Chapter 1

General Introduction
And
Literature Review

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1. Introduction to Pesticides

Pesticide is a broad term that defines all chemicals used for killing, destroying, repelling and mitigating the insects and pests (EPA[1]). Pesticides play a vital role in crop protection against weeds, insects and fungal diseases during the production, storage, transport and distribution of farm produces. Pesticides are also used to control the pest injurious to human health. Pesticides are essential for realizing the full economic benefits of modern farming. In the absence of pesticides, even the hybrid quality seeds, fertilizers and irrigation system will not deliver their potential productivity. The pesticides help farmers to increase crop productivity by 20-50%. It enables farmers to produce more crops per unit area with less tillage and is therefore a valuable and indispensable tool for the sustainable production of high quality agricultural products. However, due to indiscriminate use of chemical pesticides during the crop protection and public health safety, its residues and degradation products, often cause contamination of soil, water, air and food affecting the environment. Humans can be exposed to pesticides during handling, re-entry to treated areas, contact with environmental residues, and by dietary intake. There is a need for judicious and safe use of pesticides. Society expects manufacturers to develop safe and environment friendly active ingredients and to train the farmers to use these products judiciously and in responsible manner, for the sustainable production of quality food and fibers.

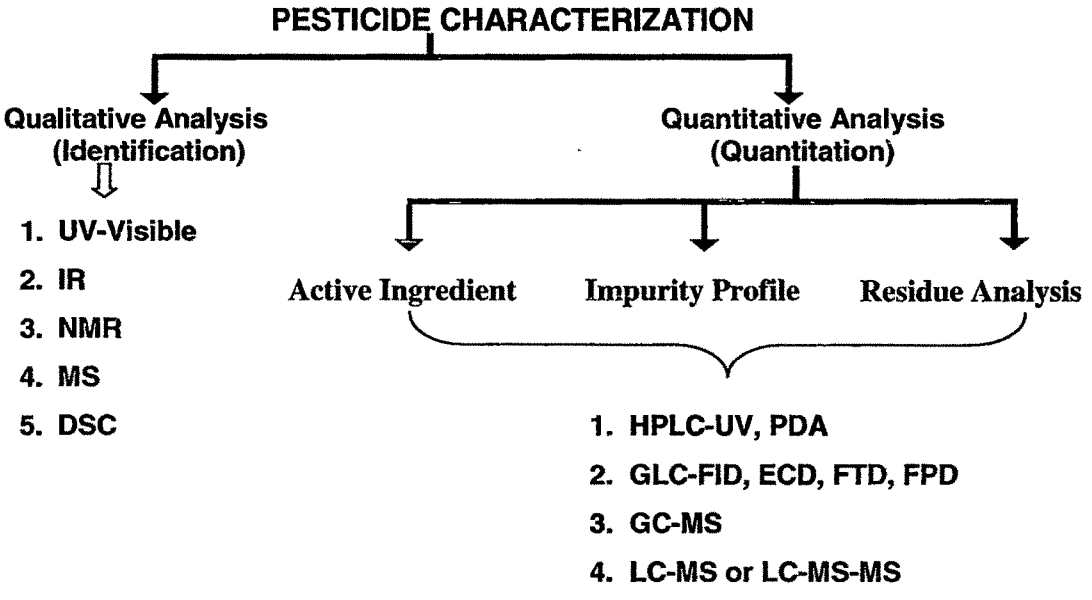
About 48000 metric tons of technical grade chemical pesticides are used to control the pests in our country. Insecticides constitute almost 60% of the total pesticides used, followed by fungicides and herbicides. The Insecticide Act, in India regulates the import, manufacture, sales, transport and use of insecticides in the country with the aim to prevent risk to human being, animals and the environment. The organochlorine insecticides are important historically as a major class of synthetic insecticides. Organophosphorous compounds form the largest single insecticidal group, which are

the derivatives of ortho-phosphoric acid. The synthetic pyrethroides are analog of the pyrethrines, the naturally occurring insecticide of plant origin i.e. the pyrethrum flower [Tanacetum cineraliaolium].

Several organochlorine pesticides viz., aldrin, dieldrin, endrin, heptachlor, chlordane, hexachlorocyclohexane (HCH) etc. have been banned due to their longer persistence and bioaccumulation. The ministry of agriculture and fisheries has approved many organophosphorous and synthetic pyrethroid compounds for agricultural uses, due to their ready biodegradability. Attention is usually focused on contamination by organochlorine pesticides (OCPs) due to their toxicity and persistence in environment; and contamination by common pesticides, such as organophosphorous pesticides (OPPs) and synthetic pyrethroids (SPs) due to injudicious use and runoffs[2,3].

The safety concerns for the environment and public health have spawned more stringent analytical testing requirements, to ascertain the chemical composition and concentration of active ingredients of technical and formulated pesticides and the determination of pesticide residues in environmental samples at very low levels as well as their confirmation. Pesticides are among the most rigorously regulated chemicals in the world, and re-registration processes ensure that their safety is regularly assessed based upon latest science. The cost of research, development and registration of a new pesticide is approx. US $ 150-200 million.

In the present study, an attempt has been made to develop the suitable analytical methods for characterization of selected pesticides belonging to different classes using various states of art modern sophisticated analytical instruments. Pesticide Characterization involved both qualitative and quantitative analysis of pesticides including identification / confirmation and macro- as well as micro-quantitation (active ingredient analysis, impurity profile analysis and residue analysis).



1.1 Analysis of Pesticides

The selected pesticides belonging to various classes have been characterized using suitable analytical methods.

Insecticides:

- **Organochlorine Pesticides:**
  α-HCH, γ-HCH (lindane), p,p'-DDT, Aldrin, Dieldrin, Heptachlor, Endosulfan-I and Endosulfan-II, Endosulfan Ether, Endosulfan Sulfate and Endrin.
- **Organophosphorous Pesticides:**
  Chlorpyrifos and Acephate.
- **Synthetic Pyrethroids:**
  Bifenthrin, Lambda-cyhalothrin, Cypermethrin, Fenvalerate, Permethrin and Deltamethrin.

Herbicides:

- Napropamide, Metribuzin, Trifluralin, Ethofumesate, Alachlor and Butachlor.

Fungicides:

- Chlorothalonil, Metalaxyl, Captan, Hexachlorobenzene, Hexaconazole and Tebuconazole.

The instrumental techniques used for the characterization of pesticides are provided below:

- **Gas Liquid Chromatography (GLC)**

  Gas Liquid Chromatography is a dynamic method of separation and detection of volatile and semi-volatile organic compounds in a mixture. GC involves the partitioning of gaseous solutes carried through an inert gas and a stationary liquid or solid phase. Due to different affinities or interactions of various components with stationary phase, the components passed through the column at different rates and are separated as distinct peaks by a suitable detector. The major components of a gas chromatograph are the gases, the injection port, the column, the detector and the data acquisition system, consisting of an electrometer and recorder. Several types of detectors (universal or specific) and columns are used for more accurate analysis of substances through improved resolution and detection limits. The gas chromatograph performs the qualitative analysis by retention time and peak area normalization and quantitative determination of compounds in mixtures by comparing the peak areas with the standards of known concentrations.

- **High-Performance Liquid Chromatography (HPLC)**

  HPLC is a widely accepted separation technique for qualitative, quantitative analysis and purification of nonvolatile and thermally labile organic or inorganic molecules[4]. HPLC has eight basic components: mobile phase reservoir, solvent delivery system, sample introduction device, column, detector, waste reservoir, connective tubing and a computer, integrator, or recorder. In HPLC, mobile phase consists of a solvent or mixture of solvents and separation of solutes is governed by the competitive interactions and distribution between the mobile and stationary phases. The eluting components are detected by suitable detectors either universal nature viz., UV absorbance detectors (fixed- wavelength, variable-wavelength and photodiode array), refractive index detector or specific detectors viz., fluorescence detector, conductivity detector, electrochemical detector etc.

- **Gas Chromatography/Mass Spectrometry (GC/MS)**

  GC/MS is a very sensitive and widely used hyphenated technique having powerful applications of both GC and MS. Therefore it is used for separation of complex mixtures with higher resolution, identification based on the molecular weight, detailed structural information based on fragmentation pattern and quantitation of all components in one system. Since mass spectrometer is a universal detector, it is a very useful technique for impurity profile analysis or multi-residue analysis of various compounds at very low levels as well as their confirmations. The gas chromatograph has a carrier gas at a pressure of about 760 torr, while the mass spectrometer operates at vacuum of about 10^{-6} to 10^{-5} torr. In mass spectrometry, the sample components are ionized either by electrical, chemical or thermal means. The resulting ions are separated in various types of mass filters viz., quadrupole, ion trap or time-of flight analyzer by their mass to charge ratios (m/z) and detected by an electron multiplier device[5]. The mass spectrum, which is a plot of the number of ions detected (abundance) against the mass of the ions (m/z), forms a unique fingerprint, which identifies each individual chemical component on the basis of mass and fragmentation pattern.

- **Liquid Chromatography/Mass Spectrometry (LC/MS)**

  The LC/MS along with electron spray ionization technique (LC-ESI-MS) is one of the most exciting developments of recent times in analytical techniques. It is widely used for separation and identification of complex mixtures of non-volatile and thermally labile biochemical and organic compounds and determination of the molecular weights of macromolecules. An LC/MS instrument consists of three major components: an LC (to resolve various components of compound), an interface (to transport the analyte into the ion source of a mass spectrometer), and a mass spectrometer (to ionize and mass analysis of the individually resolved components).

Unlike GC/MS, the LC/MS interface should be able to handle the aqueous content, organic and ionic modifiers and buffers of the mobile phase, as the liquid flow is not compatible with the MS vacuum system. Several different LC/MS interfaces have been developed for ionization of nonvolatile components viz., atmospheric pressure electron spray ionization (AP-ESI), atmospheric pressure chemical ionization (APCI) and atmospheric pressure photo ionization (APPI). The sample constituents are ionized and the ions are separated by their mass to charge ratio (m/z) by a quadrupole or ion trap analyzer. The molecular spectrum obtained is relatively simple and identification is generally based on molecular weight of the compound. If required, additional fragmentation can be obtained using a technique called collision induced dissociation (CID).

- **Fourier Transform Infrared Spectroscopy (FTIR)**

  The FTIR is one of the most common spectroscopic techniques used for the identification of functional groups in a substance. Simply, it is the absorption measurement of different IR frequencies by a sample positioned in the path of an IR beam. Different functional groups absorb characteristic frequencies of IR radiation. Using various sampling accessories, IR spectrometers can analyze a wide range of samples such as gases, liquids and solids. The identification of a compound is performed either by matching its spectrum with reference spectrum (fingerprinting) or by interpretation of spectrum using theoretical values. Thus, IR spectroscopy is an important and popular tool for structural elucidation and compound identification. FTIR can also be used for quantitative analysis but sensitivity is poor.

- **UV-Visible Molecular Absorption Spectrometry**

  The UV-Visible spectrophotometer is an important tool for monitoring the concentrations of specific organic contaminants and for the identification of various organic molecules. The molecules possessing chromophore groups and transition

metals commonly exhibit absorption bands in UV/Vis region of the spectrum due to various types of electronic transitions. A spectrophotometer consists of an internal light source, grating or prism monochromator and a photoelectric detector capable of providing transmittance or absorbance data as a function of wavelength. The spectrophotometer having double-beam capability has two matched quartz/silica cells of 1 cm thickness for sample and reference solutions. A very dilute solution of the compound (1 mg/100 mL approx.) in a suitable solvent is analysed over the whole wavelength range of UV/Vis region. Spectrum is therefore defined in terms of λ-max value (wavelength at which maximum absorption takes place), which is specific for a particular compound. Identification of compounds is based on matching the sample spectra with reference spectra.

- **Nuclear Magnetic Resonance Spectroscopy (NMR)**

  The nuclear magnetic resonance spectroscopy is a technique widely applied for identification and structure elucidation of chemical compounds. It provides detailed information on the spatial orientation of nuclei in a molecule. The main components of a NMR spectrometer are a super-conducting solenoid magnet, radio-frequency transmitters and power amplifiers for generating B_1 fields, a probe for delivering B_1 fields to the sample and receiving the NMR signals, a radio-frequency reception system and a computer for data processing. Nuclear magnetic resonance occurs when a magnetically active nuclei is placed in a stable magnetic field and subjected to electromagnetic radiation of appropriate frequency. Nuclei with an uneven atomic mass and atomic number such as ^{1}H, ^{13}C and ^{15}N has $I \neq 0$ with a characteristics magnetic moment and will absorb the radio frequency radiation for transition from one nuclear spin state to another. But the surrounding electrons shield the nucleus due to an induced circulation by the applied filed, so the effective magnetic field felt by the nucleus does not remain same as the applied field. The separation of resonance frequency of nuclei in different structural environment from an internal

standard viz., tetramethylsilane (TMS) is termed the chemical shift. The chemical shift value is proportional to the applied field strength. The tetramethylsilane (TMS) gives a single sharp absorption line at zero cps or ppm. In a NMR spectrum, the absorption frequencies of various absorption bands due to ^1H of different groups of sample molecules are compared with the internal standard and their integration value based on their areas, corresponds to the number of protons present in each group. Under higher resolution the splitting patterns (multiplet) of peaks are observed because the magnetic field experienced by the proton of one group, is influenced by the spin arrangements of the protons in the adjacent group. Therefore a NMR Spectrum gives valuable information about the molecular structural of a compound and is used as a powerful tool for identification[6].

- **Differential Scanning Calorimetry (DSC)**

DSC is the oftenly used instrument for identification of compounds, because it is rapid, easy to operate, and readily available. In DSC, the sample holder, and an empty reference holder are placed in the DSC cell at the sample and reference positions. Power is applied to heaters in the system to ramp the temperature of the DSC at a specified rate (heat-flux DSC) or to hold the DSC isothermally at a given temperature (power-compensated DSC). The DSC measures the difference in the heat flow between the sample and the reference. A typical DSC experiment involves the encapsulation of a small portion (usually 1 to 10 mg) of the desired sample into a sample pan/holder often made of aluminum. A gas purge is usually applied when the sample is placed into the DSC cell and the temperature program is adjusted with a low heating rate (generally 2 to 10 °C/min). Most commercial DSCs are provided with various types of software packages, which allow to determine the melting point, glass transition temperature and purity of compounds by integrating peaks to quantify the heat from exotherm and endotherm.

2. Literature Review

Work done earlier and proposed work in the following areas is presented in this section:

- Analysis of active ingredients in commercial pesticides.
- Impurity profile analysis in technical grade pesticides.
- Purity analysis of standard pesticides.
- Structural elucidation of pesticides.
- Pesticide multi-residues analysis in groundwater and soil.
- Pesticide multi-residues analysis in fruits and vegetables.

2.1 Pesticide: Active Ingredients Analysis

The pesticides play a vital role for the country to achieve self-sufficiency in food grain production. Pesticide plays very specific and critical role when applied appropriately. Therefore, before application, the appropriate analysis of pesticide quality (technical and formulated marketing products) is also vital to study the fate of the pesticides in/on target crops and environmental media (soil, water, air). Several classical and instrumental methods are employed for evaluating pesticides quality of technical and formulations materials.

Collaborative International Pesticide Analytical Council (CIPAC)[7] has published several analytical methods for analysis of technical and formulated pesticides. Association of Official Analytical Chemists (AOAC)[8] has also published official methods for analysis of active ingredients as well as residues of pesticides in various samples. Several methods for active ingredient content analysis in various commercial pesticide samples have been published by Bureau of Indian Standard (BIS)[9]. Various chromatographic techniques viz., gas liquid chromatography (GLC) and high performance liquid chromatography (HPLC) have found enormous applications in the field of pesticides active ingredient analysis viz., DDT[10], deltamethrin[11,19], lindane[12,21,26], trifluralin[13], heptachlor[14], alachlor[15,19], endosulfan[16] cypermethrin[17,18], fenvalerate[19], butachlor[19],

metalaxyl[20], acephate[22], ethofumesate[23], malathion[24] and dichlorvos[25]. The separation and estimation of individual isomers of commercial pesticides also has vital importance. Several methods have been reported for estimation of isomeric ratios of pesticides viz., DDT[20], endosulfan[26], permethrin[17,26], cypermethrin[26], alpha-cypermethrin[22] and lambda-cyhalothrin[20]. Majority of these methods have been reported for active ingredient analysis or isomeric analysis of single pesticide using either a gas chromatograph with flame ionization detector (GC-FID) or high performance liquid chromatograph with UV detector (HPLC-UV) using different analytical conditions and columns with a suitable internal standard. Therefore, it is difficult and time-consuming job to change the columns and conditions every time for analyzing different pesticides in the various products.

Proposed Work

An attempt has been made to develop a simple and efficient gas chromatographic method capable of determining the active ingredient contents and isomeric contents of twenty chlorinated pesticides with suitable internal standards. The proposed GLC method equipped with a capillary column and flame ionization detector was useful for estimation of active ingredient contents of heptachlor, aldrin, dieldrin, alachlor, trifluralin, bifenthrin, hexaconazole and hexachlorobenzene using different internal standards. The proposed GLC-FID method was also efficient for estimation of isomeric ratios of DDT, HCH, endosulfan, chlorothalonil, lambda-cyhalothrin, permethrin, cypermethrin, fenvalerate and deltamethrin in various commercial formulated and technical pesticides.

2.2 Impurity Profile Analysis of Technical-grade Pesticides

Technical pesticides although by definition being 'pure active ingredient' also may contains complex mixture of other minor components due to process variables, side reactions and impurities in starting materials. The technical pesticide may have various impurities viz., isomers, raw materials, intermediates, by-products or metabolites arise

either during synthesis or storage. The impurities may contribute to the toxicity of pesticide or may alter the physical properties of the product. IUPAC[27] has reported the significance of impurities in the safety evaluation of crop production products. Therefore, regular control of relevant impurities during the manufacturing and formulation process as well as during storage and handling of commercial products is very important.

The impurity profile analysis of a technical grade pesticide is a matter of great concern of regulatory authorities for the registration. The FAO regularly publishes the FAO Specifications for Plant Production Products[28], which included the permissible maximum concentrations of relevant Impurities of pesticide products based on their toxicological significance. WHO data sheet[29] on pesticides also listed the various impurities of several technical pesticides. Several papers have been published for identification of relevant toxic impurities of technical grade pesticides viz., metalaxyl[30], malathion[31,32], acephate[31], fenthion[32] and quinalphos[33]. Baron and coworkers[34] have predicted several impurities in various technical pesticides, based on theoretical considerations viz., Methomyl, monuron, parathion, simazin, trifluralin, aldicarb, captan, chlorpyrifos, diazinon, dimethoate, folpet, fensulfothion, malathion etc. Dureja and coworkers have identified impurities present in commercial samples of quinalphos[33], monocrotophos[35] and metalaxyl[30].

Unfavorable storage conditions may lead to the decomposition of pesticide to produce degradation products more toxic than the active ingredient. The classic example is the nongenotoxic carcinogen ethylenethiourea (ETU), which is formed from the widely used ethylene bisdithiocarbamates (EBDCs) in the presence of moisture, oxygen and elevated temperature[36]. A two-year-old sample of chlorpyrifos containing 13.8% trichloro pyridinol (TCP), 0.65% solfotep and traces of chlorpyrifos oxon was reported as the cause of death of 50 bulls treated directly with the product for eco-parasite control[37].

Some of the impurities are more persistent than the active ingredient, which may result in residue in food. The insecticide dicofol or tetradifon may contain DDT or DDT related compounds[38] as impurities, which are more persistent than dicofol. Similarly, hexachlorobenzene (HCB) is an impurity in chlorothalonil and quintozene[39], which has more environmental persistence than active ingredient. Sulfotep, a highly toxic impurity that may occur in formulation products of diazinon[40] and many other organophosphorous pesticides[41], is more resistance to hydrolysis than active ingredients.

Public confidence in the quality of pesticides required analysis and testing in a government monitoring and surveillance program. Independent laboratories may play an important role in improving safety of the use of pesticides by undertaking research on their composition and identifying potentially toxic impurities.

The identification of unknown impurities in a pesticide product at the mg/kg level is a very difficult analytical task because of the complex nature of technical products. The best approach is to first predict the possible impurities that may occur, based on manufacturing process, main and side reactions, impurities of starting materials, and taking consideration of known impurities in pesticides of similar structure, which have been published. Then second phase of effort is to look for probable impurities in technical products by using combination of various chromatographic and spectroscopic methods.

Impurities of interest are usually analyzed by gas liquid chromatography (GLC), HPLC, GC/MS and LC/MS, alone or in combination with enrichment of chromatography columns[33,42] or initial separation on TLC plates[37]. High resolution GC/MS (upto 18000) is required for the separation of dioxins from interfering compounds[43]. In many cases, ^{1}H, ^{13}C, ^{31}P NMR[44-46], and other spectroscopic methods have been successfully applied for the identification of unknown impurities. The thermal energy analyzer (TEA) is more

selective and sensitive than thermal ionization detector (TID), UV detector or conventional nitrogen specific GC detectors can be used at maximum sensitivity for the analysis of complex samples[47]. Capillary column GC/MS has a detection limit superior to that of TEA by comparing a large number of samples, which showed good qualitative agreement[48,49].

The most countries with advanced registration systems require the identification and positive structural characterization of impurities present in technical grade pesticides at or above 0.1% level. Therefore, the routine analytical methods for analysis of technical and formulated products must be designed to distinguish between the various isomers and impurities with a universal detector having better sensitivity for simultaneous analysis of active ingredient and associated impurities. GC/MS, being a universal detector is most suitable for impurity profile analysis of heat stable technical pesticides, since it can characterize and quantify impurities at ppb levels.

Proposed Work

An attempt has been made to develop the suitable GC/MS analytical methods for identification and simultaneous quantification of active ingredient and associated impurities of eight technical-grade pesticides viz., tebuconazole, acephate, chlorpyrifos, metalaxyl, ethofumesate, HCH, chlorothalonil and metribuzin. First the technical grade pesticides were scanned by GC/MS, the impurities were qualitatively identified and their standards were either synthesized or procured. The GC/MS methods were validated for active ingredient and associated impurities. The proposed GC/MS methods are very useful for impurity profile analysis to establish the chemical composition of technical grade pesticides. The proposed analytical methods can also be employed for the routine quality-monitoring program associated with the manufacturing and formulation processes.

2.3 Purity Analysis of Pesticide Standards

Majority of the pesticides are analysed by using a suitable chromatographic method viz., GLC or HPLC. These methods required a standard of known purity viz., reference standard for analyzing the active ingredient of pesticide. Both sample and reference standard solutions of known concentrations are analysed in identical conditions and peak areas of sample is compared with peak areas of standard to calculate the purity of sample. Therefore a certified reference standard (primary standard) with an defined purity plays a vital role in establishing the accuracy of analysis. These primary standards are very costly and are available only with authorized suppliers[50,51]. Therefore, technical pesticides after purification are often used as secondary standards for routine analysis of commercial pesticides. CIPAC[52] have published guidelines for the definition, preparation and determination of purity of reference materials for analysis of pesticide products. The purity of reference standards is determined qualitatively using an analytical method viz., GC, HPLC, GC/MS (area normalization) or differential scanning calorimeter (DSC)[53,54]. The analytical methods, which are not sufficiently sensitive to detect all associated impurities, will show higher purity. The accuracy of analysis totally depends on the purity of the standard, therefore the purity of standards should be determined very carefully using an appropriate method.

Proposed Work

The suitable analytical methods have been developed for qualitative purity analysis of selected pesticide standards using HPLC, GLC, GC/MS and DSC instruments. The reference standards and purified technical pesticides of known purities (% w/w purity) were analysed using above methods to evaluate the purity and data was compared for the suitability and reliability of the method for establishing the purity of pesticide standards.

2.4 Structure Elucidation of Unknown Pesticides

As per FAO Specifications Manual[55], the identity test for active ingredient of pesticides is very important prior to physico-chemical analysis. There must be proper qualitative procedures to confirm the pesticide identity in the sample. Several methods have been published for identification of pesticides in various commercial samples[56] using chromatographic[57-61] or spectroscopic techniques[62,63]. In case of formulated pesticides, the active ingredient was extracted from sample with suitable solvents[64] or using suitable techniques viz., thin layer chromatography (TLC), liquid chromatography (LC) or gas liquid chromatography (GLC). The extract or isolated fraction after concentration was evaluated subsequently using infrared spectroscopy (IR)[64], mass spectroscopy (MS), nuclear magnetic resonance spectroscopy (NMR) or by UV-Visible spectroscopy[65] for identification of the compound. CIPAC have published several techniques for identification of pesticides. Majority of the procedures[19-26] employed the identification of pesticides by analyzing the technical grade pesticide along with a certified standard under similar analytical conditions using one or more techniques either chromatographic viz., TLC, HPLC, GLC and GC/MS or spectroscopic viz., IR, MS, NMR and UV-Visible spectroscopy and identification was performed by matching the sample values with standard values. The characteristic values used for structural identification of different pesticides with various instruments are: R_f value in TLC for deltamethrin[19], methamidophos[20], tebuconazole[22]; retention time (RT) and elution pattern in GLC for butachlor[19], alachlor[19], metribuzin[19], DDT[20], metalaxyl[20]; and HPLC analysis for deltamethrin[19], monocrotophos[20], imidacloprid[22], ethofumesate[23], chlorpyrifos[26]; fragmentation pattern and mass of specific ions in GC/MS or MS analysis[56]; specific bands in IR analysis for cartap hydrochloride[19], tebuconazole[22], malathion[24], endosulfan[26], dichlorvos[25]; chemical shift values and splitting patterns in NMR analysis for deltamethrin[19], lambda-cyhalothrin[20], imidacloprid[22]; or λ_{max} values in UV-Visible

analysis for alachlor, metalachlor, diazium and dichlorvos[65]. In case of absence of an authentic certified standard, the identification of secondary standard or samples may be performed either by comparing the data with published standard data viz., for IR[66,67], NMR[68] MS [NIST[69]] or by interpreting the data independently based on the different structural informations obtained by various instrumental analysis.

Proposed Work

An attempt is made to develop the suitable methods for identification of three commonly used pesticides viz., metalaxyl, tebuconazole and napropamide using various spectroscopic instruments viz., mass spectrometer of GC/MS, IR, UV-Visible, ^{1}H-NMR and differential scanning calorimeter (DSC). The data was interpreted independently based on the structural informations obtained from various instrumental analyses to confirm the structure of the compound. The results were compared for the most reliable method for the identification of an unknown compound.

2.5 Pesticide Multi-residue Analysis in Soil and Water

Pesticides are integral part of modern agriculture, but their indiscriminate use results in the presence of undesirable residues in water, air and soil, and in food commodities. The adverse effects of pesticides for human health and the environment are a matter of public concern. Therefore, the pesticides and their degradation products (metabolites) in/on water, soil and agricultural products should be extensively monitored.

European Union (EU)[70] has recently developed a regulatory policy related to persistent pesticides or soil bound residues of pesticides. Several methods have been published for residue analysis of pesticides from soils[71-81]. Pesticide analysis in soil represents serious problem mainly due to high interfering compounds such as humic and fulvic acid. These methods employed the liquid- liquid extraction followed by extensive clean-up procedures to remove interferences prior to analysis. The most prevalent clean-up

procedures are solid phase extraction (SPE)[72-76], supercritical fluid extraction[77] and solid phase micro extraction (SPME)[71,78,81,82]. Some methods used ultrasonic extraction (USE)[79] for extraction of pesticides from soil samples.

An increasing number of pesticide compounds are being detected in surface water and ground water supplies worldwide. Therefore, an efficient analytical method for routine analysis of water samples is also needed. During rainy season, the pesticides from soil may enter into the ground water by leaching or enter surface water via runoff after adsorption to soil or sediments[83,84]. Foster and coworkers[85] have described the mechanisms of groundwater pollution by pesticides. High concentrations of organochlorine pesticides were reported in water in some regions of India[86-88], seawater[89-91] and in many human consumable fishes of Turkey[92], China[93] and Bay of Bangal[94,95]. It is known that synthetic pyrethroids possess apparent toxicity to fish and other aquatic organism[84,96]. DDT and many other organochlorine pesticides are extremely persistent in both the environment and the human body and are harmful for human health[97].

Several methods have been reported for extraction and preconcentration of pesticides from water samples using various modern extraction techniques viz., solid phase extraction (SPE)[83,98-101] using C8/C18 columns/membranes, solid phase micro extraction (SPME)[102-106] or LPME with HFM[107-109]. Many methods using liquid-liquid extraction technique[110,111] employing various solvents to extract pesticide residues from water samples have also been reported. The official analytical methods currently used for monitoring pyrethroids and organochlorine pesticides in water samples, employed the extraction of water samples with ethyl acetate or dichloromethane[112] solvents. The sample concentration is more (5 to 10 fold) in liquid-liquid extraction (LLE) in comparison to other extensive extraction techniques.

Majority of the published methods employed large volume of solvents for preparing clean pesticide residue extracts from the soil and water samples, prior to analysis. Various types of solvents were used either for solid-liquid or liquid- liquid extraction techniques, prior to preconcentration and extensive clean-up procedures or during elution from various columns of SPE, SPME, MSPD extraction or Florisil clean-up techniques[111]. Therefore the extraction efficiencies of various organic solvents should be studied for efficient extraction of different pesticides from various types of soils and water samples. The solubility of soil-bound pesticide residues in solvents and characteristics of soils viz., composition, particle size, pH and salt contents have significant influence on extraction efficiency. Degradation of DDT may take place during solid-liquid extraction from soil and sediment samples[113]. Few papers have been published to predict the optimal solvents based on solubility parameters for extraction of organochlorine pesticides from red soil[80].

Proposed Work

In the present work, an attempt has been made to study the extraction efficiencies of various solvents viz., methanol, acetone and dichloromethane for extraction of twenty selected chlorinated pesticides representing various chemical classes viz., organochlorine, pyrethroids, fungicides and herbicides from different types of soil samples (sandy, clay, red and black). Similarly extraction efficiency of n-hexane, ethyl acetate and dichloromethane solvents were also studied by comparing the recoveries of these pesticides from water samples. An easy, rapid and sensitive gas chromatographic analytical method using an electron capture detector (^{63}Ni) was developed for the simultaneous analysis of pesticides in extracted samples. The proposed method could analyse all twenty pesticides within 21 minutes with limit of detection between 0.0002 to 0.005 mg/kg from soil samples and 0.0001 to 0.005 µg/ml from water samples, which complies with the maximum residue levels (MRLs) set by regulatory organizations for

pesticides in different matrices. The proposed analytical technique showed good sensitivity and required minimal analysis time, and had significant advantage over conventional analytical methods.

2.6 Pesticide Multi-residue Analysis in Fruits and Vegetables

On reviewing the published literature of contaminants contained in traditional food in northern North America and Europe[114], a variety of environmental contaminants were found in various food species, which are the major issues of risk assessment and management. Various organizations like Food and Agriculture Organization (FAO), World Health Organization (WHO), United States Environmental Protection Agency (US EPA) and European Union (EU) have prescribed the maximum residue limits (MRL's) of pesticides in various raw agricultural commodities. EU has also published a review article on food contamination by metals and pesticides in the European Union[115]. The toxicological evaluation of FAO/WHO Joint Meeting on Pesticide Residues[30], based on a specified pesticide are carried out to assess the safety of food containing pesticide residues. The WHO has reviewed the dietary intakes of chemical contaminants[116]. Therefore, the pesticides and their degradation products (metabolites) should be extensively monitored in/on various raw agricultural food and feed products and environmental samples.

The European Union has established various European standard methods for pesticide residue analysis in food[117] for quality control in food industry as well as in official food inspection. Number of papers has been published for residue analysis of pesticides in various raw agricultural commodities viz., fruits and vegetables.

The pesticide residues were extracted from matrix by employing various extraction techniques and clean-up procedures. The cleaned extracted residue samples were concentrated and screened using suitable instruments for identification and quantification of pesticide residues. The clean-up of extracted pesticide residues from

potentially interfering co-extractives, which are generally present at higher concentrations than the pesticide residues is very tedious and time consuming task. Ahmed[118] has explained the importance of matrix pretreatment, sample extraction and clean-up procedures in multi-residue methods for pesticide analyses.

The most conventional method for extraction of pesticide residues from different foodstuffs is liquid-liquid extraction (LLE) or liquid-solid extraction (LSE) using a large volume of organic solvents (150 to 500 mL) with multi-step extraction using either single solvent (viz., acetone[111,56], acetonitrile[119,120], n-hexane[121,122,132], ethyl acetate[123,130,155], methanol[128] and dichloromethane[112] or mixture of solvents, such as hexane-dichloromethane[145], toluene-acetonitrile[124], acetone-dichloromethane[125], acetone-dichloromethane-hexane[126], acetone-hexane[127] and petroleum ether-2-propanol[129].

But now a large number of alternative new techniques are employed worldwide to remove interferences of fruit and vegetable matrices with pesticide residues, which are solid phase extraction (SPE) using ENVI carb[121,130] or C-8/C-18/florisil carridges/columns[119,127,131-142], solid phase micro-extraction (SPME) [143,144], matrix solid phase dispersion (MSPD)[145-154], dispersive solid phase extraction[120], ultrasonic extraction[126], supercritical fluid extraction and gel permeation chromatography[125,155-159]. The majority of published methods used these extraction techniques either in combination of liquid-liquid extraction (LLE) or followed by a clean-up step using a column filled with silica gel[123], florisil[108,122,128,139,140,159] or activated charcoal[124,127,128], prior to chromatographic analysis. Therefore these extraction techniques are very costly, tedious and time consuming.

A large numbers of analytical instruments have been used for screening the cleaned, concentrated extracts up to lower ppb levels, among them most common instruments are gas chromatography[160] coupled with different specific detectors viz., electron capture detector (GC-ECD)[135,161], flame photometric detector (GC-FPD)[129,135,144,162] or

nitrogen phosphorus detector (GC-NPD)[129,134,161]; high performance liquid chromatography with UV detector (HPLC-UV)[161], diode array detector (HPLC-DAD)[163,154] or fluorescence detector (HPLC-FD); gas chromatography/mass spectrometry (GCMS) with electron impact ionization in Scan and SIM analysis mode; GC/MS with Chemical ionization[164] or GC/MS/MS[165,110]. In majority of the cases, the multi-residue detection of chlorinated pesticides is performed by GC-ECD[85,123,143,146,150,166], which detects the residues of organochlorine pesticides at very low levels (0.1-1250 ng/g[127], 0.01 mg/kg[156], 3.0 ng/g[121], 174 ng/kg[143] and 0.5-8.0 ng/g[132]), but lacks the confirmation data for residues identification except elution pattern, retention time (RT) and spiking based identification. A gas chromatograph coupled with mass selective detector (GC-MSD), in scan mode (total ion monitoring mode) is very useful for identification and confirmation based on mass spectral information, but not sufficiently sensitive for residue analysis. Therefore, several authors have also used a GC/MS-scan system along with GC-ECD[127,130,132,136,140,148,152,156,167] for confirmation of residues, which requires double work for method development, validation and analysis of residue samples on two different instruments. Therefore, several multi-residue methods employing GC-MS in selected ion monitoring (SIM mode)[118,122,124,126,131,138,142,145,159,168,169] have been developed for simultaneous identification and quantification of pesticide residues, which were very sensitive due to selective ions detection.

Proposed Work

Present work was aimed to develop a simple, quick and efficient extraction technique and easy analytical method for multi-residue analysis of thirteen commonly found chlorinated pesticides in/on brinjal (an excessively used vegetable of India). Attempt has been made to extract the residues of chlorinated pesticides from crushed and homogenized brinjal samples, using a specific combination of solvents (acetone-dichloromethane-hexane 40:30:30 v/v/v) with ultrasonic extraction (USE) technique followed by centrifugation. The method required no further clean-up or derivitization

step, as the extracts were quiet clean without any background interference of co-extractives with sufficient recovery varying 73 to 112% on analyzing by gas chromatograph/mass spectrometer (GC/MS) in SIM mode. The limit of detection of proposed GC/MS analytical method ranged from 0.001 to 0.005 mg/kg. On comparing the method with other conventional methods, the proposed GC/MS method showed better sensitivity and selectivity and therefore, appeared to be quiet suitable and low cost approach for routine multi-residue analysis of selected chlorinated pesticides in/on brinjal samples.

3. Objectives of Present Study

i) Development of a simple and efficient gas chromatographic method to estimate the active ingredient contents and isomeric ratios of several pesticides in various commercial products using suitable internal standards.

ii) Development and validation of different GC/MS analytical methods for impurity profile analysis of technical grade pesticides.

iii) Development of suitable methods for purity analysis of pesticide standards.

iv) Structure elucidation and identification of pesticides with various spectroscopic techniques.

v) Evaluation of extraction efficiencies of various organic solvents to extract the twenty selected pesticides belonging to different chemical classes from soils and groundwater samples using gas chromatography coupled with electron capture detector.

vi) Development of a sensitive and selective GC/MS-SIM mode method with an easy, fast and efficient extraction technique for multi-residue analysis of thirteen chlorinated pesticides in/on brinjal samples.

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