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Method Validation of Pesticides by Gas Chromatograph

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ABSTRACT

The organochlorine Pesticides are more potent due to their persistence and stability and pesticides like Aldrin, Endosulfan and Heptachlor have been classified as persistent organic pollutant (POP), with restrictive use. The pesticides are banned and not in use. Central Agmark Laboratory, Nagpur 10 rice flour samples collected from all over to detect the presence of these pesticides in GC. Method validation of these pesticides was done. The LOD for the validated method was 0.00030, 0.00013 and 0.00015mg/ml respectively for Endosulfan, Aldrin and Heptachlor. The LOQ as per S/N ratio was 0.000055mg/ml, 0.000028mg/ml, 0.0000267 mg/ml respectively for Endosulfan, Aldrin and Heptachlor. Endosulfan was not detected in any of the sample, Aldrin was detected in one sample as 0.004 ppm. Heptachlor was detected in all the analysed samples and 60% of the samples were more than 0.01 ppm.

Keywords: Endosulfan, Aldrin, Heptachlor, Rice flour

INTRODUCTION

Pesticides constitute a very important group of chemical compounds that have to be controlled due to their high toxicity and their widespread use in agricultural practice for field and postharvest protection [1]. The use of pesticides in crop production and preservation had increased the food security interms of food quantity, but it terms of food quality, it is questionable.

The pesticide residues causing food contamination have become increasingly frequent in recent years raising question about their human health and economic consequences [2]. Widespread contamination of water, air, and soil by chemicals and industrial pollutants means that the crops that we grow and the animals we use for food are often exposed to toxic substances [3]. In addition, agricultural plants used for feeding livestock may be contaminated and, consequently, pesticides may become exposed to human consumption via animal feed. In recent years, increasing attention has been paid to the risks posed to consumers by chemical contaminants or residues in foodstuff [4].

Among the major groups of pesticides, organochlorines (OCPs) are more potent due to their persistence and stability. Universally important organochlorine pesticides (OCPs) are p,p' DDT, BHC, chlordane, heptachlor, aldrin, dieldrin, and endrin [5]. Aldrin, endosulfan and heptachlor epoxide have been classified as persistent organic pollutant (POP), with restrictive use [6]. Aldrin is also a cyclodiene insecticide used formally against

termites, it can volatilise and adhere to soil particles. It is converted to dieldrin in the environment, which is lost in tropical climate slowly and contaminate surface water from run off in agricultural land.

Endosulfan is a cyclodiene organic sulphite with a broad-spectrum insecticidal activity [7]. Due to its lipophilic nature, endosulfan can persist in the environment and food for 60 to 800 days [8]. At high doses increased activity, involuntary shaking, reduced breathing, dyspnoea, increased secretion of saliva, convulsions [9] and eventually death may be observed. Low doses of endosulfan stores in the adipose layer of animal over-time, this have been linked to genotoxicity and cancer [10]. Heptachlor epoxide is a white powder insecticide, practically insoluble in water, bind strongly to soil. It is obtained from break down product of heptachlor [11]. It can be found in polluted soil, air, surface water, ground water and on seeds [12]. In humans, adverse effects were observed on the alimentary, neurological, and respiratory systems, three times higher in women than in men. [13,14].

Residue concentrations have decreased in monitored foods since these chemicals were banned in most countries, although trace levels are still detected in many foodstuffs. The study had the objective of detection of pesticides like Aldrin, Heptachlor and Endosulfan in rice flour samples.

MATERIALS AND METHOD

Central Agmark Laboratory, Nagpur analysed three pesticide residues - Aldrin, Heptachlor and Endosulfan in Rice flour by GC. Method validation was done for the three pesticides.

Chemicals used: Acetonitrile, Acetic Acid, n-hexane, Standards of Aldrin, Heptachlor, Endosulfan.

The instrumental conditions adopted in Gas chromatograph are mentioned in Table-1

TABLE-1 INSTRUMENTAL CONDITIONS

Description	Instrumental condition
Column	DB-35 ms
Oven Temp	160ºC
Initial temperature	160 C at hold time and run time of 2 minutes
Ramp 1 temperature	At the 5˚C with temperature value of 290 C with a hold time of 1 minute and run time of 29 minutes
Back inlet temp	225 ºC
Back inlet pressure	20 psi
Auxiliary Detector temp	300 ºC
Flow rate	2.3 mL/Min
Run time	29 Min
Mobile Phase	Nitrogen gas
Detector	ECD detector

PREPARATION OF STANDARD SOLUTIONS-

10 mg of each standard (Aldrin, Endosulfan, Heptachlor, Endosulfan are made upto 10 ml with HPLC grade n-Hexane for the preparation of stock solution. Mixed intermediate solution is prepared with 1 ml of each stock solution and made upto 100ml with n-Hexane (HPLC grade).

Serial dilution of intermediate standard solutions was done to prepare working standard solutions for the concentrations of 0.004 mg/ml, 0.002 mg/ml, 0.001mg/ml,0.0005 mg/ml,0.0001 mg/ml.

SAMPLE PREPARATION FOR ANALYSIS OF PESTICIDE RESIDUES:

10 samples were labelled and following procedure was carried out at Central Agmark Laboratory, Nagpur for each samples individually. 10g of rice flour samples were taken in 50ml of centrifuge tubes. 20ml of HPLC grade water was added and vortexed for 1 minute. The

samples were allowed to hydrate for a period of 10 minutes. 15 ml of 1% Acetic acid in Acetonitrile (hplc grade) was added and vortexed for about 1 min. QuEChERS kit was added and vortexed for 3 minutes. Centrifuged at 8000rpm for 10 minutes. 10ml from upper layer was collected and 15ml of dispersive kit was added and vortexed for 3minutes. Centrifuged at 8000RPM for 10 minutes. 4ml of upper layer was collected and evaporated to dryness.

CALCULATION:

$$\text{Pesticide content in ppm (mg/kg)} = \frac{C_{Std} \times A_{Sample} \times V_{residue} \times V \times 1000}{A_{std} \times V_{drying} \times W_{sample}}$$

Where,

C_{Std} =Concentration of working standard. (mg/ml), A_{Sample} =Area of sample, A_{std} =Area of standard.

$V_{residue}$ =Residue dissolve in final volume. (ml), V =initial volume required for QuEChERS kit. (ml)

V_{drying} =volume taken from dispersive kit for drying. (ml), W_{sample} =Weight of the sample. (g)

RESULTS AND DISCUSSION

10 no. of Rice samples were received from all over India for the analysis of pesticide residues. Method validation was carried at Central Agmark Laboratory, Nagpur for three pesticide residues namely Endosulfan, Aldrin and Heptachlor. Table -2 represents the Method validation of Pesticide residues.

The recovery percentage of Endosulfan, Aldrin and Heptachlor were 115.22 mg/ml, 77.35mg/ml and 82.04mg/ml respectively. The LOD for the validated method was 0.00030, 0.00013 and 0.00015mg/ml respectively for Endosulfan, Aldrin and Heptachlor. The LOQ as per S/N ratio was 0.000055mg/ml, 0.000028mg/ml, 0.0000267 mg/ml respectively for Endosulfan, Aldrin and Heptachlor. The method was linear and precise for all the pesticides with a correlation coefficient > 0.99 and RSD<4.

TABLE- 2 METHOD VALIDATION OF PESTICIDE RESIDUES

Sl No.		Acceptance criteria	Endosulfan	Aldrin	Heptachlor
1	Specificity	1.Area %RSD= NMT 10%	A)0.049 B)0.070	0.0693	3.010
		2.RT%RSD= NMT 2%	0.00006, 0.006	0.032	0.034
2	Linearity Range				
	i) Range (mg/ml)	The Area/Height response should be linear w.r.t increase of concentration of analyte	0.000104-0.00416	0.000105-0.0042	0.0001-0.004
	ii) Linearity Regression	Not less than 0.99	0.998	0.999	0.999
3.	LOD (mg/mL)	From Calibration Curve	0.00030258	0.0001372	0.000153389
4.	LOQ (mg/mL)	From Calibration Curve	0.000916913	0.0004159	0.000464814
		From S/N Ratio	0.0000055	0.000028	0.0000267
	1) S/N	$\geq$ 10:1	58.53	35.18	70.62
	2) % RSD	NMT 20%	19.8	14.77	9.78
	3) Recovery	Not less than 70%	115.22	77.35	82.04
5.	Accuracy (% Recovery)	Not less than 70%	115.22	77.35	82.04
6.	Repeatability % RSD	NMT 20%	A. 6.04	0.693	3.010
			B. 1.919	1.832	0.823

7.	Reproducibility (Ruggedness) % RSD	NMT 20%	14.365	11.621	15.104
8.	System Suitability Test				
	a) Capacity Factor	Not less than 2	9.44	6.341	5.715
	b) Theoretical Plate Count	Not less than 5000	590594.2188	226149.98	162246.90
		%RSD-NMT 20%	--	2.9290	1.234
	c) Resolution Factor	Not less than 2	31.5042	9.760	13.180
	d) Tailing Factor	Between 0.8 to 1.5	1.026	0.956	0.937

TABLE-3 PESTICIDE RESIDUES IN RICE SAMPLES

COMMODITY	SAMPLE CODE	ENDOSULFAN (ppm)	ALDRIN, (ppm)	HEPTACHLOR, (ppm)
Rice Flour	FSP-24	ND	ND	0.04
Rice Flour	FSP-25	ND	ND	0.04
Rice Flour	FSP-26	ND	ND	0.02
Rice Flour	FSP-27	ND	0.004	0.01
Rice Flour	FSP-28	ND	ND	0.01
Rice Flour	FSP-29	ND	ND	0.01
Rice Flour	FSP-30	ND	ND	0.02
Rice Flour	FSP-31	ND	ND	0.02
Rice Flour	FSP-32	ND	ND	0.03
Rice Flour	FSP-33	ND	ND	0.01

The samples were not detected for Endosulfan and only one sample was detected for aldrin as 0.004 ppm. Heptachlor was detected in all samples with a minimum of 0.01 in 4 samples, 0.02 ppm in 3 samples, 0.03 ppm in one sample and 0.04 ppm in 2 samples. 60 per cent of the analysed samples are more than the residual limit of 0.01ppm.

The study conducted in Hawaul river basin in Nigeria in seven locations indicated the presence of Aldrin and Heptachlor in all locations. The MRLs of 0.01 µg/L (WHO, 2011[15]) of both Aldrin and Endrin were exceeded at all sampling areas since the minimum values recorded for Aldrin and Endrin are 0.3mg/L and 0.04mg/L [16].

A study with Experimental rats fed with diets containing 80 mg/kg of heptachlor epoxide caused 100 % death in 14-140 days [17]. Rats fed with 40 mg/kg, mortality of the female rats was total within a year, while some of the male rat began to experience mortality after two years [18]. Diets rich in less than 20 mg/kg of heptachlor epoxide result in increase in weight of liver in male rats [19].

A study conducted during 2013 in India, reveals that 10 nos. of rice samples collected in Hyderabad were not detected for OCPs like Aldrin, Heptachlor and Endosulfan sulfate [20]. A study conducted in Bangladesh during 2022 for the analysis of OCPs concluded that Aldrin was detected in 10 vegetables with a maximum of 5.42±1.32 µg/kg in Radish and minimum of 0.95± 0.39 µg/kg in chilli. Five nos. of fruits were not detected for aldrin and other five fruits were detected with a maximum of 3.67±1.98 µg/kg in watermelon and minimum of 0.93±0.47 µg/kg in grape fruit. The study opines that farmers might have not followed Good Agricultural practices [21]. A study conducted during 2021 in cowpeas at southwest Nigeria concluded that out of Nineteen markets analysed; aldrin was detected in twelve, heptachlor epoxide in all and endosulfan in seventeen markets. The authors conclude that the pesticides are still being used after being officially outlawed [22].

CONCLUSION

The rice flour samples analysed from Central Agmark Laboratory, Nagpur indicates the presence of Heptachlor in all the analysed samples and aldrin in one sample. FSSAI in its regulation Contaminants, Toxins and Residues Regulations, dated 1st August 2011 [23] has

specified the limit of Aldrin and Heptaclor as 0.01 ppm in foodgrains and not specified the limit of endosulfan in food grains. However, in the third amendment regulations of FSSAI dated 24th December, 2018[24], the pesticides limits are not given as they are banned. The authors intend that although three pesticides are banned, it might be still used or may be due to intentional or non intentional contamination during processing from rice-to-rice flour.

Declaration by Authors

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