

Comparative Evaluation of Extraction Procedures and Chromatographic Techniques for Analysis of Multiresidue Pesticides in Honey

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Abstract

Honey is under great experimentation now-a-days due to presence of pesticide residues in it. Consumers of the honey products are at stake because pesticide residues are hazardous to health. There is a clear evidence of adverse health related issues due to the pesticides being sprayed. To ensure the food safety for the consumers in compliance with the EC and Codex Alimentarius regulations, highly sensitive, selective and reliable analytical methods are required. Honey is a complex matrix, so the choice of extraction procedure should be appropriate one. This work is focused upon the review of extraction methods and instrumentation used for the analysis of pesticide residues in honey.

Keywords: Honey; Extraction methods; Multiple pesticide residues; Chromatographic; Spectroscopic techniques

Introduction

Honey is considered as pure natural product and it is being used for curing diseases like asthma, gastrointestinal disorders, burns and infectious wounds [1]. As it is of primary importance for human health, one should be conscious of its purity. With the growing world population, demand for food has increased enormously; agriculture sector has also boosted up to meet with the requirement. To enhance the production of the food and combat with the agents harmful for the plants different strategies and techniques have been used amongst them fertilizers and pesticides are of greater concern. As far as protection of the crops from the damaging insects, weeds and other agents is concerned, a huge number of pesticides are available in market and are being used frequently [1]. Especially, in developing countries trend of using pesticides has increased to greater extent to multiply production in order to meet up with the needs and reduce losses due to pest infestation [2]. Recently, it has become of greater concern as it is a direct challenge for food safety standards.

There are two major sources of contamination in honey, first when honeybees take contaminated nectar along with them and can contaminate the colony, consequently transferred to food chain [3,4]. In direct contamination, certain chemical therapeutic agents like coumaphos, flauvinate, flumethrin, amitraz are applied on hive to combat against larvae diseases and mites [5]. The principle ways of pesticides contamination in honey are shown in Figure 1.

Since 1998 scientists from Spain [6,7], Greece [8,9], Portugal [10], Brazil [11], France [12], Italy [13], Serbia [14], Iran [15], Egypt [16], Belgium [17], USA [18], China [19], Argentina [4], Poland [20] have determined pesticide residues in honey and reported the presence of enormous levels of residues above MRLs. Different national organizations have established MRLs for honey, but there is no homogeneity among the MRLs set by different countries which is a big hurdle for analyst but most importantly for traders. According to the European Union (EU) regulations, honey as a natural product must be free of chemicals; maximum concentration of pesticides in honey could be 10 ng/g (http://europa.eu/legislation_summaries/food_safety/). Codex alimentarius have't established MRLs for any pesticide in honey till now.

Analytical methodology for determination of residue of pesticides from honey includes extraction, enrichment, and isolation of pesticides from matrix greatly influences the reliability and precision of the analysis. Different extraction methods are being used now a days are liquid-liquid extraction [17], supercritical fluid extraction [18], solid-phase extraction [21,22], solid phase micro extraction [13,21] stir bar sorptive extraction [23] single drop microextraction, dispersive liquid liquid microextraction [15,9] ultrasound-assisted emulsification microextraction, coacervative microextraction technique (alternative to organic solvents microextraction) [4] QuEChERS [16,24]. Gel permeation and adsorption chromatography are also used to minimize the matrix effect in pesticides analysis from honey [11]. The details of these methods are available in Table 1.

For the identification and quantification of low levels (ppb or sub ppb) of pesticides residues more sensitive and selective chromatographic methods (GC-ECD, GC-MS, GC-MS/MS, GC-NPD, LC-APCI-MS, LC-MS, LC-MS/MS, GC*GC-TOFMS) [23,17,20,29-31] are in use now a day's Table 2. This work would be focusing upon the critical review of conventional and advanced analytical techniques used for pesticide residue determination in honey with emphasis upon the extraction procedures.

Sample preparation

The selection of method for pesticides extraction from honey should be cautious one, as honey is complex matrix. Extraction with organic solvents, followed by some form of purification to eliminate the co-extracted fat is a sequence usually applied. Sample preparation for honey includes, homogenization, extraction, pre-concentration, cleanup, and final concentration prior to analysis. An efficient sample preparation technique should be easy, rapid and efficient in sample cleanup and with operational cost. Different sample preparation procedures were developed and applied during last decades, amongst them are 1) LLE/SE 2) LLME 3) SFE 4) SPE 5) MSPD 6) SPME 7) SBSE 8) QuEChERS 9) SBSE etc.

Conventional pesticides extraction/cleanup techniques

(i) **Liquid liquid extraction (LLE):** Among the classical techniques,

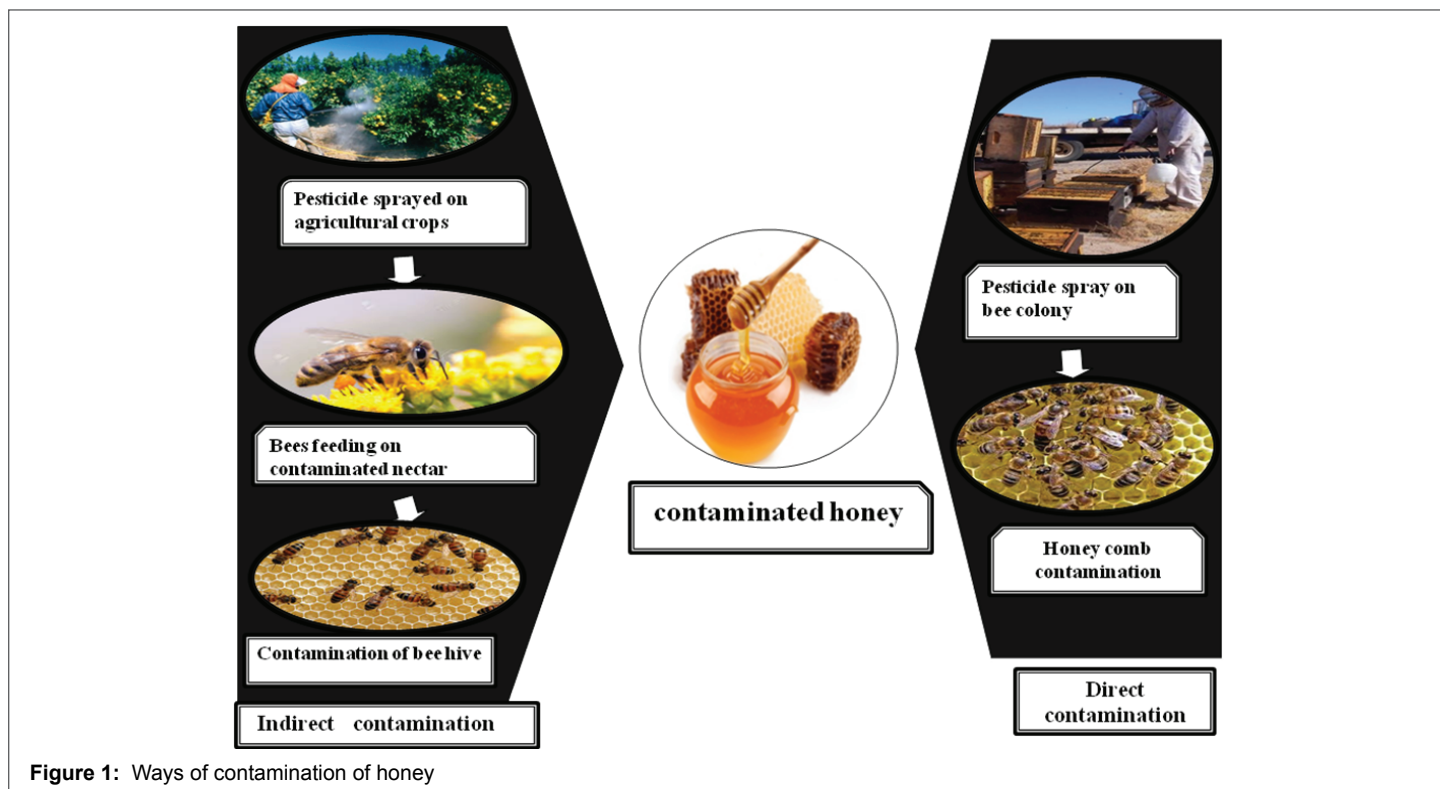


Table 1: Conventional extraction methodologies for multi-pesticides residues analysis

Pesticides	Extraction/Clean up technique	Solvent system	Recovery (%)	Reference
Organochlorine, Pyrethroids	LLE	ethylacetate, ethylacetate : water (80:20 v/v)	68-126	[23,25,26]
Chlorpyrifos, Cypermethrin, λ-Cyhalothrin, Deltamethrin	LLE-LTP (low temperature purification)	acetonitrile + ethyl acetate	84-100	Pinho et al. [10]
Amidosulfuron atrazine carbofuran, chlorotoluron Diethofencarb, diethofencarb Dimethoate Fipronil Imidacloprid Isoxaflutole Linuron Methiocarb Methiocarb sulfoxide Metosulam PirimicarbRimsulfuron Simazine Hydroxyterbutylazine	OCLLE (on column liquid liquid extraction , using Chem Elute cartridge)	2.5ml acetonitrile + 1.25ml water	71-90	Pirard et al [17]
Atrazine, simazine, Boscalid, imidacloprid, tau-fluvalinate, thiacloprid	Ultrasonic LLE Extraction time 20min	benzene+water(1:1),Ethyl acetate	69-92	[27,28]
Organochlorine, organophosphorus, organonitrogen and pyrethroid)	SFE with SFX-220 extraction system+syphonated CO2 cylinder and cleanup with SPE Extraction time 20min	for SPE clean up preconditioned with 5 ml (1:1) ethyl acetate/n-hexane two portions of 5mL each, methylene chloride/n- hexane (80:20, v/v) and n-hexane/acetone (60:40, v/v).	75-94	Rissato, et al. [18]
Organochlorines, Organophosphorus, Carbamates Fenoxycarb Phenthoate Fonofos Diazinon Phosalone Pyrazophos Chlorpyrifos methyl, Profenofos Pirimiphos ethylTemephos Bromophos ethyl Chlorpyrifos ethyl	SPE with C18,HLB,RAM-MISPE, MISPE, Florisil,C18,RP-C18	preconditioned with 10ml methanol and 10ml water eluted with ethyl acetate 10 ml, methanol 4 ml, dichloromethane 1ml.	80-120	[10,21,22,8]

one is solvent extraction termed as liquid liquid extraction (LLE) in which different water-immiscible solvents and solvent mixtures are used for extraction depending upon the polarity of the respective pesticides. The method is based on the partition of analytes between the aqueous and organic phase. The polarity of the solvent is a trade-off between acceptable recovery and good measurements. Blasco, Fernández et al. [23] used this technique to extract organochlorines with three different solvents n-hexane, ethyl acetate and light petroleum and found ethyl acetate was

the best solvent for extraction of these compounds with mean recovery 68-126% [Table 1].

The LLE efficiency was improved when single extraction solvent was replaced with solvent mixture (ethyl acetate to ethyl acetate and water mixture (5:1). Louca Christodoulou, Kanari et al. [25] use this approach and extracted 13 organochlorines, 8 pyrethroids and 146 pesticides belonging to different groups (organophosphorus, carbamates, triazoles, amides, neoinconoids, strobilurines, phenylureas, bendimidazoles and

Table 2: Advanced extraction techniques for analysis of multi-pesticides residues

Pesticides	Extraction technique (Extraction time, fiber type)	Solvent system	Recovery	reference
Chlopyriphos-methyl, Diazinon Fonofos Phenthoate Phosalone Pirimiphos ethyl, Phorate Fenitrothion Malathion Parathion Quinalphos	SBSE, PMDS/PVA	methanol, Acetonitrile	40-64%, 81-124%	[23,19]
Demeton-S-methyl, α -HCH, Lindane, Vinclozolin, Aldrin Chlorpyrifos Malathion Parathion, Chlorfenvinphos (Z isomer) Endosulfan A, 4,4'-DDE, Captan, 2,4'-TDE, Endrin, Ethion 4,4'-DDT Acrinathrin Methoxychlor, Tetradifon Phosalone, Fluvalinate 1 Fluvalinate 2, chlorfenvinfos, Diazinon, ethion, pirimiphos methyl, terbufos, (Amitraz, bromopropylate 2,4-dimethylaniline coumaphos, cymiazole fluvalinate), Dichlorvos, phorate, dimethoate, diazinon, methyl parathion, ethion, fenitrothion, malathion, triazophos, fenthion, chlorpyrifos, Fenoxycarb Phenthoate Fonofos Diazinon Phosalone Pyrazophos Chlorpyrifos methyl Profenofos Pirimiphos ethyl Temephos Bromophos ethyl Chlorpyrifos ethyl, Chlopyriphos methyl Diazinon Fonofos Phenthoate Phosalone Pirimiphos ethyl	SPME polydimethylsiloxane fibers		5-127%	[6,19,13,21,23]
	CME-UABE	60 μ l n-hexane	\geq 90%	Fontana et al. [4]
Amitraz, Organochlorine compounds, neonicotinoids	DLLME	Disperser solvent Acetonitrile, acetone, Extraction solvent CCl ₄ , chloroform	69.2-119%	[15,20,9,14,
Five triazole pesticides (penconazole, hexaconazole, diniconazole, tebuconazole, and difenoconazole)	Elevated temperature -DLLME/ET-DLLME PH 4-8 Temperature 75°C Centrifugation time 5mint Speed 4000 rpm	Extraction solvent: 1,2-DBE = 130 μ l Disperser solvent: DMF = 1.5 ml	97-100%	- [30]
Amitraz	HF-LPME	Extracting solvent 1-undecanol	90-98%	Yamini, Faraji et al. [32]
Diazinon, lindane, chlorpyrifos methyl, α -endosulfan, β -endosulfan, 4,4-DDT	HS-SDME		74-102% BQL for chlorpyrifos and β -endosulfan	Amvrazi, et al. [33]
	D-SDME		n.g	
Triazoles and Triazines	AALLME PH 4-8, extraction number 3 centrifugation speed and time 4000 rpm and 5 min	extraction solvent 1,2-DBE (70 μ l)	61-95%	Farajzadeh, Mogaddam et al. [30]
OCP's OP's Pyrethroids, neonicotinoids and certain organonitrogen Trichlorfon Trifluralin Hexachlorobenzene Lindane Chlorpyrifos methyl Chlorothalonil Kresoxim methyl Heptachlor Malathion Chlorpyrifos ethyl Bromophos methyl Fipronil Heptachlor epoxide Endosulfan alpha 4,4-DDE Dieldrin Endrin I Endosulfan beta Endrin II Trifloxystrobin Endosulfan sulfate Endosulfan sulfate Tetradifon Lambda-cyhalothrin	dSPE QuEChERS kits	Acetonitrile, Acetonitrile acedified with acetic acid	70-120%	[24,34,29,14,31,21]
Organophosphorus. carbamate, amide	MSPD	5 ml n-hexane-ethyl acetate mixture (90 + 10 v/v)	>80% for organophosphorus 60% for carbamate & amide	Sanchez-Brunete, et al. [35]

others) and found recoveries in the range of 70-120%, 73-111% and 71-101% respectively.

The efficiency of LLE is further enhanced and matrix effect is reduced when it was coupled with low temperature purification. The aqueous phase, containing the sample components is frozen while pesticides extracted with the organic phase. de Pinho, Neves et al. [11] applied this modified method for the extraction of OPPs and pyrethroids pesticides from honey and found good recoveries with minimal matrix effect [11]. This technique has certain drawbacks as it consumes huge quantities

of solvent, emulsion formation, time consuming, laborious and large amounts of waste is generated which is not environment friendly [27,5].

(ii) **Ultrasonic solvent extraction:** This technique has been employed to reduce the consumption of solvent, lower the time, and to enhance the recovery. Alehagen, et al. [28] used sonication technique for the extraction of Boscalid, imidacloprid, tau-fluvalinate and thiacloprid from honey by using ethyl acetate as extractant solvent with recovery within acceptable range (69.4-91.8%) [28].

(iii) **Supercritical fluid extraction (SFE)** involves the unique property

of supercritical fluids for the extraction of the analyte. It has gained potential importance over conventional SE method because of being fast, using minimum solvent amount, little sample volume and selective extraction can be done through it. Commonly used supercritical fluid is SC CO₂, which is a good replacement for halogenated solvent because of CO₂ being less toxic [36,18] determine organochlorines, organophosphorus, organonitrogen and pyrethroids while cleanup was done with SPE and found recoveries 75-94% SFE with SFX fibers and sulfonated CO₂ cylinder to [18]. Among the limitations, it is not economical and pesticides dissolved in water can't be treated through this procedure due to low solubility of CO₂ in water [5].

(iv) SPE (solid phase extraction) have advantage over SE for less solvent consumption, its robust, rapid and comparatively simple method. Blasco et al., [10] used SPE by using C18 as sorbent for the extraction of 9 organochlorines, 5 carbamates, and 28 organophosphorus and found recoveries within acceptable range (73-95%) for all the selected pesticides except Dimethoate with recovery <50% [10]. The extraction efficiency of SPE for Dimethoate and other Ops was enhanced with sorbent was change to RAM-MISPE (Restricted access material-molecularly imprinted polymer solid phase extraction) (Table 1). HLB, MISPE, Florisil, C18, RP-C18, PSA, GCB are also used as sorbent for different classes of pesticides to minimize the matrix effect by different scientists globally [8,10,21,22]. All the sorbents have different affinities with different classes of pesticides and proved efficient with recoveries greater than 70% in all cases. Its shortcoming include lack of selectivity, large sample volumes and cartridges as made of plastic which can absorb the analyte, interference problems [4,5].

Modern extraction/cleanup techniques

(i) SPME (Solid phase micro extraction) is a popular technique as it reduces steps by doing extraction and pre-concentration simultaneously. In this technique, fused silica coated fiber is dipped in sample and then analytes were either directly desorbed from the fiber into the injection port of a gas chromatograph or by using a polar organic solvent, such as methanol or acetonitrile. The sensitivity and selectivity of extraction by SPME is dependent on type of SPME fiber type. Comparative assessment of extraction efficiency of SPME fibers in terms of extraction time and mean percent recovery was presented in Figure 2. Sol-gel crown ether fiber had greater recovery and least extraction time for analysis of multiple pesticides from honey. It eliminates the problems associated with SPE, as described previously, it retains following advantages: (i) it's a solvent free system, (ii) largely reduced extraction time, (iii) provides good results over a wide range of analyte concentrations, and (vi) can be easily automated. However it shows input sample limitations and relatively high LOD's. It is relatively expensive technique due to the fibers used in it [23].

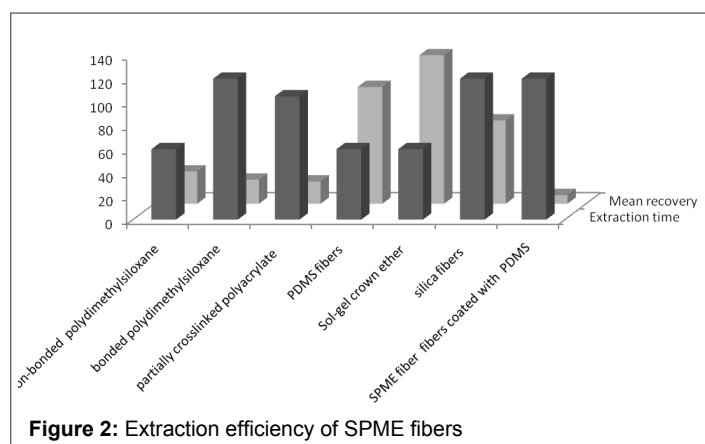


Figure 2: Extraction efficiency of SPME fibers

(ii) SBSE (Stir-bar sorptive extraction) a relatively new technique, it is similar to SPME. In SBSE sample is stirred with a stir bar coated with PDMS fibre, and extraction of analyte is done by partitioning between the polymer and aqueous phase based on the distribution constant. After extraction the solute are injected into the analytical system either through liquid desorption (LD) or thermal desorption (TD). SBSE was used for extraction of organophosphates from honey samples by using methanol as extraction solvent with PDMS fibers and obtained recoveries within 40-64%. [23]. The extraction efficiency of SBSE for OPP's and OCP's was enhanced with polyvinyl coating to PDMS and acetonitril as extraction solvent [19]. SBSE uses larger volumes and surface area coating (50-200 times) and brings higher sensitivity and better reproducibility. It is more accurate and sensitive technique and matrix effect of honey in quantification is lower in it as compared to SPME [23].

(iii) MSPD (Matrix solid-phase dispersion) is a new extraction and clean up technique which was developed to avoid the issues encountered in SE and SPE. It requires less solvent and time as compared to conventional methods. The polar compounds and pigments are retained on adsorbent and analyzed directly in this technique. The extraction and cleanup steps are performed in a single step by utilizing small amount of organic solvent. It eradicates the diluting step for solid or semi-solid samples [35,5].

(iv) CME-UABE (Coacervative microextraction ultrasound-assisted back-extraction technique) was introduced in 2010 by AR Fontana [4] and coworkers for OPP's extraction. This extraction/pre-concentration technique is supported on micellar organized medium based on non-ionic surfactants, analyte is back extracted with hexane to make compatible with GC, because surfactants are highly viscous and have low volatility [4]. Its economical, easy to operate and environment friendly, uses surfactants thus lowers the consumption of organic solvents [4].

(V) LPME/LLME: liquid phase microextraction/liquid-liquid microextraction technique similar to the LLE but with reduced solvent volume, it has certain types like single drop microextraction (SDME), dispersive liquid liquid microextraction (DLLME) [14], air-assisted liquid-liquid microextraction (AALLME) [30], hollow fiber protected liquid phase microextraction (HF-LPME) [32].

(Va) DLLME (Dispersive liquid-liquid microextraction) is an advanced technique in which water-insoluble extracting solvent is dissolved in a water-soluble dispersive solvent such as acetone. The obtained mixture is then injected into the centrifuge tube containing water sample. Extraction solvent being insoluble in water creates emulsion, increasing contact area between the phases and establishes extraction equilibrium quickly as compared to conventional liquid-liquid extraction. After centrifugation a particular amount of extraction solvent is taken from the tube and injected into the instrument [15,20,9] proposed dispersive liquid-liquid microextraction protocol for the determination of residues of 15 organochlorine pesticides in honey and compared it with SPE, SFE, QuEChERS, SPME LLE, LLE-LTP and results proved that DLLME is best technique with respect to sensitivity, time and low operational cost [9]. It's simple, economical method with high extraction efficiency while taking short time for extraction but it uses large volume of solvent especially halogenated solvent (highly toxic nature that is difficult to handle in the laboratory) which is major shortcoming of this technique [9,30].

(Vb) HF-LPME (Hollow fiber liquid phase microextraction) is a micro-extraction technique in which hollow fibers containing the extractants inside the lumen are used. Thus sample is vigorously stirred without loss of extractant. HF-LPME is the most robust and reliable alternative for typical LPME. There are two modes of this system, two-phase HF-LPME and three-phase HF-LPME. Generally two-phase HF-LPME is applied when the analytes have high solubility in non-polar organic solvents and three-phase is applied in case of basic/acidic analytes

containing ionizable functionalities. Selection of extractant solvent is crucial step in this technique. It must have following characteristics (i) it should be immiscible with water to reduce the losses, (ii) it must have compatibility with the fiber and should be immobilized easily in the pores of hollow fiber; (iii) it must show good chromatographic behavior. Its fibers are not very costly so it can be widely used as far as economical point of view is concerned. It is simple to use, efficient and excellent for cleanup and involves less solvent consumption. Simple LPME provides lesser recoveries as compared to HF-LMPE [32].

(Vc) AALLME (Air assisted liquid-liquid microextraction) a relatively novel technique, introduced by Farajzadeh et al in [30]. A small amount of extractant solvent is added into the aqueous phase containing analyte. This mixture is taken into a syringe and pushed out into a tube for predetermined cycles in order to produce a cloudy mixture. This mixture contains the extractant dispersed as minute droplets into the aqueous phase. Phase separation is done by centrifugating the tube containing mixture and next step is proceeded with the sediment phase. Farajzadeh et al [30] compared the proposed method's results with previously reported protocols for the determination of the same pesticides e.g. SPME, SPE-DLLME, SBSE-DLLME and concluded that AALLME has good repeatability than others. It's a disperser solvent free technique hence completely rapid. It is more or less similar to DLLME, having advantage of using smaller amounts of solvent. Solvent chosen must have different and higher density than sample, good gas chromatography behavior, less soluble in water and most importantly must have high extraction efficiency with analyte [37,30].

(vi) SDME (single drop micro extraction) is a solvent miniaturized microextraction technique, in it single microdroplet of organic solvent is suspended at the tip of the microsyringe needle and sample solution either can be directly pre-concentrated through D-SDME (direct SDME) or through headspace SDME(HS-SDME). It's convenient to use and reduces cost in comparison with SPME and HF-LPME, highly sensitive and eliminates matrix effect greatly [33].

(vii) QuEChERS: This method is acronym for easy, cheap, quick, rugged, safe and effective first introduced in 2003 and was further modified during recent years. Now-a-days it has become most frequently used technique for the determination of multi-residue pesticides. Its principle is based upon the dispersive solid phase extraction. The analyte is extracted with an organic solvent or mixture of organic solvents, water is removed by salting out, afterwards the extract is cleaned by passing through SPE sorbent kit rather than SPE column, and finally the extract is analyzed through a suitable technique. Blasco et al., [21] extracted honey samples with QuEChERS and compared its extraction efficiency with SPE, SPME, and PLE. Results indicate that QuEChERS gave the highest recoveries in comparison with other techniques [21]. A number of other researchers also analyzed different pesticides by using this method and found it reliable method with adequate clean up, satisfactory recoveries and repeatability [16,29,24,31,34]. It is considered as an advanced technique due to less time consumption, less waste generated and matrix interference, low financial cost and also introduced ease in operation thus minimizing the potential source of error. [31].

A number of extraction & clean up protocols have been developed by analysts so as to reach the maximum easiness and robustness and above all economical. To date quick, robust and effective extraction and cleanup methods have been proposed and successfully practiced as shown by the results given.

Analytical techniques used for pesticide residues determination

Another important step in analysis of pesticides residue from honey after extraction and cleanup is separation of selected analytes through

chromatography. Gas chromatography as well as liquid chromatography is being used as separation technique coupled with some detector. Ideal detectors used for the detection and quantification of pesticide residues would respond only to target analyte, while other coextracted elements remain transparent. Table 3 summarizes all available analytical methods with corresponding references.

Gas chromatography: Gas chromatography has been used with different detectors like electron capture detector (ECD) [24] ECD is particularly a popular technique due to sensitivity and specificity for electronegative chlorine atoms. It is highly sensitive for halogenated pesticides and nitro compounds. Zacharis et al [9] detected 15 organochlorine pesticides using GC-ECD with LOD (0.02-0.15 ug/L) and linearity (0.986-0.996). It has lower linearity range and widely varying response. GC with micro-ECD (μ -ECD) has revolutionized the trace level detection of halogenated pesticides. It is highly sensitive and reliable detector with low quantification limits. It has broad linearity range for confident quantification, which are lacked by ECD [40,33]. GC-NPD (nitrogen phosphorus detector) is specific for nitrogen-phosphorus containing compounds [24,30].

Flame ionization detector (FID) is a non-specific detector, Amitraz a member of formamidine pesticide family was analyzed by using DLLME-GC-FID approach. The method is linear in range of 0.01-1 mg/kg with limit of detection was 0.0015 mg/kg proving it an efficient method [15]. Mass spectrometric detector (MSD) [20] is termed as the universal detector on the basis of its non-specific properties. MSD being versatile and selective detector is preferred by analyst [5].

Mass spectrometry is frequently used technique for detection, identification and quantification of pesticides due its sensitivity, high selectivity, and low limits of detection, employing atmospheric potential ionization in positive and negative mode [5]. Every mass spectrometer is made up of three main components: (i) an ion source (ii) an analyzer for the separation of ions according to mass-to-charge ratio (iii) a detector to count ions.

Among ion sources electron spray ionization (ESI) and atmospheric pressure chemical ionization (APCI) [23] are mostly used, these both are based on atmospheric pressure ionization. For the determination of multiresidue in honey following analyzers are in common use; (i) ion trap mass analyzer (IT) [9] (ii) time of flight (ToF) [29] (iii) **quadropole** [41]. A quadropole analyzer has less separation efficiency not exceeding 3000 now replaced by triple quadropole analyzer. TOF has characteristics of broad range of measurement, high sensitivity and high scanning speed. Its Separation efficiency exceeds 40,000, while the separation efficiency and m/z range of the IT is similar to that of a typical quadropole [41].

There are different MS techniques on the basis of arrangement/combination of analyzers. Following are the frequently used modifications of the system Tandem mass spectrometry (MS/MS); it's a combination of two same or different type of analyzers, and characterized with high separation efficiency, sensitivity and selectivity as compared to single analyzer. It has certain types on the basis of kind of analyzers (a) triple quadropole system (QQQ) (b) quadropole-time-of-flight (Q-TOF) (c) quadropole-linear-ion-trap (Q-Trap). Amongst three, triple quadropole tandem mass spectrometry is most popular due its higher separation efficiency, higher selectivity and sensitivity [41].

Though gas chromatography is widely used technique with variable detectors but it is only suitable for volatile and less polar compounds or for the compounds which are amenable to derivatization to ensure the volatile properties. Compounds with low thermal stability or low volatility cannot be analyzed by GC. e.g. fluralinate can be determined through GC-ECD/FID but it is decomposed easily due to higher temperature in GC injector or column, for such compounds liquid chromatography is a preferable technique [5].

Table 3: Analytical techniques for analysis of residue of pesticides

INSTRUMENT	ANALYTE	LOD	reference
GC-ECD	Organochlorines, Organophosphorus, pyrethroids, Trichlorfon Trifluralin	0.1-30 ug/kg	[6,8,18,16,11,9,31,24]
GC- μ ECD	Organochlorines, Organophosphorus	0.07-19 ug/kg	Amvrazi, et al. [33]
GC-NPD	Organophosphorus, Pyrethroids, certain organonitrogen and triazole pesticides	0.05-0.21 ng /g	[30,33]
Capillary GC-NPD	Ops, carbamate, amides	6-15ug/kg	Sanchez-Brunete, et al. [35]
GC-FPD	Dichlorvos, phorate, dimethoate, diazinon, methyl parathion, ethion, fenitrothion, malathion, triazophos, fenthion, chlorpyrifos, Quinalphos, ethoprophos, terbufos and fenamiphos	4-80 ng/kg	[19,38,22]
GC-FID	Amitraz, Triazoles and Triazines	1.2-5 ug/kg	[15,30,32]
GC-MS	Organochlorines; Fenitrothion, chlorpyrifos, parathion, methidathion	0.3-2.5 ug/kg	[10,26,4]
GC-IT/MS	chlorfenvinfos, , Diazinon, ethion, pirimiphos methyl, terbufos	0.2-3 ug/kg	[13,20]
GC*GC-TOFMS	Alachlor, Bifenthrin, Diazinon, Organochlorines, Pyriproxyfen, Quinalphos, Vinclozoline	2.5-16 ng/g	(Barganska, Olkowska et al. [29])
GC-MS (quadropole)	Amitraz, bromopropylate 2,4-dimethylaniline coumaphos, cymiazole fluvalinate	0.3-10 ug/kg	
LC-APCI-MS	Chlorpyrifos-methyl, Diazinon, Fonofos, Phenthoate, Phosalone, Pirimiphos ethyl	0.3-1 mg/kg	(Blasco, Fernández et al.[23])
LC-MS/MS	OPP's & carbamates Amidosulfuron atrazine carbofuran, chlorotoluron Diethofencarb, diethofencarb Dimethoate Fipronil Imidacloprid Isoxaflutole Linuron Methiocarb Methiocarb sulfoxide Metosulam Pirimicarb Rimsulfuron Simazine Hydroxyterbuthylazine, Boscalid, tau-fluvalinate, thiacloprid	0.2-1232 ng/kg	[17,28,25,14]
LC-IT-MS/MS	Fenoxycarb Phenthoate Fonofos Diazinon Phosalone, Pyrazophos Chlorpyrifos methyl, Profenofos Pirimiphos ethyl, Temephos Bromophos ethyl Chlorpyrifos ethyl	0.024-1.155 mg/kg	Blasco, Vazquez-Roig et al. [21]
LC-MS/MS (SRM)	Neonicotinoids (dinotefuran, nitenpyram, thiamethoxam, clothianidin, imidacloprid, acetamiprid and thiacloprid) in honey liqueur	0.5-1.5 ug/L	(Jovanov, Guzsvány et al. [14])
UHPLC-MS/MS	79 pesticides ()	0.03-1.51 ug/kg	(Orso, Floriano et al. [34])
TLC and video densitometry	Atrazine, simazine	80, 90 ng/spot respectively	I. Rezic et al [27]
ELISA	Imidacloprid and thiamethoxam		Huixin Ma et al [39]

Liquid chromatography (LC): To deal with the pesticides which are labile, have not been derivatized, more polar and their metabolites are even more polar and less volatile than the parent compound, for such class of pesticides, liquid chromatography (LC) is used [41-43]. Now-a-days HPLC and UHPLC are commonly employed for the separation of analytes with ultraviolet (UV) [5], diode array detector (DAD) [12] variable wavelength detector (VWD), MS [34] detectors is in common practice. However UHPLC is preferred over conventional HPLC to achieve high eluent flow rate in column and has much greater separation efficiency to determine multicomponent mixtures [41].

LC-MS and LC-MS/MS is an ideal, extremely specific and highly sensitive technique used for identification and quantification of pesticides residues. It provides information about analyte without derivatizing, it can compensate sample purity and it enables simultaneous analysis of the compounds with varying polarity. The only drawback of LC is that it has greater matrix effect thus increasing signal to noise ratio. This problem can be rectified by matrix-matched calibration, internal standard addition and extended the duration of analysis [41].

LC-MS and LC-MS/MS is an ideal, extremely specific and highly sensitive technique used to detect a wide range of chemicals and a preferred technique over GC-MS because LC-MS involves simple sample preparation and can detect much wide range of pesticides on the other hand GC-MS is limited only for non-polar and volatile class of compounds.

ELISA (Enzyme-linked immunosorbent assay): is a technique in which multiresidues are determined just by simple dilution of the samples, no extraction and clean steps are required. Its results are in comparison

LC-MS. Huixin Ma et al., [39] determine the residue of imidacloprid and thiamethoxam in honey through ELISA. In an indirect ELISA microplate wells were coated overnight at 4°C with coating antigens (4 ng of thiamethoxam-BSA or 6ng of imidacloprid-BSA in 100 ml per well of 0.05M carbonate/bicarbonate buffer, pH 9.6) and found recoveries 96-122% proved it an effective method [39].

Thin layer chromatography (TLC): TLC has been used to determine pesticide residues. It involves extraction of sample with a solvent mixture and separation of the components into blocks with a suitable coating material (e.g. Silica gel) and finally elution with suitable solvents. Rezic et al. [27] detected residues of herbicides atrazine and simazine in honey by this technique with estimated recoveries 92.3% and 94.2% respectively. It is less specific and sensitive technique and requires special equipment for visualization and quantification of results [27].

Conclusion and Final remarks

Microextraction techniques are preferred over the conventional procedures due to less time; reduce solvent consumption and minimal matrix effect. Among the analytical techniques, GC- μ ECD is best technique for outline analysis of pesticides from honey, while MS detector with either GC or LC is suitable for identification of acaricides and neonicotinoid pesticides from honey. This review helped to judge the suitable technique for determination of volatile and labile pesticides from honey.

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