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Rapid Estimation of Chlorpyrifos in Feed Samples using Gas Chromatograph-Micro Electron Capture Detector (GC-µECD)

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ABSTRACT

In this research paper, we have standardized a rapid method for estimation of chlorpyrifos residues in feed samples using Gas Chromatograph- Micro Electron Capture Detector(GC-µECD). The extraction method used for this study was QuEChERS viz. quick, easy, cheap, effective, rugged and safe. Validation was done by performing the following parameters; Linearity, Limit of detection (LOD), Limit of quantification (LOQ), Matrix effect and Recovery percentage. A regression equation with regression coefficient (r2) of 0.9913 was obtained indicating excellent linearity. LOD and LOQ of the method were obtained as 0.1 mg/L and 0.4 mg/L respectively. Recovery percentage of the spiked concentration 0.1 ppm was obtained as 85.7% and for 0.5 ppm it was 77.7%. The method qualified the required parameters for analysis of chloropyriphos in the different classes of feeds. Different classes of feedstuffs were analyzed using this method.

KEYWORDS: Chloropyrifos, Gas Chromatograph-Electron Capture Detector, Feeds, QuEChERS.

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INTRODUCTION

Chlorpyrifos (CPF) is a widely used pesticide which comes under the group organophosphorus (OP) (Callahan et al., 2014). They are commonly available in the form of white crystalline-like solids, slightly hydrophobic in nature and having affinity towards oily liquids. In the year 1965, chlorpyrifos was brought into market to be used against insects, termites, mosquitos and beetles, particularly their larval stage (George et al. 2014 and Dua and Joshi, 2014).Chlorpyrifos kill the pest by malfunctioning their nervous system. When the pest is infested with chlorpyrifos, they block the enzyme acetylcholinesterase which results in the accumulation of acetylcholine in the synapse. This leads to overstimulation of neuronal cells and muscle spasms which eventually causes the death of pests (Saunders et al., 2012).

In the present scenario, agriculture practices to increase the crop yield pesticides are still used even though they have some drawbacks if not used properly. The pesticide CPF is mainly used to control soil pests, foliar treatments and directly applied in cattle, sheep etc to control fever ticks, ear ticks, lice and so on (Supreeth et al., 2016; Katuri et al., 2017; Arevalo and Stansly., 2019). The prolonged usage of these pesticides can lead to deposition of its residue in the environment. However, if the residue content is below the MRL (maximum residue limit) it is safe to use(Mackay et al., 2014). The higher dose of lipophilic CPF on agriculture crops may increase the chances of bioaccumulation of pesticide; the leftover residue will remain on vegetables, crops and fruits hence entering into the food/feed chain through the consumption of crop-by products by livestock. Higher doses of CPF exposure can cause several health hazards in humans as well as in animals. Intake of crops and vegetables containing higher amounts of CPF residue can cause metabolic abnormalities, chronic neurotoxicity, gastrointestinal effects, musculoskeletal effects etc. Eventually, over usage of these pesticides can also lead to the damage of aquatic and terrestrial ecosystems. Some of the American and European countries have already restricted the domestic usage of CPF due to the human health risk(Lim et al., 2011; Osterloh et al., 1983; Eaton et al., 2008; Nandi et al., 2022). Thus, proper evaluation and monitoring of these pesticide residues in the natural vegetation and crops is inevitable.

Various methods are available for isolation and detection of CFP such as immunization assays (Hongsibsong et al., 2020), flow cytometry(Zhang et al., 2018) and chromatography. Commonly used chromatographic methods include, Thin Layer Chromatography (TLC), Liquid Chromatography (LC), High Performance Thin Layer Chromatography (HPTLC) and Gas Chromatography (GC). Coupled with specific chromatography, various detectors such as mass spectrometry, Flame ionization detector, Electron capture detector, Near-Infrared spectroscopy etc. are used (Chauhan et al., 2017).

Extraction and clean up methods prior to the analysis is an important step. There are many conventional methods available such as Soxhlet extraction, sonication extraction, homogenization with solvents etc. However, these traditional methods have many drawbacks like wastage of solvents, time consuming procedures and so on. Many advanced techniques are available today in order to overcome such drawbacks. Among the advanced methods, solid phase extraction (SPE), solid phase microextraction (SPME) and the quick, easy, cheap, effective, rugged and safe (QuEChERS) dispersive solid phase extraction are the widely used effective methods (Obuseng et al., 2013).Even though SPE offers higher selectivity, it is a time consuming method and have challenges such as batch to batch variability and clogging issues with dirt samples (Rawa-Adkonis et al., 2006). Similarly, SPME which is noted for its simplicity, speed and minimal use of solvents, lacks reproducibility and may cause matrix affects whiles using complex samples(Fernández-Amado et al., 2016). In contrast, QuEChERS method has revolutionized pesticide residue analysis especially in terms of food sample analysis. This method is developed by Anastassides and his team which have many advantages like its simplicity to use, minimal solvent use, good extract recovery and can be used for wide ranges of pesticides (Anastassiades et al., 2013).

In this study we have used QuEChERS extraction methods and Gas chromatography coupled with a micro electron capture detector to estimate the presence of chlorpyrifos pesticides in the feed samples. Since chlorinated organic pesticides contain high electron affinity, detection and measurement of chloropyriphos with GC-ECD was more accurate. Electron capture detector with gas chromatography (GC-ECD) is a suitable method for determining low concentrations in feeds below maximum residue limits.

MATERIALS AND METHODS

Standard preparation

Stock solution (1000 ìg /ml) of Chlorpyrifos ($C_9H_{11}Cl_3NO_3PS$) (Sigma-Aldrich)standard was prepared by accurately weighing 0.01 g of chlorpyrifos (Shimatzu Analytical Balance with minimum 0.00001 g accuracy) in a 10 ml volumetric flask and volume made up to the mark by adding HPLC grade acetonitrile. A working solution of chlorpyrifos (10 ìg /ml) was prepared from the stock solution. The working solution was then diluted to six concentrations (0.01, 0.1, 0.5, 0.25, 0.75, 1ìg /ml) to prepare a calibration curve for quantitative analysis. For each level of concentration in the calibration curve, four replicates of the standards were injected and chromatograms were obtained to find out the peak area and retention time. . Storage of standards wasdone at -20^{0}C in deep fridge (Velfrost Co.)

Extraction and sample preparation

The sample extraction was performed following the QuEChERS extraction protocol combined with solid phase extraction. From the properly grounded and homogenized feed sample, 2 g was transferred to a 50 ml centrifuge tube and 10 ml of milli Q water was added. 10 ml of acidified acetonitrile was added, the water was then shaken manually for 1 minute. Followed by that, QuEChERS salt mixture (6 g MgSO4 and 1.5g Na Acetate) was added to the tube and vortexed thoroughly. Subsequently the tubes were centrifuged at 5000 rpm for 5 minutes at room temperature. After 5 minutes pH was adjusted 4.5 to 4.8 and again centrifuged at 5000 rpm for 3 minutes at room temperature. From the supernatant 1 ml was transferred into a 2ml dispersive SPE tube (150 mg MgSO4, 50 mg C18EC, 50 mg PSA, 7.5 mg GCB) and further centrifuged at 13000 rpm for 2 minutes at room temperature and dried under a stream nitrogen (99.9995%) in nitrogen evaporator (Speedovap-LV Takahe analytical instruments). Finally, the dried extract in the SPE tube has been dissolved in 1 ml of ethyl acetate and transferred into an autosampler vial and ready for injection.

GC-µECD system

Concentration of Chlorpyrifos pesticides in the samples were determined using an Agilent 7890A

gas chromatographic system coupled with an electron capture detector. DB-5MS (Agilent Technologies, #123-5531) column (30 m x 0.320 mm x 0.10 µm, max. Temp: 325°C) was used for separation of pesticide. Nitrogen was used as the carrier gas (1 mL/min). Inlet temperature was held at 250°C in splitless mode and the purge flow rate was 3mL/min (Table 1).

Table 1. The set values for Inlet, Detector, column and oven parameters.

Inlet		Detector		Column
Temp.	250°C	Temp.	340°C	Mode: Constant Pressure
Mode	Splitless	Makeup flow	60 ml/min	Pressure: 6 psi(1.1846 ml/min)
Purge Flow	3ml/min	Signal	50Hz/0.004	
		Oven programming		
Stage	Rate °C/min	Temperature (°C)	Hold time (min)	Run time
Initial		60	1	1
Ramp1	30	180	0	5
Ramp 2	3	220	0	18.333

Validation of method

Linearity

To determine the linearity of the method 6 different concentrations (0.01;0.1; 0.25;0.50;0.75;1.0 ppm) of chlorpyrifos standards were taken and performed analysis. Linearity equations used for analysis is as given below,

$y=mx+b$

Where, m=Slope

b= y intercept (value of y where x=0)

y= area of peaks plotted on y axis

x= concentration of analyte plotted on x axis

Regression coefficient (r^2) value also calculated to see the goodness of fit of a model

LOD and LOQ. LOD and LOQ were calculated using the following equation.

LOD = 3.3*SD intercept / slope

LOQ =10*SD of intercept / slope

Recovery studies

Determination of the recovery percentage of the method was done using green para grass fodder which is not contaminated with chlorpyrifos. The desired quantity of the samples spiked with 0.1 ppm and 0.5 ppm of chlorpyrifos and further extractions were followed as same as the extraction method along with a blank sample.

Matrix effects

Percentage of matrix effects were calculated using following equation:

$\% ME = 100 - (100 * A_m / A_s)$

Where, A_m = Peak area of analyte with matrix (extract)

A_s = Peak area of analyte without matrix (Standard)

RESULTS AND DISCUSSION

Linearity of the method

Concentration of standards in X axis were plotted in scatter plot against the area of standards (Hz*sec) in Y axis (Figure 1.) and a calibration curve was prepared.

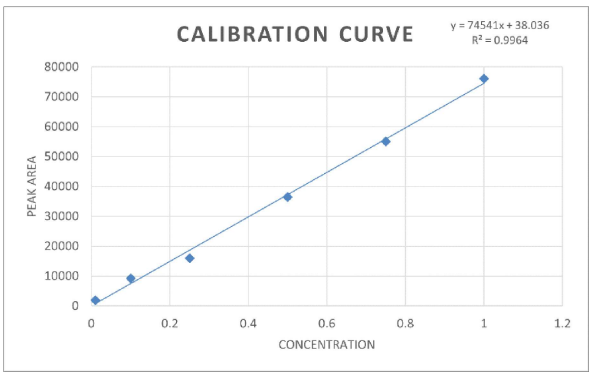

Figure 1: Linearity graph of chlorpyrifos with different concentration of pesticides

A regression equation with regression coefficient (r^2) of 0.99 was obtained indicating excellent linearity. Similar to our findings, several researchers obtained good linearity (0.99) using GC-ECD coupled with QuEChERSmethod in different samples like soil, vegetables, etc. (£ozowicka et al., 2017; Cho et al., 2013).

Limit of detection and Limit of quantification (LOD and LOQ)

LOD and LOQ are the two important parameters that need to be considered to define the performance of the method at lower concentrations. LOD is the lowest quantity or concentration of the analyte that can precisely analyze with the given method whereas LOQ is the smallest concentration of the analyte that can be determined with accuracy and repeatability. In this study, LOD of the method was obtained as 0.1 mg/L and LOQ as 0.4 mg/L (Table 2). Similar values were reported in other studies (Zhang et al., 2018; Schwantes et al., 2020). Some have reported lower and higher values than our studies and those variations due to differences in extraction procedure or instrumentation Tay and Wai 2021)

Table 2. LOD and LOQ values for standardized method of chlorpyrifos estimation in animal feeds

	Coefficients	Standard Error	t Stat	P-value
Intercept	38.0360588	1256.706746	0.030266455	0.97730449
X Variable	74540.85772	2242.034932	33.24696535	4.88E-06
		Calculations		
SD Intercept	SE of intercept * SQRT N			3078.3
LOD	3.3*SD intercept/slope			0.1
LOQ	10*SD of intercept/slope			0.4

Recovery studies

Recovery percentage of the spiked concentration 0.1 ppm was obtained as 85.7% and for 0.5 ppm it was 77.7%. Details of spiking and recovery calculations are given in Table 3. Similarly, 73% recovery at 0.1 ppm in rice straw was obtained (Lee et al., 1993). Chromatograms of 0.5 ppm standard of chlorpyrifos and spiked in the sample have been given in Figure 2 and 3.

Table 3. Spiking and recovery calculations

Spike Concentration (ppm)	Sample RT	Sample Area	Std RT	Std Area	Recovery %
0.1 ppm	12.882	8332.2	12.881	9767.5	
	12.88	8202.5	12.878	10583	
	12.895	10527.3	12.876	10823	
	12.893	9666.6	12.901	11680	
		9182.15		10713.375	85.7
0.5 ppm	12.867	39934.8	12.888	44381.9	
	12.9	33624.4	12.884	48781.4	
	12.897	34859	12.883	47526.6	
	12.899	37299.6	12.911	46801	
		36429.45		46872.725	77.7

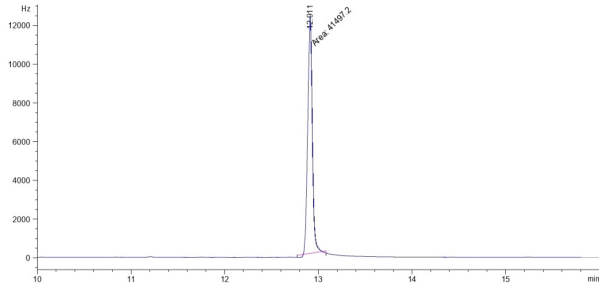

Fig 2. Chromatogram of 0.5 ppm Standard chlorpyrifos

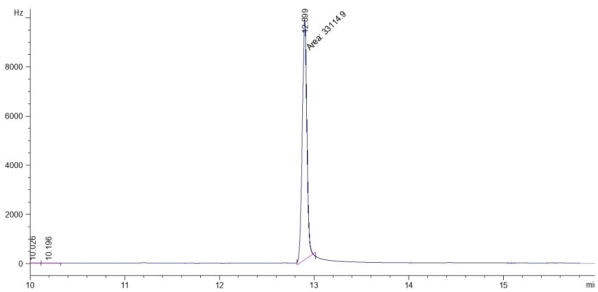

Fig 3: Chromatogram of Chlorpyrifos recovered after extraction

Matrix effect

In Order to determine the influence of undetected matrix components from the sample on the analyte measurement, percentage of matrix effects were calculated. A negative value indicates the suppression of the matrix and the positive value indicates the enhancement. As per the criteria set by SANTE/ 11321/2021 matrix effect less than 20% signal enhancement or suppression need not be addressed in the calibration. In our study we obtained -16% indicates matrix suppression which is a negligible matrix effect.

$\% ME = 100 - (100 * A_m / A_s)$

Where, A_m = Peak area of analyte with matrix (extract)

A_s = Peak area of analyte without matrix (Standard)

Validation of method using field samples

A total of 565 samples collected from dairy farmers from small, medium and large enterprises have been analyzed using Gas Chromatograph- Micro Electron Capture Detector (GC-µECD). Only 37 samples out of 565 samples collected were positive for chlorpyrifos indicating a positivity rate of 6.55%.

CONCLUSIONS

We have described a rapid extraction and analysis of chlorpyrifos residues using Gas chromatography-µElectron Capture Detector. The method qualified all the standardized parameters prescribed for the analytical method and tested using variety of feedstuffs.

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