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RP-HPLC ANALYSIS OF CHLORANTRANILIPROLE PESTICIDE IN CAULIFLOWER VEGETABLE AT HESARAGHATTA VILLAGE BENGALURU

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Abstract:

The present work is aimed to develop a simple and rapid RP-HPLC method for the determination of chlorantraniliprole pesticide residue in cauliflower vegetables collected from different markets at Hesaraghatta village Bengaluru. The QuEChERS extraction method was employed to extract pesticides. The chromatographic separation was carried out using C_{18} column with mobile phase consisting of acetonitrile:water (40:20 v/v) at a flow rate of 0.6 ml/min. The chromatographic peak was measured at 254 nm. The obtained chromatogram showed a well-resolved chlorantraniliprole peak within run time of 4.30 min. The proposed method was validated as per US-FDA guidelines and found to be linear, accurate, precise, selective and robust. The proposed method was applied for the estimation of chlorantraniliprole pesticide residue in real sample. There was no chlorantraniliprole pesticide residue peak was observed. The study revealed the safety consumption of cauliflower vegetables. The proposed method can be adopted for the routine analysis of chlorantraniliprole pesticide residue in cauliflower vegetables.

Keywords: Chlorantraniliprole, Cauliflower, HPLC, QuEChERS extraction method, US-FDA guidelines

INTRODUCTION

Cauliflower, *Brassica oleracea* L. var. botrytis is one of the popular vegetables and known as 'Phoolgobi. It belongs to the family Cruciferae. It is among attractive vegetables due to its nutritional importance. It consists of micronutrients such as vitamin A, B and C and minerals, phosphorus, potassium, calcium, sodium and Iron[1]. In India it is popularly used as vegetable in curries, soups and pickles. In addition, this vegetable contains rich dietary fiber and is low in fat. It is cultivated from various parts of India. During the cultivation, insect pests' such as Diamond Back Moth (*Plutellaxylostella*), Tobacco caterpillar (*Spodopteralitura*), Stem borer (*Hellulaundalis*), Aphids, *Brevicornea brassicae*, and *Lipaphiserisimi* affect the qualitative and quantitative production of cauliflower[2].

Among these Diamond Back Moth and Tobacco caterpillar are resistance DDT and many synthetic pesticides[3]. Chlorantraniliprole (CTP) is the most effective insecticide having broad spectrum of foliar insecticide activity against a range of insect pests, pod borer. CTP is widely used for controlling the insects in vegetables in cultivated in green houses[4]. CTP is chemically known as 3-bromo-4'-chloro-1-(3-chloro-2-pyridyl)-2'-methyl-6'-(methyl carbamoyl) pyrazole-5-carboxanilide.The formers are resorting frequent applications of CTP pesticide for the pest control.

The survey was conducted at Hesaraghatta village located at Karnataka about the cultivation of cauliflower vegetable. Our survey revealed that the CTP is the most commonly used pesticide during the cultivation. The usage of CTP causes problem to human health. Keeping in view, the present work was aimed to develop a method for the analysis of CTP to quantify the amount of CTP from the sample collected from different markets of Hesaraghatta village.

MATERIALS AND METHODS:

The technical grade analytical standards of CTP (purity 98.1%) were supplied by M/s Sigma Aldrich, India. Solvents ethyl acetate, acetonitrile (HPLC grade) and water (HPLC grade) were obtained from Merck, Darmstadt, Germany. Sodium chloride (ASC reagent grade C99.9%; NaCl) were received from Merck, Darmstadt, Germany. Sodium sulfate (Na_2SO_4) anhydrous (AR grade) was supplied by S. D. Fine Chemicals, Mumbai. Analytical grade activated anhydrous $MgSO_4$ was also obtained from Merck, Darmstadt,

Germany. Primary Secondary Amine (PSA) Sorbent and activated graphitized carbon black (GCB, 400 mesh) were purchased from Sigma-Aldrich, Mumbai, India. hesperetin is used as internal standard (IS)

Pesticides selection:

Based on the survey conducted at Hesaraghatta village, Karnataka, we have selected CTP pesticide residue. CTP pesticide was one among the most commonly used pesticide by the cultivator.

Samples collection:

Sample in the form of raw cauliflower vegetable were collected from different sites at Hesaraghatta village, Bengaluru (Karnataka) named as Site A, site B and site C. Before collection of cauliflower it was confirmed from the working farmers about the uses of CTP pesticides. Cauliflower flower which has not been sprayed with CTP pesticide during cultivation was obtained from different source of cultivator. The standard CTP was applied at different concentration in the range of 10 -100 µg /ml.

Extraction methodology:

The cauliflower samples were prepared by QuEChERS method for the determination of CTP residues. Cauliflower were chopped into small pieces and homogenised by mixer grinder. A 15g portion of homogenised sample was weighed into a 50ml polypropylene screw cap tube and added15 ml of acetonitrile containing 1% acetic acid (v/v). Then 6g $MgSO_4$ and 2.5g sodium acetate trihydrate (equivalent to 1.5g of anhydrous form) were added for phase separation and the sample was shaken vigorously for 4 min and kept in ice bath. The samples were then centrifuged at 4000 rpm for 5min then 6ml of supernatant was transferred to a 15ml polytetrafluoro ethylene tube to which 900 mg $MgSO_4$ and 300mgof PSA were added. The extract was shaken using vortex mixer for 20s and centrifuged at 4000 rpm for 5min, approximately 2ml of supernatant were taken in vials. The aliquot were evaporated to dryness under stream of nitrogen.

Analytical Technique by HPLC:

The quantification of CTP residues were done by using high performance liquid chromatography (HPLC). The high performance liquid chromatography (Model DGU-2045) equipped with reverse phase (RP) C_{18} column and photo diode array (PDA) detector, dual pump was supplied by M/S Shimadzu Corporation, Kyoto, Japan. The

HPLC analyses were carried out at column temperature 25ºC under isocratic condition acetonitrile: water (40:20 v/v) with pump flow rate 0.6 ml/ minute. The instrument was set at wavelength of 254 nm. An injectable volume of 20 µL was used in all experiments. Residues of CTP were quantified by comparison of peak height/peak area of standards with that of unknown or spiked samples run under identical conditions. Under these operating conditions the retention time of CTP was found to be 4.3 min.

Cauliflower extraction and sample preparation:

Cauliflower extraction was performed by liquid-liquid extraction technique without applying derivatization.500µl volume of cauliflower sample was transferred to a 15ml polypropylene tarson tube, and then 25µl of IS was spiked. After vortexing 1min, 1ml phosphate buffer (p^H 2.14, 0.01M) was added to the tarson tubes. After vortex mix well for 30sec, 5ml of tert -butyl methyl ether (TBME) was added to the sample tubes. After that the sample was vortex-mixed for 5min and then centrifuged at 5000r.p.m for 10min. Then obtained supernatant organic layer 3.0ml was transferred to a 15ml plastic tarson tube and evaporated to dryness at 40^0C under a steam of nitrogen. Then the dried extract was reconstituted in 200µl of diluents (mobile phase) containing methanol: water (50:50) and a 10µl aliquot was injected into chromatographic system.

Stock solution and calibration standards preparation:

Stock solutions of CTP were prepared by dissolving accurately weighed samples in the DMSO to obtain concentrations of 1mg/mL. The stock solutions were then gradually diluted with methanol: water: 50: 50 (v/v) to obtain calibration samples of 5.86, 11.72, 23.44, 46.87, 93.75, 187.5, 375, 750, 1500ng/ml for CTP

Method validation:

The method validation parameters such as selectivity, sensitivity, linearity, precision, accuracy, recovery and robustness were performed as per US-FDA guidelines. The selectivity of the assay was illustrated by the chromatograms of mobile phase run and extract of blank cauliflower extract recorded for samples near the C_{max} for 2.00 to 2.50hr for CTP. The linearity of the calibration curve was determined by an least square regression analysis. Representative calibration curves of CTP from human cauliflower were depicted in the linearity graph.

The precision and accuracy of the method was determined by selecting three concentration range of quality control (QC) sample such as low(LQC), medium (MQC) and high (HQC)samples. A total of 5 replicates of each QC concentration were assayed on day 1 and a total of 5 replicates each QC concentration were assayed on day 2 and 3. The QC samples concentrations were determined from three different calibration curves that were assayed with QC samples. Within-run precision and accuracy were determined from a total of 5 replicates of each QC concentration. The low, medium and high QC samples (LQC, MQC and HQC) were assayed on day 2. The same was carried out separately for CTP and IS. The QC samples concentrations were determined from calibration curves for CTP. Precision was expressed as percent variation (%CV), while accuracy was measured as the percent nominal.

The percentage recovery was determined by measuring the peak areas of the analyte and IS from the prepared cauliflower extract low, medium and high quality control samples. The peak areas of the cauliflower extract low, medium and high quality control samples were compared to the absolute peak area of the unextracted standards containing the same concentrations of the analytes and IS.

RESULTS AND DISCUSSION

The RP HPLC method development involves selection of column, mobile phase system and other chromatographic condition for the separation of analytes. Prior to method development, the extraction method for CTP pesticide residue from cauliflower was carried out because, many organic acids and sugars that might act as instrumental interferences. In the literature, the QuEChERS method has been commonly employed for extraction of pesticides from vegetable and fruits[5-7]. In addition, the application QuEChERS method has the advantage of high recoveries of pesticides of wide polarity and volatility range compared to other traditional methods of extraction[8]. Therefore, in this study, QuEChERS (Association of Analytical Communities (AOAC) Official Method 2007.01) approach was applied for the extraction of CTP residue.

Preliminary screening for the chromatographic separation of CTP was carried out in order to select a suitable chromatographic condition such as type of stationary phase and mobile phase system. On the basis of literature search and chemistry of the compound, we have selected C_{18} column for the analysis. Initial screening studies was carried out by using Phenomenex C_{18} (250 mm × 4 mm, 5 µm) analytical column with

various ratios of mobile phase composition containing water, MeOH and ACN. However there was no peak was observed for CTP.

In reversed phase liquid chromatographic separation, pH of the mobile phase, buffer type and concentration, and mobile phase additives plays a major role for the separation compounds [9-10].With regarding to the chemical structure of CTP, it is a nitrogenous based compound. The effect of pH was tested for the separation of CTP. The chromatographic peak of CTP was observed at pH 3. However, poor peak shape was observed. Addition of basic additives such as diethylamine (DEA) triethylamine (TEA) etc can improve the peak shape of the analyte. The effect of basic additives such DEA and TEA was tested. A satisfactory separation and good peak symmetry for CTP was obtained with a mobile phase consist of acetonitrile: Water, 0.2% triethylamine at pH 3.0 adjusted using ortho-phosphoric acids (40:60) (v/v). The CTP peak was detected at a wavelength of 258 nm. The effect of mobile phase flow rate from 0.5 to 1ml/min was tested. The mobile phase flow rate of 0.8 ml/min was selected for the study.

The applicability of developed method for the regular analysis of CTP residue was assessed by performing validation as per US-FDA guidelines.

Method Validation:

Validation of the method was performed by following US-FDA guidelines to ensure adequate selectivity, linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ) and robustness.

Selectivity

Selectivity of the method was assessed by comparing the chromatograms of placebo sample containing a CTP with that of selected analytes; no interfering peaks were noticed in the chromatogram. The respective chromatogram was shown in Fig.1. The linearity was established by analyzing five working solutions of CTP (10-50 µg/ml). Calibration curves were plotted using peak area ratios of chlorantraniliprole. The obtained regression equations for CTP are summarized in Table 1. The obtained correlation coefficients for CTP was >0.9 (Fig.2) that indicated high linearity over the entire concentration range.

The accuracy of the method was tested at three concentration levels of 80, 100 and 120 % of the expected value. The % recovery of CTP (n = 3) and mean % recovery (n = 9)

were determined, and results were found to lie within the acceptable criteria of the bias 2 %. The method precision was evaluated by injecting six replicates of CTP at three concentration levels for intra-and inter-day precision and the results were expressed as % RSD. The %RSD was found to be less than 4 that indicate the method precision.

Limit of detection (LOD) and limit of quantification (LOQ) values for CTP was estimated by plotting calibration curves at five levels ranging from 0.05 to 1.0 % of the nominal concentration. The stock and the sample solutions were stable throughout the period of study (30 d). No significant degradation was found within the period of evaluation, indicating that solutions are stable. Peak areas of all the analytes were almost identical to that obtained during initially prepared solutions, and additional peaks were not observed. The LOD and LOQ chromatogram was given in Figure 3. The robustness of the method was evaluated. The method was more robust within the normal operating range i.e. ACN (20 ±2) flow rate (0.6 ± 0.05 ml/min) that demonstrating the robustness of the method.

Application of the method to determine pesticide:

The applicability of the developed method for the determination CTP was assessed by analyzing the real sample (cauliflower extract).The pesticide extract of the sample collected from the different places were injected into HPLC and there was no CTP peak was observed. The study revealed the safety consumption of cauliflower vegetables. The obtained chromatogram for the real sample collected from site 1, 2 & 3 are represented in Figure 4, 5 & 6 respectively. The proposed method can be adopted for the routine analysis of chlorantraniliprole pesticide residue in cauliflower vegetables.

Conclusion:

The present study, a simple RP-HPLC method was developed for the determination of chlorantraniliprole pesticide residue in cauliflower vegetables The CTP pesticide residue from cauliflower was extracted by QuEChERS method. The chromatographic separation was achieved by using C_{18} column with mobile phase containing acetonitrile: water(40:20 v/v). The CTP peak was measured at 254 nm. The run time of the method was found to be 4.30 min. The proposed method was validated as per US-FDA guidelines and found to be linear, accurate, precise, selective and robust. The proposed method was applied for the estimation CTP pesticide residue in real sample. There was

no CTP pesticide residue peak was observed. The study concluded the safety consumption of cauliflower vegetables in Hesaraghatta village.

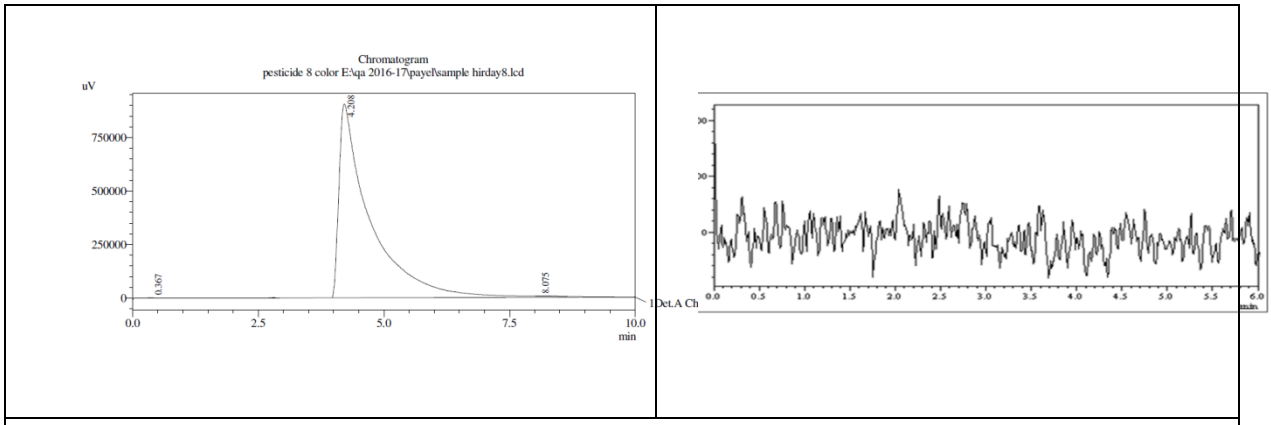


Fig: 1. Selectivity of the pesticide :Retention time was found at 4.2 min for injected sample vs no peak was found in blank sample

Table no 1: Calibration table for different range of concentration:

S. No	Concentration(µg /ml)	Peak area
1	10	580743
2	20	1033296
3	30	1598048
4	40	2230030
5	50	2721554

Fig 2: Linearity curve for different range of concentration of chlorantraniliprole

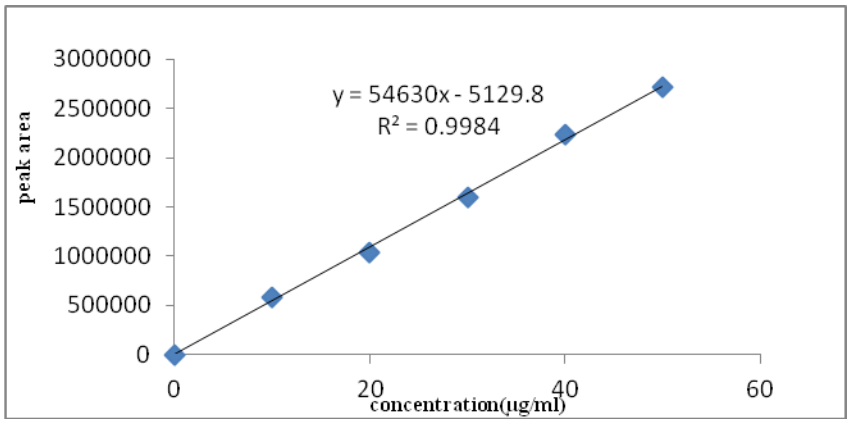


Figure 3: Chromatogram of chloroanthraniprole at the concentration of Lower limit of detection(LOD)

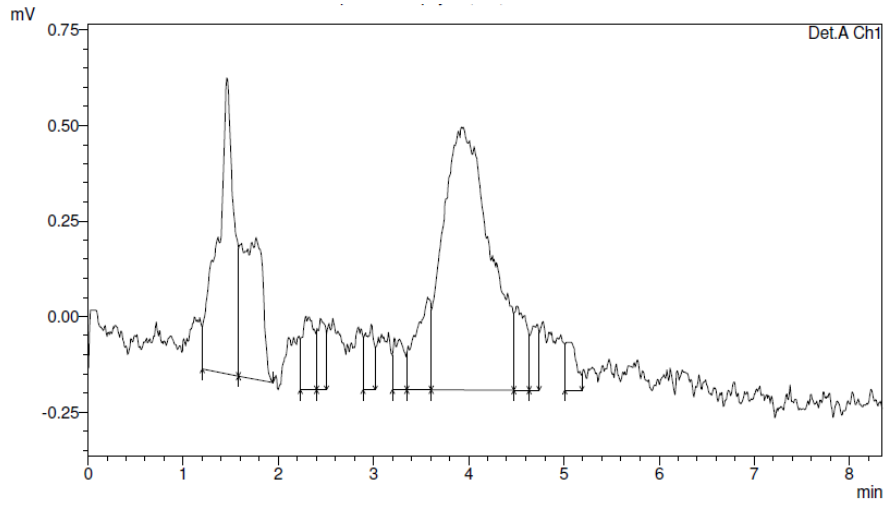


Fig 4: Representative chromatogram of the cauliflower collected from Site 1.

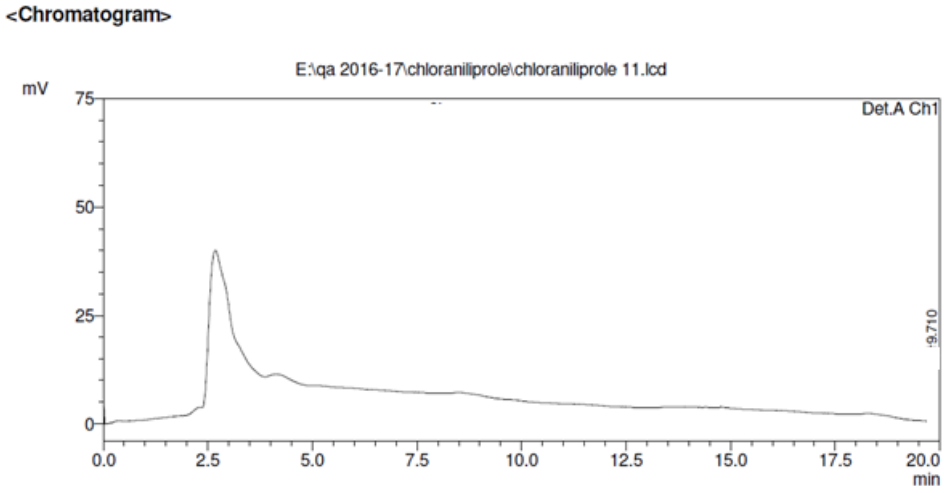


Fig 5: Representative chromatogram of the cauliflower collected from Site 2.

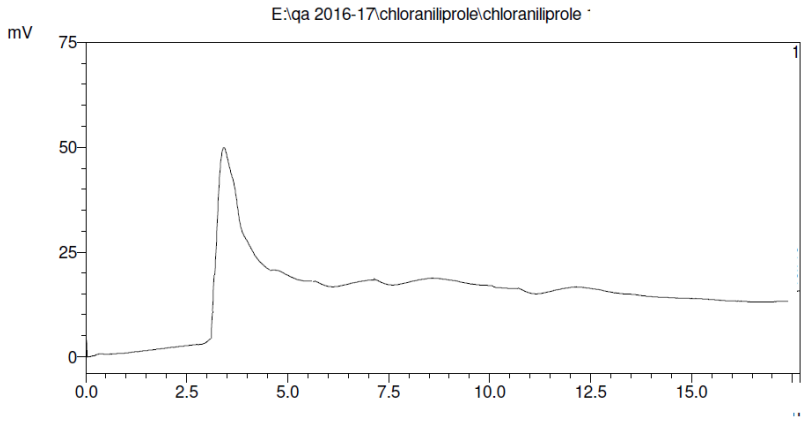
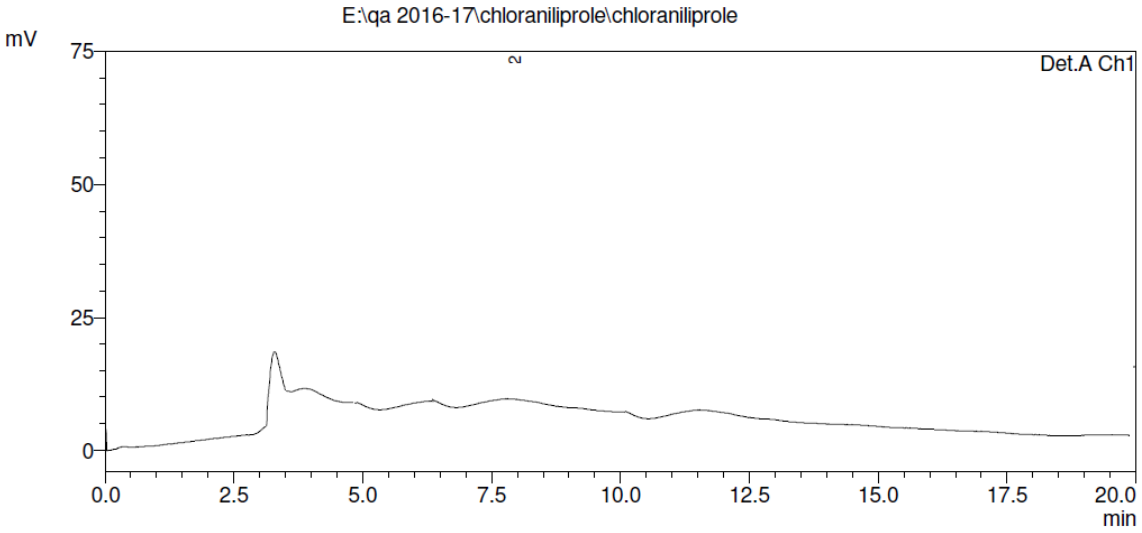


Fig 6: Representative chromatogram of the cauliflower collected from Site 3.



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