Development of HPLC Based QuEChERS Method for High Recovery of Fungicides From Vegetable Samples

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Data Article

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Abstract

Vegetables are the most essential part of our diet. Several pesticides are used to kill the pests. These pest cause different diseases on vegetable plants. As a result of which they are getting more and more production of vegetables but there are many hazards for humans. To get rid of this problem, a simple QuEChERS method is used which is based on solvent extraction method in which different salts and acetonitrile were used. In this method clean-up process and extraction were important HPLC technique is used to determine the accurate results. Despicable recoveries of fungicides were ranges between 0.63% to 107.68% at spiking levels and at different factors through percentage RSD between 3.6% to 4.7%. LOD of two fungicides were in the range of 0.0021mg/kg to 0.00181mg/kg and LOQ of two fungicides were in the range of 0.0706mg/kg to 0.00606mg/kg. By varying many factors the recoveries of fungicides may vary. The recoveries of two fungicides may increase by increasing temperature, pH and concentration but decrease with the passage of time due to degradation of fungicides. By this QuEChERS method we get different concentration of fungicides in some vegetables but in Methi vegetables they are absent. And determinations and extraction of fungicides were completed by HPLC technique. QuEChERS method is very efficient and good method for determination of pesticides in various vegetables. Hence our experimental results were validated with this efficient method.

Introduction

Azoxystrobin is a wide range pesticide that is used as fungicide for many diseases of crops with different trade names including Amistar, Abound, Quadris, Bankit, and Heritage. It works by constraining mycelial growth, spore germination and mitochondrial respiration. Though, Azoxy shows less toxic effects for bees, birds and mammals and other terrestrial organisms [1]. Azoxystrobin is fungicide that strongly works against various diseases of numerous ornamental and edible crops. A latest study illustrate that azoxystrobin degradation product R234886 can percolate via loamy soils for a long period of time following application of the pesticide [2] and thereby pose a potential threat to vulnerable aquatic environments and drinking water resources [3]. Some diseases controlled or prevented are late blight, rice blast, Septoria, rusts, apple scab, powdery, red spider mite [4] and downy mildew [5]. No numerical proliferation in the figure of animals containing tumor, animals having benign tumors, malignant tumors, single or multiple tumors or metastic tumors in testing individuals administered with azoxystrobin at dose levels of up to 117.1 mg/kg body weight per day for about two years [6]. Azoxystrobin also remained low sub chronic in dermal testing of twenty one days [7]. The study was also established as gross swollenness of the bile duct convoyed by histological alteration. It showed no toxicity of development in the rabbit or rat by dose level of up to and levels displayed to be maternally toxic [7]. Azoxystrobin showed a weak response of clastogenic effect in vitro in mammalian cells at cytotoxic dosages and in vivo it implies no genotoxicity [7].

It is a non-volatile compound and does not leach down, it is highly vulnerable to photolysis. It is not present in groundwater or drinking water because Azoxystrobin does not leach down in soil water [8]. As it does not leach it is very improbable to go into water reservoirs except by direct spray or accidentally.

Though, in flooded anaerobic soils the compound is despoiled with a half-life of about seven weeks during laboratory tests [9]. In plants it is metabolized having complex with more than fifteen identified metabolites. These metabolites are existing in very less amounts, usually considerably fewer than 5% of the TRR (Total Recoverable Residue).

Thiophenate methyl is class of fungicide which is used for different crops and vegetables to kill different germs causing different disease [9]. Thiophenate methyl has a colorless crystals,[10] having melting point 342° F [11]. It is frugally resolvable in many of organic solvents at 25 °C like Acetone, methanol ,ethyl acetate, dichloromethane, n-octanol ,Xylene, n-hexane. It is somewhat resolvable in many organic solvents [12]. Thiophenate methyl is strongly soluble in methanol, acetone and chloroform [13]. Its solubility in water is 26.6 milligram/liter at 20 °C [14]. When excited to disintegration it releases exact poisonous gases and oxides of nitrogen and Sulphur [15]. It is removed from the body in twenty four hour that gone in muscles afterward 24 hours is mostly abolished within 96 hour[12].

Determination and quantification of pesticides from crops is usually done by QuChERS method [16]. The process of QuEChERS method has several steps. This comprises of sample research and abstraction method. At that step samples are mixed regularly [17]. Now different acidic solution and buffers are used to enhance the efficiency of extraction[18]. This is the clean out method of extraction of sample using solid phase extraction method [19].

In this research we propose a multi residue QuEChERS method [20] [21] **for the determination of** Azoxystrobin and Thiophenate Methyle from two vegetables Spinach and Fenugreek (Methi). Structures of both fungicides is given in Figure S1 (supporting information). Vegetables are crushed and homogenized and treated with acetonitrile in centrifuge tube for extraction [22]. Then sodium sulphate and sodium chloride are added to maintain pH of the sample solution and acetonitrile is added to remove matrix by cleanout method by dSPE. Finally HPLC analysis is carried out [23]. The proposed method is fast, easy and efficient for fungicide analysis with high recovery, low limit of detection and limit of quantification [24].

Experimental

Materials And Methods

Sodium sulphate 99–100%, Azoxystrobin 95%, Thiophenate Methyle 70%, Acetonitrile 99.9%, Sodium Chloride 99.9%, Magnesium Sulphate 98%, Charcoal 95% were purchase from Sigma Aldrich and MERK.. All the chemicals were of analytical grade.

Two types of vegetables Fenugreek (Methi) and Spinach were bought from native market MuzaffarGarh (Pakistan). A blank and spiked sample was prepared for analysis. These vegetables had been eroded by means of water and dried out in sun light

Sample Preparation

Samples of spinach and Fenugreek were kept in sun light for some days for water evaporation. These vegetables were dried, crushed and filtered the powder from 25 meshes and put the vegetables in distinct envelope. Weighed up 1.0 gram of each crushed vegetable and placed into 15 mL centrifuge tube and saturated with 4 ml of deionized water for 2 minutes. 7 ml of various working solutions were prepared, agitated and vortexed strongly for 3 minutes. Then put 7 ml analytical grade ACN in every centrifuge tube and agitated for 3 min. Then put 1.0 g sodium chloride and 4 gram anhydrous Na_2SO_4 in every tube and shaken for 3 minutes. Then centrifuged in TGL-16 high speed centrifugal device at 4000 rpm for 5 mints. Detached the upper ACN layer and added 0.05 gram activated carbon, 0.150 gram Na_2SO_4 and centrifuged again at 4000 rpm for 7 mints. Acetonitrile organic layer was strained by Whatman filter paper and HPLC analysis was carried out.

1 g dried vegetable (Spinach and Fenugreek) was taken in 15 ml tube, added 4 ml deionized water and saturated for 2 minutes. Then put 10 ml Acetonitrile and agitated strongly for 3 minutes and vortexed. 4 g anhydrous $NaSO_4$ and 1 gram sodium chloride were added in every centrifuge tube and agitated strongly for 3 minutes. Centrifuged it for 5 mints at 4000 rpm and detached the upper organic acetonitrile layer in separate tube. 0.05-gram activated charcoal and 0.150 gram $NaSO_4$ were added and agitated again followed by centrifugation at 4000 rpm. Finally, HPLC analysis was carried out.

Hplc Analysis

Series 1500 Lab Alliance C18 Agilent 5 HPLC was used for analysis of fungicide residues. Reverse phase C 18 column of length and width 150 and 4.6 mm respectively, was used for analysis. Mobile phase for samples was the combination of Organic phase (Acetonitrile) and water at 80:20 ratio. Flow rate was set 1 mL/minute. UV -visible Lab Lines sensor Model No. 8266–9868 was used as detector in HPLC, fixed at 245 nm.

Result And Discussion

Two different types of vegetables (spinach and fenugreek) are studied by HPLC based Qucher method. Firstly, blank solution of both vegetables are analyzed by HPLC with different concentration of standard solution of fungicides. Concentration of Thiophenate and Azoxystrobin in blank solution of Spinach was 8.84 mg/Kg and 6.96 mg/Kg respectively. On the other hand, both pesticides were absent in blank solution of Fenugreek (Methi). The results show that different fungicides assimilate in vegetable or accumulate in soils.

Then solutions of various concentrations 10, 20, 30, 40 and 50 (ppm) of Azoxystrobin and Thiophenate methyl, are analyzed by HPLC (Fig. 2). Peaks under the area of two fungicides are determined and calibration curves for both fungicide standards are plotted (Fig. 3). A straight-line graph is obtained with

correlation coefficient (R^2) of 0.9990, 0.9998. By using the Y value of peaks under the area in straight line equation the determined amount in mg/kg of azoxystrobin and thiophenate methyl in Spinach and Fenugreek sample is obtained. After separate analysis of each standard solution of both pesticides, mixture of each concentration is analyzed to check the effect of one pesticide on the other and results are presented in Fig S2 (supporting information).

Obtained RSD, LOD and LOQ for Azoxystrobin are 3.6%, 0.00211 and 0.0706 respectively. Similarly, RSD, LOD and LOQ for Thiophenate Methyl are 4.7%, 0.00181 and 0.00606. The detailed information is given in Table 1.

Table 1
Correlation coefficient (R^2), RSD, LOD and LOQ of Fungicides

Compound	R^2	RSD	Intercept of Y	Slop	LOD	LOQ
Azoxystrobin	0.9990	3.6%	10101	66011	0.00211	0.0706
Thiophenate Methyl	0.9998	4.7%	10159	76635	0.00181	0.00606

Quchers Method Validation

The removal of fungicides and method authentication is based on QuChERS method which is very simple and easy method to determine the fungicide residue in vegetables without internal standard. Different concentration such 50, 75 and 100 ppm of both pesticides are spiked in vegetable samples. Aacetonitrile is used as solvent for extraction of fungicides and water is used for the soaking of samples. Chromatograms showing the retention times and peak areas for Thiophenate Methyl and Azoxystrobin at 50, 75 and 100 ppm spiking concentrations in Spinach and Fenugreek are shown in Fig. 4 and Fig. 5 respectively.

Extraction efficiency of the method for both pesticides is given in Table 2. Recovery (%) both pesticides range from 63.98% to 107.68% at different spiked concentrations. % recoveries of azoxystrobin in Spinach and Fenugreek sample are 65.38, 87.56 respectively in 50 ppm spiking solution. While % age recoveries of Thiophenate Methyl in Fenugreek and Spinach are 107.68 and 73.36 correspondingly which shows positive results. % recovery of Thiophenate Methyl and Azoxystrobin in Spinach is 73.82 and 63.98 while in Fenugreek is 77.32 and 92.42 in 75 ppm spiking solution which are higher values. Recovery % of azoxystrobin in Spinach is 75 and in Fenugreek is 65.42 and of thiophenate methyl in Fenugreek is 74.74 and in spinach is 72.45 which show best results by spiking different concentration of fungicides, we get different vegetables. These results indicate that both pesticides show excellent recoveries in vegetables samples using HPLC based QuChERS method.

Table 2
Recovery (%) of fungicides within vegetable of 50, 75 and 100 ppm spiking level.

Pesticide	Vegetable	Peak area	Rt (min)	Conc. after Extraction (milligram/kg)	Recovery %
Azoxystrobin	Spinach	2168622	4.590	32.69	65.38
	Fenugreek	2900330.500	4.423	43.78	87.56
Thiophenate Methyl	spinach	2821241.250	3.573	36.68	73.36
	Fenugreek	4136603.750	3.498	53.84	107.68
Azoxystrobin	Spinach	3178465.750	4.553	47.99	63.98
	Fenugreek	3838471	4.432	57.99	77.32
Thiophenate methyl	Spinach	4254090.500	3.540	55.37	73.82
	Fenugreek	5323110	3.507	69.32	92.42
Azoxystrobin	Spinach	4961264	4.530	75.00	75
	Fenugreek	4329061.500	4.457	65.42	65.42
Thiophenate methyl	Spinach	5562824.500	3.518	72.45	72.45
	Fenugreek	5738472	3.532	74.74	74.74

Conclusion

QUECHERS method consists of clean-up process in which different salts and activated charcoal are used for clean-up. The recoveries of Azoxystrobin and Thiophenate in vegetable are best to some level. % Mean recoveries of two fungicides were extended between 0.63%- 107.68% at different factors and spiking levels through% age RSD between 46.63% – 46.64%. LOD of two fungicides had been varied in the range of 0.00606 mg/kg and0.00606.LOQ of azoxystrobin and thiophenate in the series of 0.0706 mg/kg and 0.00606 mg/kg. By this QuChERS method we get different fungicides in some vegetables but in some vegetables they are absent. And determination of fungicides was done by HPLC technique. Hence this method is very easy for determination of fungicides in vegetables. QuChERS method may vary % recoveries of two fungicides in two vegetables by varying different factors like concentration, temperature, time, and PH (effect).By varying concentration, time, PH and temperature, % age recoveries of two fungicides by HPLC smethod vary between 63.98-107.68, 26.36–57.49, 2.31-11.903, 1.001–30.44 respectively. These two vegetables were planted in different areas of Muzaffargarh (Pakistan) and are available in different markets. Hence QuChERS HPLC scheme is extremely quick, susceptible and easy process for extraction of fungicide residues in Spinach and Fenugreek sample. This removal scheme is

not simply precise and extra well-organized as well as saves currency, chemical reagents and period. This method is very sensitive in extraction of two pesticides in different vegetables at parts per million.

List Of Abbreviations

“Not Applicable” in this section.

Declarations

Compliance with Ethical Standards

Authors declare no conflict of interest in this study. All the authors contributed equally to this research. The corresponding author designed the study and revised the manuscript. The manuscript is submitted with the approved consent of all authors.

Consent for Publication

“**Not Applicable”** in this section.

Availability of data and materials

The complete data that support the findings of this study are openly available in the online library of Higher education commission Pakistan (HEC).

Competing interest

The authors whose names are listed immediately below certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers’ bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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Credit authorship contribution statement

Muhammad Sajid: Conceptualization, Methodology, Supervision. **Abdul Majid:** Data collection, Writing - original draft, Validation, Methodology. **Zeeshan Ali:** Conceptualization, Writing – review & editing. **Dilshad Hussain:** Software, Formal analysis, Writing – review & editing.

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Figures

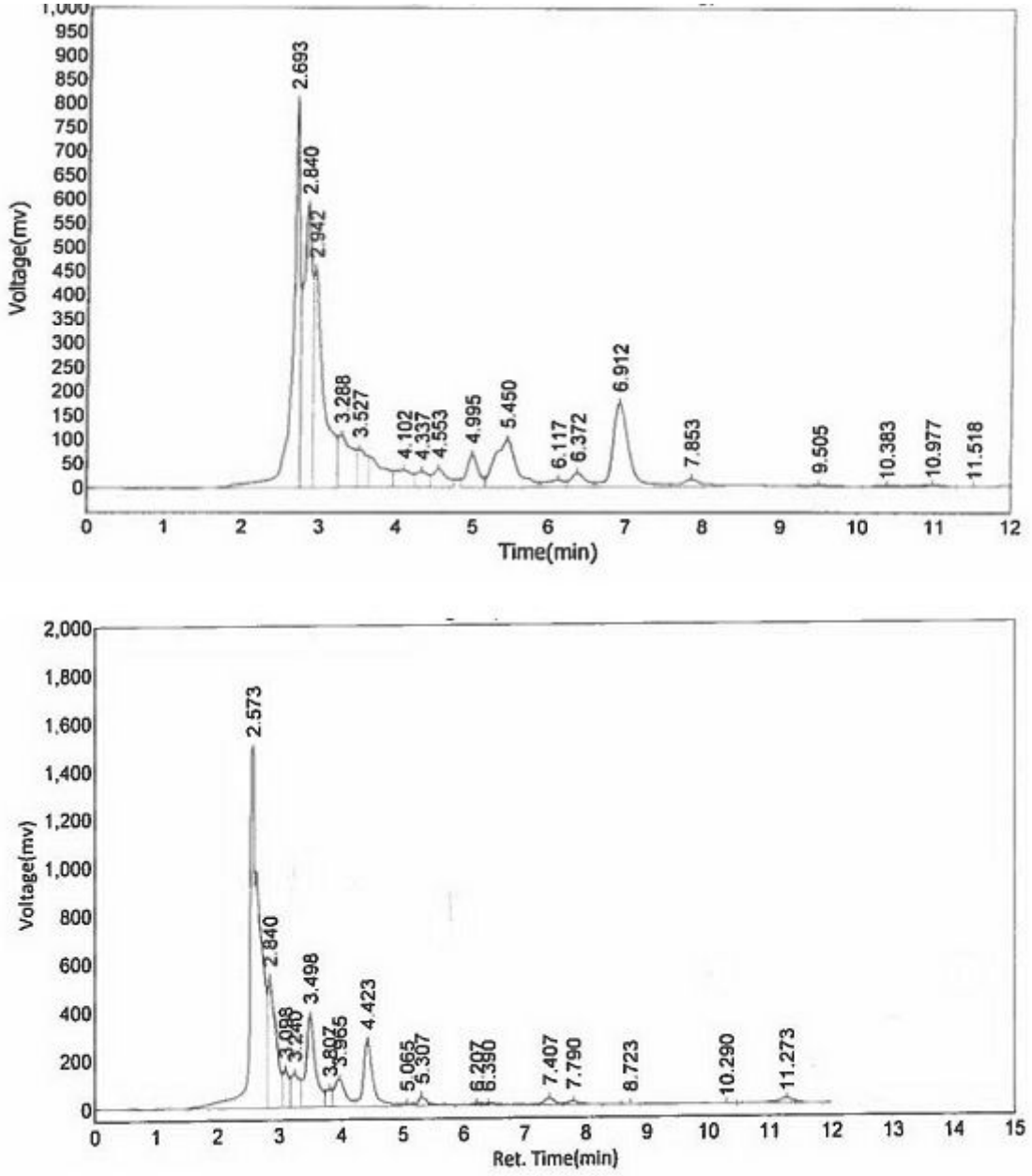


Figure 1

(A) Blank solution of Spinach and (B) Fenugreek (Methi)

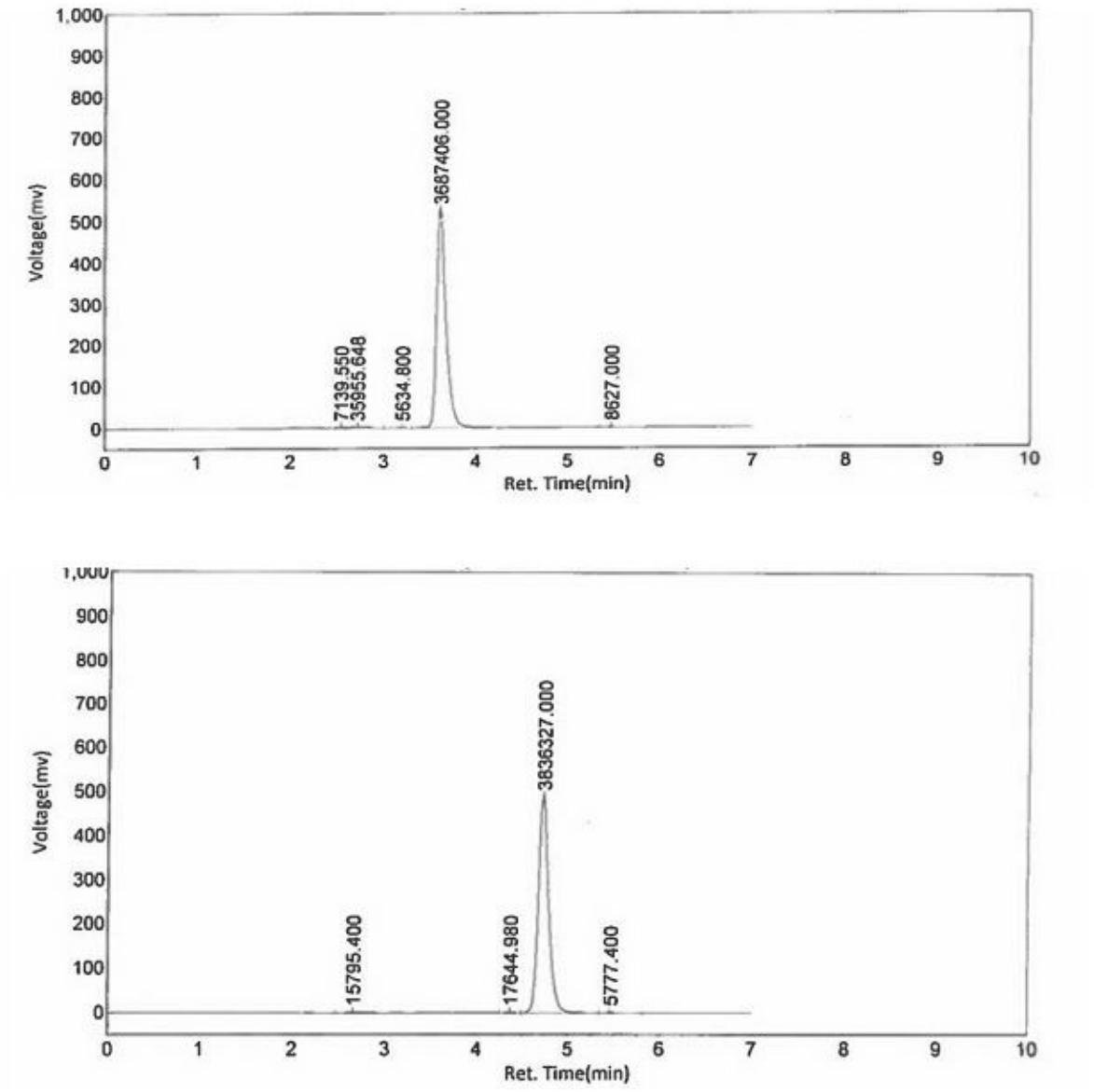


Figure 2

HPLC Chromatogram of (A) Thiophenate Methyl standard solution and (B) Azoxystrobin standard solution

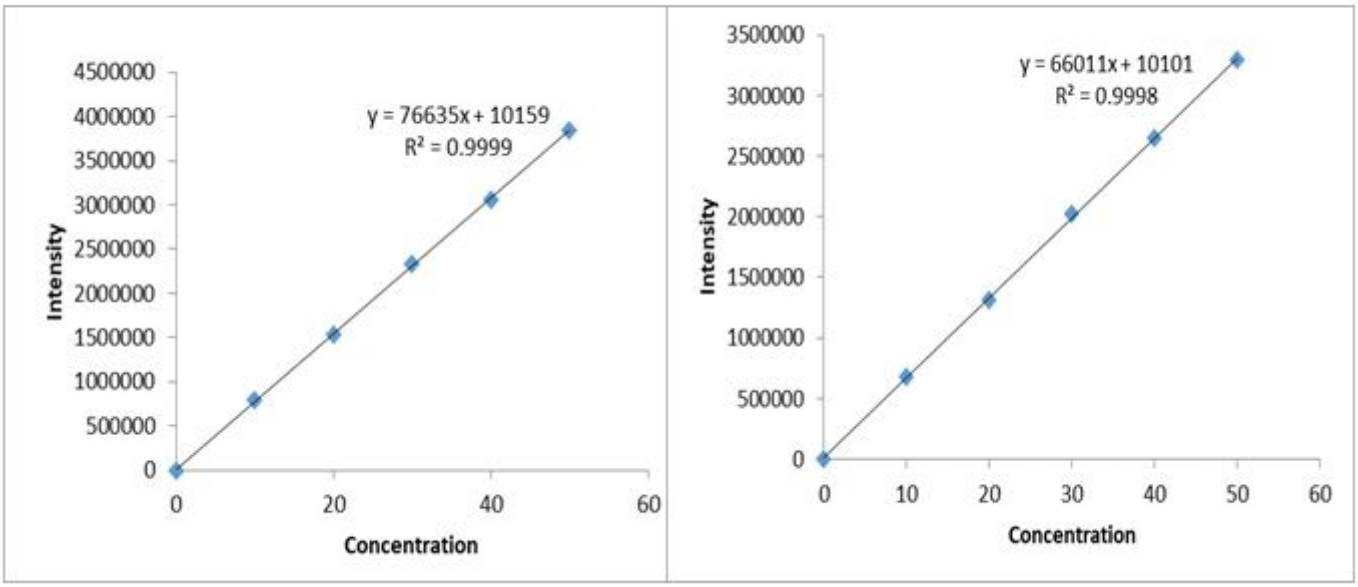


Figure 3

Calibration Curve of (A) Thiophenate and (B) Azoxystrobin

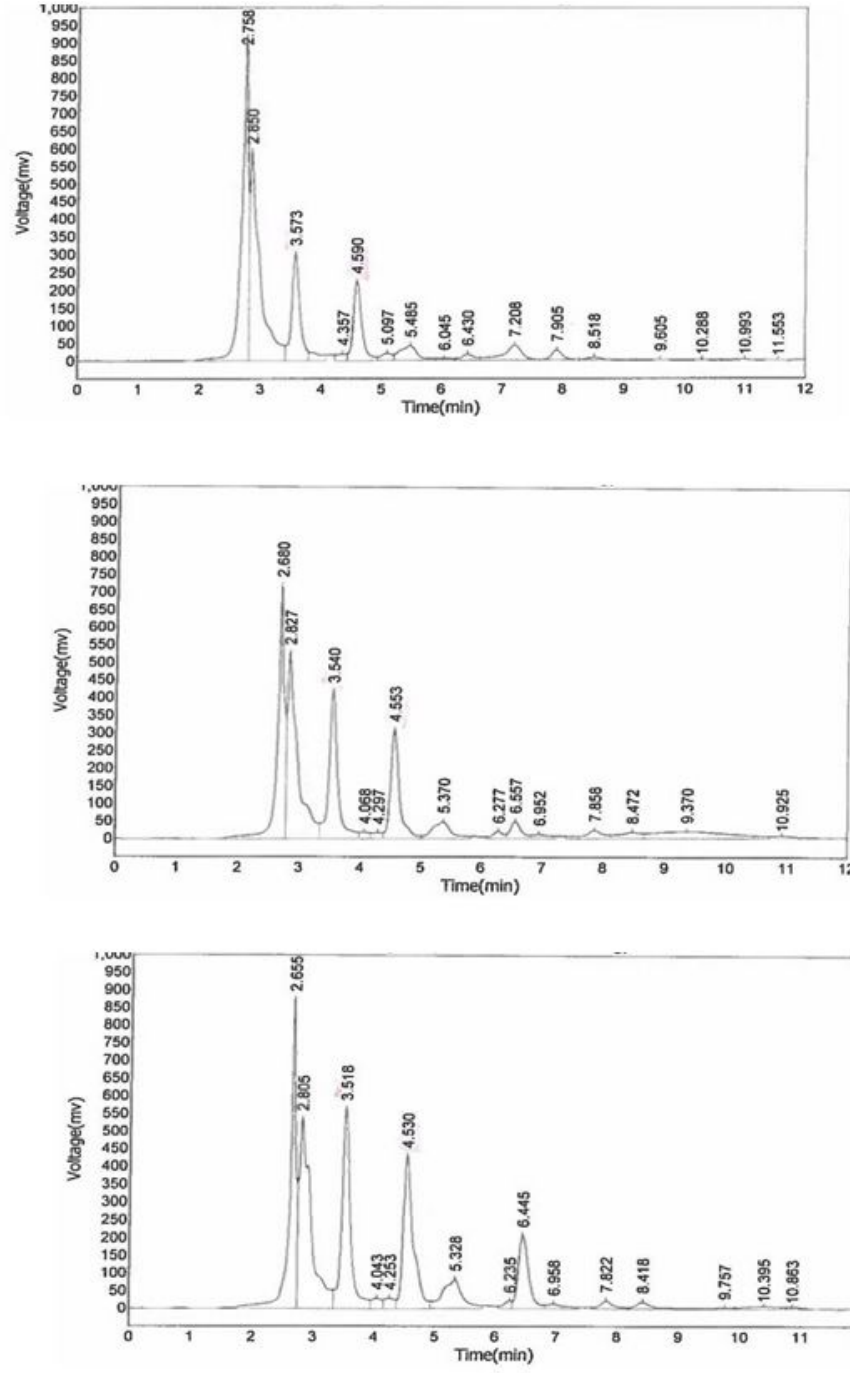
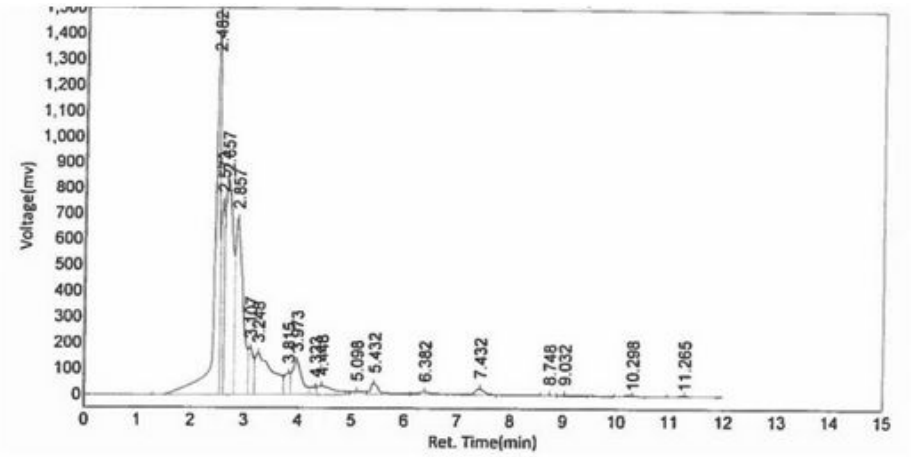
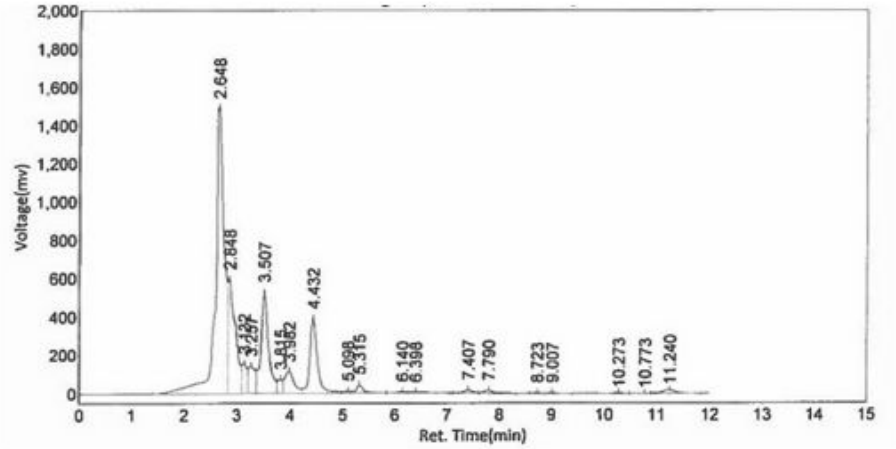


Figure 4

Chromatogram of Azoxystrobin and Thiophenate methyl in spinach at (A) 50 ppm, (B) 75 ppm and (C) 100ppm





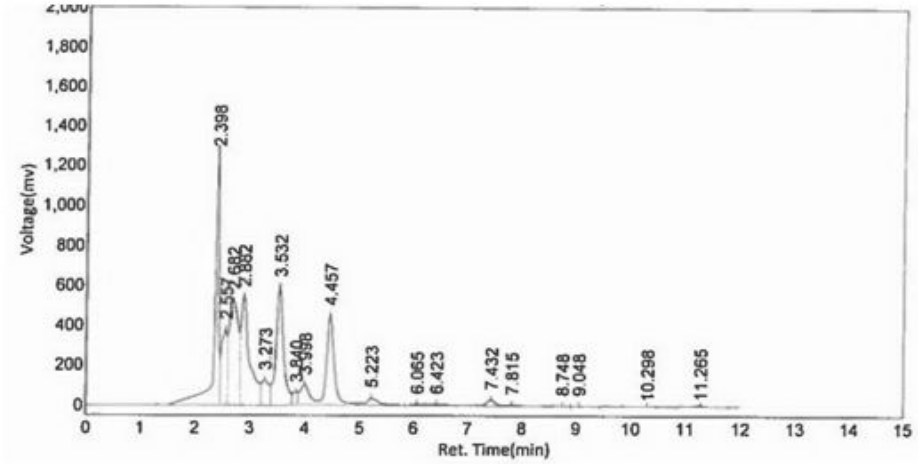


Figure 5

Chromatogram of Azoxystrobin and Thiophenate methyl in Fenugreekat at (A) 50 ppm, (B) 75 ppm and (C) 100ppm

Supplementary Files

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