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Determination of Toxic Pesticide Residues in Crops

Muralidhar Reddy Avuthu[1], Tentu Nageswara Rao[2]*, B. Venkata Reddy[3], SNVS Murthy[4], Prathipati Revathi[2]
[1]Department of Chemistry, SVKP& Dr.K.S Raju Arts and Science College, Penugonda, AP, India
[2]Department of Chemistry, Krishna University, Machilipatnam, AP, India
[3]Department of Animal Science and BK21 PLUS program, Chonbuk, South Koria
[4]Department of Chemistry, DLR P.G College, Gollalamamidada, Andhra Pradesh, India

***Corresponding Author**
Name: Tentu Nageswara Rao
Email: tentu6581@rediffmail.com

Abstract: A simple and inexpensive method was developed using solid-phase extraction, together with high performance liquid chromatographic method with UV detection for determination of pesticide residues (Valifenalate, Kresoxim-methyl, Ethoxysulfuron, Topramezone and Tolfenpyrad) in different crop commodities. The evaluated parameters include the extracts by phenyl, silica gel and alumina solid phase extraction cartridges using methanol, ethyl acetate, distilled water and tetrahydrofuran solvents. The method was validated using croup samples spiked with Valifenalate, Kresoxim-methyl, Ethoxysulfuron, Topramezone and Tolfenpyrad at different fortification levels (0.01 and 0.1 µg/g, 0.05 µg/g and 0.5 µg/g, 0.03 µg/g 0.3 µg/g 0.01 and 0.1 µg/g and 0.03 µg/g 0.3 µg/g). Average recoveries (using each concentration six replicates) ranged 84-95%, with relative standard deviations less than 2%, calibration solutions concentration in the range 0.01- 10.0 µg/mL and limit of detection (LOD) and limit of quantification (LOQ) range were 0.003 to 0.02µg/g and 0.01 to 0.05µg/g respectively. Subsequently the crop residue samples have been analyzed through HPLC.
Keywords: Valifenalate, Kresoxim-methyl, Ethoxysulfuron, Topramezone and Tolfenpyrad, HPLC-UV and LOQ

INTRODUCTION

The term pesticide includes herbicide, insecticide, insect growth regulator, nematicide, termiticide, molluscicide, piscicide, avicide, rodenticide, predacide, bactericide, insect repellent, animalrepellent, antimicrobial, fungicide, disinfectant (antimicrobial) and sanitizer [1]. India is an agricultural country and our economy is depending on the agriculture sector. 60% of the population was depending on agriculture. India loses nearly 30% of its potential crop to insects, weeds and rodent attacks. The Pesticides/Crop Protection/Agrochemicals industry plays a crucial role in protecting crops from damage by weeds, pests, insects and fungus, both before and after harvest. This helps to increase crop yields, which is important given the rate at which cultivable land is shrinking. In this way the use of pesticides become necessary for harvesting. A pesticide poisoning occurs when chemicals intended to control a pest affect non-target organisms such as humans, wildlife, or bees.Now a days, the farmers have spraying the pesticides in higher side to protect the crop and for the better yield. But, the pesticide traces are remaining as residues in the crop which is harmful to human life and showing the adverse on human health. Independent analytical method were developed for Valifenalate, Kresoxim-methyl, Ethoxysulfuron, Topramezone and Tolfenpyrad to determine the content of residue present in crop/fruit and the analytical methods were validated successfully by performing specificity linearity, precsion and accuracy in tomato fruit, red chilli, sugarcane juice, maize and mango fruit. Storage stability test was conducted for the stability of the residue for a period of 30 days in different condition [2, 3]. Valifenalate is an acylamino acid fungicide. It acts systemically. Valifenalate once absorbed and trans located throughout the plant, gives a long term protective and curative effect. It acts on cell wall synthesis and thus affects all growth stages of the pathogen, reducing spore germination and mycelia growth. The fungicide is used to control late blights in tomato's and potato's, downy mildews in vines ad in tobacco. Thus, the fungicide controls many pathogens belonging to the class Oomycetes, particularly viral diseases may be difficult to identify. The symptoms can vary from one plant to the next and also with age and growing conditions [4].

Fungicides are the essential part of agriculture crop management for better yields. In this process several new molecules have been introduced for the potential control of pests and diseases. Fungicides can be divided into protectant and specific types. Protectants are the older type and includes copper and sulfur based products. They form a protective film on the plant surface and inhibit the germination of fungal spores. Specific type fungicides are so called because

they act on one specific chemical reaction in the fungus. Strobilurin compounds, they inhibit the respiratory electron transport is fungus and thereby killing fungus [5,6]. They act as efficient inhibitors. One of the most commonly used strobilurin fungicides; kresoxim methyl is mainly used for the control of powdery mildew and scab in apples, pears, grapes, strawberries and vegetables. It is one of the most frequently used fungicides in Indian viticulture, where application is done by foliar spray and also through drip irrigation [7 - 10].

Sulfonylurea herbicide a modern class of herbicides, are extensively used to control a wide range of weeds in many crops. These herbicides exhibit a simple but effective biological mode of action through inhibiting acetolactate synthase, a key enzyme that participate in the protein synthase of plants. Ethoxysulfuron is synthetic herbicide it has the unique characteristic of exhibiting herbicidal activity against weeds that show resistance to commercialized sulfonylurea herbicides [11-14].

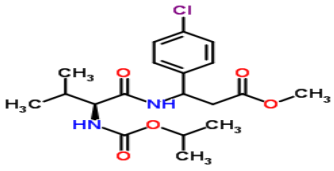
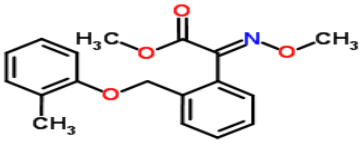
Topramezone is the first herbicide belonging to a new chemical class called pyrazolones. In sensitive plant species topramezone inhibits the enzyme 4-hydroxyphenyl-pyruvat-dioxygenase. As a result, the biosynthesis of lastochinones and indirectly of carotinoides discontinues, leading to a discruption of the synthesis and function of chloroplasts. Consequently, chlorophyll is destroyed by oxidation. This process is expressed in pronounced bleaching symptoms of the growing shoot tissue and subsequent necrosis of the aboveground plant matter. The pronounced selectivity in maize consists of a lower sensitivity of enzymatic target and a faster metabolic decomposition in maize compared to sensitive species. Topramezone is taken up by the shoot and the roots, the distribution within the plants is both akro and basipetally. Uptake by and distribution within the shoot is significantly increased with asuitable adjuvant [15-18]. Topramezone has favorable oxicological and ecotoxicological properties. Water solubility and persistency in the soil are in a medium range, which results in weed control also through soil uptake. However, due to the strongly pronounced foliar activity of this compound even against advanced weed growth stages and the very good crop safety, topramezone is intended to be used postemergence of the crop in a range from 1 to 8 leaf stage of maize.

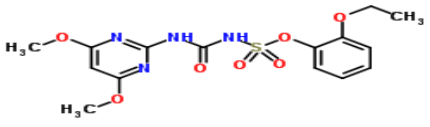
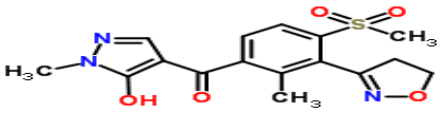
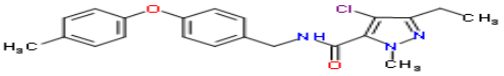
Tolfenpyrad is a pesticide developed by Mitsubishi chemical Co. That was first approved in 2002 in japan under the trade name of Hachi-hachi. It is used against a broad range of pests such as hemiptera, coleopteran, Diptera, Lepidoptera, tysanoptera and acarina. It is especially effective against pests that are resistant to existing insecticides such as organophosphates and carbametes, because it supposedly possesses a new mode of action: inhibition of complex in the respiratory electron-transfer chain of mitochondria.

MATERIALS AND METHODS

The Structure, Molecular formula, Molecular Weight and Description of Valifenalate, Kresoxim-methyl, Ethoxysulfuron, Topramezone and Tolfenpyrad are given in Table 1.

Table 1: Structure, Molecular formula and Molecular Weight of Valifenalate, Kresoxim-methyl, Ethoxysulfuron, Topramezone and Tolfenpyrad

Pesticide	Structure	Molecular Formula	Molecular Weight
VALIFENALATE		$C_{19}H_{27}ClN_2O_5$	398.881
KRESOXIM-METHYL		$C_{18}H_{19}NO_4$	313.348

ETHOXYSULFURON		$C_{15}H_{18}N_4O_7S$	398.391
TOPRAMEZONE		$C_{16}H_{17}N_3O_5S$	363.388
TOLFENPYRAD		$C_{21}H_{22}ClN_3O_2$	383.871

VALIFENALATE:

Extraction procedure for Tomato fruit

Accurately weighed 50 g of representative tomato fruit. The sample was homogenized with 100 mL extraction solvent (80 mL of 80: 20 (v/v) acetonitrile: triethylamine (0.02 M)) using an homogenizer for 15 min at about 3000 rpm. After decanting, the liquid was filtered under vacuum through a Buchner funnel using Whatman filter paper. The extraction was repeated with solid residue using 80 mL aliquot of extraction solvent and eventually the solvent was collected through filtration.

Purification

The 250 mL pooled liquid extract was transferred in to a 1.0L separatory funnel. After adding 25 g of sodium chloride and 200 mL of n-hexane saturated with acetonitrile, the solution was shaken vigorously for 1 min at least. The separatory fennel was left to stand (at least 1 hour) until the three phases (water, acetonitrile and n-hexane, in ascending order) were separated. The lower aqueous layer (containing un-dissolved NaCl at the bottom) was discarded and the intermediate acetonitrile phase was transferred quantitatively in a round bottomed flask. The upper organic phase (n=hexane) was discarded. Acetonitrile was reduced to small volume by Buchi rotavapour at 30°C maximum temperature and filtered through 0.45 micron in order to get rid of possible sodium chloride. The filtered acetonitrile was evaporated to dryness firstly by Buchi Rotavapour as above and at last by gentle nitrogen stream and analysed by HPLC.

KRESOXIM - METHYL:

Extraction Procedure

A 25g of red chilli sample taken into 500 mL Erlenmeyer flask then added 100 mL of Acetone. Kept in an end-over end shaker for 30 minutes and filtered. The residual material was once again extracted with 50 mL of Acetone and filtered. The flask was rinsed with 50 mL of Acetone and filtered. The combined filtrate was concentrated to 2-5 mL in a rotary vacuum evaporator.

Partitioning

Above extract was transferred into 1000 mL separating flask then added 100 mL of 10% sodium chloride solution. Partitioned thrice with Dichloromethane (100, 75, 75mL). Collected Dichloromethane layer through sodium sulphate and evaporated to near dryness.

Clean-Up

Above residue was dissolved in 5% Ethyl acetate in hexane. Transferred it into column which was packed in silica gel using 100 mL of 5% Ethyl acetate in Hexane and discarded the washings. Finally, eluted the column with 100 mL mixture of 10% Ethyl acetate in Hexane. Concentrated the collected eluate to dryness on a rotary vacuum evaporator and dissolved the residue in suitable volume of mobile phase (Acetonitrile: Water, 80 : 20, v/v) for HPLC analysis.

ETHOXYSULFURON

Extraction procedure

1 mL methanol and 1 mL 0.2% HCl was added to the 50 mL of sugarcane juice and mixture was allowed to stand for 2-3 hours. Then the mixture was made alkaline by sodium hydroxide solution. The above solution was transferred into 1000 mL separating funnel and partitioned with 100 mL methylene dichloride. The methylene dichloride layer was collected over anhydrous sodium sulphate and repeated partition with 100 mL dichloromethane and collected dichloromethane layer over anhydrous sodium sulphate. Combined dichloromethane was concentrated under vacuum using a buchi rotary vacuum evaporator.

Clean-up procedure

The concentrated material was transferred on a glass column pre-packed with silica gel in dichloromethane. 50 mL dichloromethane was eluted through the glass column and discarded. Finally the column was eluted with 100 mL acetone and collected the elute into a round bottom flask. Concentrated to dryness and then re-dissolved in 20 mL of acetonitrile. The final extract solutions were analysed by HPLC and LC-MS/MS.

TOPRAMEZONE

Extraction and clean up

The representative homogenized sample (maize 50g) was taken in a 500 ml stoppered conical flask and extracted with 100 ml of water and methanol (1:1) using an end-over-end mechanical shaker for about 30 minutes and filtered. Extraction was repeated twice with 50 ml of same solvent. Combined filtrate was passed through celite filter and concentrated to 5 ml using vacuum rotary evaporator.

Solid Phase extraction

A phenyl solid phase extraction cartridge was conditioned with 10 ml of methanol and water (1:1). Concentrated extract was percolated through the cartridge and eluate was discarded. Attached the phenyl SPE cartridge column to a conditioned Envicarb Cartridge column with an adapter. Eluted the residues from upper to lower cartridge with 10ml of water/methanol (1:1). Then phenyl cartridge column was removed and residues were eluted from Envicarb Cartridge with 10 ml of water/ tetrahydrofuran (9:1). Evaporated the residues to near dryness and then re-dissolved in 20 mL of acetonitrile. The sample was filtered through 0.45 µm filter and analysed by HPLC-UV.

TOLFENPYRAD

Extraction and clean up

The representative homogenized mango fruit 50 g was taken into a 500 mL erlenmeyer flask and added 5.0 mL water followed by 95 mL ethyl acetate : cyclohexane (90:10) and kept in end-over-end mechanical shaker for about 15 minutes. The sample was centrifuged for 15 minutes at 6000 rpm and then 12 mL of supernatant was passed through glass column packed with fluorosil6 material and eluted the residue with ACN:H2O (90:10) and elute was collected into a flask and Concentrated to dryness and then re-dissolved in 20 mL of acetonitrile. The sample was filtered through 0.45 µm filter and analysed by HPLC-DAD.

Specificity:

Valifenalate	Kresoxim-methyl	Ethoxysulfuron	Topramezone	Tolfenpyrad
Specificity was confirmed by injecting the tomato fruit control and valifenalate sample solution	Specificity was confirmed by injecting the red chilli control and kresoxim-methyl sample solution	Specificity was confirmed by injecting the sugarcane juice control and exthoxysulfuron sample solution	Specificity was confirmed by injecting the maize control and topramezone sample solution	Specificity was confirmed by injecting the mango fruit control and tolfenpyrad sample solution

Linearity:

Valifenalate	Kresoxim-methyl	Ethoxysulfuron	Topramezone	Tolfenpyrad
Different known concentrations of standards (0.01, 0.1, 0.5, 1.0, 2.0 and 5.0 µg/mL) were prepared in methanol by diluting the stock solution of 200µg/mL. Each standard solution were prepared in triplicate and injected into HPLC	Different known concentrations of fungicides (0.05, 0.1, 0.5, 1.0, 2.0 and 5.0 µg/mL) were prepared in acetonitrile by diluting the stock solution 522.72 g/L. Each standard solutions were directly injected into a HPLC	Different known concentrations of standard solutions (0.03, 0.1, 0.5, 1.0, 2.0 and 10.0 µg/mL) were prepared in acetonitrile by diluting the stock solution of 3000 µg/mL. Each standard solutions were directly injected into a HPLC	Different known concentrations of standard solutions (0.03, 0.1, 0.5, 1.0, 2.0 and 10.0 µg/mL) were prepared in acetonitrile by diluting the stock solution of 3000 µg/mL. Each standard solutions were directly injected into a HPLC	Different known concentrations of standard solutions (0.03, 0.1, 0.5, 1.0, 2.0 and 10.0 µg/mL) were prepared in acetonitrile by diluting the stock solution of 3000 µg/mL. Each standard solutions were directly injected into a HPLC

Accuracy and Precision:

Valifenalate	Kresoxim-methyl	Ethoxysulfuron	Topramezone	Tolfenpyrad
Recovery studies were carried out at 0.01 and 0.1 µg/mL fortification levels for valifenalate in tomato fruit. Accuracy and precision was carried out by injecting from six replicate analyses of given sample (valifenalate) made by a single analyst on one day. The repeatability of method will be satisfactory when RSD <2 %.	Recovery studies were carried out at 0.05 and 0.5 mg/kg fortification levels for Kresoxim-methyl in red chilli Accuracy and precision was carried out by injecting from six replicate analyses of given sample (Kresoxim-methyl) made by a single analyst on one day. The repeatability of method will be satisfactory when RSD <2 % .	Recovery studies were carried out at 0.03 and 0.3 µg/mL fortification levels for Ethoxysulfuron in sugar cane juice Accuracy and precision was carried out by injecting from six replicate analyses of given sample (Ethoxysulfuron) made by a single analyst on one day. The repeatability of method will be satisfactory when RSD <2 % .	Recovery studies were carried out at 0.01 and 0.1 µg/g fortification levels for Topramezone in maize Accuracy and precision was carried out by injecting from six replicate analyses of given sample (Topramezone) made by a single analyst on one day. The repeatability of method will be satisfactory when RSD <2 %	Recovery studies were carried out at 0.03 and 0.3 µg/g fortification levels for Tolfenpyrad in mango juice Accuracy and precision was carried out by injecting from six replicate analyses of given sample (Tolfenpyrad) made by a single analyst on one day. The repeatability of method will be satisfactory when RSD <2 %

Detection and Quantification Limit:

Valifenalate	Kresoxim-methyl	Ethoxysulfuron	Topramezone	Tolfenpyrad
This quantification limit also reflects the fortification level at which an analyte peak is consistently generated at approximately 10 times the baseline noise in the chromatogram. The limit of detection was determined to be 0.01 µg/mL at a level of approximately two times the back ground of control injection around the retention time of the peak of interest.	This quantification limit also reflects the fortification level at which an analyte peak is consistently generated at approximately 10 times the baseline noise in the chromatogram. The limit of detection was determined to be 0.02 µg/mL at a level of approximately three times the back ground of control injection around the retention time of the peak of interest.	This quantitation limit also reflects the fortification level at which an analyte peak is consistently generated at approximately 10 times the baseline noise in the chromatogram. The limit of detection was determined to be 0.03 µg mL-1 at a level of approximately three times the back ground of control injection around the retention time of the peak of interest.	This quantitation limit also reflects the fortification level at which an analyte peak is consistently generated at approximately 10 times the baseline noise in the chromatogram. The limit of detection was determined to be 0.01 µg/g at a level of approximately three times the back ground of control injection around the retention time of the peak of interest	This quantitation limit also reflects the fortification level at which an analyte peak is consistently generated at approximately 10 times the baseline noise in the chromatogram. The limit of detection was determined to be 0.03 µg/g at a level of approximately three times the back ground of control injection around the retention time of the peak of interest.

Stability:

Valifenalate	Kresoxim-methyl	Ethoxysulfuron	Topramezone	Tolfenpyrad
A storage stability study was conducted at -20 ± 1°C with tomato fruit samples spiked with 0.1 µg/mL of Valifenalate Samples were stored for a period of 30 days at this temperature. Analysed for the content of Valifenalate before storing and at the end of storage period.	A storage stability study was conducted at refrigerator condition (5 ± 3°C) and Ambient temperature (25 ± 5°C) of 0.1 mg/kg level fortified fruit samples were stored for a period of 30 days. Analysed for the content of kresoxim-methyl before storing and at the end of storage period	A storage stability study was conducted at refrigerator condition (5 ± 3°C) and Ambient temperature (25 ± 5°C) of 0.1 µg mL-1 level fortified juice samples were stored for a period of 30 days at this temperature. Analysed for the content of ethoxysulfuron before storing and at the end of storage period.	A storage stability study was conducted at refrigerator condition (5 ± 3°C) and Ambient temperature (25 ± 5°C) of 0.1 µg/g level fortified fruit samples were stored for a period of 30 days at This temperature. Analysed for the content of topramezone before storing and at the end of storage period.	A storage stability study was conducted at refrigerator condition (5 ± 3°C) and Ambient temperature (25 ± 5°C) of 0.1 µg/g level fortified fruit samples were stored for a period of 30 days at this temperature. Analysed for the content of tolfenpyrad before storing and at the end of storage period

Chromatographic Condition for HPLC (High Pressure Liquid Chromatography):

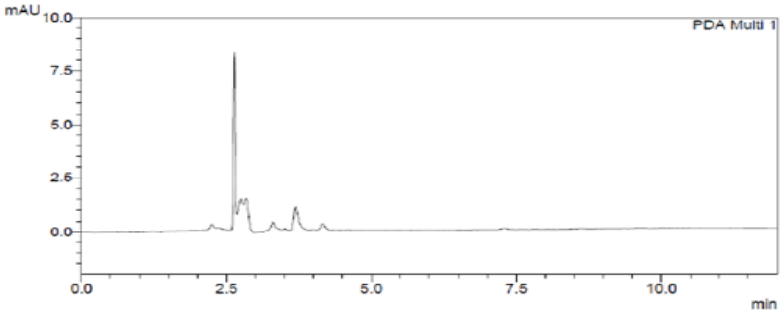
Conditions	Valifenalate	Kresoxim-methyl	Ethoxysulfuron	Topramezone	Tolfenpyrad
Instrument	HPLC-PDA system used, consisted shimadzu high performance liquid chromatography with LC- 20AT pump and SPD-20A interfaced with LC solution software				
Column	Phenomenex C18 (250 mm x 4.6 m i.d x 5µ particle size)				
Mobile phase	Acetonitrile : 0.1% trifluro acetic acid (85:15 ,v/v)	Acetonitrile : 0.1% trifluro acetic acid (80:20 ,v/v)	Acetonitrile : 0.1% trifluro acetic acid (80:20 ,v/v)	Acetonitrile : 0.1% Orthophosphoric acid in water (80:20 ,v/v)	Acetonitrile : HPLC Water (90:10 ,v/v)
Column temperature (°C)	30	30	30	30	40
Flow rate (mL/min.)	0.7	1.0	0.8	0.9	0.8
Wave length (nm)	220	230	235	225	230
Injection Volume (µL)	20	20	20	20	20
Retention Time (minutes)	6.410	5.210	5.410	5.321	6.270

RESULTS AND DISCUSSION

Specificity:

Valifenalate	Kresoxim-methyl	Ethoxysulfuron	Topramezone	Tolfenpyrad
There were no matrix peaks in the chromatograms to interfere with the analysis of fungicide residues confirms the specificity of the method (Refer Figure 1)	There were no matrix peaks in the chromatograms to interfere with the analysis of fungicide residues confirms the specificity of the method (Refer Figure 2).	There were no matrix peaks in the chromatograms to interfere with the analysis of herbicide residues confirms the specificity of the method (Refer Figure 3)	There were no matrix peaks in the chromatograms to interfere with the analysis of herbicide residues confirms the specificity of the method (Refer Figure 4)	There were no matrix peaks in the chromatograms to interfere with the analysis of pesticide residues confirms the specificity of the method (Refer Figure 5).

(a) Representative chromatogram of fruit control



(b) **Representative chromatogram at fortification level of 0.01µg/m**

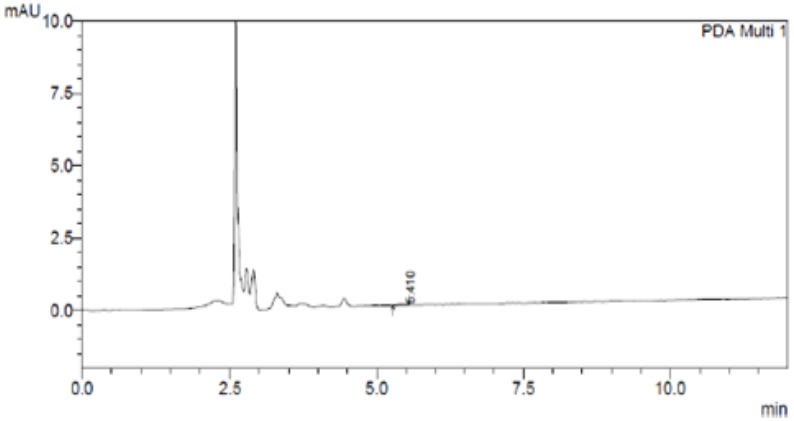
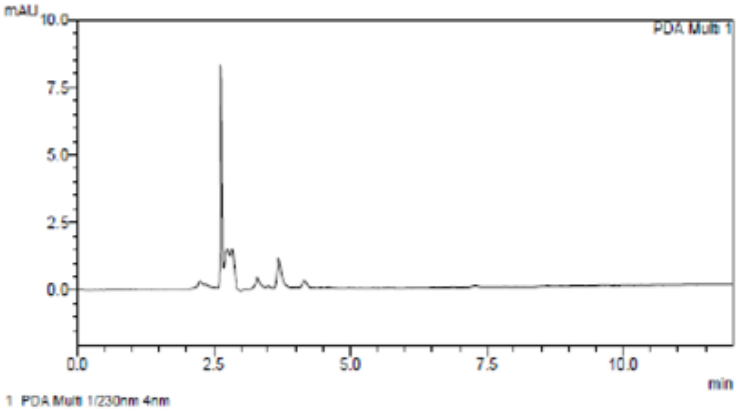


Fig 1:Representative Chromatogram for specificity test - Valifenalate

(a) Representative chromatogram of red chilli control



(b) Representative chromatogram at fortification level of 0.05mg/kg

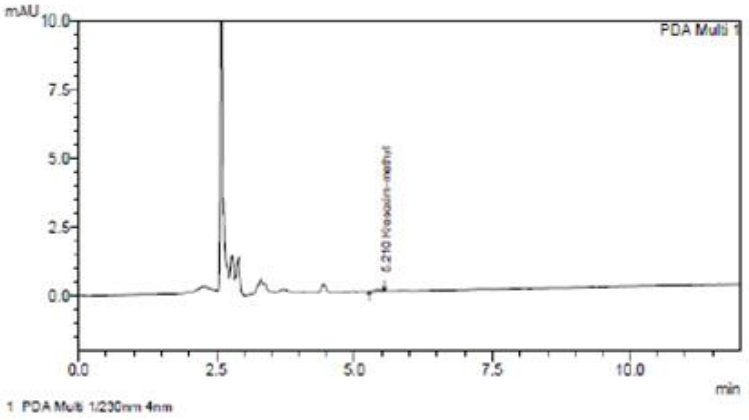
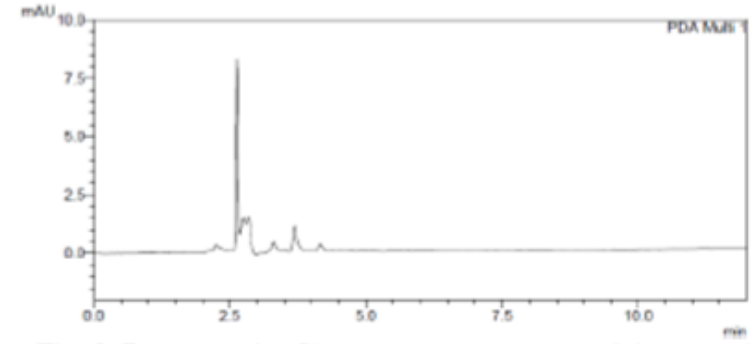


Fig 2: Representative Chromatogram for specificity test and Calibration Curve- Kresoxim-methyl

(a) Representative chromatogram at sugarcane juice control



(b) Representative chromatogram at fortification level of 0.05µg/mL

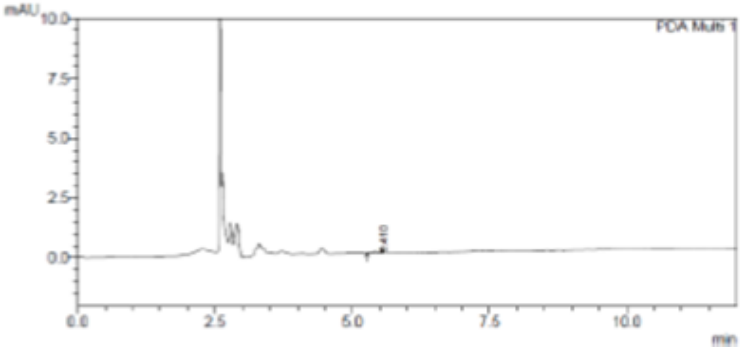
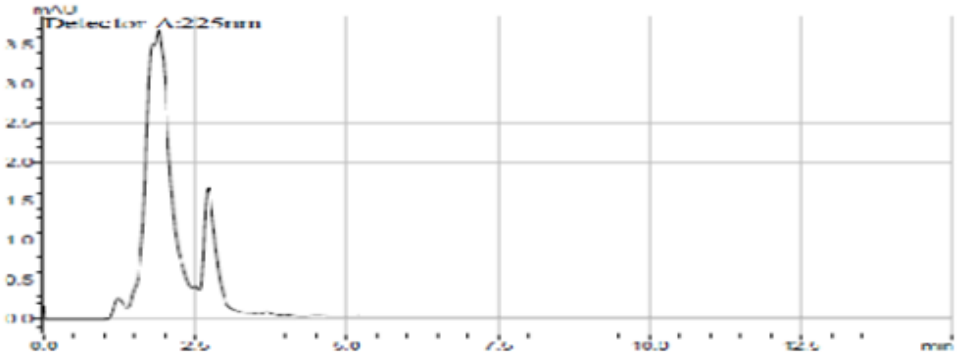


Fig 3: Representative Chromatogram for specificity test - Ethoxysulfuron

(a) Representative chromatogram at maize control



(b) Representative chromatogram at fortification level of 0.01µg/g

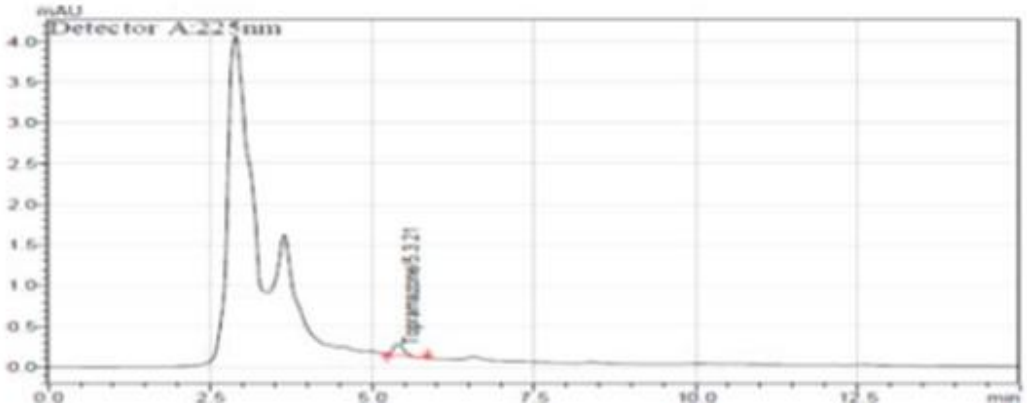
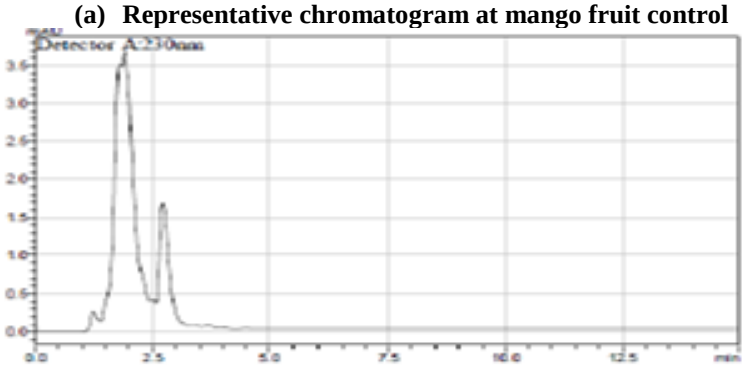


Fig 4: Representative Chromatogram for specificity test – Topramezone

(a) Representative chromatogram at mango fruit control



(b) Representative chromatogram at fortification level of 0.03µg/g

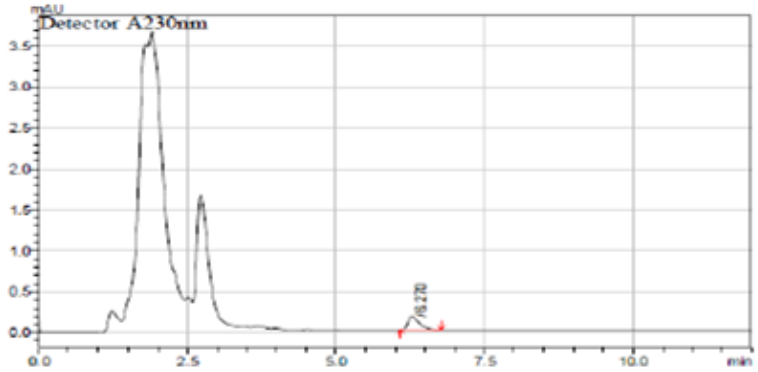


Fig 5: Representative Chromatogram for specificity test and Calibration Curve- Tolfenpyrad

Linearity:

A calibration curve has been plotted for concentration of the standards injected versus area observed and the linearity of method was evaluated by analyzing six standard concentration solutions. The peak areas obtained from different concentrations of standards were used to calculate linear regression equation (Refer Figure 6 to Figure 10)

Valifenalate	Kresoxim-methyl	Ethoxysulfuron	Topramezone	Tolfenpyrad
Y= 22253.72X + 44.92 r = 0.9999	Y=30799.81X + 9.11 r = 0.9998	Y=10666.52X + 12.56 r = 0.9999	Y=17184.03X + 36.38 r = 1.0000	Y=10407.80X + 30.44 r = 1.0000

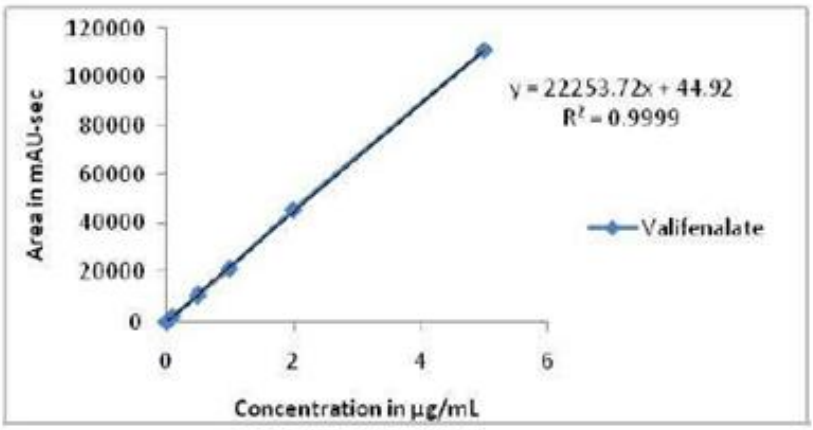


Fig 6: Representative Calibration Curve of Vifenalate Standard

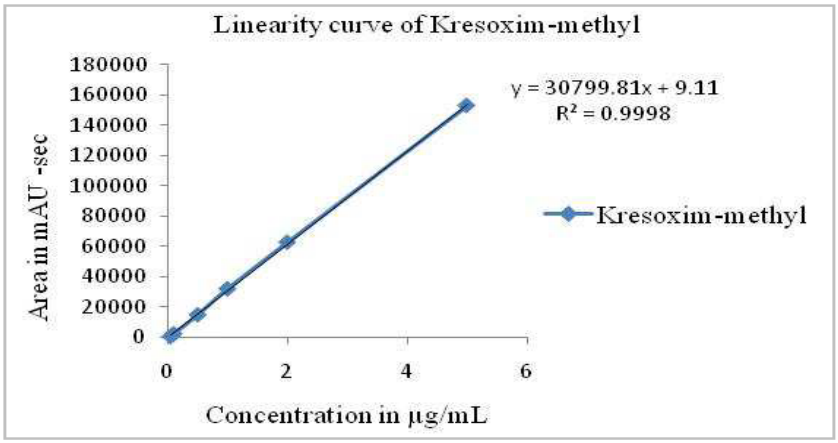


Fig 7: Representative Calibration Curve of Kresoxim-methyl Standard

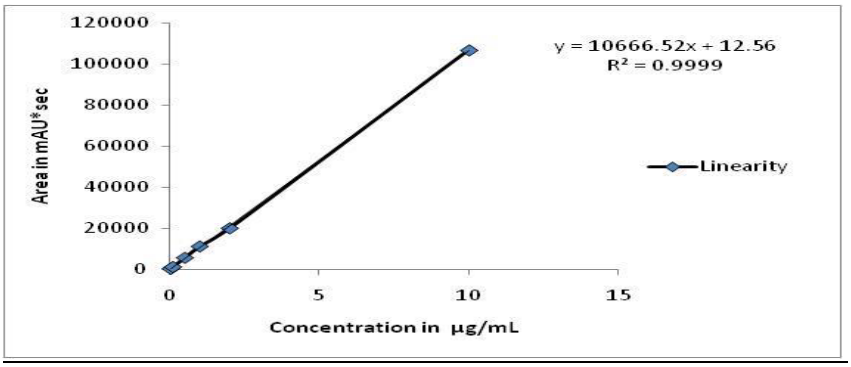


Fig 8: Representative Calibration Curve of Ethoxysulfuron Standard

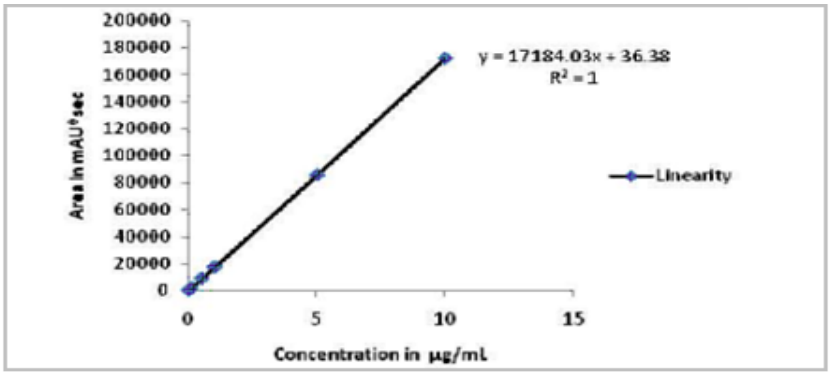


Fig 9: Representative Calibration Curve of Topramezone Standard

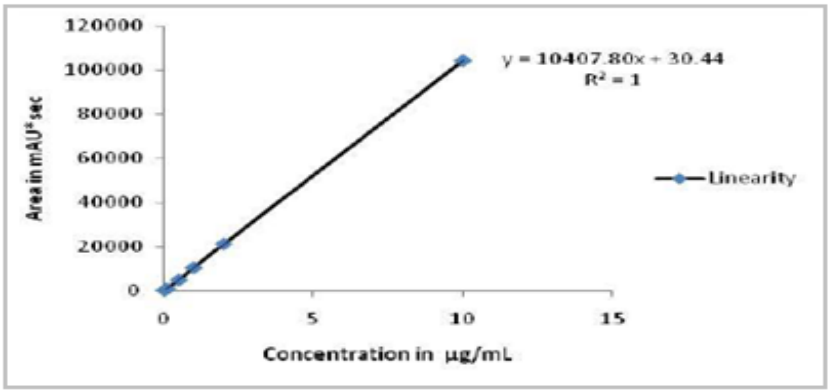


Fig 10: Representative Calibration Curve of Topramezone Standard

Accuracy and Precision

These numbers were calculated from six replicate analyses of given samples made by a single analyst on one day. The repeatability of method was satisfactory and RSDs ≤ 2 % for all the pesticides. The results are presented in **Table 2**.

Table 2: Recoveries of pesticides in different Crops

Replication No.	Valifenalate (µg/mL)		Kresoxim-methyl (mg/L)		Ethoxysulfuron (µg/mL)		Topramezone (µg/g)		Tolfenpyrad (µg/g)	
	0.01	0.1	0.05	0.5	0.03	0.3	0.01	0.1	0.03	0.3
R1	88	90	87	95	84	92	85	93	84	92
R2	87	89	86	95	85	91	84	94	84	94
R3	90	90	87	94	85	92	85	94	86	93
R4	89	89	85	96	86	94	84	96	83	95
R5	88	90	86	95	88	93	86	96	84	96
R6	88	90	87	96	86	92	86	95	85	94
Mean	88	90	86.33	95.17	85.67	92.33	85.17	94.67	84.33	94.00
% RSD	1.17	0.58	0.95	0.79	1.59	1.12	0.88	1.28	1.22	1.50

Stability

The pesticides were fortified with fruits/crops and stored in prescribed climatic conditions and checked the recovery on different occasion. The stability data are given in **Table 3**.

Table 3: Storage stability

Pesticide (Spiked Conc.)	Temperature	Sampling Occassion	R1	R2	R3	R4	R5	R6	Mean	% RSD
Valifenalate (0.1 µg/mL)	- 20±1°C	Day '0'	94	93	94	92	94	95	94	1.10
		30 Days	92	90	91	93	90	91	91	1.28
Kresoxim-methyl (0.1 mg/kg)	5±3°C	Day '0'	96	95	95	95	96	94	95.17	0.79
		30 Days	92	94	93	92	91	90	92.00	1.54
	25±5°C	Day '0'	94	96	95	95	96	94	95.00	0.94
		30 Days	90	90	91	91	92	90	90.67	0.90
Ethoxysulfuron (0.1 µg/mL)	5±3°C	Day '0'	95	95	94	95	94	96	94.8	0.79
		30 Days	91	90	92	90	91	92	91.0	0.98
	25±5°C	Day '0'	93	92	94	93	92	93	92.8	0.83
		30 Days	90	89	90	91	90	91	90.2	0.83
Topramezone (0.1 µg/g)	5±3°C	Day '0'	95	94	95	93	93	95	94.2	1.04
		30 Days	92	92	89	91	90	90	90.7	1.34
	25±5°C	Day '0'	94	93	92	93	94	94	93.3	0.87
		30 Days	89	90	89	91	90	90	89.8	0.84
Tolfenpyrad (0.1 µg/g)	5±3°C	Day '0'	95	92	94	93	92	93	93.2	1.25
		30 Days	90	89	90	91	90	91	90.2	0.83
	25±5°C	Day '0'	94	92	91	93	93	92	92.5	1.13
		30 Days	89	90	89	90	91	89	89.7	0.91

CONCLUSION

This paper describes a fast, simple sensitive analytical method based on HPLC-UV to determine the pesticide residues in crops. The SPE extraction procedure is very simple and inexpensive method for determination of pesticide residues in crops. The mobile phase composition showed good separation and resolution and the analysis time required for the chromatographic determination of the crops were very short (around 15 min for a chromatographic run). Satisfactory validation parameters such as linearity, recovery, precision and LOQ were established by following South African National Civic Organization (SANCO) guidelines. Therefore, the proposed analytical procedure could be useful for regular monitoring, residue labs and research scholars to determine the pesticide residues in different commodities (cereals, seed, oil, fruit, and water and soil samples).

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