ANALYSIS OF PESTICIDE RESIDUES IN CURRY LEAVES AND RED GRAM IN TIRUPATI REGION, CHITTOOR BY GAS CHROMATOGRAPHY

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Abstract

Medicine is food and food is medicine" is the best way to describe on how the ailments were cured by using the plants during the ancient period of time. The "Magical plant of Indian Spice" (Murraya koenigii) has served human kind not only as food enhancer but also as village or folk medication to cure many disorders, the tribal communities has used many parts of the Murraya koenigiito to cure diseases. Pigeon pea [Cajanuscajan (L.)] Millsp] Pigeon pea (Arhar) commonly known as Red gram or tur is a very old crop in India. The term 'Pigeon pea' was coined in Barbados. The present study is to determine the pesticide residue levels in Curry leaf (Murraya koenigii) and Red gram (Pigeon Pea) in Tirupati, Chittoor region.

Key-words: *Curry leaves, Gas Chromatography, Pesticide Standards, Red Gram.*

Introduction

Murraya koenigii, commonly known as curry leaf or karivepaku or Kari patta in Indian dialects, belonging to Family Rutaceae which represents more than 150 genera and 1600 species [1]. Fresh leaves, dried leaf powder, and essential oil are widely used for flavoring soups, curries, fish and meat dishes, eggs dishes, traditional curry powder blends, seasoning and ready to use other food preparations [2] . It is called as 'Magical plant of Indian spice'. It is not only used as food enhancing purpose but also shows medication for many diseases [3]. The essential oil is also utilized by soap and cosmetic aromatherapy Industry. Pigeon Pea which belongs to the family Fabaceae is commonly known as Red gram, in Andhra Pradesh we called as Kandi pappu, other names like Tur, arhar, Dhal etc. This is very old crop and major cultivated crop in India [4]. These crops were contaminated with pesticides, not only these two; almost all crops are contaminated with pesticides which are sprayed on crops. So the present work is aimed to determine the pesticide residue levels in Curry leaves and Red gram in Tirupati region, Chittoor by using Gas Chromatography. Pesticides which are determined in this article are chlorpyrifos, profenofos, and triazophos. Chlorpyrifos is a broad spectrum organophosphate pesticide, kills the insects and worms on crops, animals, buildings [5]. Profenofos is a non-systemic broad spectrum, foliar insecticide and acaricide. It shows excellent translaminar action and effective against chewing and sucking insects, mites on cotton, rice, maize, sugar beet, soybeans, vegetables, tobacco, oil seeds etc.,[6]. Triazophos is a broad spectrum, non systematic contact organosphosphorus pesticides used to control pests, insects, acarids,

some nematodes in okra, cotton, maize, rice paddies etc., [7].Pesticide residues in curry leafs was determined in different markets of Andhra Pradesh & Telangana by using LC-MS/MS [8].

Materials and Methods

Market samples of curry leaf and Red gram were collected from local market Tirupati region Chittoor. Samples were extracted for pesticide residues following the validated QuEChERS method to give best results.

Sample Extraction

Curry leaf samples were analyzed for pesticide residues following the AOAC official method (QuEChERS) which is the best method in the laboratory. The samples were collected from local Tirupati market in polythene bag. Curry leaves was homogenized separately with robot coupe Blixer. 5g of sample was weighed and taken in 50 ml centrifuge tube and 10ml distilled water; 15 ml acetonitrile was added to sample tube. The sample was homogenized at 14000-15000rpm for 2-3 min. 6g sodium chloride was added to sample, mixed thoroughly by shaking gently followed by centrifugation for 3min at 2500-3000rpm to separate the organic layer. The top organic layer of about 9ml was taken into the 15ml centrifuge tube and added with 1.4g magnesium sulphate to remove the moisture content and added 1g PSA sorbent (for dispersive solid phase d-SPE cleanup), and 0.05g of GCB (Graphitized Carbon Black) shaken gently followed by centrifuge for 2min at 2500rpm. The sample tube was vortexed for 30 sec then followed by centrifuge for 5 min at 2500-3000rpm. 2ml supernant layer was transferred into 10ml tube for evaporation using water bath and reconstitute with 1ml of n-hexane. Now pour it in GC vials and inject it in GC having FPD detector. Same procedure was followed for Red gram. Weigh individual reference standard around 15mg in 25ml volumetric flask. Dissolve in n-hexane for standards to be used on GC-FPD and adjust to 500ppm removing required quantity and making up to 25ml. The working standards are prepared by serial dilutions from stock solution i.e. 0.1, 0.25, 0.5, 0.75, 1ppm by suitable solvent n-hexane and used as standard check in analysis linearity studies (Swarupa Rani et al., 2016).

Instrument Analysis

The chromatographic system was SHIMADZU AOC-20S 2010 plus, equipped with an Auto sampler and EB_1 Column 0.25mm×0.25µm, 30m length. The determination of pesticide residue analysis was carried out by following conditions - Injection volume 1µl, Run time 20min, Detector Flame photometric detector (FPD), Injection temperature 260^0c, Column flow 6ml/min, column programming 60^0c, Column EB_1, Detector, temperature 280^0c the run time will change for standards.

Result and Discussion

The pesticide residue levels are calculated by following formula:

Residues (µg/g):

$$= \frac{\text{height or area of sample}}{\text{height or area of the sample}} \text{x} \frac{\text{µl of sample injected}}{\text{µl of the standard injected}} \text{x} \frac{\text{conc. of standard}}{\text{weight of sample}} \text{x final volume}$$

Equation (1)

Pesticides are applied to Vegetables, leafy vegetables, fruits and various crops at various stages of cultivation and for protection against a range of pests during harvest before they become available to consumer.

To ensure the safety of food for consumers and regulate international trade, legislations such as European Union directives has established maximum residue limits for pesticides in food stuff. Thorough monitoring of pesticide residues is crucial for proper risk assessment of human exposure through food. Compared with other available methods, the QuEchERS method is believe to give the best results.

GC Determination of Pesticide Standards

The pesticide residue was identified by comparing its retention time (RT). The quantitative determination was carried out with help of a calibration curve. A good linearity was established by a correlation coefficient (R^2) value (0.97).

Correlation coefficient is a statistical tool used to measure the degree of this type relationship and here a high correlation value (A value very close to1.0) indicates a high level linear relationship between the concentration of standards and peak area. For quantification an external calibration curve with four different concentration matrix matching were made.

The standards are dissolved in n-hexane it was injected in GC as a blank. In blank chromatogram we observed only one peak i.e. solvent peak as shown in Fig.1

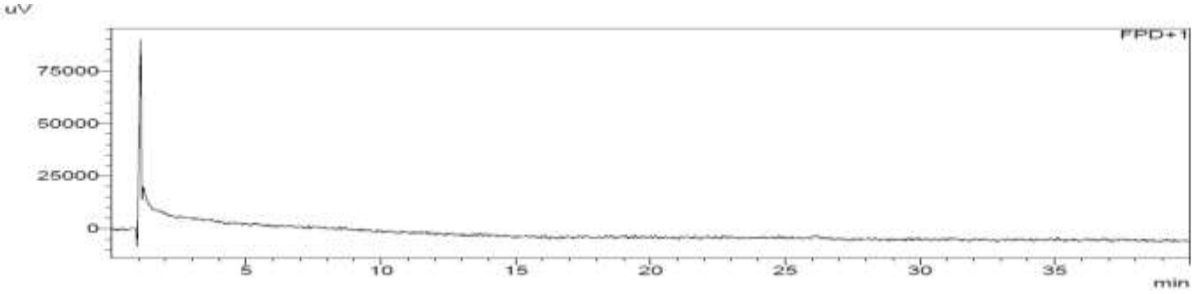

Fig. 1: The blank chromatogram with solvent peak

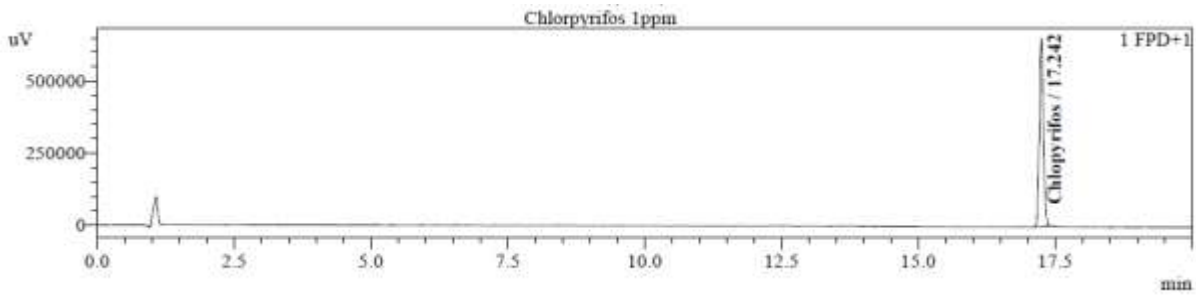

Fig. 2: The Standard chromatograms of Chlorpyrifos

Peak	Retention time	Area	Height	Name
1	17.242	3383918	649767	Chlorpyrifos
Total		3383918	649767	

Linearity for the Chlorpyrifos

The linearity was established by analyzing standard solutions of the insecticides at five concentrations. The concentrations were 0.1, 0.25, 0.5, 0.75, 1µg/ml. Graphs were constructed from the GC chromatograms based on the average peak area of the signal response versus the concentration plot of

the analytes. The parameters such as the slope of the regression line, y-intercept and the correlation coefficient were evaluated.

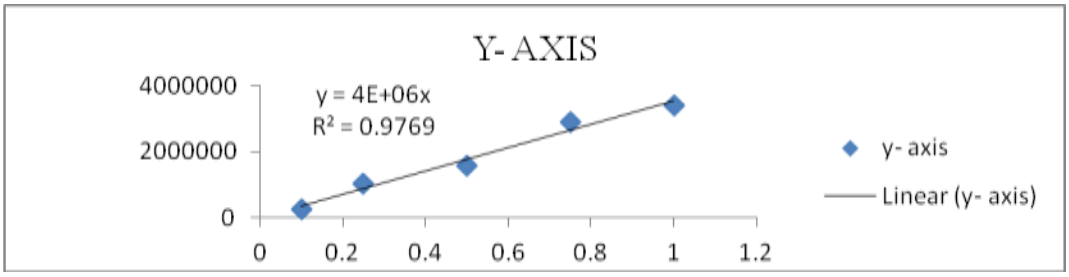

Fig. 3: Linearity of chloropyrifos

Table: 1

Sl no.	Concentration(µg/ml)	Area
1	0.1	256874
2	0.25	1022717
3	0.5	1572715
4	0.75	2910537
5	1	3383918
6	Slope	4E
7	Y-intercept	06x+4E
8	Correlation coefficient	0.976

The linearity of the method was determined at five concentration levels ranging from 0.1-1ppm for Chlorpyrifos. The regression line equation for Chlorpyrifos was Y=4E+06X and the regression coefficient value were 0.967 respectively.

The regression data for the calibration curve for chlorpyrifos showed good linear relationship over a concentration range 0.1-1ppm with respect to peak area.

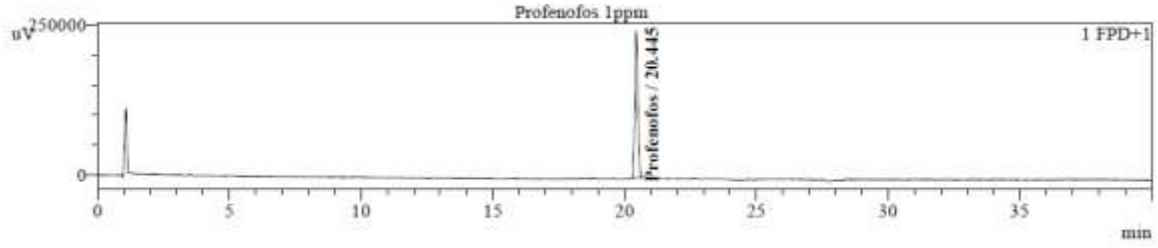

Fig. 4: Standard chromatogram of profenofos

Peak	Retention time	Area	Height	Name
1	20.445	1985369	243885	Profenofos
Total		198569	243885	

Linearity for Profenofos

The linearity of the method was determined at five concentration levels ranging from 0.1-1 ppm for profenofos. The regression line equation for Profenofos was Y=2E+06Xand the regression coefficient value was 0.994 respectively.

The regression data for the calibration curve showed good linear relationship over a concentration range 0.1-1ppm for Profenofos with respect to peak area.

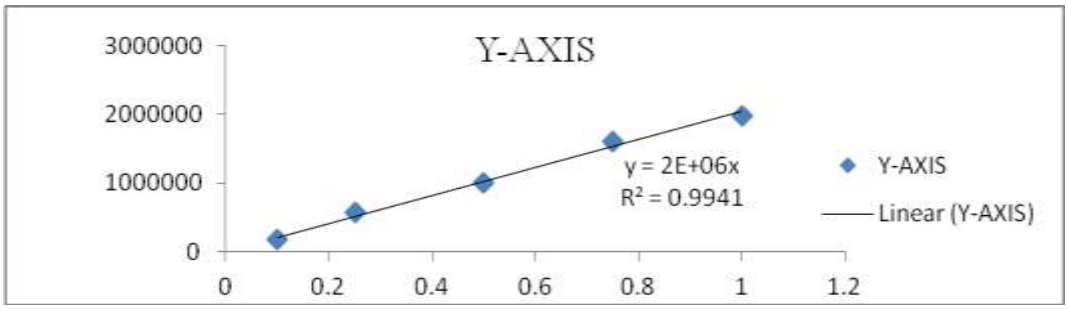

Fig. 5: Linearity of profenofos

Table: 2

Sl no	Concentration(µl/ml)	Area
1	0.1	175354
2	0.25	570827
3	0.5	997618
4	0.75	1600129
5	1	1985369
6	Slope	2E
7	y-intercept	2E+06X
8	Correlation coefficient	0.994

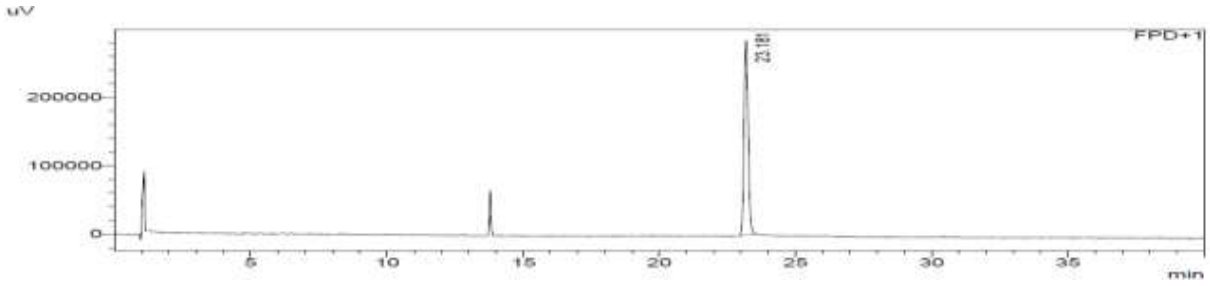

Fig. 6: Standard chromatogram for triazofos

Peak	Retention time	Area	Height	Name
1	23.181	3032415	284816	Triazofos
Total		3032415	284816	

Linearity for Triazofos

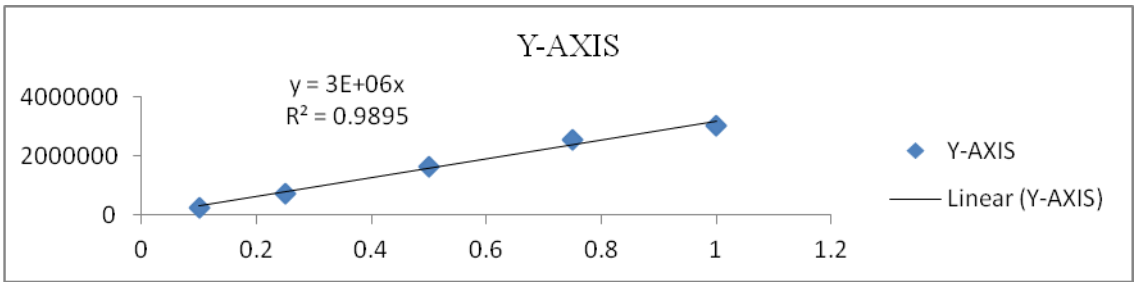

Fig.7: Linearity of triazofos

Table: 3

Sl. No	Concentration	Area
1	0.1	237780
2	0.25	743255
3	0.5	1634619
4	0.75	2545213
5	1	3032415
6	Slope	3E
7	Y-intercept	06X+3E
8	Correlation coefficient	0.989

The linearity of the method was determined at five concentration levels ranging from 0.1-1 ppm for Triazofos. The regression line equation for Triazofos was Y=3E+06Xand the regression coefficient value was 0.989 respectively.

The regression data for the calibration curve showed good linear relationship over a concentration range 0.1-1ppm for Triazofos with respect to peak area.

Chromatograms of curry leaf samples

Unwashed curry leaves

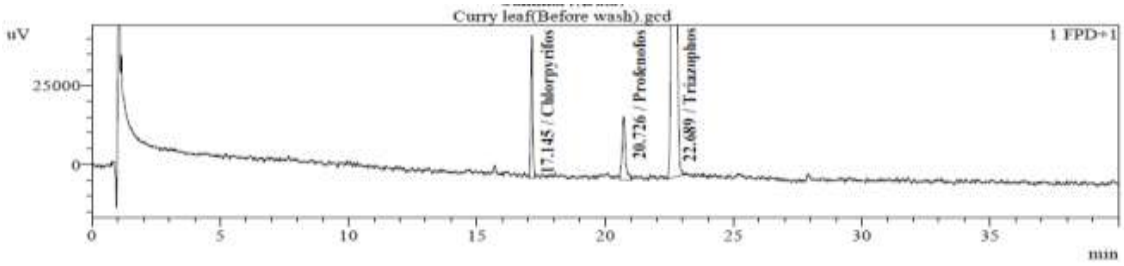

Fig. 8: Sample chromatogram of unwashed curry leaves

Sample	Detected pesticide	Retention time	Area	Height
Unwashed curry leaves	Chlorypyrifos	17.145	233414	45295
	Profenofos	20.726	191030	20284
	Triazofos	22.697	4574265	452562

Observation

The Curry leaf samples were analyzed in GC for the presence of pesticide residue to know the different pesticides eluted in washed curry leaves samples, were compared with standard chromatograms with their retention time i.e. Chlorpyrifos RT was 17.242, Profenofos RT was 20.445 and Triazofos RT was 23.181. Then the presence of pesticide residue levels is compared with standard MRLs fixed by prevention of food Adulteration Act (PFA), Govt. Of India was followed by European Union MRLs.

In Unwashed curry leaves the Chlorpyrifos was found to be 0.069 mg/kg, Profenofos was found to be 0.096mg/kg and Triazofos was found to be 1.508mg/kg. In this present study we observed that Triazofos was detected above the MRLs i.e. is 1.508mg/kg respectively. But it should be 0.01mg/kg as per European Union (Eu).

Washed curry leaves

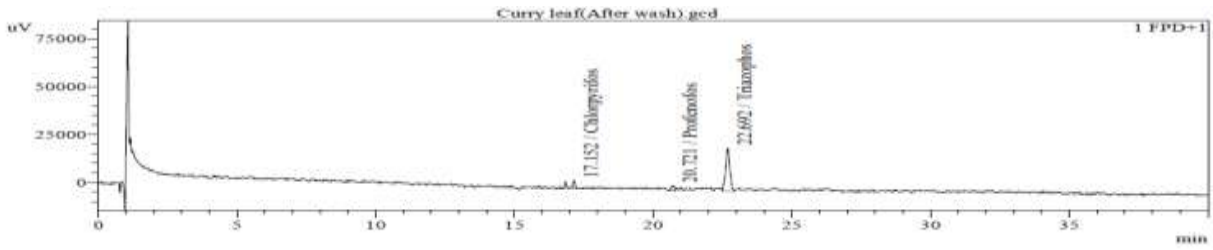

Fig. 9: Sample chromatogram of washed curry leaves

Sample	Detected pesticide	Retention time	Area	Height
Washed curry leaves	Chlorypyrifos	17.152	23422	4212
	Profenofos	20.721	20501	2642
	Triazofos	22.692	240020	22350

Observation

In washed Curry leaves the Chlorpyrifos was found to be 0.0069 mg/kg, Profenofos was found to be 0.01mg/kg and Triazofos 0.079mg/kg. So, in washed curry leaves the pesticide residue levels are decreased when compared with unwashed curry leaves residue levels (Chlorpyrifos was 0.069 mg/kg, Profenofos was 0.096mg/kg and Triazofos was 1.508mg/kg). So water might have a capacity to minimize the pesticide residues.

Curry leaves soaked in salt water

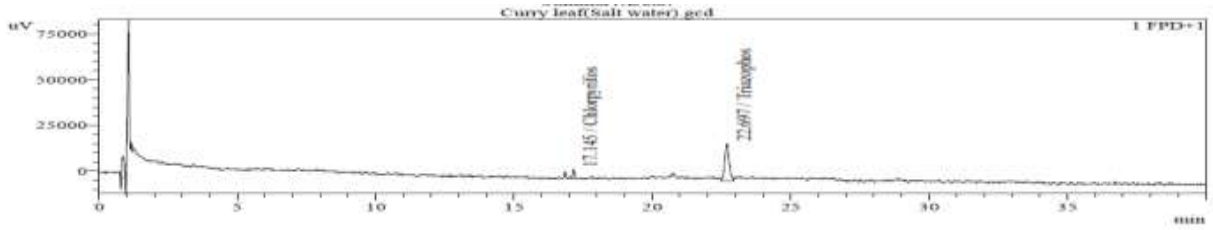

Fig. 10: sample chromatogram of curry leaves soaked in salt water

Sample	Detected pesticide	Retention time	Area	Height
Curry leaves soaked with salt water	Chlorypyrifos	17.145	21996	5042
	Profenofos	Not detected	-	-
	Traizofos	22.697	228521	20446

Observation

In this sample, the Chlorpyrifos was found to be 0.0065 mg/kg, Triazofos 0.075mg/kg and Profenofos were not detected. The Curry leaves were soaked in salt water about half hour, the pesticide residue levels was reduced (Chlorpyrifos was 0.0065 mg/kg, Triazofos 0.075mg/kg and Profenofos was not detected) when compared with both washed (Chlorpyrifos was 0.0068 mg/kg, Profenofos was 0.01mg/kg and Triazofos was 0.079mg/kg) and unwashed (Chlorpyrifos was 0.0069 mg/kg, Profenofos was 0.096mg/kg and Triazofos was 1.508mg/kg) curry leaves samples. In soaked curry leaves the Profenofos was not detected, so the salts may have capacity to remove pesticide after soaking curry leaves in salt water and it may be advisable to the people.

The Chromatograms of Red gram sample was carried out by GC

Major cultivation crops in pulses are red gram. Red gram is found to be contaminated with more number of pesticides the most commonly detected pesticide in cereals and pulses is Chlorpyrifos which has effect on health and safety of mammals. Poisoning with this compound can affect the central nervous system, cardiovascular and respiratory system elucidated the percent contamination of pesticides in the pulses.

Unwashed red gram

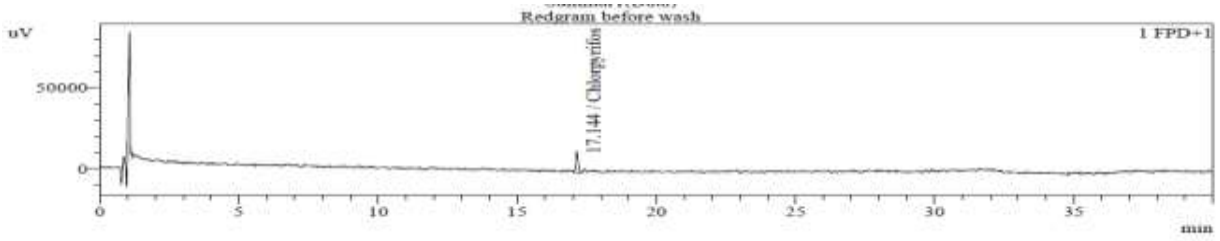

Fig. 11: Chromatogram of unwashed red gram sample

Sample	Detected pesticide	Retention time	Area	Height
Washed red gram	Chlorpyrifos	17.144	74396	13069

Observation

In Unwashed red gram the Chlorpyrifos was found to be 0.024mg/kg. It was within the limits according to European Union (EU) i.e. Chlorpyrifos MRL is 0.05mg/kg

4.3.2 Washed red gram:

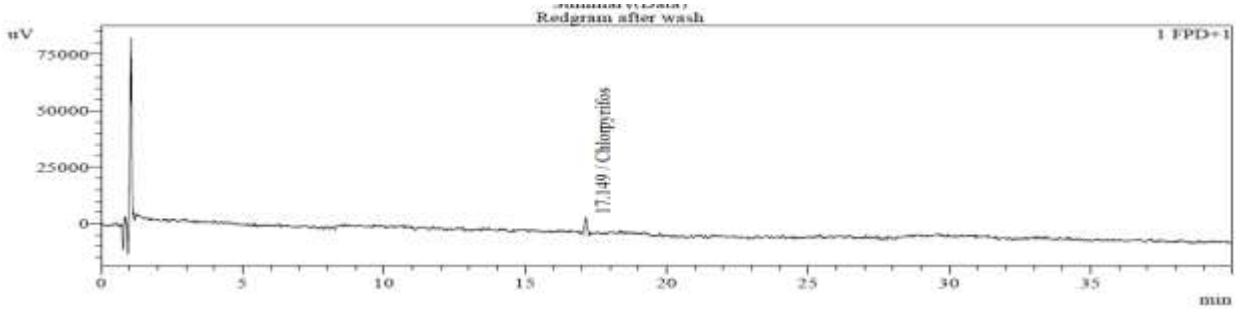

Fig.12: Sample chromatogram of washed red gram

Observation

In washed red gram the Chlorpyrifos was found to be 0.014mg/kg which was within the limits according to European Union (EU). The Chlorpyrifos was decreased in washed red gram when compared to unwashed red gram sample i.e. about 0.02mg/kg, in washed with tap water the residue levels was 0.014mg/kg.

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