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REVIEW

Methods of sample preparation for determination of pesticide residues in food matrices by chromatography–mass spectrometry-based techniques: a review

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Abstract Much progress has been made in pesticide analysis over the past decade, during which time hyphenated techniques involving highly efficient separation and sensitive detection have become the techniques of choice. Among these, methods based on chromatographic separation with mass spectrometric detection have resulted in greater likelihood of identification and are acknowledged to be extremely useful and authoritative methods for determination of pesticide residues. Even with such powerful instrumental techniques, however, the risk of interference increases with the complexity of the matrix studied, so sample preparation before instrumental analysis is still mandatory in many applications, for example food analysis. This article summarizes the analytical characteristics of the different methods of sample-preparation for determination of pesticide residues in a variety of food matrices, and surveys their recent applications in combination with chromatographic mass spectrometric analysis. We discuss the advantages and the disadvantages of the different methods, address instrumental aspects, and summarize conclusions and perspectives for the future.

Keywords Pesticides · Sample preparation · Chromatography–mass spectrometry · Food analysis

Introduction

Analysis of pesticides in food matrices is a difficult task, because of the complexity of the matrix and the low concentrations at which these compounds are usually present. Thus, despite advances in the development of highly efficient analytical instrumentation for their final determination, sample pretreatment remains an important part of obtaining accurate quantitative results. Many choices have been proposed for pretreatment and/or extraction of pesticide residues in foods. In most of these the extraction procedure usually involves sample homogenization with an organic solvent, alone or mixed with water or pH-adjusted water, using a homogenizer, blender, or sonicator [1, 2]. In addition to these classical extraction techniques, other more recent approaches [3], for example QuEChERS [4–9], supercritical fluid extraction (SFE) [10], pressurized-liquid extraction (PLE) [11–13], microwave-assisted extraction (MAE) [14], matrix solid-phase dispersion (MSPD) [15], solid-phase extraction (SPE) [16], solid-phase microextraction (SPME) [17], and stir-bar-sorptive extraction (SBSE) [18–20], have resulted in new possibilities in sample treatment and advantages such as a substantial reduction of the extraction time and incorporation into on-line flow-analysis systems. Each technique has its advantages and disadvantages and the choice should depend on the analytical problem.

There is a wealth of scientific literature on the applications of these extraction techniques for preparation of different food samples (e.g. fruits, juices, vegetables, milk, grain, plant matrices, etc.) for determination of pesticide residues. Because of increasing interest in elucidation of the structures of the pesticides, however, this article will focus on applications of chromatography–mass spectrometry (MS) techniques, covering relevant publications between 2002 and 2006. After an introduction summarizing modern preconcentration techniques, emphasis will be on recent developments and trends.

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Table 1 Operational characteristics of SE applications based on GC–MS methods for determination of pesticides in foods

Analyte	Matrix	Extraction method	Extraction solvent	Clean-up	Ionization mode	Detection	GC column	LOD (g kg^{-1})	LOQ (μg kg^{-1})	Recovery (%)	RSD	Ref.
Chlorpyrifos	Vegetables	SE	Acetone	no	EI	Q-MS(SIM)	HP-5 MS	n.r.	n.r.	89–108	1.0–8.2	[48]
72 Multiclass pesticides	Vegetables	SE	DCM	no	EI	IT-MS(MS–MS)	CP-Sil 8 CB	0.02–4	0.06–13	70–130	4.7–19.7	[50]
9 OPPs, OCLs, pyrethroids, triazines, ureas	Olive oil	SE	Petroleum ether - MeCN	no	EI	Q-MS(SIM)	ZB-5 MS	3–60	n.r.	73–91	3.3–9.9	[58]
31 Multiclass pesticides	Fruits, vegetables	SE	DCM	no	EI or PCI	MS(SIM)	DB-5 MS	0.01–2.60	0.02–8.60	71–119	6–19	[51]
20 Modern pesticides	Peach extracts	SE	EtAc	no	EPS	TOF-MS	DB-5 MS	n.r.	0.5–25	n.r.	2.7–8.1	[37]
5 OPPs, 1 acaricide	Apricots and peaches	SE	Acetone/DCM-light petroleum	no	NCI	IT-MS(MS–MS)	RTX-5 MS	10–100	n.r.	n.r.	n.r.	[57]
20 Pesticides (OPPs, Pyrethroids, triazoles, triazines, others)	Baby food	SE	MeCN	no	EI	QMS(SIM)	CP-Sil 8 CB	n.r.	0.07–1.44	70–110	0.59–1.28	[9]
18 Pesticides (OPPs, Pyrethroids, triazoles, triazines, others)	Baby food	USE	MeCN	SPE (NH2)	EI	MS(SIM)	CPSil 8 MS	0.07–18.9	70–110	n.r.	<20	[32]
20 Multiclass pesticides	Fruits	SE	EtAc	GPS	ESI^+	TOF-MS	DB-XLB×DB-17	0.2–140	n.r.	n.r.	4.2–6.6	[45]
51 Multiclass pesticides	Honey	SE	Water/MeOH	SPE (C_{18})	EI	Q-MS	ZB-5 MS	<6	n.r.	86–101	1.6–9.2	[53]
90 Multiclass pesticides	Fruits	SE	Acetone	SPE (SDB)	EI	MS(SIM)	DB-35 MS	10	20	72–147	1–19	[49]
17 Multiclass pesticides	Vegetables, fruits, baby food	SE	MeOH	SBSE (PDMS)	EI	MS(SIM)	HP-5 MS	n.r.	n.r.	43–100	<10	[60]
78 Multiclass pesticides	Vegetables	SE	EtAc	DSPE (NH2)	EI	MS-TQ(SIM)	RTX-5	1.1–19.8	n.r.	96–113	1.6–6.6	[36]
78 Multiclass pesticides	Vegetables	SE	EtAc	DSPE (NH2)	EI	MS(SCAN)	DB-5	0.3–2.5	n.r.	93–109	4.1–9.1	[36]
78 Multiclass pesticides	Vegetables	SE	EtAc	DSPE (NH2)	EI	MS-TQ(SIM)	DB-5	0.1–0.4	n.r.	95–110	2.1–5.5	[36]
78 Multiclass pesticides	Vegetables	SE	EtAc	DSPE (NH2)	EI	MS(SIM)	DB-5	0.1–0.6	n.r.	94–104	1.0–4.2	[36]
20 Pesticides (OPPs, Pyrethroids, triazoles, triazines, others)	Baby food	SE	MeCN	SPE (NH2)	EI	QMS(SIM)	CP-Sil 8 CB	n.r.	0.07–3.47	70–110	1.14–2.20	[9]
Multiclass pesticides	Fruits, vegetables	QuEChERS	MeCN	DSPE(PSA)	EI	Q-MS(SIM)	DB-5 MS	n.r.	<10	85–101	<5	[4]
229 Multiclass pesticides	Fruits, vegetables	QuEChERS	MeCN	DSPE(PSA)	EI	Q-MS(SIM)	DB-5 MS	n.r.	<10	70–120	<10	[6]
32 Multiclass pesticides	Milk, eggs, avocado	QuEChERS	MeCN	DSPE(PSA)	EI	Q-MS(SIM)	DB-5 MS	n.r.	<10	>95	<10	[7]
32 Multiclass pesticides	Fatty food (Milk, eggs, avocado)	QuEChERS	MeCN	DSPE(PSA)	EI	Q-MS(SIM)	DB-5 MS	n.r.	<10	>27	n.r.	[8]
12 Priority pesticides	Baby food	QuEChERS	MeCN	DSPE(PSA-C_{18})	EI	Q-MS–MS (MRM)	ZB-50	n.r.	n.r.	60–113	<28	[35]
18 Pesticides (OPPs, pyrethroids, triazoles, triazines, others)	Apples	QuEChERS	MeCN	DSPE(PSA)	EI	MS(SIM)	CP-Sil 8 CB MS	<5	n.r.	n.r.	<14	[34]
43 Herbicides	Barley samples	QuEChERS	MeCN	DSPE (PSA)	ESI^+	TOF-MS	RTX-CL	1.0–2.3	n.r.	62–78	1.1–9.3	[33]
20 Pesticides (OPPs, Pyrethroids, triazoles, triazines, others)	Baby food	QuEChERS	MeCN	DSPE (PSA)	EI	QMS(SIM)	CP-Sil 8 CB	n.r.	0.15–3.97	70–110	1.93–2.67	[9]
27 OCLs	Fish tissue	USE	Acetone-Hexane	SPE(Florisil)	EI	Q-MS(SIM)	DB-5 MS	0.5–20		78–115	3–15	[25]
Chlordimeform and their metabolites, 4-chloro-o-toluidine, *N*-formyl-4-chloro-*o*-toluidine	Honey	USE	Acetone-Hexane	no	EI	Q-MS(SIM)	DB-17	n.r.	n.r.	n.r.	n.r.	[22]

o-Phenylphenol	Citrus	USE	DCM	no	EI	IT-MS(SCAN)	DB-17	n.r.	n.r.	n.r..	1–5	[23]
o-Phenylphenol	Citrus	USE	DCM	no	EI	Q-MS(SIR)	HP-5	n.r.	n.r.	n.r..	1–2	[23]
22 Pesticides (OPPs, OCHs, Pyrethroids, others)	Plant	SFE	CO_2 (50°C, 123 bar or 80°C, 202 bar)	-	EI	Q-MS(SCAN)	DB-5 MS	n.r.	n.r.	n.r.	n.r.	[73]
Multiclass pesticides	Apple, green bean, and carrot	SFE	CO_2 (60°C, 360 atm)	-	EI	Q-MS(SIM)	-	n.r.	n.r.	>50	<15	[72]
33 Pesticides (OPPs, OCLs, pyrethroids, organonitrogen)	Honey	SFE	CO_2 (60°C, 200 atm, acetone)	Florisil	EI	Q-MS(SCAN)	LM-5	n.r.	n.r.	n.r.	n.r.	[70]
22 GC-amenable pesticides	Rice	SFE	CO_2 (50 °C, 200 atm, MeOH)	Aminopropyl	EI	IT-MS(SIM)	DB-5 MS	n.r.	n.r.	n.r.	n.r.	[71]
Bifenthrin, acrinathrin, λ-cyhalothrin, deltamethrin	Strawberries	MAE	(5 min, 30 W, MeCN-Water)	SPME (PDMS 100 m)	EI	Q-MS(SIM)	DB-5 MS	0.9–13.8	2.8–41.3	n.r.	1.2–14.2	[84]

USE, ultrasonic extraction; n.r., not reported

Sample pre-concentration techniques

Analysis of pesticide residues in food samples is usually hampered by interfering compounds present in the complex matrix. Thus, the challenge for analysts is to maximize recovery of the analytes and minimize the accompanying interferences by use of appropriate extraction and clean-up procedures. Several modern techniques based either on solvent or sorptive membrane extraction have been tested the recent years to achieve this objective. A brief description of these methods is given in the next sections, with some relevant applications in which they are used in conjunction with chromatographic MS analysis (Tables 1, 2, 3, 4, 5, 6).

Solvent extraction procedures

Solvent extraction

Solvent extraction (SE)—which may be followed by solid-phase extraction (SPE)—is still the most widely used technique, mainly because of its ease of use and wide-ranging applicability (Tables 1 and 2). The extraction process varies slightly, depending on whether the sample is liquid or solid. Analysis of liquid samples has an advantage over analysis of solid samples that one fewer pretreatment step is usually required, because of their liquid state. The latter are usually repeatedly extracted with an immiscible organic solvent. Occasionally, very little sample preparation may be required if the liquid is sufficiently free from matrix interferences, for example dilution with water or filtration. An interesting study in this field was recently reported by Goto et al. [21], who simply diluted and filtered samples of juices and wines before direct injection into the ESI LC–MS–MS system for the determination of *N*-methyl carbamate pesticides. Solid samples are usually homogenized before extraction, by mechanical grinding, mixing, rolling, agitating, stirring, chopping, crushing, macerating, mincing, pressing, pulverizing, or any other reasonable means of comminuting the sample. A portion is then blended or stirred with an organic solvent which is then homogenized with sodium sulfate to bind water present in the sample. The dried powder is then centrifuged and the supernatant is either concentrated or injected directly in the chromatographic system. Sample clean-up is usually performed before final chromatographic analysis. Although the scale of the extraction varies, the most common SE configuration uses approximately 50 mL solvent with 5–50 g sample. SE extraction is occasionally performed with sonication to increase the extraction yield and increase the speed of the procedure [22–25]. A notable example was recently presented by Granby et al. [24]. The method was based on extraction of carbamates and other relatively polar

Table 2 Operational characteristics of SE applications based on LC–MS methods for the determination of pesticides in foods

Analyte	Matrix	Extraction method	Extraction solvent	Clean-up	Ionization mode	Detection	LC column	Mobile phase	LOD (g kg^{-1})	LOQ (μg kg^{-1})	Recovery (%)	RSD	Ref.
31 Multiclass pesticides	Fruit and vegetables	SE	EtAc	no	ESI^+	TQ-MS(SIM)	Polaris 3 m C_{18}-A and precolumn Polaris 3 m C_{18}-A	MeOH/Ammonium formate	0.011–6400	0.038–21000	72–104	4–22	[38]
9 OPPs, OCLs, pyrethroids, triazines, ureas	Olive oil	SE	Petroleum-MeCN	no	ESI^+	IT-MS(MS–MS)	Zorbax Eclipse XDB-C_8	Water-HCOOH/ MeCN	0.2–3	n.r.	84–104	4.6–7.6	[58]
74 Multiclass pesticides	Fruits, vegetables	SE	EtAc	no	ESI^+	TQ-MS(SRM)	Nucleosil 100−5 C_{18} HD	Water-HCOOH/ HCOOH-MeOH	n.r.	10	63–133	2–109	[39]
16 Multiclass pesticides	Vegetables	SE	EtAc	no	ESI	TQ-MS(MRM)	Polaris C_{18}-A and precolumn	NH_4COOH-HCOOH/ MeOH-MeCN	0.5–5.0	n.r.	70–105	<28	[40]
OPP, DP, TBZ, IMZ, IMZ-M	Citrus	SE	Diethyl ether	no	APPI	MS(SIM)	Inertsil ODS-3	Water/MeOH	0.01–0.05	n.r.	67–100	2–8	[47]
Propamocarb	Vegetables	SE	MeOH	no	ESI^+	MS(SIM)	Shodex RSpak DE-613	MeOH-$AcNH_4$	25	n.r.	92–11	1.2–10.3	[46]
Abamectin and azadirachtin	Oranges	SE	MeCN	no	ESI^+	TQ-MS(MS–MS)	Nucleosil C_{18}	Water/MeOH	2–7	10	53–103	2–32	[28]
10 Pesticides	Oranges	SE	EtAc	no	$APCI^+$	MS-Q(SIM)	Luna C_{18} and precolumn	Water/MeOH	2–200	n.r.	32–98	<18	[41]
6 Pesticides	Oranges	SE	EtAc	no	$APCI^+$	IT-MS(MRM)	Luna C_{18} and precolumn	Water/MeOH	n.r.	1–300	72–94	7–16	[42]
9 Pesticides	Fruits	SE	EtAc	no	ESI^+	IT-MS(MRM)	Luna C_{18} and precolumn	Water/MeOH	n.r.	10–400	59–101	8–17	[43]
17 Semi-polar multiclass pesticides	Apples	SE	MeCN	no	ESI^+	MS-Q(MRM)	Acquity UPLC BEH C_{18}	Water-MeOH	n.r.	0.5–8.0	n.r.	n.r.	[30]
17 Semi-polar multiclass pesticides	Apples	SE	MeCN	no	ESI^+	MS-Q(MRM)	Discovery C_{18}	Water-MeOH	n.r.	2–8.0	n.r.	n.r.	[30]
6 Fungicides	Fruits	SE	Acetone	no	ESI^+	MS-Q(MS-MS)	Nucleosil C_{18}	Water-AcNH4/ MeOH-HCOOH	5–25	50	75–99	3–13	[16]
24 New pesticides	Fruit and vegetables	SE	Acetone and EtAc/ cyclohexane	no	ESI^+	MS-Q(MRM)	Synergy Polar-RP	Water-HCOOH/ MeCN	n.r.	n.r.	76–106	3–15	[56]
Carbosulfan and its metabolites	Oranges	SE	DCM	no	TIS	TQ-MS(SRM)	Zorbax Bonus-RP	Water-$AcNH_4$/ MeOH-$AcNH_4$	0.4–3	1–10	n.r.	n.r.	[61]
6 Pesticides	Oranges	SE	EtAc	no	ESI^+ or ESI-	QIT-MS(SRM) or (SCAN)	Luna C_{18} and precolumn	Water-MeOH	5–200	1–300	72–92	12–19	[44]
6 Pesticides	Oranges	SE	EtAc	no	ESI^+ or ESI-	TQ-MS(SRM) or (SCAN)	Luna C_{18} and precolumn	Water-MeOH	5–200	n.r.	70–94	8–19	[44]
19 Carbamates and others	Fruit, vegetables and cereals	USE	MeOH-$AcNH_4$-CH_3COOH	no	ESI^+	Q-MS(MRM)	Genesis C_{18} column with Phenomenex C_{18} ODS precolumn	$AcNH_4$-CH_3COOH-Water/ $AcNH_4$-CH_3COOH-MeOH	10–20	n.r.	70–120	4–20	[24]
Carbosulfan and its metabolites	Oranges	PLE	DCM	no	$APCI^+$	IT-MS(MRM)	Luna C_{18} and precolumn	Water/MeOH	0.01–0.07	0.1–0.7	55–90	8–19	[76]
7-*N*-Methylcarbamate pesticides	Citrus fruits	SE	Cyclohexane	GPS	ESI^+	MS(MRM)	-	-	50[a]	n.r.	67–129	4.1–15.9	[59]
10 Pesticides	Oranges	SE	MeOH/Water	SBSE	$APCI^+$	MS-Q(SIM)	Luna C_{18} and	Water/MeOH	1–50	n.r.	8–84	n.r.	[41]

				(PDMS)			precolumn					
13 Carbamates	Apple-Based Infant Foods	SE	MeCN	SPE (Oasis HLB)	ESI^+	MS-Q(MRM)	YMC ODS-AQ S-3 and precolumn	MeCN/AcNH4: MeCN:Water/Water	0.09–0.2	n.r.	75–95	0.9–13	[29]
Chlormequat and mepiquat	Tomato, pear, and wheat flour.	SE	Water/MeOH	SPE (LiChrolut SCX)	ESI^+	MS-Q(MRM)	n.r	Water-Ammonium formate - MeOH	< 3	< 6	n.r.	n.r.	[54]
17 Sulfonylurea herbicides	Grain, potatoes	SE	Water/MeOH	DSPE (IAs)	ESI^+	MS-Q(MS-MS)	Inertsil C_{18} ODS 3	MeCN:Water-HCOOH/MeCN	1.1– 6.9	n.r.	80–116	0.5–31.9	[55]
43 Multiclass pesticides and nine metabolites	Fruit and vegetables	SE	MeOH/H_2O with (0.1% HCOOH)	SPE (Oasis HLB)	ESI^+	MS-TQ(MS–MS)	Atlantis C_{18}	Water-HCOOH/ MeOH-HCOOH	n.r.	10	70–110	<15	[64]
4 Neonicotinoid pesticide	Fruit and vegetables	SE	Acetone	SPE (Extrelut-NT20)	ESI^+	MS-Q	LiChroCart 125–4 and precolumn LiChrospher 100	Water-CH_3COOH/ MeOH-CH_3COOH	20–100	100–500	74.5–105	1.5–15	[16]
5 Neonicotinoid pesticides	Fruits and vegetables	SE	MeOH	SPE (EnviCarb)	$APCI^+$	MS-Q	Cadenza CD-C_{18}	Water/MeOH	10–20	n.r.	70–95	2–10	[63]
16 Priority OPPs	Baby food	QuEChERS	MeCN	DSPE (PSA)	ESI^+	Q-MS(MS–MS)	Sunfire C_{18}	Water/$AcNH_4$-MeOH	n.r.	1000	85–113	2–10	[26]
16 Priority OPPs	Baby food	QuEChERS	MeCN	DSPE (PSA)	ESI^+	Q-MS(MRM)	Acquity UPLC BEH C_{18}	Water/$AcNH_4$-MeOH	n.r.	1000	92–119	1–17	[26]
43 Herbicides	Barley samples	QuEChERS	MeCN	DSPE (PSA)	ESI^+ or-	TQ-MS–MS(SRM)	Symmetry C_{18} and precolumn Xterra MS C_{18}	Water-HCOOH/ MeOH-HCOOH	0.2–23.2	n.r..	37.4–135	1.0–19.5	[33]
15 Multiclass pesticides	Fruit and vegetables	QuEChERS	MeCN	DSPE (PSA)	ESI^+	TOF-MS(SCAN)	Zorbax Eclipse XDB-C_8	MeCN/Water-HCOOH	0.5–30	n.r.	n.r.	0.8–11	[31]
Imazalil and prochloraz	Citrus	QuEChERS	MeCN	DSPE (PSA)	ESI^+	TOF-MS	Zorbax Eclipse XDB-C_8	Water-HCOOH/ MeCN	n.r.	n.r.	n.r.	n.r.	[27]
229 Multiclass pesticides	Fruits, vegetables	QuEChERS	MeCN	DSPE(PSA)	ESI^+	IT-MS–MS	Luna C_{18} and precolumn	Water-HCOOH/ MeOH-HCOOH	n.r.	<10	70–120	<10	[6]
10 Pesticides	Fruits	PLE	EtAc	MSPD (Acidic Alumina) sample)	$APCI^+$	IT-MS(MRM)	Luna C_{18} and precolumn	Water/MeOH	n.r.	25–250	58–97	5–19	[77]
6 Carbamates	Bovine milk	PLE	Water (90 ^{0}C)	MSPD (Sand)	ESI^+	MS-Q(SIM)	Alltima C_{18} and precolumn	MeOH-HCOOH/ Water-HCOOH	1–4	3–8	85–105	<9	[78]

OPP, *o*-phenylphenol; DP, diphenyl; TBZ, thiabendazole; IMZ, imazalil (and its major metabolite R14821 (IMZ-M)), USE, ultrasonic extraction; n.r., not reported
[a] μg mL^{-1}

Table 3 Operational characteristics of MSPD applications based on GC–MS methods for the determination of pesticides in foods

Analytes	Matrix	Support	Solvent elution	Ionization mode	Detection	GC column	LOD ($g\ kg^{-1}$)	LOQ ($\mu g\ kg^{-1}$)	Recovery	RSD	Ref.
266 Multiclass pesticides	Apple juices	Diatomaceous earth	Hexane–DCM	EI	MS(SIM)	DB-5 MS	3–16	n.r.	64 –117	2–23	[90]
Endosulfan isomers and endosulfan sulfate	Tomato juices	Florisil	EtAc	EI	MS(SIM)	ZB-5 MS	1	3	81–101	<6	[89]
16 Pesticides	Honey	Florisil	Hexane–EtAc	EI	MS(SIM)	HP-5 MS	<12	n.r.	60–112	<10	[88]
9 OPPs, OCLs, pyrethroids, triazines, ureas	Olives	Aminopropyl (clean-up with Florisil)	MeCN	EI	Q-MS(SIM)	ZB-5 MS	8–80	n.r.	n.r.	3.5–6.1	[58]
20 Pesticides (OPPs. Pyrethroids, triazoles, triazines, others)	Baby food	Florisil	EtAC	EI	QMS(SIM)	CP-Sil 8 CB	n.r.	0.06–5.03	70–110	n.r.	[9]

n.r., not reported

pesticides from fruits by ultrasonication with MeOH–ammonium acetate–acetic acid buffer. After centrifugation the samples were filtered in Miniprep filter HPLC vials and analyzed by LC–MS–MS [25].

It is essential to match solvent polarity to analyte solubility, and a combination of non-polar, water-immiscible solvents (e.g., dichloromethane (DCM) or hexane) with solvents of different polarity have been used to achieve the desired viscosity and solvent strength for the particular extraction. Among the solvents tested acetonitrile (MeCN) [4–9, 26–35] and ethyl acetate (EtAc) [36–46] are the most commonly used. MeCN is a polar solvent, miscible with water but with sufficient dispersive (hydrophobic) properties to effectively extract both polar and nonpolar pesticide residues from non-fatty foods. MeCN extracts usually also contain smaller amounts of co-extractives compared with extracts obtained with other solvents, e.g. DCM, especially in the analysis of complex matrices that often contain many different raw ingredients and additives. Finally, the suitability of MeCN extracts for direct analysis by LC–MS or MS–MS is another advantage. Because of its distinct properties, MeCN is highly suitable for use in the QuEChERS method (see below). Seven methods based on use of EtAc as solvent have been validated and used for determination of different groups of pesticides in fruit and vegetables [36–46]. Diethyl ether (DEE) [47], which has lower boiling point than EtAc and is readily removed by rotary evaporation at a lower temperature without analyte losses, has also been chosen as extraction solvent for post-harvest fungicides, *o*-phenylphenol, diphenyl (DP), thiabendazole (TBZ), and imazalil (IMZ) and its major metabolite R14821 (IMZ-M) in citrus fruits. It is, however, interesting to note that, because of its low ignition point and tendency to form explosive peroxides, DDE must be used with care. Use of medium-polarity solvents, for example acetone [16, 48, 49] and DCM [23, 50, 51], has also been preferred, although the latter is a possibly carcinogenic to humans (Group 2B according to the IARC [52]), and its use is not recommended. Štajnbaher and Zupančič-Kralj [49] described rapid SE with acetone using vortex mixing for multiresidue determination of 90 pesticides in fresh fruits and vegetables by use of GC–MS. Vidal's research group performed SE of several classes of pesticide from fruit and vegetables with DCM, and without clean-up, before GC–MS–MS determination [50]. Apart from the solvents mentioned above, mixtures such as MeOH–water [53–55], acetone–hexane [22, 25], EtAc–cyclohexane [56], DCM–light petroleum [57], and petroleum ether saturated with MeCN [58] have been also used, with satisfactory results.

After extraction with solvents of low polarity, for example EtAc or DCM, the polarity of the extract is usually increased before LC analysis, to prevent band broadening, which will hinder separation and reliable

Table 4 Operational characteristics of MSPD applications based on LC–MS methods for the determination of pesticides in foods

Analytes	Matrix	Support	Solvent elution	Ionization mode	Detection	LC column	Mobile phase	LOD (g kg^{-1})	LOQ (μg kg^{-1})	Recovery (%)	RSD	Ref.
6 Pesticides	Citrus	C_{18}	DCM-MeOH	ESI^+	MS-Q	Luna C_{18} and precolumn	Water/MeOH	n.r.	10–20	51–97	5–19	[86]
9 OPPs, OCLs, pyrethroids, triazines, ureas	Olives	Aminopropyl (clean-up with Florisil)	MeCN	ESI^+	IT-MS (MS–MS)	Zorbax Eclipse XDB-C_8	Water-HCOOH/ MeCN	0.4–4	n.r.	81–111	3.7–6.1	[58]
9 Pesticides	Fruits	C_{18}	DCM-MeOH	ESI^+	IT-MS(MRM)	Luna C_{18} and precolumn	Water/MeOH	n.r.	50–200	52–108	4–15	[43]
8 Carbamates	Fruit, vegetables	Sand	Water (50°C)	ESI^+	MS-Q(SIM)	Alltima C_{18} and precolumn	MeOH-HCOOH/ Water-HCOOH	2–7	n.r.	84–110	<9	[92]
6 Pesticides	Citrus	C_{18}	DCM-MeOH	ESI^+	MS-Q	Luna C_{18} and precolumn	Water/MeOH	n.r.	n.r.	68–92	5–19	[86]
3 Dithiocarbamates and two metabolites	Plants	Carbon	DCM-MeOH	$APCI^+$	MS-Q-(SIM)	Phenomenex C_8 and precolumn C_{18}	Water/MeOH	n.r.	250–2.500	33–109	4–21	[92]
10 Pesticides	Oranges	C_8	DCM	$APCI^+$	MS-Q(SIM)	Luna C_{18} and precolumn	Water/MeOH	8–20	n.r.	47–96	<15	[41]
5 Fungicides	Fruit, vegetables	C_8	DCM	$APCI^-$	MS-Q(SIM)	Phenomenex C_8 and precolumn C_{18}	Water/MeOH	10–100	n.r.	52–92	6.1–11.9	[87]
10 Pesticides	Fruit juices	Diatomaceous earth	EtAc	TIS^{-a}	MS-Q(MRM)	Alltima C_{18}	MeCN-HCOOH/ Water-HCOOH	0.07–0.9	n.r.	77–102	<9	[91]

[a] TurboIonSpray source
n.r., not reported

quantitative analysis. This is usually achieved by partial or complete evaporation of the eluate and dissolution of the compounds in a more polar solvent, for example methanol (MeOH) [16, 30, 31, 55, 59–61], MeCN [58], or mixtures of these with water [55, 58] or ultra-pure water [62]. Evaporation to a volume not less than 50–100 μL has been performed when volatile compounds are being determined, because complete evaporation may lead to substantial loss of these compounds. In contrast, for some GC–MS applications polar extracts have been re-dissolved in non-polar solvents, for example cyclohexane [37, 50], toluene [32], EtAc [45], or octane–toluene [57].

Extraction conditions, for example pH, must sometimes be adjusted to enhance analyte extraction. Yoshioka et al. [47] demonstrated that adjustment of the pH to 10 is necessary for simultaneous determination with high extraction yields of analytes with different physicochemical characteristics (*o*-phenylphenol is acidic, TBZ, IMZ, and IMZ-M are basic, and DP is neutral) from citrus fruits. To ensure the pH of the aqueous phase was not reduced to below 10 during extraction with DEE, as a result of the strong acidic buffer action of citrus fruit extracts, the authors decided, however, to adjust sample pH to 12 before shaking. In another study [56] adjustment of fruit and vegetable samples to pH 6 seemed to improve analytical performance for all the new-generation pesticides tested, especially benfuracarb and imidachloprid.

Although SE can be performed without clean-up [22, 23, 28, 30, 37–43, 47–51, 56, 57, 59, 61], especially for samples sufficiently free from matrix interferences, in most of the work cited further clean-up of the solvent extracts was necessary to improve quantitative results in subsequent chromatographic analysis. SPE, classic [9, 16, 29, 32, 49, 53, 54, 63–65], and dispersive SPE [4–9, 26, 27, 31, 33–36, 55] are the methods most commonly used for this purpose, although gel-permeation chromatography (GPS) [45, 59] and SBSE (enrichment and clean-up) [41, 60] have also been performed successfully.

SE methods have several disadvantages—they are laborious, time-consuming, expensive, and subject to problems arising from evaporation of large volumes of solvent and the disposal of toxic or inflammable solvents. Despite these disadvantages, SE is still among the most popular method for routine sample preparation, mainly because of its simplicity, robustness, efficiency, and the wealth of analytical data available. To overcome the drawbacks mentioned above new trends in sample treatment and miniaturization of time-tested SE have resulted in improved SE techniques that use much smaller amounts of organic solvent, enable more efficient extraction, enable on-line coupling to analytical measurement techniques, and enable easier automation and higher extraction throughput. A good example of this is the QuEChERS method which,

Table 5 Operational characteristics of sorptive extraction applications based on GC–MS methods for the determination of pesticides in foods

Analytes	Matrix	Extraction method	Adsorbent	Ionization mode	Detection system	GC column	LOD (g kg^{-1})	LOQ (μg kg^{-1})	Recoveries	RSD	Ref.
7 OPPs	Strawberries and cherries	HS-SPME	PDMS 100 m	EI	Q-MS(SIM)	DB-5 MS	6.3–12.7	21–42.3	74–91	7–15	[104]
7 Pyrethroids	Strawberries	MAE-SPME	PDMS 100 m	EI	Q-MS(SIM)	DB-5 MS	0.9–13.8	n.r.	n.r.	1.2–14	[84]
6 Phenylurea herbicides and their homologous anilines	Vegetables	SPME	PA 85 m	EI	Q-MS(SIM)	BP-10	0.1–0.7	n.r.	76–95	<10	[106]
4 Triazoles	Wine and strawberries	SPME	PA 85 m	EI	Q-MS(SIM)	SPB-5	0.03–0.1	n.r.	n.r.	7–28	[107]
9 OCls	Honey	SPE	C_{18}	EI	Q-MS(SIM)	DB-5	n.r.	< 100	79–98	3–18	[95]

n.r., not reported

because of increasing interest by many laboratories in recent years, is described in detail in the next section.

QuEChERS

QuEChERS is a quick and convenient replacement for LLE which furnishes high-quality results in a minimum number of steps and with low consumption of solvent and glassware. QuEChERS stands for quick, easy, cheap, effective, rugged, and safe, and as the newest-generation method for analysis of pesticide residues in food matrices it lives up to its name [4]. The original procedure consists in extraction of the homogenized sample by hand-shaking or vortex mixing with the same amount of MeCN to furnish a final extract sufficiently concentrated to remove the need for solvent evaporation. Gram quantities of salts (4 g anhydrous magnesium sulfate, $MgSO_4$, and 2 g sodium chloride, NaCl, a combination which affords well-defined phase separation without dilution with hazardous non-polar organic solvents) are then added to the sample, with mixing, to drive partitioning of the analytes between the aqueous residue and the solvent. After simple vortex mixing and centrifugation, which results in perfect physical separation of the phases, clean-up and removal of residual water is performed simultaneously by use of a rapid procedure, called dispersive solid-phase extraction (DSPE), in which a primary–secondary amine (PSA) adsorbent and more anhydrous $MgSO_4$ are mixed with the sample extract. Dispersive SPE is based on SPE methodology, but the adsorbent is added directly to the extract without conditioning and the clean-up is easily performed by shaking and centrifugation. The latest procedure requires less time than traditional SPE and simultaneously removes residual water and many polar matrix components, for example organic acids, some polar pigments, and sugars.

Because of its distinct advantages, MeCN is the solvent of choice for the QuEChERS method (see previous section). Using this solvent the technique was successfully assessed for extraction of several classes of pesticides from different food matrices, for example barley, fruit, and baby-food [4–9, 32–35] (Tables 1 and 2). The dispersing adsorbent most frequently used was PSA, a weak anion-exchanger which removes fatty acids, sugars, and other matrix co-extractives that form hydrogen bonds. Mixed-mode materials containing two adsorbents, for example C_{18} and PSA, have also been tested, and have been found to give contradictory results, depending on the matrix and the class of the pesticide tested. For example, Leandro et al. [26] used DSPE for clean-up after QuEChERS extraction of seven priority OPPs and nine transformation products from baby food. PSA (50 mg), C_{18} (100 mg), and their mixture (50 mg PSA+100 mg C_{18}) were tested as adsorbent materials. Recoveries obtained without clean-up or with addition of 50 mg of PSA were very similar and close to 100%. When C_{18} was used recoveries were reduced to, for example, ca 38% for oxydemeton-*S*-methyl, 31% for fensulfothion-oxon, and 15% for cadusafos. The same was observed for the mixed adsorbent. PSA was therefore chosen because it furnished relatively clean extracts, and peak shape and signal-to-noise ratio (*S*/*N*) were improved compared with crude extracts, resulting in satisfactory calibration plots in both HPLC–MS–MS and UPLC–MS–MS. In contrast, the same research group [35] evaluated the effectiveness of different amounts (100–300 mg) of C_{18} with a constant amount (50 mg) of PSA with regard to the quantity of co-extractives remaining after evaporation of the solvent. Quantification using 100 mg C_{18} resulted in a lack of reproducibility among the diverse range of matrices investigated, occasionally giving non-linear calibration plots, and lower recoveries were obtained when 300 mg

Table 6 Operational characteristics of sorptive extraction applications based on LC–MS methods for the determination of pesticides in foods

Analytes	Matrix	Extraction method	Adsorbent	Elution solvent	Ionization mode	Detection system	LC column	Mobile phase	LOD ($g\ kg^{-1}$)	LOQ ($\mu g\ kg^{-1}$)	Recovery	RSD	Ref.
6 Pesticides	Grapes	SPE	C_{18}	DCM-MeOH	$APCI^+$	MS-Q(SIM)	Phenomenex C_{18}	Water/MeOH	n.r.	3–10	60–100	7–17	[60]
3 Dithiocarbamates and 2 metabolites	Plants	SPE	EnviCarb	DCM-MeOH	$APCI^+$	MS-Q-(SIM)	Phenomenex C_8 and precolumn C_{18}	Water/MeOH	n.r.	n.r.	8.7–67.4	n.r.	[93]
42 OPPs, OCLs and carbamates	Honey	SPE	C_{18}	EtAc, MeOH, DCM	$APCI^-$	MS-Q(SIM)	Luna C_{18} and precolumn	Water/MeOH	n.r.	< 100	40–98	6–19	[95]
22 OPPs	Honey	SPE	C_{18}	EtAc, MeOH, DCM,	$APCI^+$ or $^-$	MS-Q(SIM)	Spherisorb C1 and precolumn LiChrosorb RP-18	Water/MeOH	< 240	n.r.	16–102	<17	[96]
6 OPPs	Honey	SPME	PDMS 100 m	–	$APCI^+$ or $^-$	MS-Q(SIM)	Luna C_{18} and precolumn	Water/MeOH	< 500	n.r.	52–75	3–10	[110]
6 Pesticides	Grapes	SBSE	PDMS 1 mm	–	$APCI^+$	MS-Q(SIM)	Phenomenex C_{18}	Water/MeOH	n.r.	10	15–100	10–19	[60]
6 OPPs	Honey	SBSE	PDMS 1 mm	–	$APCI^+$ or $^-$	MS-Q(SIM)	Luna C_{18} and precolumn	Water/MeOH	< 100	n.r.	75–115	5–9	[110]

n.r., not reported

C_{18} was used. Compared with the other two amounts, 200 mg C_{18} resulted in cleaner extracts, improved signal-to-noise ratio, less variation in calibration plots, and consistent response; they concluded the appropriate adsorbent was between 100 and 200 mg C_{18}.

The QuEChERS method has the advantages of high recovery, accurate results, high sample throughput, low solvent and glassware usage (no chlorinated solvents), less labor and bench space, lower reagent costs, and ruggedness [4–9]. Organic acids and other potential contaminants are removed during clean-up. The main disadvantage of QuEChERS is that for 1 g sample per milliliter of final extract the concentration of the extract is lower than for the concentrated extracts obtained by use of most traditional procedures. Thus, the final extract must be concentrated to a greater extent to furnish the necessary sensitivity and to achieve the limits of quantification (LOQ) desired. Despite this drawback, the quantitative results obtained from a large number of pesticides indicate that combination of QuEChERS with hyphenated methods of detection provides scientists with the capability to achieve efficient and effective monitoring of pesticide residues in food.

Instrumental solvent-extraction methods

Supercritical-fluid extraction (SFE)

Enhanced extraction methods are usually instrumental techniques, and the enhanced efficiency of these methods is because of the elevated solvent temperatures used. This temperature elevation increases the speed of extraction of analytes from solid matrices, as a result of increased solubility, better desorption, and enhanced diffusion. The new generation of enhanced extraction techniques is based on use of temperatures above the atmospheric boiling point of the extracting solvent. One such emerging technique is SFE, which resembles Soxhlet extraction in which the solvent used is a supercritical fluid (SF), i.e. a substance above its critical temperature and pressure, which results in an unusual combination of properties. SFs diffuse through solids like gases, but dissolve analytes like liquids, so the rate of extraction is enhanced and less thermal degradation occurs [10, 65, 66]. Much sample pretreatment can also performed with non-polluting, non-toxic SFs, which are an excellent alternative to the potentially hazardous and expensive solvents used in Soxhlet extraction.

SFE in food analysis is usually performed with carbon dioxide (CO_2) as extracting solvent [67–73], because its critical conditions are easily achieved (critical temperature, T_c, 31 °C and critical pressure, P_c, 1073 psi), and it is non-toxic, non-flammable, relatively inexpensive, and easily obtained commercially (Table 1). Use of pure CO_2 in multiresidue pesticide analysis is, nevertheless, limited,

because it is a nonpolar solvent with solvent properties similar to those of hexane. For quantitative extraction of moderately polar and polar pesticides, therefore, precisely known amounts of "modifiers" and/or complexing agents are usually added to obtain satisfactory results [67, 68]. Aguilera et al. [69] showed that the extractability of many polar pesticides from rice or Gazpacho (a table-ready food composite of plant origin) is very poor if the extraction is performed without a modifier and that use of MeOH or EtAc as static modifier seems to be a critical in achieving acceptable recovery. In another study [70], MeCN and acetone were compared as modifiers for SFE extraction of 32 pesticides from different classes (OPPs, organochlorine (OCPs), organonitrogen (ONPs), and pyrethroids) from honey. The results showed that the extraction yield of some pesticides (tetradifon, etaconazole, hexaconazole, imazalil, metolachlor, prochloraz, propiconazole, triadimenol, chlorpyrifos, diazinon, dichlorvos and dimethoate) is greatly improved (from 32% to 61%) with MeCN as modifier compared with CO_2 modified with acetone. In contrast, for other pesticides (e.g. OCPs) recovery was lower or no effect was observed. The increase in average recovery indicated that MeCN increased the solvating power of CO_2 sufficiently for extraction of several classes of pesticides. Because analytes of different polarity were recovered better by use of fluid containing MeCN, the effect of the modifier might, furthermore, be related not only to the change in polarity of the extraction fluid but also to its interaction with the matrix.

In SFE the solvating power of the supercritical fluid can be manipulated simply by changing the pressure (*P*) and/or temperature (*T*), so remarkably high selectivity can be achieved. SFE of fruit and vegetables usually, therefore, furnishes clean extracts that can be analyzed directly by GC without further clean-up. After SFE extraction of high-fat foods and very complex matrices, for example honey, however, a clean-up step (post-extraction clean-up or "in-situ" clean-up) should be included before chromatographic determination to remove co-extracted interfering compounds (e.g. lipids, sugars, etc.). Rissato et al. [70] described an SFE method for extraction of several classes of pesticide from honey and illustrated the effectiveness of SPE clean-up in its simplest form by use of Florisil cartridges. Although this type of purification procedure is simple and gives satisfactory results, additional manipulation throughout the process results in increased total extraction time and volume of solvent consumed. Analytical methods that incorporate "in-situ" or "in-line" clean-up are, therefore, of particular interest. Such sample clean-up can be achieved "in-situ" by placing a fat retainer (adsorbent) inside the extraction thimble, between the sample and a restrictor, so that the lipids are retained and the pesticides are carried in the supercritical CO_2. By use of this approach, Aguilera et al. [71] evaluated the effectiveness of different adsorbent materials (Celite, Extrelut, Hydromatrix, Florisil, and aminopropyl) at retaining the fat from wild rice during SFE with 15 mL CO_2 at 200 atm and 50 °C. The results showed that the first three were unsuitable materials for "in-line" SFE clean-up of fat (the amounts of fat extracted per 100 g wild rice using Celite, Extrelut, or Hydromatrix were 1.84, 1.80, and 1.62 g, respectively). With use of Florisil the amount of fat extracted per 100 g wild rice was reduced up to 0.36 g, and fat-free SFE extracts were obtained only when "in-line" clean-up was performed with a 1-g layer of aminopropyl. From these results it can be seen that "in-line" clean-up with aminopropyl is an effective method for obtaining fat-free SFE extracts of rice samples. Information on the applicability of this "in-line" clean-up technique for removing fats from food samples by SFE is not extensive, and an adequate number of analytes and adsorbents should be studied to confirm that this combination is generally applicable for different groups of pesticides.

Because SFE has several distinct characteristics it has attracted increasing attention in recent years as a potential alternative to conventional extraction methods, and several authors have shown its extraction efficiency to be higher than of SE. A good example of this type of comparison is in the extraction of 32 pesticides (OPPs, OCPs, ONPs, and pyrethroids) from honey [70]. When SFE was compared with SE-GPS the sensitivity obtained was comparable but SFE was more precise and recovery, as measured by ECD and TCD, was higher. SFE also has the advantages of saving organic solvent, less time consumption, much less solvent evaporation, and simplified clean-up.

In all these publications MS detection was, unfortunately, used for confirmatory analysis only and there is lack of information about quantitative MS data, i.e. no about linearity, precision, and method LODs. This lack of data makes it difficult, to say the least, for a reader to evaluate or adopt a method. Quantitative data relating to the specific detectors used (ECD, NPD, TCD) are, nevertheless, more than satisfactory which should encourage coupling of SFE with MS detection. The last objective is also strongly encouraged by results from a collaborative study conducted by seventeen laboratories from seven different countries to determine pesticide residues from several classes in apples, green beans, and carrots by SFE with GC–MS [72]. To the best of our knowledge this is the only report in the period of this review which uses SFE in combination with MS detection. The report illustrates quite well the advantages of SFE–GC–MS over traditional methods, especially with regard to sample preparation (the selectivity of SFE and GC–MS eliminates the need for post-extraction clean-up, and conversion of the CO_2 solvent to a gas after SFE eliminates the solvent-evaporation step). When reporting

the results from the collaborative study the authors concluded that although the proposed method resulted in poor recovery of the most polar analytes and the non-polar analytes, it could be useful for many monitoring programs for pesticide residue analysis.

In summary, despite its demonstrated advantages, the high cost of the technology used and the onerous operating conditions of SFE have restricted its application to some very specialized fields, for example extraction of essential oils, decaffeination of coffee, and university research. In pesticide analysis most published SFE work deals with combination with GC by use of specific detectors, for example ECD or NPD; SFE coupled with GC–MS or LC–MS has followed quite slowly. Rapid developments in SFE techniques for analysis of food contaminants are expected in the future, however.

Pressurized-liquid extraction (PLE)

This technique, also known as accelerated solvent extraction (ASE), is one of the most recent solid and semisolid sample-extraction techniques. The fundamental difference between SFE and PLE is that SFE uses solvents near or above their critical point (usually CO_2-based fluids), whereas PLE uses traditional aqueous and organic solvents. At high temperature the rate of extraction increases because the viscosity and the surface tension of the solvent decrease whereas its solvent strength and rate of diffusion into the sample increase. Pressure keeps the solvent below its boiling point and forces its penetration into the pores of the sample. The combination of high temperature and pressure results in better extraction efficiency, thus minimizing solvent use and expediting the extraction process. The time required for extraction is almost independent of sample mass and the efficiency of extraction is mainly dependent on temperature [74, 75].

PLE has been successfully used for determination of pesticides in different food matrices [16, 76–82]. There has been particular interest in application of the technique to analysis of lipid-containing foods—for example organochlorine compounds have been isolated from cod liver and fish fillets [78] and different pesticides have been extracted from baby foods [80]. PLE has also been found useful for rapid analysis of pesticide residues in fruits and vegetables. The technique has been applied to the determination of fungicides in oranges and bananas [81], OPPs in apples and carrots [12], and a variety of pesticide residues in fresh pear, cantaloupe, white potato, and cabbage [13]. As for SFE, however, PLE–MS applications are still under development and only three papers have been published during the review period (Table 1). Blasco and coworkers described an analytical procedure that combines PLE with LC–IT–MS^3 for determination of a variety of pesticides from different classes (benzimidazoles and azoles, OPPs, carbamates, neonicotinoids, and acaricides) in oranges and peaches [77]. The effect of extraction conditions, for example solvent composition, temperature, pressure, and static extraction time has been tested. The method was tested for its applicability to both types of fruit and was compared with conventional SE with EtAc and anhydrous sodium sulfate. The results showed the precision of PLE was similar to that of SE, even though PLE is automated and software-controlled. Recoveries obtained by use of PLE methods were better for all pesticides except trichlorfon in both matrices. Under optimum conditions the proposed method enabled rapid and accurate determination of pesticide residues in the fruit with LOQs in the range 1–50 g kg^{-1}, which are below the MRLs established by the EU.

In more recent work Soler and coworkers [76] examined the use of PLE to extract carbosulfan and seven of its metabolites from oranges by use of 40 mL DCM as extraction solvent at 100 °C and 2000 psi with 100% flush volume, 2 min heating time, and two cycles of static extraction for 5 min each. LC–MS^3 was used for identification and confirmatory analysis. The authors concluded that the matrix of the samples affects quantitative analysis of the target compounds by substantially enhancing the response to early-eluting metabolites. The magnitude of the effect was only slightly dependent on the particular orange extract analyzed, however, because RSDs were never higher than 14%. They suggested an analyte-added control orange extract could be used as standard to improve the accuracy of the analysis. Because of its simplicity and sensitivity (limit of quantification (LOQ)<0.07 mg kg^{-1}), the method enabled efficient determination of carbosulfan and its metabolites in oranges.

Although PLE is usually performed with organic solvents, for example hexane and DCM, pressurized hot water or subcritical water can also be used in a PLE apparatus. The technique is commonly referred to as subcritical-water extraction (SWE), because the practitioners of this approach come from an SFE background; other terms, for example hot-water extraction (HWE) or superheated-water extraction, are found in the literature. The benefit of using subcritical water for analytical extraction is that the solvent strength can be tuned by varying the extraction temperature and/or by addition of a co-solvent. Water as a solvent is easily obtained and disposed of, being benign to the laboratory worker and the environment [75]. Aqueous extractions of food samples are also convenient, because the sample matrix does not need to be dried before the extraction. Application of this relatively new technique to food analysis has been limited and the only work reported during the review period was determination of carbamate pesticides in bovine milk before ESI LC–MS–MS analysis [76]. Nevertheless, although hot

water extraction has the advantage of the complete elimination of organic solvents, whether the technique finds increasing use in analytical laboratories will depend on the reluctance to use temperatures in the 150–250 °C range and the more lengthy solvent (water) evaporation times.

In summary, commercially available PLE systems, in addition to the advantages of SFE and MAE, have the capability to be easily automated for sequential unattended extraction of up to 24 samples. The amount of time spent on method development can therefore be substantially reduced compared with other techniques. Relatively matrix-independent methods can, furthermore, be developed by using high temperature and suitable solvents. Compared with Soxhlet extraction, use of pressurized fluids has the advantages of reducing solvent consumption and extraction time with the disadvantage of using expensive specialized equipment. The main disadvantage is that a sample clean-up is still required after extraction. Particular attention should also be paid to PLE performed at high extraction temperature, which may lead to degradation of thermally labile compounds.

Microwave-assisted extraction (MAE)

In recent years, MAE has attracted increasing interest, because it enables rapid extraction of solutes from solid matrices by using microwave energy as the heat source, with extraction efficiency comparable with that of classical techniques. The partitioning of the analytes from the sample matrix into the extractant depends on the temperature and the nature of the extractant. Unlike classical heating, microwaves heat the entire sample simultaneously without heating the vessel, so the solution reaches its boiling point very rapidly, leading to a very short extraction time [14].

To develop a successful MAE method, several conditions that affect the extraction yield, for example solvent composition, solvent volume, extraction temperature, extraction time, and matrix characteristics, including water content, are usually studied. The extraction solvents used usually have high dielectric constant to absorb microwave energy efficiently; examples include MeOH, water, and ethanol. Non-polar solvents with low dielectric constants, for example hexane and toluene, are not potential solvents for MAE, but their extracting selectivity and efficiency can be modulated by using mixtures of solvents. One of the most commonly used mixtures is hexane–acetone [83]. During extraction, the solvent volume must be sufficient to ensure the solid matrix is entirely immersed. In conventional extraction techniques a higher ratio of solvent volume to solid matrix mass usually increases recovery. In MAE, however, a higher ratio may lead to lower recoveries, probably because of inadequate stirring of the solvent by the microwaves. Although temperature is another important condition contributing to recovery—elevated temperatures usually result in improved extraction efficiency—for extraction of thermally labile compounds high temperatures may lead to degradation. In such circumstances the power selected during MAE must be set correctly to avoid excessive temperatures.

The main advantages of microwave pretreatment are the low temperature requirement, high extraction efficiency, complete automation, and the possibility of simultaneously extracting different samples at the same time without interference. It is interesting to note that, in contrast with other heating techniques, the extraction vessel is not heated directly, which reduces the extraction time required. It is also believed that high efficiency is achieved because of destruction of the macrostructure of the matrix. Use of closed vessels enables use of an operating temperature higher than the boiling point of the solvent, further reducing extraction time. The main disadvantage of MAE seems to be lack of selectivity compared with SFE for comparable extraction efficiency; this results in the co-extraction of significant amounts of interfering compounds. Additional clean-up is therefore needed before chromatographic analysis. A minor step could be simple filtration of the extract using glass wool, glass microbore filters, or membrane syringe filters. Instead of filtration, a centrifugation step, with or without cooling, can be performed to separate the extract from particles. More extensive clean-up procedures have been performed using SPME [84] and disposable SPE cartridges packed with C_{18}, silica, or ion-exchange material for removal of interfering compounds [14]. Extracts from fatty tissue and highly contaminated samples have been cleaned by GPC. Apart from the additional clean-up step, the poor efficiency of microwaves when either the target compounds or the solvents are non-polar, or when they are volatile, can be regarded as another disadvantage.

Surprisingly, in the period covered by this review there has been only one report of use of MAE coupled with chromatographic techniques and MS for determination of pesticides in food matrices. One reason might be the need for sample filtration and clean-up after extraction, something that is almost impossible to circumvent, compared to SFE and PLE for example, in which on-line clean-up and filtration are possible. The only reported study was that of the Montury group [84] who analyzed residues of the four pyrethroid pesticides authorized for strawberry cultivation by using focused microwave-assisted extraction (FMAE) coupled with SPME before chromatographic analysis by GC–MS. In this study [84] the authors established the best instrumental and FMAE-SPME conditions, including use of a co-solvent to increase the transfer of the analytes into the solution analyzed by SPME, and validated the method. According to the results obtained, addition of a co-solvent to

the extraction solution always enhanced the observed signal compared with use of pure water. Of the three co-solvents tested (MeCN, MeOH, and ethanol), MeCN was most efficient and a 50:50 mixture of ACN and water resulted in maximum sensitivity. For three (acrinathrin, bifenthrin, and λ-cyhalothrin) of the four compounds the proposed method enabled efficient, rapid routine analysis, without blending and centrifugation, at concentrations corresponding to the MRLs and below. For the other compound, deltamethrin, the LOD was close to the MRL. Although this should enable effective detection of overloaded samples, it is clear the LOD for this compound by use of this method must still to be improved. This report illustrates quite well the advantages of both MAE and SPME, especially in terms of sample preparation and speed of analysis.

MAE coupled with GC–MS or LC–MS has not yet been well established in pesticide analysis, as is evident from the lack of publications in the open literature during the review period. The applicability of this approach to the analysis of pesticide residues in environmental samples such as soils and sediments suggests, however, this trend may be reversed in the next few years.

Sorptive extraction methods

Matrix solid-phase dispersion (MSPD)

Matrix solid-phase dispersion (MSPD) is a new SPE-based extraction and clean-up technique developed for pesticide multiresidue analysis [15]. The main difference between MSPD and classic SPE is that, in SPE, samples must be in liquid state before application to the column whereas MSPD can handle solid or viscous liquid samples directly. Interactions of the components of the system are greater in MSPD and different, in part, from those in SPE. This technique enables extraction of analytes from samples dispersed homogeneously in a solid support, usually Florisil or C_{18}. The homogenized mixture is placed in a column in which the adsorbent works as abrasive compound breaking the physical structure of the sample and enabling its fractionation and adsorption of the compounds of the matrix. The column is finally eluted with an appropriate solvent and the extract can then be analyzed directly. Interferences, for example pigments or other polar compounds, are retained on the adsorbent and so sample extraction and clean-up are performed in the same step with good recovery and reproducibility, reducing the analysis time and the amount of solvent used [15, 85].

Reversed-phase materials such as C_8 and C_{18}-bonded silica are two of the most commonly used adsorbents, because their lipophilic character enable good disruption, dispersion, and retention of lipophilic species (Tables 3 and 4). Several methods of food analysis based on use of these adsorbents as dispersing agent [41, 43, 86, 87] have been validated and applied to the determination of several classes of pesticides including pyrethroids, OPPs, OCPs, carbamates, pyrazoles, strobilurin, benzimidazoles, ureas, and conazoles. Florisil [88, 89] has also been used, with good results (recoveries >60%), for determination of endosulfan sulfate and endosulfan isomers in tomato juices and sixteen other pesticides in honey, by fortifying or diluting the sample with an organic solvent (acetone or MeOH) for better sample distribution through the column, which was finally eluted with EtAc. Inert adsorbents, for example diatomaceous earth [90, 91] and sand [92], have also been used, because they enable early elution of interferences that would not be retained by any adsorbent during elution of the target analytes. One interesting application is the MSPD extraction of 266 pesticides from apple juice [90]. Samples (10 g) were mixed with 20 g diatomaceous earth and the analytes were eluted with a 160 mL 1:1 hexane–DCM before GC–MS (SIM) determination. Finally, two more adsorbents, aminopropyl [58] and carbon [93], have also been used successfully. The first was used as a selective adsorbent for quantitative analysis of insecticides and herbicides in olive oil (with preliminary LLE of olive oil samples) and in olives. The second was used for determination of dithiocarbamates and metabolites in plants by LC–APCI–MS. In general, choice of one adsorbent or another depends on analyte polarity and on the interferences possibly co-extracted from the sample matrix. From the papers reviewed here it is clear that in most LC–MS-based methods reversed-phase materials, for example C_8 and C_{18}-bonded silica, have been used as the solid support; Florisil, sand, and more selective adsorbents, for example as aminopropyl, are used less frequently. For GC–MS based methods Florisil, diatomaceous earth, and aminopropyl have been tested and applications of reversed phase material are still scarce.

The nature of the elution solvent is also crucial for obtaining efficient desorption of pesticides from the adsorbent while retaining interferences on the column. Most adsorbents have been tested in combination with a large variety of solvents, for example MeCN [58], EtAc [89, 91], DCM [41, 87], or mixtures of these with MeOH [43, 86, 92] or hexane [88, 90]. Although some MSPD extracts are clean enough for direct instrumental analysis [41, 43, 87–91], a clean-up step is often required. To achieve the latter objective the column is usually washed with a suitable solvent before elution of the target analytes. This step can occasionally be accomplished with "co-column" clean-up, to achieve better removal of the matrix, by packing a co-column material (for example Florisil or silica) at the bottom of the same adsorbent column. The sample is thus cleaned as it elutes from the MSPD adsorbent–matrix mixture. A good example is the method

developed by Ferrer et al. [58], in which the power of the MSPD method to provide clean extracts is illustrated in the extraction of pesticides from high-fat-content matrices, for example olive oil and olives, by using aminopropyl as adsorbent material and Florisil as co-column material for further clean-up.

From the papers reviewed the main conclusion that can be drawn is that MSPD has become a well-established sample-preparation technique in food analysis. In many of the studies reported here MSPD was compared with other extraction techniques for a variety of pesticide compounds and matrices. The performance of MSPD was usually similar or superior. It has several advantages, including simplifying and speeding up the sample-treatment process, reducing the use of large amounts of toxic solvents, eliminating emulsion formation, and increasing reliability, selectivity, and sensitivity. The primary advantage of MSPD is that sample extraction and clean-up are performed in the same step by use of small amounts of adsorbent and solvent, thus reducing cost and analysis time. Solvent evaporation remains a problem, however, and literature reports of on-line coupling of MSPD to LC or GC instruments are scarce.

Solid-phase extraction (SPE)

SPE is widely accepted as an alternative extraction/clean-up method to LLE for determination of pollutants in liquid samples. In SPE the sample is passed through a cartridge or a packed column filled with a solid adsorbent on the surface of which the analytes are adsorbed while the other sample components pass through the bed (or vice versa, if clean-up is necessary). When the analytes have been retained on the SPE adsorbent they are then eluted with an organic solvent. Advantages of SPE are that the analytical procedure is much simpler, small volumes of solvents are used, and much cleaner extracts and greater recoveries are usually obtained. SPE also enables avoidance of the emulsion formation often encountered in LLE, and automation is also possible [94].

Fruit juices are typically processed for SPE without pretreatment or are centrifuged before reversed-phase or ion-exchange SPE. If centrifuged, the resulting supernatant is used for the SPE procedure. Viscous liquid samples (e.g. honey) must be diluted with water or organic solvents (mixed or not mixed with water) to facilitate passage of the sample through the solid phase. Plant tissues, fruit, vegetables, and commodities such as grain are homogenized and pre-extracted with water, polar organic solvents (e.g. MeOH or MeCN), or mixtures of these solvents and water, before SPE enrichment and/or clean-up.

Method development in SPE is usually accomplished by investigating pH, type and solvent strength of the sample matrix, polarity and flow rate of the elution solvent, and physicochemical characteristics of the adsorbent bed. Sample pH can be crucial to obtaining high pesticide retention on the adsorbent. Occasionally, therefore, sample pH modification can be necessary to stabilize the pesticides and increase their absorption by the solid phase [93].

A variety of adsorbents are available, each of different selectivity. The adsorbent of choice depends on the food matrix, the analytes of interest, and interferences. Materials such as normal phase (Florisil) [55], Oasis HLB [29, 65], graphitized carbon [62, 91], adsorbents with weak anion-exchange and polar capabilities (NH_2) [32, 36], mixed-mode phases, and polystyrene–divinylbenzene supports [49, 54] have been shown to be valuable adsorbents for sample enrichment and clean-up of a variety of pesticides in food matrices; the most commonly used material, however, is the reversed-phase octadecyl silica (RP-C_{18}) [59, 53, 95, 96] because it is sufficiently reactive to enable its surface to be modified by chemical reaction and yet sufficiently stable to enable its use with a wide range of solutions.

The adsorbents mentioned above are rather non-specific in nature, however; there is, therefore, increasing interest in the development of alternative adsorbents with high extraction selectivity for single analytes or classes of compounds which enable efficient sample clean-up for the monitoring of trace analytes in complex environmental or food samples. To meet these requirements highly selective SPE adsorbents (known as immunosorbents, ISs) involving antigen–antibody interactions have recently been developed [94]. Antibodies produced against a target compound are immobilized on a support to form an IS that is used just as a classical SPE adsorbent. Because of the high affinity and high selectivity of the interactions, extraction and clean-up of complex aqueous matrices are achieved in the same step. Degelmann et al. [55] reported an interesting application of two different immunoaffinity supports (IAS), crushed sol–gel monoliths and sol–gel-coated highly porous silica, for quantitative SPE enrichment of sulfonylurea (SU) herbicides in water and food samples. Both kinds of support had similar characteristics and, therefore, enabled reliable and rapid analysis of SUs at trace levels in complex matrices. The high selectivity for group-specific recognition of SUs, compared with other, non-specific, SPE materials, was the main advantage of the prepared IAS; this proved that IA extraction could be another feasible SPE approach for trace analysis of organic contaminants. Despite the potential of the method, however, its use in food analysis is much less widespread than in environmental analysis, possibly because of the high production cost of IA columns.

After an adsorbent has been selected, on the basis of its retention efficiency for the target pesticides, the second step consists in determining the best solvent or mixture of solvents to disrupt this link and to displace the analytes

from the SPE materials. An eluent is usually chosen on the basis of its high-performance, low volume, weak toxicity, non-interference with compounds, and compatibility with the chromatographic system used (GC or LC). Tables 5 and 6 summarize selected SPE applications for liquid and solid food samples (in addition to the examples of SPE clean-up of solvent extracts given in Tables 1 and 2). Different kinds of solvent have been tested for elution of priority pesticides, including herbicides, insecticides, fungicides and their degradation products from different adsorbents. The most widely used were MeOH [29, 63, 96], EtAc [96], acetone [32], and DCM [96], or mixtures of these [49, 53, 60]. Di Muccio and co-workers [16] reported a rapid, sensitive, and accurate LC–ESI–MS method for determination of acetamiprid, imidacloprid, thiacloprid, and thiamethoxam in fruit and vegetables; after a single extraction with diatomaceous earth (Extrelut-NT20) cartridges from aqueous acetone extracts the target compounds were eluted with DCM. Obana et al. [63] described a rapid screening process based on SE-SPE clean-up using MeOH as extraction and elution solvent for simultaneous analysis of five neonicotinoid insecticides by LC–MS in the APCI positive-ion mode. MeOH was also used as SPE eluent by Wang et al. [29], who developed and validated a method for identification and quantification of trace levels of thirteen pesticides in apple-based infant food by LC–ESI-MS–MS . The food was extracted with MeCN and the extracts were cleaned and concentrated on Oasis HLB cartridges. In addition to DCM and MeOH, acetone has also been found useful for elution of eighteen pesticides from an NH_2 adsorbent used for clean-up, before analysis by rapid GC–MS, of a solvent extract from baby food [32]. Most of the examples cited are multiresidue, including several groups of pesticides, and so mixtures of solvents are usually used to ensure high recoveries for all the target compounds. Different mixtures have been used, including DCM–MeOH [60], MeOH–H_2O [54], EtAc–hexane [53], and EtAc–acetone [49]. Finally, use of acidified solvents, to prevent problems related to incomplete elution of analytes from the adsorbent and losses because of degradation of the compounds in organic solvents or during the evaporation step, has also been reported in the literature. For example, Hernández et al. [64] recently showed that the mixture MeOH–methyl *tert*-butyl ether (MTBE), 10:90 (*v*/*v*), acidified with 0.1% HCOOH, was suitable for elution of 52 non-GC-amenable pesticides and metabolites from Oasis HLB cartridges, resulting in satisfactory recoveries, minimizing degradation of the target analytes, and enabling sensitive and selective determination by use of LC–MS–MS with a triple quadrupole. There have also been reports of stepwise elution of pesticides through a C_{18} adsorbent for multiresidue analysis by LC–MS. This approach is usually used for analysis of pesticides in several classes because of the high recoveries obtained without extraction of large quantities of interferences. Two papers published by Fernández et al. [96] and Blasco et al. [95] explored the sequential elution of 22 OPPs and 42 OPPs, OCLs, and carbamate pesticides, respectively. In both procedures 5 g honey diluted with 50 mL water was passed through a C_{18} packed-column and the retained pesticides were eluted by passage of 10 mL EtAc, then 4 mL MeOH, and finally 1 mL DCM. Recovery was >76% for all the analytes tested except for highly polar compounds, for example omethoate and dimethoate, and enabled sensitive and selective determination by LC–APCI–MSD in positive and negative-ion modes.

Although the potential of SPE for enrichment or clean-up in the extraction of pesticides from food samples is clearly now recognized, some features must still be improved. Difficulties choosing an adsorbent and elution solvent for multiresidue analysis of compounds with a very wide range of physicochemical characteristics, high blank values, substantial variation in the performance of the products offered by different manufacturers, and the small sample volume that can be extracted with some SPE adsorbents are among the main problems researchers are trying to overcome.

Solid-phase microextraction (SPME)

SPME is an easily automated, simple, one-step, rapid, solvent-free method of extraction. The technique is based on establishment of equilibrium between analyte in a sample and analyte adsorbed by a fused-silica fiber coated with a stationary phase, which can be a liquid polymer, a solid adsorbent, or a combination of both. SPME is increasingly being used instead of classical and time-consuming extraction and leaching processes. Most SPME applications have been performed in combination with GC—after extraction the analytes are thermally desorbed from the fiber into the injector of the chromatograph. To widen its range of application to nonvolatile and thermally unstable compounds, SPME has recently been interfaced with high-performance liquid chromatography (HPLC) and LC–MS. Instead of thermal desorption in the injection port of the GC, an SPME–HPLC interface equipped with a special desorption chamber is used for solvent desorption before LC separation. A new SPME–HPLC system, known as in-tube SPME, uses an open-tubular fused-silica capillary column as the SPME device instead of an SPME fiber. In-tube SPME is suitable for automation, which not only reduces analysis times but often results in better accuracy and precision than manual techniques [97, 98].

The main advantages of SPME are good analytical performance, simplicity, and low cost. SPME produces relatively clean and concentrated extracts, and is ideal for MS applications. This technique does not suffer from the

plugging or channeling problems encountered with SPE. It also completely eliminates use of organic solvents. A relatively long equilibration time (up to 1 h) is needed, and methods such as sample stirring, sample sonication, fiber vibration, and fiber rotation have been used to reduce this absorption time [17, 99]. An inherent disadvantage of SPME is that quantitative work is still rather laborious because carry-over between samples can be severe.

A variety of SPME methods have been used for analysis of acaricides, OCPs, OPPs, and ONPs in a range of different foods. Most of these methods have focused on liquid samples, for example fruit juices, wine [99–101], and honey (the last of which are usually analyzed after dilution with water) [102, 103], usually in the direct mode but sometimes in the headspace mode [104, 105]. As with SFE, PLE, and MAE, however, SPME–MS applications are still under development and few studies have been reported during the review period. Berrada et al. [106] described use of SPME with a PA fiber for analysis of phenylurea herbicides and their aniline homologs in carrots, onions, and potatoes. Juice obtained from food samples was diluted and an aliquot was extracted after addition of sodium chloride (14%) and adjustment of the pH. Analysis was performed by GC–MS. Zambonin et al. [107] developed an SPME–GC–MS method for rapid screening of wine and strawberries for triazole residues. The method was fully and thoroughly validated. LODs of the method were well below maximum residue limits (MRLs) fixed for wine (or grapes) and strawberries by European legislation [108, 109]. Because of its simplicity, rapidity and remarkable analytical characteristics (linearity, reproducibility and LODs) the method is a useful means of assessing contamination. Blasco et al. [110] compared SPME and stir-bar-sorptive extraction (SBSE), in combination with liquid chromatography – atmospheric pressure chemical ionization mass spectrometry (LC–APCI–MS) for analysis of six OPPs in honey. In both SPME and SBSE enrichment was performed using a polydimethylsiloxane (PDMS) coating. Conditions affecting the adsorption process, for example sample volume, adsorption and desorption times, ionic strength, elution solvent, and extent of sample dilution (water:honey) were optimized and discussed. Both techniques were simple, economical, did not require preliminary sample preparation, and reduced the volume of (toxic) solvents used. The honey matrix barely affected SBSE but had a substantial effect on SPME. Linearity and precision obtained by SBSE and SPME were similar but SBSE was shown to be more accurate and more sensitive than SPME. Finally, headspace SPME combined with GC–MS has been used for analysis of OPPs in strawberries and in cherry juice [104]. For most of the analytes use of a PDMS fiber resulted in the more efficient extraction than use of a polyacrylate (PA) fiber. Addition of salt (15%) and water to the sample increased the amounts of analytes extracted by both fiber coatings. The HS-SPME approach was also found to be useful for GC–MS determination of oxadiazon residues in wines [111].

It is clear that the number of applications of SPME MS is substantially less than those with specific detectors, despite its potential. The significance of SPME, and its nearly ideal combination with MS, has rapidly been recognized; a further rapid increase in the GC–MS and LC–MS applications should be expected in the near future.

Stir-bar-sportive extraction (SBSE)

Another very elegant enrichment extraction technique based on the same principle as SPME is the recently developed SBSE. This technique was developed to extract organic analytes from liquid samples and is based on adsorption of analytes on to a thick film of PDMS coated on to an iron stir bar. The stir bar is placed in a liquid sample and analytes are adsorbed on this as the samples is stirred for a given time. The stir-bar is then either thermally desorbed on-line for capillary GC–MS or extracted with organic solvent. The stir bars, commercialized under the name "Twister" (Gerstel, Mulheim a/d Ruhr, Germany) result in 500-fold greater enrichment, and thus sensitivity, than SPME with 100-m PDMS fibers. The only adsorbent yet used for SBSE is PDMS, although development of new stir bars coated with polar adsorbents is predicted in the literature.

Analysts usually encounter no problems in the application of SBSE to food matrices if the fat content is less than 2–3%; otherwise dilution is necessary. For samples that contain large levels of alcohol, dilution to a maximum ethanol concentration of 10% is recommended. Although SBSE was developed only recently it has already been used for analysis of dicarboximide fungicides in wine [24], OPPs and carbamates in oranges [26], OPPs in honey [27], and OCPs in fruit and vegetables. Apart from the work (described in the previous section) by Blasco et al. [110], however, during the period covered by this review there has been only one report of the combination of SBSE with LC–MS. In this study Juan-García et al. [60] developed an LC method for determination of six fungicides and compared the detection limits obtained by use of ESI and APCI in positive and negative ionization modes. They selectively optimized the MS conditions in detail for each ionization method used. APCI in positive-ion mode proved most sensitive, resulting in low-picogram detection limits for all the analytes. ESI was between 25 and 100 times less sensitive than APCI for the compounds studied. These authors also developed and compared two sorptive techniques, SBSE and SPE, for quantitative analysis of the target compounds in grapes. Although both methods can be used

to determine residues of the fungicides, SPE resulted in higher recovery, lower RSDs, and better LOQs and was more rapid than SBSE. SBSE had the advantages of lower organic solvent consumption and cleaner extracts, however.

The most important benefits of SBSE are the same as for SPME. SBSE is, nevertheless, regarded as superior to SPME in terms of sensitivity and accuracy. Despite these advantages, its disadvantages have restricted its widespread application in food analysis. The most important of these is the desorption step, because analyte loaded on coated stir bars cannot be desorbed directly in the injection port of a gas chromatograph. The analyte must therefore be back-extracted into a suitable solvent, which adds an additional step to the overall analytical method, or a specially designed thermal desorption unit must be used. This desorption unit is usually a relatively sophisticated instrument, because of problems with high dead volume. Another disadvantage is that the stir bar must be transferred manually to the desorption unit. This may cause partial loss of the sensitivity gained by use of an extended adsorbent surface.

Chromatographic determination

The variety of fields of application described in this literature review show that, because of their versatility, chromatographic MS techniques have been proved successful in virtually any analytical challenge; this makes them robust and effectively applicable options for analysis of pesticides in food. Many pesticides in different chemical groups have been analyzed by GC–MS, LC–MS, or MS–MS. The analytical MS methods used in the pesticide food publications on which this review is based are listed in Tables 1, 2, 3, 4, 5, 6.

Separation of GC-amenable pesticides has been conducted with a variety of capillary columns, with helium as carrier gas. Non-polar stationary phases for capillary GC, for example polydimethylsiloxane or phenylmethylpolysiloxane (DB-5, HP-5, ZB-5, RTX-5, HP-5, SPB-5) have been most frequently used, as is apparent from Tables 1, 3 and 5. Use of the Rapid-MS (10 m×0.53 m i.d., 0.25 μm film thickness, wall-coated open tubular (WCOT) fused-silica CP-Sil 8 CB low bleed) analytical column has also been proposed for high-speed LP–GC–IT-MS–MS analysis of several classes of pesticide [50] and 14% cyanopropylphenyl+86% dimethylpolysiloxane (BP-10) has been used as stationary phase for analysis of phenylureas herbicides [106]. Splitless injection is the technique most commonly used before GC separation, because of its robustness, although limitations of splitless injection include low sample capacity (up to 2 μL) and, for samples with complex matrices, retention of non-volatile co-injected compounds in the liner, which affects sensitivity. To overcome these analytical difficulties with splitless injection, programmed-temperature vaporization (PTV) injectors can be an alternative, because they enable use of sample volumes up to 5 μL and eliminate matrix effects by releasing high-boiling co-extracted compounds through split vent and/or by trapping them on a liner. For example, Kirchner et al. [34] demonstrated that a PTV inlet in the cold splitless mode under optimized conditions enabled sample vaporization and sample transfer into the column with excellent repeatability compared with conventional splitless GC. Hercegová et al. [32] came to the same conclusions after use of rapid PTV-GC–MS (SIM) for analysis of pesticides in baby food. PTV in cold splitless mode was more efficient than classical hot splitless injection at preventing problems connected with matrix effects and elimination of less volatile matrix constituents which caused deterioration of GC system performance.

Because of the identification power afforded by the electron impact (EI) spectrum, because of the number of fragment ions and their relative abundance, EI has been used as ionization technique in almost all of the studies described in this review; only two reports based on chemical ionization (CI) have been described. LODs obtained in EI ionization and SIM mode by use of a quadrupole as mass analyzer are at sub to low μg kg^{-1} levels (0.01–60 ppb) (Table 1). Similar or higher μg kg^{-1} LODs (0.02–140 ppb) have also been reported for use of IT-MS in MS–MS mode and TOF-MS in full-scan mode (Table 1). Negative chemical ionization (NCI) with the identification power of IT-MS–MS, with heptacosafluorotributylamine-$(C_4F_9)_3N$ as chemical reagent, has been shown to be an alternative method for analysis of OPPs [57]. NCI is recognized as favorable for electron-capturing compounds (compounds with sufficient electron affinity) such as OPPs, because the background does not ionize and few ions of high abundance are usually observed in the relevant mass spectrum; this enhances analyte detectability. Apart from NCI, PCI (MeOH used as reagent) was also performed as a complement to EI for determination of 31 pesticides in different classes by use of tandem MS [51].

Although GC–MS, especially with EI ionization, furnishes fingerprint spectra, qualitative and quantitative GC–MS analysis of pesticides can be complicated by interference from matrix components co-eluting with the analytes of interest. Analytes with low and, hence, unspecific *m*/*z* value ions in their mass spectra are especially troublesome. Conventional GC–MS methods may, therefore, fail to identify and quantify these analytes at sufficiently low concentrations. This problem becomes critical if a MRL is set for a particular commodity, e.g. MRL in baby food are 0.01 mg kg^{-1}. One means of overcoming this problem is to improve the GC separation. A new approach to chromato-

graphic separation, known as comprehensive two-dimensional gas chromatography (GC×GC), has recently been introduced as alternative to conventional GC separation because of its outstanding separation potential and capability of solving demanding analytical tasks [112]. In this approach a second column, coated with a stationary phase different from that of the primary column, is used for rapid chromatography with TOF-MS detection. This technique uses only TOF-MS as the detector because it has the most sensitivity for fast-eluting peaks [112, 113]. Zrostlíková et al. [45] described the use of GC×GC coupled with TOF-MS for determination of residues of 20 modern pesticides in apple and peach baby food. Good separation was achieved on a DB-XLB×DB-17 column set and most of the analytes tested could be identified reliably in fruit at levels below 0.01 mg kg^{-1}. Despite its potential, little attention has been devoted to trace-level determination of pesticides in food and very few studies have been reported in the literature. This is probably at least partly because there are many detailed procedures for their precise and accurate analysis by 1D-GC–MS [112, 113].

From the work discussed in this literature review it is clear that GC–MS has proved itself successful for analysis of non-polar, semi-polar, volatile, and semi-volatile pesticides in food. Nevertheless, for polar, nonvolatile, and thermally unstable pesticides, for example phenylureas, carbamates, pyrimidines, triazoles, phenoxyalkanoic acids, and most pesticide transformation products use of GC is impossible and LC coupled to MS is the technique of choice [114]. Today, as is apparent from the increasing number of LC–MS applications being published on analysis of pesticides in food, the technique has left the experimental stage and has firmly established itself [115, 116]. Under optimized conditions, both electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) interfaces, operating in negative-ion (NI) or positive-ion (PI) modes, have worked well and been found complement each other with regard to polarity, molecular mass of analytes, and chromatographic conditions for determination of pesticide residues in food. In most studies positive ion mode has been the ionization mode of choice for both interfaces. MS conditions promoting limited fragmentation and a single predominant ion have been selected as optimum to furnish maximum sensitivity under SIM conditions. These predominant ions correspond either to the protonated molecular ion ($[M+H]^+$) or to adducts of the analyte molecule with one sodium atom ($[M+Na]^+$). Occasionally, however, an additional signal for m/z corresponding to K^+ or NH_4^+ adducts appear as the base peak in the spectra and can be selected as the predominant ion. For example, in LC–APCI-MS analysis of carbamates and other polar pesticides in fruit and vegetables [24], ammonium adduct ions are observed as base peaks. In this particular study some of the pesticides were detected as protonated molecules and others as adduct ions.

ESI in PI mode was the interface of choice for most of the studies cited in this review, and resulted in sensitive, robust, and accurate methods (Tables 2, 5 and 6). In general, ESI is the ionization technique recommended for polar, ionized, and high-molecular-weight compounds, and so is frequently used for analysis of pesticides containing sulfonic acid or carboxyl groups in the chemical structure. Selection of an appropriate mobile phase is crucial for the ionization process in ESI; it should always contain at least a small amount of a volatile buffer, acid, or base. For some compounds better results are obtained by use of APCI (Tables 2, 5 and 6), suggesting that both API sources should be considered when establishing new methods for analysis of food. APCI enables very sensitive analysis of weakly basic compounds, and pesticides such as triazines and phenylureas and can be easily protonated by gas-phase, mobile-phase ions, depending on their proton affinity. Because ionization is CI, however, this is a soft ionization technique and no informative fragmentation occurs. Blasco et al. [92] compared ESI and APCI interfaces in both ionization modes (NI and PI) for determination of dithiocarbamates and their metabolites in plants. At the concentrations studied, the analytes (thiram, disulfiram, dazomet, ETU, and PTU) were detected in PI mode but not in NI mode. Comparison of APCI and ESI revealed sensitivity differences. When ESI was used sensitivity for ETU and PTU was a factor of 5–10 less than when APCI was used; ESI was much more sensitive than APCI for thiram and disulfiram, however. The APCI interface was eventually selected by the authors because of the better sensitivity for ETU and PTU and because its greater robustness resulted in reproducible spectra of the compounds without adduct formation. Another interface, called atmospheric pressure photo-ionization (APPI), has recently been proposed for complex sample analysis, because it can overcome the suppression problems encountered with APCI and ESI sources. The APPI was recently used for LC–MS analysis of carbamate pesticides in fruits and vegetables [47]. No applications of APPI-MS–MS have been reported in the literature.

Of mass spectrometers enabling MS or MS–MS experiments, most of research work reviewed here was performed with quadrupole (single or triple) and ion-trap instruments. This is principally because of their greater ease of operation, their greater robustness for routine analysis, and their relatively low cost compared with TOF instruments. Tandem MS (MS–MS), or in-source collision-induced dissociation (CID), is required to obtain structural information, to improve selectivity and sensitivity, and to confirm the identity of pesticides. In most instances, among the analyzers capable of MS–MS, triple-quadrupoles operating

in MRM mode for improving sensitivity have been most frequently used, proving they were most suitable for achieving the strict MRLs introduced for pesticides in foods. The sensitivity of ion-trap instruments is usually similar to or less than that of triple-quadrupole analyzers. Because of the possibility of obtaining product-ion scans (PIS) without loss of sensitivity, and the ability to perform multiple-stage fragmentation (MS^n) ion traps have, however, been selected for screening purposes. More recent approaches to MS–MS analysis, including linear traps, new-generation triple quadrupoles, and hybrid instruments, for example Q-TOF and Q-linear traps can be good alternatives, because of their high scan speeds, accurate mass measurement (QqTOF), and higher sensitivity (linear traps and new-generation triple quadrupoles). Soler et al. [61] recently compared LC–TQ-MS and LC–QIT-MS and discussed the advantages and disadvantages of both for analysis of pesticides in oranges. The results indicated that precision, linearity, and robustness were better for the TQ, which was better than the QIT for quantitative analysis, although both mass spectrometers could be used for both qualitative and quantitative analysis of conventionally treated oranges.

LC of target pesticides in extracts obtained from food samples has been performed with different columns. Pesticides have usually been separated by reversed-phase chromatography on C_{18} columns (4.6 mm i.d.). Column type is, nevertheless, always critical and other types of column have been proposed for more specific separations (Tables 2, 5 and 6).

MeCN–water or, most often, MeOH–water mixtures at different pH have been used as mobile phases (Tables 2, 5 and 6). The mobile phase is occasionally modified in attempts to improve the sensitivity of MS detection; this has been accomplished by addition of acetate [16, 24, 26, 54, 61], formate [38, 40], and formic acid [6, 27, 31, 33, 39, 56, 58, 64, 91, 92]. Limits of detection are in the low $\mu g\ kg^{-1}$ range for all the pesticides under investigation, emphasizing the good performance of the methods reported here.

Finally, it worth remarking that the problem of exact quantitative determination in LC–MS methods is particularly important for pesticide residue analysis in food, because of the high variability of the matrices. An important issue in this respect is the so-called matrix effect, which is usually apparent as (unexpected) suppression or enhancement of response to the analyte because of co-eluting matrix constituents. Matrix effects are known to be both compound and matrix-dependent. To reduce the extent of matrix interference, the standard addition method, labeled internal standards, and/or external calibration plots have been used in almost all the studies reviewed here. For multi-residue analysis the matrix-matched method is recommended, because labeled standards are not available for all analytes. Occasionally, to allow for matrix interferences, isotope dilution has been used as the most reliable method for correct quantification of the analytes, because of its advantage of taking into account intrinsic matrix responses by using a deuterated or carbon-13-labeled internal standard with the same chemistry as the pesticide being analyzed (i.e. carbaryl-d_7, methomyl-d_3, fenobucarb-d_3 for carbamate analysis) [62]. Detailed descriptions of the consequences and extent of matrix effects in quantitative pesticide analysis by LC–MS are available in the reviews and textbooks cited; these should be consulted for detailed analytical planning and better understanding of the problems associated with variable ionization and matrix effects [115, 117–119].

Conclusions

In recent years important improvements in sample-preparation techniques for liquid and solid foods have led to adaptation of existing methods and the development of new techniques to save time and chemicals, to improve overall performance, and, if possible, to hyphenate the different steps of the analytical process. In this respect several rapid, low cost, environmentally friendly, and readily automated methods of extraction are now available. Because of the complexity of the matrices, extraction is usually followed by very specific clean-up procedures to achieve accurate sample quantification. The method selected will involve a compromise between cost, selectivity, and sensitivity, particularly because different compounds require different instrumental optimization techniques, especially polar and thermally unstable compounds. Among the extraction techniques tested (SE, SFE, PLE, MAE, SPE, MSPD, SPME, and SBSE) SE remains the most frequently used, although occasionally an additional clean-up step, usually SPE, is performed. In the many publications on analysis of pesticides in food, subsequent GC or LC coupled to MS or MS–MS clearly shows the well established performance of all these extraction techniques. Nevertheless, future developments in all areas of analytical sample preparation are expected to continue to be application-driven in a quest for improved recovery, higher sample throughput, and consumption of less organic solvent.

Important progress has been made in the sensitivity and selectivity of chromatographic analysis. Most applications employ LC rather than GC, because many polar, nonvolatile, and thermally unstable pesticides are detected in food matrices. Of the different LC methods, LC–ESI-MS–MS seems to be the technique of choice, because it provides reliable results at levels of subnanogram per liter or per gram. Obviously, LC–MS in pesticide food analysis has reached a level of maturity that makes it a robust and

routinely applicable alternative. It is fair to say that powerful analytical methods have been developed that can challenge any other analytical approach, for example the much-acclaimed GC–MS. Despite the high sensitivity and selectivity of LC–MS-based methods, however, and of LC–MS–MS in particular, there is a need to know more about the importance in pesticide analysis of the effects of the matrix of foods such as vegetables and fruits.

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