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Review on Extraction Procedures and Chromatographic Techniques for Analysis of Multiple Pesticides Residues in Honey

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Abstract

Global consumption rate of honey increased due to its high nutritional and therapeutic values. It is under great experimentation now-a-days. Consumers of the honey products are at stake due to presence of pesticide residues which are hazardous to health. Honey is a complex matrix and pesticides are present in sub-ppb levels, so reliable analytical method for identification and quantification of multiple pesticides is required to ensure the food safety for the consumers in compliance with the EC and Codex Alimentarius regulations. In this review, different sample preparation and detection techniques used globally are discussed especially focusing on honey matrix components-pesticides interference. Future perspectives for minimization of these matrix interferences will also be presented.

Keywords: Honey, Multiple Pesticide Residues, Extraction Methods, Chromatographic and Spectroscopic Techniques .

Introduction

Honey is considered as pure natural product and favorite of man of all ages especially for children due to its sweet flavor and aroma. Antioxidant power is the prominent characteristics of honey along with other properties like wound healing, anti-inflammatory, antimicrobial, used for gastrointestinal disorders and skin diseases [Al-Waili, Salom et al. 2012]. As it is of primary importance for human health, one should be conscious of its purity. A huge number of pesticides are available in market and are being used frequently for protection of the crops from the damaging insects and weeds (Al-Waili, Salom et al. 2012). Especially, in developing countries unwise use of pesticides create an alarming situation (Tilman, Cassman et al. 2002).

There are two major sources of contamination in honey, first when honeybees take contaminated nectar along with them and contaminate the colony, consequently transferred to food chain (Kujawski and Namieśnik 2008, Fontana, Camargo et al. 2010). In direct contamination, certain chemical therapeutic agents like coumaphos, fluvalinate, flumethrin, amitraz are applied on hive to combat against larvae diseases and mites (Rial-Otero, Gaspar et al. 2007). The principle ways of pesticides contamination in honey are shown in Fig.1.

Pesticides monitoring programs should include determination of directly applied acaricides, (used to control Varroa) and insecticides, fungicides, herbicides and rodenticides (used to protect crops from disease infestation) indirectly introduced by bees in the hive. Since 1998 scientists from Spain (Jimenez, Bernal et al. 1998, Campillo, Viñas et al. 2015), Greece (Tsipi, Triantafyllou et al. 1999, Zacharis, Rotsias et al. 2012), Portugal (Blasco, Fernández et al. 2003), Brazil (de Pinho, Neves et al. 2010), France (Martel and Zeggane 2002), Italy (Volante, Galarini et al. 2001), Serbia (Jovanov, Guzsvány et al. 2014), Iran (Bashiri-Juybari, Mehdinia et al. 2011), Egypt (Barakat, Badawy et al. 2007), Belgium (Pirard, Widart et al. 2007), USA (Rissato, Galhiane et al. 2004), China (Yu and Hu 2009), Argentina (Fontana, Camargo et al. 2010), Poland (Kujawski, Pinteaux et al. 2012) have determined pesticide residues in honey and reported the presence of enormous levels of residues above MRL's. Different national organizations have established MRL's for honey, but there is no homogeneity among the MRL's set by different countries which is a big hurdle for analyst but most importantly for traders.

The residues of pesticides in honey are usually at trace levels and several pesticides can be present simultaneously, so highly selective and efficient extraction techniques are required (Tette, Guidi et al. 2016). Researcher have reported different extraction methods globally such as, liquid-liquid extraction (Pirard, Widart et al. 2007), supercritical fluid extraction (Rissato, Galhiane et al. 2004), solid-phase extraction (Blasco, Vazquez-Roig et al. 2011, He, Song et al. 2015), solid phase micro extraction (Volante, Galarini et al. 2001, Blasco, Vazquez-Roig et al. 2011) stir bar sorbtive extraction (Blasco, Fernández et al. 2004) single drop microextraction, dispersive liquid liquid micro-extraction (Bashiri-Juybari, Mehdinia et al. 2011, Zacharis, Rotsias et al. 2012)

ultrasound-assisted emulsification microextraction, coacervative microextraction technique (alternative to organic solvents microextraction) (Fontana, Camargo et al. 2010) QuEChERS (Barakat, Badawy et al. 2007, Eissa, El-Sawi et al. 2014). Gel permeation and adsorption chromatography are also used to minimize the matrix effect in pesticides analysis from honey (de Pinho, Neves et al. 2010).

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) (Blasco, Fernández et al. 2004, Pirard, Widart et al. 2007, Kujawski, Pinteaux et al. 2012, Barganska, Olkowska et al. 2014, Farajzadeh, Mogaddam et al. 2014, Orso, Martins et al. 2014) are in use now a days. Different scientist reviewed the analytical methodologies for pesticide analysis in honey. Rial-Otero et al. [21] and Tette et al., 2016 reviewed and discussed various extraction and analytical techniques, but no one focused on honey matrix components-pesticides interference/hinderence in the pesticides detection and quantification. So in this review special focus was given on sample preparation and separation/detection methods for individual pesticides as well as multiple, multiclass pesticides.

Matrix Inference- Hindrance in Pesticides Analysis

Honey Matrix Composition

Honey contains more than 200 substances and its composition especially its secondary metabolites are influenced by some external factors either environmental or seasonal. Processing, handling, and storage and quality of honey are also determining factors of its composition. The main substances present in honey are sugars (major components: fructose 38%, glucose 31%), proteins, moisture (10–20%), vitamins (ascorbic acid, niacin, etc.), mineral salts (potassium, calcium, sodium, phosphorus, etc.), organic acids (acetic acid and gluconic acid, etc.), 5-hydroxymethyl furfural (HMF), enzymes (phosphatases,

glucoseoxidase, invertase and catalase), flavonoids, phenolic acids and volatile compounds [{Ahmed, 2016 #5}]. Moreover, honey has acidic pH, (3.1- 4.5) which is favorable for degradation of certain pesticides like amitraz and chlordimeform {Martel, 2002 #6}. Certain pesticides like thymol and rotenone decompose quickly when exposed to light.

Pesticides- Honey Matrix Interactions

Matrix effects, mostly observed as enhancement or suppression of the analyte signal which leads to false positive or negative results. The physical and chemical properties of pesticides which are used to characterize pesticide-matrix interactions are: vapor pressure, water solubility (S), octanol/water partition coefficient (Kow), dissociation constant (pKa) and DT 50 (degradation time). The nature of intermolecular interactions between matrix and pesticides may also vary widely due to great diversity of structures and physicochemical characteristics. These interactions can be ionic, hydrogen bond, covalent bonds, dipole, and van der Waal forces, hydrophobic interactions or partitioning. Two or more types of interaction can simultaneously occur between the same molecule and the matrix. These are particular characteristics and often so divergent that hinders the laboratory analysis of monitoring pesticide residues in food (Tette, Guidi et al. 2016). Similarly, co-eluting matrix component (s) has similar chemical properties to target compounds and cannot be removed using non-specific cleanup procedures.

Different approaches which are used to reduce matrix effects in food samples include sample clean-up techniques such as QuEChERS and solid-phase extraction (SPE), sample dilution, chromatographic separation of analytes from interfering matrix components, use of matrix-matched standards, and some other. Exhaustive sample cleanup may help remove interfering components but this is time-consuming and may result in loss of analytes of interest and variability of sample results. The use of matrix-matched calibration standards (MMCS) can be an effective approach but this requires the availability of uncontaminated sample matrix to be used to prepare calibration standards, this can be a hectic exercise. The blank matrix may not be identical to the sample matrix and still can cause biases in quantitative analysis. The use of standard addition which involves addition of a fixed amount of analytical standards to the samples in order to generate a calibration curve for each sample may be an effective approach, but is tedious and impractical for real world sample analysis, where one has to deal with a large number of samples and analytes. Sample dilution, also referred as dilute-and-shoot, is one of the most attractive solutions because the technique is simple and rapid, doesn't require its own method development, and introduces less chance of error and variation as compared to some of the other techniques {Yang, 2015 #13}. Yang et al., 2015 used High-Resolution Accurate Mass (HRAM) mass spectrometry to investigate the contribution of matrix components to pesticide residue analysis assays in honey. Dilutions of 1X, 1/10X, and 1/100X was interrogated and matrix effects were measured via principal component analysis and through slope ratios of the calibration curves {Yang, 2015 #13}.

Matrix effect in Honey extract is due to presence of carbohydrates, such as glucose and fructose and it can be affected by the floral origin of honey. Quantitative errors arising from matrix effects are minimized by using matrix-matched standards {Tomasini, 2012 #14}.

Analytical Methodology for Determination of Pesticides from Honey

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.

Extraction enrichment and isolation of pesticides from matrixes

The selection of extraction method for pesticides analysis in 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. Different sample preparation procedures were developed and applied during last decades, amongst them are 1) LLE/SE 2) SPE 3) SFE 4) QuEChERS etc.

Solvent Extraction/Liquid Liquid Extraction (LLE)

In liquid liquid extraction (LLE), 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 recov-

ery and good measurements. Blasco, Fernández et al. 2004 used this tehnique to extract organochlorines with three different solvents n-hexane, ethyl acetate and light petroleum and found ethyl actete as the best solvent for extraction of these compounds with mean recovery 68-126% {Blasco, 2004 #12}[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. 2015 use this approach and extracted 13 organochlorines, 8 pyrethroids and 146 pesticides belonging to different groups (organophosphorus, carbamates, trizoles, amides, neonicotenoids, strobilurines, phenylureas, bendimidazoles and others) and found recoveries in the range of 70-120%,73-111% and 71-101% respectively (Louca Christodoulou, Kanari et al. 2015). The choice of solvent for pesticide extraction in honey should be vigilant as few pesticides like rotenone is unstable in methanol storage at 4 ◦C {Jimenez, 2000 #7}.

The efficiency of LLE is further enhanced and matrix effect was 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. 2010 applied this modified method for the extraction of OPPs and pyrethoid from honey and found good recoveries with minimal matrix effect. (de Pinho, Neves et al. 2010).

To reduce the consumption of huge quantity of organic solvent, lower the time, and to enhance the recovery, sample was sonicated. Alehagen, 2012 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%) (Alehagen 2012). LLE was modified by using acidic extraction solvent mixture to avoid the co extraction of antibacterial sulfonamides with pesticides from honey {Gómez-Pérez, 2012 #11}.

Certain solvent miniaturized LLE techniques like single drop micro-extraction (SDME), dispersive liquid liquid microextraction (DLLME) (Jovanov, Guzsvány et al. 2014), air-assisted liquid-liquid micro-extraction (AALLME) (Farajzadeh, Mogaddam et al. 2014), hollow fiber protected liquid phase micro-extraction (HF-LPME) (Yamini, Faraji et al. 2015) have been developed and are used frequently to enhance the efficiency and reduce the organic waste generation. Tette et al., 2016 reviewed these techniques in detail {Tette, 2016 #8}.

SDME (single drop micro-extraction) is a solvent miniaturized microextraction technique, in this technique a 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 is convenient to use and reduces cost in comparison with SPME and HF-LPME, highly sensitive and eliminates matrix effect greatly(Amvrazi, Martini et al. 2012).

AALLME (Air assisted liquid-liquid micro-extraction) a relatively novel technique in which 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 preceded with the sedimented phase (Farajzadeh et al., (2012). Farajzadeh et al (2014) 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. 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 (Farajzadeh, Khoshmarram et al. 2014, Farajzadeh, Mogaddam et al. 2014).

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 (Bashiri-Juybari, Mehdinia et al. 2011, Kujawski, Pinteaux et al. 2012, Zacharis, Rotsias et al. 2012). Zacharis et al., 2012 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 (Zacharis, Rotsias et al. 2012). Zhang et al. coupled DLLME with ultrasound assisted (UA) and high temperature program and found higher enrichment factors and extraction recoveries due to better dispersion of the extraction solvent in the aqueous phase{Zhang, 2011 #9}.

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 me immiscible with water to reduce the loses,(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. Fibers used in HF-LPME are not very costly, hence 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(Yamini, Faraji et al. 2015).

SPE (solid phase extraction) has advantage over SE for less solvent consumption, being robust, rapid and comparatively simple method. Blasco et al., 2003 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% (Blasco, Fernández et al. 2003). The extraction efficiency of SPE for Dimethoate and other OP's was enhanced when 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 by different scientists globally in order to minimize the matrix effect (Tsipi, Triantafyllou et al. 1999, Blasco, Fernández et al. 2003, Blasco, Vazquez-Roig et al. 2011, He, Song et al. 2015) All the sorbents have different affinities with different classes of pesticides and proved efficient with recoveries greater than 70% in all cases. Among shortcomings of SPE are, lack of selectivity, large sample volumes and cartridges material, as made of plastic which can absorb the analyte, interference problems (Rial-Otero, Gaspar et al. 2007, Fontana, Camargo et al. 2010).

SPME (Solid phase micro extraction) is a popular technique as it reduces preparatory steps by doing extraction and pre-concentration simultaneously. In this technique, fused silica coated fiber is dipped in sample and then analytes are 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 upon type of SPME fiber. Comparative assessment of extraction efficiency of SPME fibers in terms of extraction time and mean percent recovery was presented in Fig.1. 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 (Blasco, Fernández et al. 2004)

SBSE (Stir-bar sorptive extraction) a relatively novel 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 extaction solvent with PDMS fibers, found recoveries within 40-64%.(Blasco, Fernández et al. 2004) .The extraction efficiency of SBSE for OPP's and OCP's was enhanced with polyvinyl coating to PDMS and acetonitrile as extraction solvent.(Yu and Hu 2009). SBSE consumes larger solvent 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 (Blasco, Fernández et al. 2004).

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 (Sanchez-Brunete, Albero et al. 2002, Rial-Otero, Gaspar et al. 2007)

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 (Anklam, Berg et al. 1998 Rissato, Galhiane et al. 2004) performed SFE with SFX fibers and sulfonated CO2 cylinder to determine organochlorines, organophosphorus, organonitrogen and pyrethroids while cleanup was done with SPE and found recoveries around 75-94%. (Rissato, Galhiane et al. 2004). 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. (Rial-Otero, Gaspar et al. 2007).

CME-UABE (Coacervative miroextraction ultrasound-assisted back-extraction technique) was introduced in 2010 by A.R.Fontana 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(Fontana, Camargo et al. 2010). Its economical, easy to operate and environment friendly , uses surfactants thus lowers the consumption of organic solvents (Fontana, Camargo et al. 2010).

QuEChERS: This method is acronym for quick easy, cheap, effective, rugged, safe and 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., 2011 extracted honey samples with QuEChERS and compared its extraction efficiency with SPE, SPME, and PLE. Results indicates that QuEChERS gave the highest recoveries in comparison with other techniques (Blasco, Vazquez-Roig et al. 2011). A number of other researcher also analyzed different pesticides by using this method and found it reliable method with adequate clean up, satisfactory recoveries and repeatability (Barakat, Badawy et al. 2007, Barganska, Olkowska et al. 2014, Eissa, El-Sawi et al. 2014, Orso, Martins et al. 2014, Orso, Floriano et al. 2016). It is considered as an advanced technique due to less time consumption, reduced waste generated and minimized matrix interference, low financial cost and also introduced ease in operation thus minimizing the potential source of error (Orso, Martins et al. 2014).

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.

Detection and Quantification

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 extracted elements remain transparent. Table 2 summarizes all available analytical methods with corresponding references

Gas chromatography: Gas chromatography It has been used with different detectors like electron capture detector (ECD) (Eissa, El-Sawi et al. 2014). ECD is particularly a popular detector due to sensitivity and specificity for electronegative chlorine atoms. It is highly sensitive for halogenated pesticides and nitro compounds. Zacharis et al (2012) detected 15 organochlorine pesticides using GC-ECD with LOD (0.02-0.15 ug/L) and linearity (0.986-0.996) and compared the sensitivity with GC-IT/MS, finding GC-ECD less sensitive. 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(-Karazafiris, Menkissoglu-Spiroudi et al. 2008, Amvrazi, Martini et al. 2012). GC-NPD (nitrogen phosphorus detector) is specific for nitrogen-phosphorus containing compounds (Eissa, El-Sawi et al. 2014, Farajzadeh, Mogaddam et al. 2014). Flame ionization detector (FID) is a non-specific detector; Amitraz member of formamidine pesticide family was analyzed by using DLLME-GC-FID approach compared results with SPME-GC-ITD and HSME-GC-TSD. Results showed satisfactory linear range and low detection limits of GC-FID. The method is linear in range of 0.01-1mg/kg with limit of detection was 0.0015mg/kg proving it an efficient method(Bashiri-Juybari, Mehdinia et al. 2011). Mass spectrometric detector (MSD) is termed as the universal detector on the basis of its non-specific properties. MSD being versatile and selective detector is preferred by analyst (Rial-Otero, Gaspar et al. 2007).

Mass spectrometry is frequently used technique for detection, identification and quantification of pesticides due its sensitivity, high selectivity, low limits of detection, employing atmospheric potential ionization in positive and negative mode (Rial-Otero, Gaspar et al. 2007). 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) (Blasco, Fernández et al. 2004) 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)(Zacharis, Rotsias et al. 2012) (ii) time of flight (ToF) (Barganska, Olkowska et al. 2014) (iii) quadropole (Stachniuk and Fornal 2016). Single 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 scaning 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.(Stachniuk and Fornal 2016).

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 types 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) quadruple-time-of-flight (Q-TOF) (c) quadruple-linear-ion-trap (Q-Trap). Amongst three ,triple quadruple tandem mass spectrometry is most popular due it's higher separation efficiency ,higher selectivity and sensitivity(Stachniuk and Fornal 2016).

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. fluvalinate 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 (Rial-Otero, Gaspar et al. 2007).

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 (Andreu and Picó 2004, Stachniuk and Fornal 2016). Now-a-days HPLC and UHPLC are commonly employed for the separation of analytes with ultraviolet (UV) (Rial-Otero, Gaspar et al. 2007), diode array detector(DAD) (Martel and Zeggane 2002) variable wavelength detector(VWD), MS(Orso, Floriano et al. 2016) 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(Stachniuk and Fornal 2016).

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 extending the duration of analysis.(Stachniuk and Fornal 2016).

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 comparable with LC-MS. Huixin Ma et al., (2008) determined the

residue of imidacloprid and thiamethoxam in honey through ELISA. In an indirect ELISA microplate wells were coated overnight at 4 oC 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 as an effective method for pesticide residue analysis in honey(Ma, Xu et al. 2009).

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. (2005) 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(Rezić, Horvat et al. 2005).

Conclusion and Final Remarks

Miniaturized extraction techniques are preferred over the conventional procedures due to less time; reduce solvent consumption and minimal matrix effect. Due to the complexity of the matrix, efficient sample preparation and trace-level detection and identification are important to obtain reliable results. Efficient sample preparation depends on the matrix, as well as on the properties and the analyte concentration. Among the analytical techniques, GC-μECD is best technique for routine analysis of pesticides from honey, while MS detector with either GC or LC is suitable for identification of accaricides and neonicotinoid pesticides from honey. This review helped to judge the suitable technique for determination of volatile and labile pesticides from honey.

Ethical statement

This article does not contain any studies with human participants or animals performed by any of the authors.

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