DEVELOPMENT OF NEW ANALYTICAL TECHNIQUES FOR PESTICIDES CHARACTERIZATION

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1. Introduction

Pesticide are the substances indented for preventing, destroying, attracting, repelling or controlling any pest including unwanted species of plants or animals during production, storage, transport, distribution and processing of food agricultural commodities, or animal feeds. The term pesticide covers a wide range of chemicals used to control the pest injurious to health of man or to man's economic efforts. The major classes of pesticides are insecticides, herbicides and fungicides. Pesticides are invaluable inputs for increasing agricultural production due to the fact that pests and diseases destroy up to one third of the crop during growth, harvest and storage. However, the rapidly increasing usage of pesticides often with improper extension services on research has brought many environmental problems with undesirable pesticide residues in water, air and soil, and food commodities. Therefore their judicious and safe use is necessary.

Pesticides Characterization covers the identification/confirmation as well as macro- and micro-quantitation (active ingredient analysis, impurity profile analysis and residue analysis) using various states of art modern sophisticated analytical instruments.

The thesis consists of seven chapters. Chapter 1: covers the general introduction of the pesticides, their benefits and harmful effects. The significance of various types of analyses required for quality control of pesticides and residue analysis are explained. It also includes the literature review related to the pesticide analyses. Chapter 2: describes the active ingredient content analysis of commercial products, Chapter 3: describes the impurity profile analysis of technical grade pesticides, Chapter 4: describes the purity analysis of pesticide standards, Chapter 5: describes the structure elucidation of unknown pesticides, Chapter 6: describes the multi-residue pesticide analysis in soil and groundwater, and Chapter 7: describes the multi-residue analysis of pesticides in/on brinjal.

2. Development of a capillary gas chromatographic method for estimation of active ingredient contents and isomeric contents of several commercial pesticides using internal standards

Pesticides are essential tools of modern agriculture. The efficiency of the pesticide application is greatest when biggest fraction of applied chemicals reach the specified biological target for which, the active ingredient contents of various technical and formulated pesticides should be analysed appropriately. Several classical and instrumental methods have been published in CIPAC Handbook[1], Official Methods of AOAC[2], BIS[3], FAO[4], WHO[5] etc. to evaluate the pesticide quality in various technical and formulated materials. Majority of the methods analysed only single active ingredient of the pesticide either by a gas chromatograph with flame ionization detector (GC-FID) or a high performance liquid chromatograph with UV detector (HPLC-UV) using internal standard. Therefore, It is not easy task to analyse the different pesticide active ingredient contents in various commercial products by changing instruments, columns and conditions repeatedly.

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

3. Development of Methods for Impurity Profile Analysis of Technical Grade Pesticides using Gas Chromatograph Coupled with Mass Spectrometer

The impurity profile of a technical grade pesticide is a matter of great concern for regulatory authorities. As per regulatory requirements, all the impurities of a technical compound, which are ≥ 0.1 %, need to be identified and quantified. Therefore, an appropriate sensitive and validated analytical method for impurity profile analysis of technical compounds is of vital importance. Several methods for identification of impurities in technical grade pesticides viz., metalaxyl[6], malathion[7,8], acephate[7], fenthion[8] and quinalphos[9] have been reported.

An attempt has been made to develop and validate different GC/MS analytical methods for impurity profile analysis of technical pesticides viz., tebuconazole, acephate, chlorpyrifos, metalaxyl, ethofumesate, HCH, chlorothalonil and metribuzin. The impurity profile analysis consists of three steps. First a suitable method was developed for screening of sample with satisfactory separation of all impurities and active ingredient. The impurities were identified based on its mass and fragmentation pattern and correlating with the manufacturing process, raw materials and intermediates. Standards for each of the impurities and active ingredient were procured. The identity of each of the standard was confirmed based on the mass and fragmentation pattern, separately. The standard synthetic mixture was injected onto GC/MS along with technical sample for simultaneous analysis of all the impurities and active ingredient. The GC/MS method was validated for all the impurities and active ingredient, separately prior to quantitation. The method validation covered the aspects: (i) Characterization (ii) specificity (iii) linear dynamic range, (iv) limit of detection (LOD), (v) limit of quantitation (LOQ), (vi) precision [reproducibility/repeatability] (% RSD) and (vii) accuracy (% Recovery). The method ruggedness and robustness was also verified. Some important validation results of GC/MS method for impurity analysis of a typical pesticide, tebuconazole technical is provided in **Table 1**.

Table 1: GC/MS method validation data for tebuconazole and associated impurities in tebuconazole technical sample.

Components	LOD (ppm)	LOQ (ppm)	Reproducibility		Repeatability		Mean Accuracy (%)
			SD	% RSD	SD	% RSD	
Tebuconazole	0.26	0.51	0.25	0.26	0.190	0.20	98.60
Imp-1	0.13	0.25	0.011	1.75	0.007	1.11	97.89
Imp-2	0.13	0.25	0.012	1.94	0.007	1.14	98.19
Imp-3	0.25	0.50	0.007	1.70	0.006	1.49	98.03
Imp-4	0.99	1.98	0.018	1.74	0.013	1.26	98.99

4. Development of Analytical methods for Purity Analysis of Pesticide Standards

To avoid the adverse effects of pesticides, their judicious and safe use are necessary. It is only possible when appropriate application technology is used and active ingredient content analysis of various technical and formulated pesticides have been conducted accurately. The common method for the active ingredient content analysis of pesticide is using chromatographic techniques by comparing the sample response with reference standard response.

The purity of pesticide standards plays vital role for quantitation of pesticide products. The methodology requires “primary standards” of certified purity, which are very expensive and generally not available. Therefore, purified technical pesticides of known purity may be used as “secondary standards” for routine analysis of commercial pesticides after accurate purity determination using appropriate analytical methods.

The suitable analytical method has been developed for purity analysis of standards and purified technical samples of selected pesticides, using GC/FID, HPLC/UV, GC/MS and differential scanning calorimeter (DSC) with purity software. The data was compared to study the suitability and reliability of the method to establish the purity of a pesticide standard. The results of various analytical instruments used for purity analysis indicated that the differential scanning calorimeter (DSC) was more suitable instrument for purity estimation of pesticide standards having purity more than 99%, but not suitable for pesticides with less than 97% purity. The mass selective detector (MSD) being very sensitive detected maximum impurities in the technical pesticide and therefore the gas chromatograph with mass selective detector (GC-MS) was more reliable instrument to establish the accurate purity of technical compounds (95 to 99%).

5. Structure Elucidation and Identification of Pesticides Using Different spectroscopic Instruments

As per FAO specifications[4], the identity test for active ingredient content of pesticides are very important before further analysis for physico-chemical properties. There should be proper qualitative procedure for confirmation of a pesticide in the sample.

Attempt has been made to develop suitable methods for identification of three commonly used pesticides viz., metalaxyl, tebuconazole and napropamide using various spectroscopic instruments viz., UV-Visible Spectrophotometer, Fourier Transform Infrared Spectrometer (FTIR), Mass Spectrometer coupled with Gas Chromatograph (GC/MS) and Proton Nuclear Magnetic Resonance Spectrometer (^{1}H-NMR). The data was interpreted independently based on the structural informations obtained from different instrumental analyses to confirm the structure of the compound.

The characteristic structural information obtained from various instrumental analyses for the identification of a typical pesticide, metalaxyl have been summarized in **Table 2.**

Table 2: Structural informations obtained from various instruments for the identification of metalaxyl.

S. N°	Instrument	Characteristics	Structural Information
1.	DSC	Melting Point	71.8 °C
2.	UV-Vis	λ_{max} Value	217 cm
3.	FTIR	Functional Groups	$-CH_3$, $-CH_2$, -COOH, $-CONH_2$, $-CH_2OCH_3$, aromatic amine
4.	MS	Fragments	279, 249, 234, 220, 206, 192, 174
5.	H^1-NMR	Protons of various groups	Ar-H, Ar-CH_3, -CH-N, CH_3-CH, $-OCH_3-CH_2$, $-OCH_3$-CO, $-CH_2$

The data suggested that various analytical instruments are supplementary to each other and provide significant information for structure elucidation and identification of the pesticides. The spectral and analytical data obtained by these instruments provides specific information about the structure of a pesticide but no single method was sufficient to provide complete structural information for confirmation. Therefore, the identity of the active ingredient of a pesticide should be established by comparing the data with an equivalent authentic standard using at least three instrumental techniques. At least one of which should be spectroscopic.

6. Gas Chromatographic Determination of Pesticides Multi-residues in Soil and Groundwater

The adverse effects of pesticides on human health and the environment are a matter of public concern. Therefore, the pesticides and their degradation products (metabolites) in water, soil and raw agricultural commodities should be extensively monitored. 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 (MRLs) of pesticides in/on various raw agricultural commodities. Several methods have been published for residue analysis of pesticides in different environmental samples viz., soil, water and food commodities. Majority of the published methods employed a liquid-liquid extraction using various organic solvents for extraction of pesticide residues from the soil and water matrices, followed by extensive clean-up procedures to remove interfering co-extractives prior to analysis. Therefore the extraction efficiencies of various organic solvents should be studied for extraction of different pesticides from various matrices viz., soils, plant, food, feed and water.

The present work describes the extraction efficiencies of some polar solvents viz., methanol, acetone and dichloromethane for extraction of a wide range of pesticides belonging to various classes viz., organochlorine, synthetic pyrethroids, fungicides and herbicides from different soils namely sandy, clay, red and black. The extraction of soil-bound pesticides from different soils mainly depends on their solubility in solvents and soil characteristics. The extraction efficiencies of n-hexane, ethyl acetate and dichloromethane were also studied for extraction of pesticides from water samples. The dichloromethane was an ideal extraction solvent to extract all the pesticides from sandy and red soil samples as well as ground water samples with recovery ranging from 76 to 120%, while acetone was suitable to extract pesticides from clay and black soil samples with recovery ranging from 71 to 104%.

A simple, efficient and cost effective extraction technique was developed for extraction of pesticides from various soil and water samples, which does not require any clean-up or derivatisation procedure prior to analysis. The recovery of pesticides with various organic solvents was determined by gas chromatography.

A rapid and sensitive gas chromatographic method with electron capture detector (GC-ECD) was developed for simultaneous quantitation of twenty pesticides within 21 min, with detection limits between 0.0002 to 0.005 mg/kg for soil and 0.0001 to 0.005 µg/ml for water samples. The correlation coefficients of various pesticides varied from 0.9983 to 0.9998, with concentration ranged from 0.001 to 1.0 µg/ml, and the relative standard deviation (% RSD) was between 0.59 to 4.96% for selected pesticides. On comparing the proposed analytical method with other conventional method[10] it is observed that proposed method is faster and more sensitive than reported methods used for monitoring similar pesticides using GC-ECD (0.1-20 ng/g[11], 0.01 mg/kg[12], 3.0 ng/g[13] and 0.5-8.0 ng/g[14]). Therefore, due to its simplicity, short analysis time and improved sensitivity, the proposed method is very useful for routine residue analysis of chlorinated pesticides in various soil and water samples.

7. Gas chromatography-mass spectrometry multi-residue screening of chlorinated pesticides in/on brinjal

The safety concerned for the environment and public health have spawned more stringent analytical testing requirements, for the determination the pesticide residues in/on food at a very low levels as well as the confirmation of their identity. Most of the multi-residue procedures reported for the determinations of chlorinated pesticides in vegetables and fruits, uses gas chromatographic techniques with electron capture detector (GC-ECD). The ECD detector being a specific detector detects the residues of organochlorine pesticides at very low levels. The GC data have only retention time and peak spiking based identification and lacks confirmation. Therefore, several methods had been reported using GC/MS system along with GC-ECD for the confirmation of residues. 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 have poor sensitivity for residue quantitation. Therefore GC-MS techniques with selected ion monitoring (SIM mode) have been developed for low-level identification and quantitative detection of pesticide residues. This method is selective and very sensitive due to selective ions detection. The extraction of pesticide residues from various raw agricultural commodities is the most tedious and time-consuming. The clean-up procedure removes the potentially interfering co-extractives, generally present at higher concentrations than the pesticide residues. Therefore, the majority of published methods have used multi-step extraction procedures, followed by a clean-up step prior to chromatographic analysis, using either silica gel or florisil columns, activated carbon, solid phase extraction (SPE) on C_8 or RP-C_{18} cartridges, solid phase micro-extraction (SPME) or gel permeation chromatography.

The objective of the present work was to develop a simple analytical method for screening and selective detection of thirteen commonly used chlorinated pesticide residues in/on brinjal. Attempt has been made to develop a very simple, rapid and single-step extraction procedure based on ultrasonic extraction (USE). The brinjal samples were pulverized, homogenized, sonicated and centrifuged with acetone-dichloromethane-hexane (40:30:30 v/v/v) solvent mixture. The method required no further clean-up or derivatisation step, as extract was quiet clean and satisfactory recovery (73 to 112%) was obtained. The concentrated organic extract was directly analyzed by gas chromatography with mass selective detector (GC-MS) using SIM mode. The limit of detection ranged from 0.001 to 0.005 mg/kg. The automated GC-MS system appeared quiet suitable and low cost approach for multi-residue analysis of chlorinated pesticides in various matrices, having high sensitivity, selectivity and mass spectral information.

8. Conclusion

- The GC-FID analytical method for routine estimation of active ingredients and isomeric contents of more than twenty chlorinated pesticides in various commercial pesticidal products was developed.
- GC/MS analytical methods were developed and validated for identification and quantitation of the impurities [≥0.1%] in technical grade pesticides. The impurity analysis is of great concern for regulatory authorities for quality control of pesticides.
- Suitable methods were studied for purity analysis of primary and secondary standards, which are of vital importance for active ingredient analysis of commercial pesticides using various techniques.
- The spectral informations obtained from various spectroscopic methods, using GC/MS, IR, UV-Vis, NMR instruments and DSC were evaluated and compared, which are very valuable for identification of pesticides.
- The multi-residues gas chromatographic methods using electron capture detector (GLC-ECD) and mass selective detector (GC-MS) for routine residue analysis of about twenty chlorinated pesticides in soil, groundwater and brinjal samples were developed. The proposed methods were more efficient, sensitive and rapid than methods available.

9. References

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