAb	Indirect competitive ELISA	6.5 ng/mL	Ma et al., 2009
Thiamethoxam						0.57 ng/mL	
Imidacloprid	Apple juice, grape juice, orange juice and peach juice	None	Dilution with buffer	MoAb	Indirect competitive ELISA	6.2 ng/mL	Xu et al., 2010
Thiamethoxam						0.5 ng/mL	
Imidacloprid	Honeybee	Liquid-liquid extraction with dichloromethane after homogenization with acetone and coagulation	Dilution with water	PoAb	Indirect competitive chemiluminescent ELISA	14.8 ng/mL	Girotti et al., 2010
Imidaclothiz	Water	None	Dilution with methanol/buffer (2:8, v/v)	MoAb	Indirect competitive ELISA	0.0875 mg/L	Fang et al., 2011
	Soil and cabbage	Ultrasonic extraction with	Centrifugation, evaporation and				

		dichloromethane	reconstitution with methanol/buffer (1:9, v/v)				
Dinotefuran	Rice	Mechanical shaking with methanol	Centrifugation and dilution with water (if necessary, dilution with methanol/water (1:9, v/v))	MoAb	Direct competitive ELISA kit	5.4 ng/mL	Watanabe et al., 2011

Table 3. Overview of nonchromatographic techniques with immunochemical determinations for determination of neonicotinoid insecticides.

4. Overview of analytical methods for pesticide residues including neonicotinoid insecticides

Sections 2 and 3 summarize trends in the determination methods for neonicotinoid insecticides and the problems discovered during the development of analytical methods such as the matrix effect. At present, highly accurate and sensitive multiresidue analysis by GC or HPLC equipped with MS or MS/MS as a detector is the major trend in the determination not only of neonicotinoid insecticides but also of pesticides in general. It goes without saying that pesticide residue analysis is an indispensable basic technology that is useful to secure the safety of food, including crops, as well as in various research fields such as environment and ecological impact evaluation. However, it is questionable because it is entirely dependent only on GC-MS, LC-MS, and LC-MS/MS for pesticide residue analysis. Particularly when pesticide residues are analyzed to secure food safety, two situations are assumed: (1) testing of foods on the market, and (2) testing of crops before shipment. In the former situation, the history of pesticides that have been used is often unknown. Therefore multiresidue analysis using MS or MS/MS is suitable, in which as many pesticides as possible can be tested and unknown ingredients can be identified. On the other hand, in the latter situation, the subject pesticide can be selected based on the history of use. Therefore there might be cases in which it is more sensible to choose a rapid, simple, and easy method such as ELISA described in Section 3 over multiresidue analysis which has better than necessary performance.

In any event, among the numerous analytical methods, it is necessary to make a wise choice for a suitable method via accurate comprehension of the analytical objectives.

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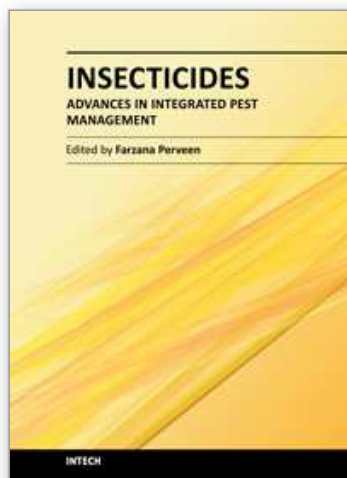
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This book contains 30 Chapters divided into 5 Sections. Section A covers integrated pest management, alternative insect control strategies, ecological impact of insecticides as well as pesticides and drugs of forensic interest. Section B is dedicated to chemical control and health risks, applications for insecticides, metabolism of pesticides by human cytochrome p450, etc. Section C provides biochemical analyses of action of chlorfluazuron, pest control effects on seed yield, chemical ecology, quality control, development of ideal insecticide, insecticide resistance, etc. Section D reviews current analytical methods, electroanalysis of insecticides, insecticide activity and secondary metabolites. Section E provides data contributing to better understanding of biological control through *Bacillus sphaericus* and *B. thuringiensis*, entomopathogenic nematodes insecticides, vector-borne disease, etc. The subject matter in this book should attract the reader's concern to support rational decisions regarding the use of pesticides.

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