Chapter 4

Development of Analytical methods for Purity Analysis of Pesticide Standards

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1. Introduction

The pesticides constitute integral part of intensive agriculture to improve quality as well as quantity. But to get wholesome food of high quality without any adverse effects on environment, the safe and judicious use of these chemicals is very important. The accurate estimation of active ingredient contents of various commercial pesticides is very essential. The quantitative analysis of pesticide is often based on analytical procedures (GC, HPLC or GC/MS) that require standards of accurately known purity called as reference materials[1]. The active ingredient content of samples are determined by analyzing both sample and reference material under same analytical conditions and comparing the sample response with standard response of known concentration. Therefore, accuracy of analysis depends on purity of reference material, which must be defined most carefully. The "Primary Standards" with a certified purity and traceability are very costly and are not easily available (supplied by only authorized supplier[2-5]). Therefore, the purified technical pesticides having high purity are often used as "Secondary Standard" for routine analysis of commercial pesticides. The purity of secondary standards should be determined very carefully either quantitatively, against the certified standard or qualitatively[6] using appropriate analytical methods viz., GC, HPLC, GC/MS (area normalization) or differential scanning calorimeter (DSC).

An attempt has been made to develop the suitable qualitative methods for purity analysis of selected pesticide samples (certified standard and purified technical samples) using HPLC, GLC, GC/MS and DSC. The data have been compared to study the suitability and reliability of the method to establish the purity of pesticide standards.

2. Experimental Procedure

2.1 Instruments and Equipments

Sr. No.	Instruments	Model	Manufacturer
1	Weighing Balance (Least Count 0.01 mg)	CP 225 D	Sartorius, Germany
2	High Performance Liquid Chromatograph with UV-Visible Detector (HPLC-UVD)	LC 2010 AHT	Shimadzu, Japan
3	HPLC Column [150 mm x 4.6 mm, 5.0 µ particle size]	ODS (C-18)	SGE, Australia
4	Gas Liquid Chromatograph with Flame Ionization Detector (GLC-FID)	Star 3350	Varian, USA
5	GC Column [30 m x 0.25 mm (i.d.) x 0.25 µm film thickness]	DB-5	SGE, Australia
6	Gas Chromatograph with Mass Selective Detector (GC/MS)	6890/5973	Hewlett Packard, USA
7	GC Column [30 m x 0.25 mm (i.d.) x 0.25 µm film thickness]	HP-5 MS	Hewlett Packard, USA
8	Differential Scanning Calorimeter (DSC) with Purity Software	Pyris 6 DSC	Perkin Elmer, UK

2.2 Solvents and Chemicals

Sr. No.	Solvents/Reagents	Grade	Supplier
1	Dichloromethane	ExcelaR	Qualigens, India
2	Acetonitrile	HPLC & Spectroscopic	s.d.fine-chem Ltd., India
3	Water	Milli Q	Milli Q Water System, USA

2.3 Pesticide Samples

Sr. No.	Pesticides	Grade	Purity (% w/w)	Source
1.	Metalaxyl	Standard	99.85%	Sigma-Aldrich, USA
2.		Tech. Sample-1	99.02%	M/S Punjab Chemicals & Crop Protection Limited, India
3.		Tech. Sample-2	98.52%	M/S Indofil Chemicals Company, India
4.	Chlorpyrifos	Standard	99.52%	Chem Service, U SA.
5.		Tech. Sample-1	98.95%	M/S Punjab Chemicals & Crop Protection Limited, India
6.		Tech. Sample-2	98.35%	M/S Punjab Chemicals & Crop Protection Limited, India
7.	Bifenthrin	Standard	99.27%	Sigma-Aldrich, USA
8.		Tech. Sample-1	98.41%	M/S United Phosphorus Limited, India
9.		Tech. Sample-2	98.15%	M/S United Phosphorus Limited, India

3. Methodology

Two types of pesticide samples (certified standard and purified technical samples) were analysed for purity determination by area normalization method using HPLC with UV detector, GLC with FID detector, GC coupled with mass selective detector (GC-MS) and DSC with purity software. The % purity values of the pesticide samples obtained by various instruments were compared with the actual purity (quantitative purity % w/w) for the most suitable and reliable instumental method for purity analysis of the pesticide standards.

The pesticide samples were homogenized by thorough mixing. The semi-liquid samples were heated gently, liquefied and stirred to homogenize. A known concentration solution of each sample was prepared into suitable solvent and injected onto HPLC/GC/GC-MS in five replicates using suitable parameters as mentioned in **Section 3.1.2 to 3.3.2**. A solvent blank was also run under similar conditions to study any interference due to solvent impurity. The purity or active ingredient content was determined by area normalization of each injection. The mean purity along with standard deviation was calculated.

3.1 Purity Analysis by High Performance Liquid Chromatography (HPLC)

The pesticide sample solutions (1000 ppm concentration) were injected onto HPLC equipped with UV detector and octadecyl silane (ODS/C-18) column. The active ingredient contents of pesticide samples were determined by area normalization using suitable wavelength and mobile phase combination.

3.1.1 Preparation of Sample Solutions

A known quantity of sample (10 mg, approx.) was weighed into a volumetric flask of 10 mL capacity, contents were dissolved in 5 mL acetonitrile and volume was made upto the mark with acetonitrile (concentration 1000 ppm). The sample solutions were injected onto HPLC using following parameters:

3.1.2 HPLC Parameters

Instrument : HPLC (Shimadzu, LC 2010 AHT) with UV-Visible Detector and Quaternary Pump

Column : C-18 (SGE, ODS), [250 mm x 4.6 mm, 5.0 μ particle size]

Detector : UV-Visible detector

Injection Volume : 20 μl

Range : 0.036 AUFS

Metalaxyl

Wave Length : 215 nm

Mobile Phase : Acetonitrile : Water (80 : 20, v/v)

Flow Rate : 1.0 mL/min

Chlorpyrifos

Wave Length : 230 nm

Mobile Phase : Acetonitrile : Water (85 : 15, v/v)

Flow Rate : 1.5 mL/min

Bifenthrin

Wave Length : 222 nm

Mobile Phase : Acetonitrile : Water (90 : 10, v/v)

Flow Rate : 2.0 mL/min

Retention Time (approx.) :
Metalaxyl 3.79 min
Chlorpyrifos 6.08 min
Bifenthrin 7.08 min

3.2 Purity Analysis by Gas Liquid Chromatography (GLC)

The pesticide sample solutions were injected onto gas chromatograph equipped with flame ionization detector (GLC-FID) using a capillary column and the active ingredient contents were determined by area normalization.

3.2.1 Preparation of Sample Solutions

A known quantity of sample (20 mg, approx.) was weighed into a volumetric flask of 10 mL capacity, contents were dissolved in 5 mL acetone and volume was made upto the mark with acetone (concentration 2000 ppm). The sample solutions were injected onto GC using following parameters:

3.2.2 GLC Parameters

Instrument : Gas Chromatograph, Varian (3350), Star Workstation software
Column : BPX-5, [30 m x 0.25 mm (i.d.) x 0.25 µm film thickness]
Temperatures (**Metalaxyl**)
Oven temp. : 230 °C (hold for 9.0 min)
Injector temp. : 260 °C
Detector temp. : 280 °C
Temperatures (**Chlorpyrifos**)
Oven temp. : 220 °C (hold for 9.0 min)
Injector temp. : 250 °C
Detector temp. : 300 °C
Temperatures (**Bifenthrin**)
Oven temp. : 250 °C (hold for 10.0 min)
Injector temp. : 280 °C
Detector temp. : 300 °C
Detector : Flame Ionization Detector (FID)
Injection mode : Split (5 : 1)
Injection volume : 1.0 µl
Carrier gas : Nitrogen
Carrier gas flow : 1.5 mL/min
Hydrogen flow : 30 mL/min
Air flow : 300 mL/min
Retention Time (approx.) : Metalaxyl 4.7 min
Chlorpyrifos 5.0 min
Bifenthrin 7.3 min

3.3 Purity Analysis by Gas Chromatography/Mass Spectrometry (GC/MS)

The pesticide sample solutions were injected onto gas chromatograph coupled with mass selective detector (GC/MS) and the active ingredient contents were determined by area normalization.

3.3.1 Preparation of Sample Solutions

A known quantity of sample (10 mg, approx.) was weighed into a volumetric flask of 10 mL capacity, contents were dissolved in 5 mL dichloromethane and volume was made upto the mark with dichloromethane (concentration 1000 ppm). The sample solutions were injected onto GC/MS using following parameters:

3.3.2 GC/MS Parameters

Instrument : GC/MS (Hewlett Packard-6890/5973)
Column : HP-5, MS; [30 m x 0.25 mm (i.d.) x 0.25 µm film thickness]
Injection mode : Splitless (Purge Flow 20 mL/min for 2.0 min)
Injection volume : 1 µl
Carrier gas : Helium
Carrier gas flow : 1.0 mL/min
Detector : Mass selective detector (MSD)
Mass range : 30 to 450 m/z
Filament delay : 4.0 min.
Quadrupole temp : 150 °C
Temperatures (**Metalaxyl**)
Oven temp. : 40 °C (hold 3.0 min) to 280 °C @ 15 °C/min (hold for 5.0 min)
Injector temp. : 250 °C
Transfer line temp: 280 °C
Temperatures (**Chlorpyrifos**)
Oven temp. : 50 °C (hold 3.0 min) to 300 °C @ 10 °C/min (hold for 2.0 min)
Injector temp. : 250 °C
Transfer line temp: 300 °C
Temperature (**Bifenthrin**)
Oven temp. : 50 °C to 230 °C @ 10 °C/min (hold for 9.0 min)
Injector temp. : 280 °C
Transfer line temp: 300 °C
Retention Time (approx.) : Metalaxyl : 15.47 min
Chlorpyrifos : 19.99 min
Bifenthrin : 23.58 min

3.4 Purity Analysis by Differential Scanning Calorimeter (DSC)

The purity of pesticide samples was determined by a heat-flux differential scanning calorimeter (DSC) using purity software based on Van't Hoff approach on melting peak. The sample was scanned by supplying power in the system to ramp the temperature at a specified rate. The transitions (melting) in the sample were studied by measuring the difference in the heat flow between the sample and the reference during DSC scanning. The purity of the sample was determined by integrating melting peaks to quantify the heat from exotherms of DSC scan.

3.4.1 Preparation of Sample Solutions

A small quantity (usually 1 to 2 mg) of the sample (finely powdered, homogenized sample) was encaped into an aluminum sample holder using a standard sample pan crimper press. Both, sample holder and an empty reference holder were placed carefully in the DSC cell at the sample and reference positions. A nitrogen gas purge was applied and samples were scanned using the following parameters:

3.4.2 Differential Scanning Calorimeter (DSC) Parameters

Instrument : Perkin Elmer, Pyris 6 DSC with Purity Software
Temperature Program
Metalaxyl : 50 °C to 90 °C @ 2 °C/min
Chlorpyrifos : 30 °C to 60 °C @ 2 °C/min
Bifenthrin : 50 °C to 100 °C @ 2 °C/min
Heating rate : 2 °C/min
Purge gas : Nitrogen
Purge gas flow : 40 mL/min

4. Results and Discussion

Three pesticide samples having different purities viz., certified standard and purified technical samples (secondary standards) were analysed for purity determination by area normalization using HPLC with UV detector, GLC with FID detector, GC coupled with mass selective detector (GC-MS) and DSC with purity software. Each sample solution of pesticide standard was injected onto HPLC/GLC/GC-MS in five replicates. A solvent blank was also run under similar condition to study any interference due to solvent impurity. The purity or active ingredient was determined by area normalization of each injection. The mean purity along with standard deviation was calculated for each standard sample. The % purity values of the pesticide standards obtained by various instruments were compared with the actual purity (quantitative purity % w/w) to find the most suitable and reliable instumental method for purity analysis of the pesticide standards (**Table 1**).

Table 1: Purity values of pesticides by various analytical instruments.

Pesticide	Grade	Actual Quantitative Purity (% w/w)	Mean % Purity (Area Normalization)			Purity by DSC
			GC-MS	HPLC-UV	GLC-FID	
Metalaxyl	Standard	99.85%	99.82	99.88	100.00	99.84
	Tech. Sample-1	99.02%	98.96	99.31	99.72	99.24
	Tech. Sample-2	98.52%	98.55	98.75	99.48	99.02
Chlorpyrifos	Standard	99.52%	99.54	99.79	99.91	99.56
	Tech. Sample-1	98.95%	98.87	99.26	99.58	99.14
	Tech. Sample-2	98.35%	98.32	98.51	98.79	98.78
Bifenthrin	Standard	99.27%	99.24	99.48	99.72	99.35
	Tech. Sample-1	98.41%	98.42	98.65	99.08	98.96
	Tech. Sample-2	98.15%	98.11	98.36	98.61	98.73

The data revealed that the % purity values of pesticides were highest by analysing with GLC-FID, followed by HPLC-UVD and GC-MSD (**Table 1**). This was due to the fact that flame ionization detector (FID) has poor sensitivity. Therefore, GLC with FID detector determined minimum impurities and maximum % purity for active ingredient by area normalization (**Fig. 2**). The mass selective detector (MSD) being very sensitive, detected maximum impurities present in a pesticide standard (**Fig. 1**) and therefore resulted in minimum % purity, which was closest to the actual purity of the pesticide determined by quantitative analysis using a certified standard. HPLC equipped with UV detector, being slightly less sensitive determined less impurities and higher % purity than GC/MS (**Fig. 3**). Although, HPLC-UVD would not respond to all the impurities at a particular wavelength, still it was sensitive than GLC-FID and estimated more impurities and % purity was less than GLC-FID. Therefore, the mean % purity of pesticide standards obtained by GC-MS was very close to the actual purity (quantitative purity % w/w), followed by HPLC-UVD and GLC-FID.

The purity of pesticide standards assessed by DSC using purity software was very close to that with GC/MS, while purity of technical samples obtained by DSC was quite higher than GC/MS purity (**Table 1**). Therefore, the DSC is ideal for purity estimation of pure compounds viz., standard pesticides, which have more than 99.0% purity, but it is not suitable for purity analysis of technical pesticides, where purity is less than 97%.

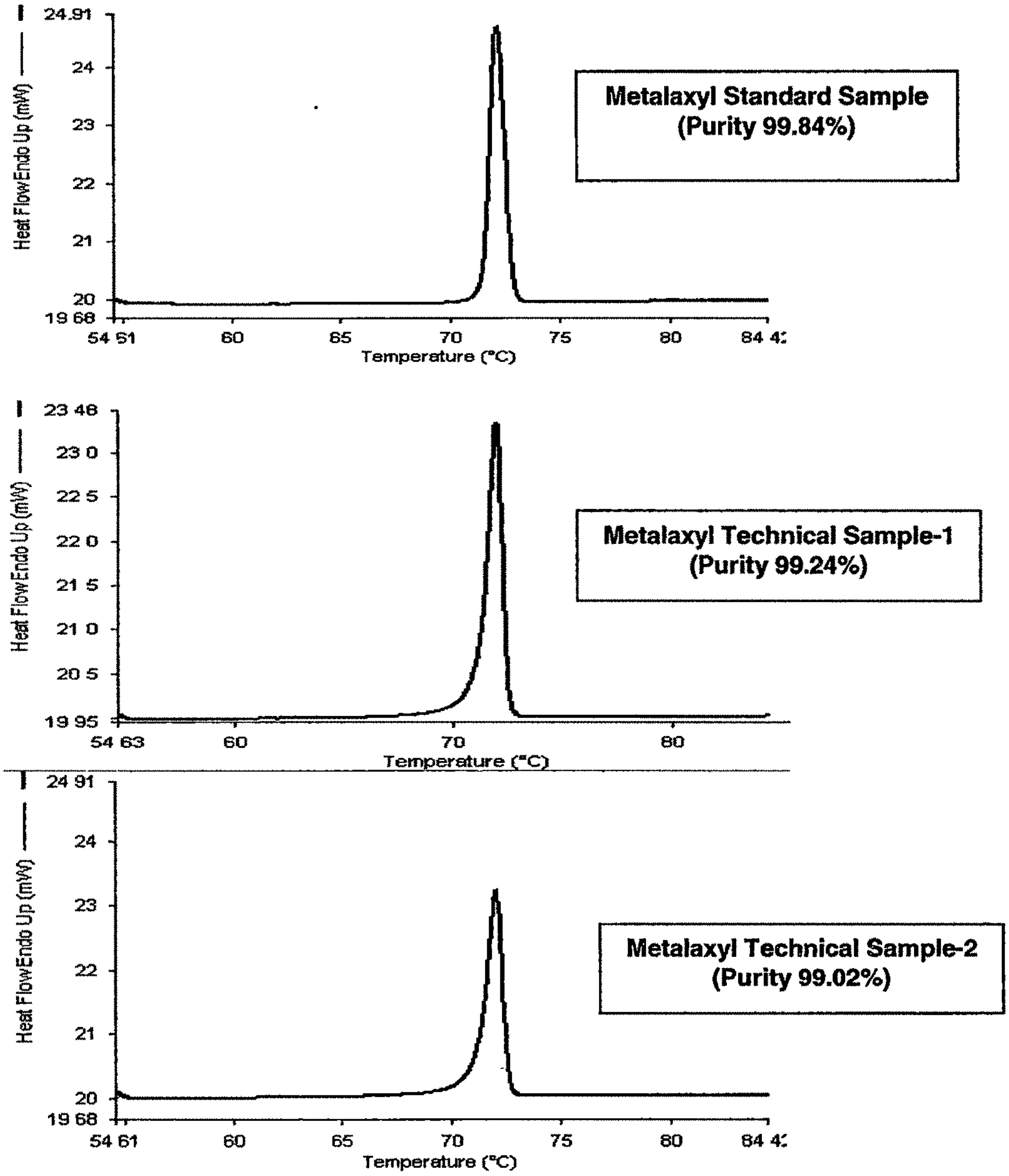

Fig. 4: Typical DSC endotherms of metalaxyl standard samples.

5. Conclusion

The purity of pesticide standards plays vital role for accurate estimation of active ingredient contents of technical or formulated pesticides, as majority of chromatographic techniques calculate the contents by comparing the sample response with a standard response of known purity. This methodology requires primary standard of certified purity, which is very expensive and mostly not available. Therefore, a technical pesticide of good purity may be used as secondary standard for routine analysis. In this study, the area normalization purity of purified technical pesticides and their standards were determined by using HPLC-UV, GLC-FID, GC/MS and DSC. The results were compared with the quantitative purities (% w/w) to find the most suitable and reliable method to establish the purity of pesticide standards. The critical evaluation of the data concluded that the gas chromatograph with mass selective detector (GC-MS) was most reliable instrument to establish the purity of technical compounds as secondary standards, since the mass selective detector (MSD) being very sensitive, detected maximum impurities present in a technical pesticide and determine very accurate purity by area normalization. While, the HPLC with UV detector, being less sensitive than MSD can be used next to GC/MS to determine the purity of standard samples. But GLC with FID detector having poor sensitivity was not suitable for purity estimation of standard compounds. The differential scanning calorimeter (DSC) was ideal instrument for purity estimation of only pure pesticide standards having more than 99.0% purity, but it was not suitable for purity analysis of technical pesticides, where purity was less than 97%.

6. References

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