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# Method Development and Validation of Pesticides Residues in Rice by GC-MS/MS

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## ABSTRACT

A GC-MS/MS method was developed for estimation of Pesticides in Rice using Restek Rtx-5MS column (30m X .25mm X 0.25µm) and a mobile phase of Helium gas with gradient GC oven programming, at flow rate of 1.0 ml/min with MS detector. The mass of Pesticides Bifenthrin and Cyfluthrin were found 226.1 and 181 respectively. The proposed method was validated for Specificity, Linearity, LOD and LOQ, Recovery, Repeatability, Ruggedness and Matrix effect. All the parameters were found within the acceptable limits. Linearity of Bifenthrin and Cyfluthrin was in the range of 2.5-100µg/kg. GC-MS/MS method was specific, accurate, rugged and suitable for the analysis of Bifenthrin and Cyfluthrin in Rice.

**Keywords:** Gas chromatography with mass spectrometry (GC-MS/MS), Residual Pesticides, Sanco guideline and Method Validation.

## INTRODUCTION

Pesticides are widely used to protect crops and livestock from losses due to insects, weeds, and diseases. Colorado uses about 1% of the 900 million pounds of conventional pesticide applied annually in the United States. The Environmental Protection Agency (EPA) has estimated that 76% of the total pesticide use nationally is for agricultural production, with the remaining 24% used in the urban, industrial, forest, and public sectors. These chemicals have helped to increase agricultural production with reduced labor. However, problems associated with improper pesticide use have led to human illness, wildlife losses, and water quality degradation.

Pyrethroids (Bifenthrin and Cyfluthrin) are organic synthetic insecticides that are widely used for the protection of crops and food storage against insects and acarids.

Rice is the most widely consumed staple food for a large part of the world's population. It is one of the important human diets as carbohydrate source obtained from paddy (oryza sativa L). It is the grain with second highest worldwide production after maize (corn) according to data for the year 2010. Pesticides are chemical compounds which are widely used in rice cultivation through different spray schedules and also during storage and transport. This results contamination of paddy of course rice with pesticide residues. Most of the analytical procedures used in the determination of pyrethroids are based on the use of chromatographic mainly gas chromatography (GC) with mass spectrometry (MS/MS).

For that reason, Food safety Standards Authority of India (FSSAI) under Food Safety and Standards (contaminants, toxins and residues) Regulations, 2011 and European union (EU) under EU Regulation (Ec) No.396/2005 have established maximum residue levels (MRLs) in products of plant origin such as rice.

The molecular structure of Cyfluthrin are shown in figure 1 and Bifenthrin in figure 2.

**Chemical Structure of Cyfluthrin**

Formula: $C_{22}H_{18}Cl_2FNO_3$[(R)-cyano-[4-fluoro-3-(phenoxy)phenyl]methyl] (1R,3R)-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane-1-carboxylat

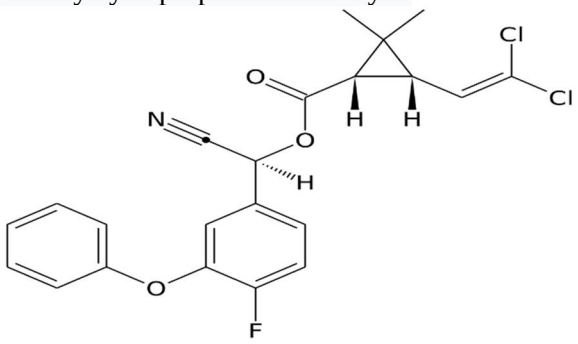
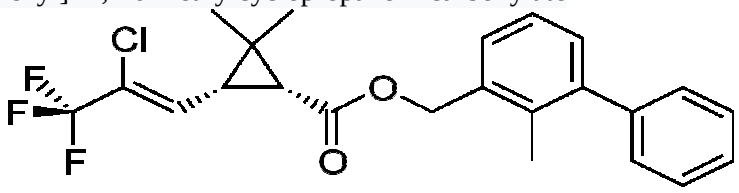



**Fig-1**

**Chemical Structure of Bifenthrin**

Formula: $C_{23}H_{22}ClF_3O_2$ (2-Methyl-3-phenylphenyl)methyl (1*S*,3*S*)-3-[(*Z*)-2-chloro-3,3,3-trifluoroprop-1-enyl]- 2,2-dimethylcyclopropane-1-carboxylate



The objective of this work was to develop a simple and rapid GC-MS/MS method which would be accurate and rugged. The method was validated according to Sanco guidelines.

## METHOD DEVELOPMENT

### Chemicals and Reagents

Acetonitrile (J. T. Baker), water (mill-Q), Sodium chloride (Sigma), Trisodium citrate (Sigma), Disodium citrate (Sigma) and Sodium sulphate (Sigma).

### Instrumentation

A Shimadzu Gas chromatography system with mass spectrometer equipped with an auto sampler with Real time analysis software.Column was employedin the method was Restek Rtx-5MS(30m X .25mm X 0.25µm).The flow rate selected was 1.0ml/min. All the weighing in the experiments was done with Sartorious electronic balance capable of measuring with an accuracy of 0.01 mg.

### Glassware

All the volumetric glassware used in the study was grade A quality Borosil.

### Chromatographic Conditions for GC

**Table-1**

| Parameters | Description |
|---|---|
| Injector Tempature | $280^0$C |
| Flow control mode | Linear velocity |
| Linear velocity | 44.9cm/sec |
| Split ratio | 10 |
| Ion source temp. | $220^0$C |
| Interface temp. | $290^0$C |
| Oven program of GC | |
| Initial Temp | $70^0$C, hold for 1.0 min |
| Ramp 1 | $20^0$C / min to $150^0$C, hold for 0.00 min |
| Ramp 2 | $5^0$C / min to $200^0$C, hold for 0.0 min |
| Ramp 3 | $3^0$C / min to $240^0$C, hold for 0.0 min |
| Ramp 3 | $10^0$C / min to $280^0$C, hold for 10.0 min |
| Run time | 42.33 minutes |

### MS Conditions

| Compound Name | Q1 MASS | Q2 MASS | CE |
|---|---|---|---|
| Cyfluthrin | 226.1 | 206 | 14 |
| | | 199 | 6 |
| | | 151 | 28 |
| Bifenthrin | 181 | 166 | 12 |
| | | 153 | 8 |
| | | 179 | 12 |

### Preparation of Bifenthrin and Cyfluthrin standard solution

Weigh 10mg of standard and dissolve in 10ml of methanol and sonicate for 5 minutes to achieve concentration of 1000 ppm. Prepare working standard solution of 1ppm from this stock solution by appropriate dilution with Diluent.

**Preparation of calibration standards**

Calibration curve for GC is prepared by using ethyl acetate.

| Sr.No | Volume taken from solution | Conc. of Std Solution | Volume taken (µl) | Volume of diluent(µl) | Volume made(ml) | Final conc. | Identification of solution |
|---|---|---|---|---|---|---|---|
| 1 | A | 1000 ppm | 100 | 900 | 1.0 | 100 ppm | B |
| 2 | B | 100 ppm | 100 | 900 | 1.0 | 10 ppm | C |
| 3 | C | 10 ppm | 100 | 900 | 1.0 | 1 ppm | D |
| 4 | D | 1 ppm | 100 | 900 | 1.0 | 100 ppb | E |
| 5 | D | 1 ppm | 50 | 950 | 1.0 | 50 ppb | F |
| 6 | D | 1 ppm | 25 | 975 | 1.0 | 25 ppb | G |
| 7 | E | 100 ppb | 100 | 900 | 1.0 | 10 ppb | H |
| 8 | E | 100 ppb | 50 | 950 | 1.0 | 5 ppb | I |
| 9 | E | 100 ppb | 25 | 975 | 1.0 | 2.5 ppb | J |

**Preparation of Rice sample solution**

- Weigh 1kg of sample and grind it in Grinder.
- Weigh accurately 10 g of Rice sample in to 50 ml centrifuge Tube.
- Add 10ml of water, 10ml of acetonitrile, 1.0gm sodium chloride, 1.0gm trisodium citrate, 0.50mg disodium citrate seq-hydrate, 5.0gm sodium sulphate, homogenize for 2 minutes at 10,000 rpm and vertex for 10 minutes.
- Centrifuge at 5000 rpm for 10 minutes.
- Take 5ml of the supernatant in to 15 ml centrifuge Tube. Add 2 gm anhydrous sodium sulphate and 50 mg of PSA and vortex for 2 mins.
- Centrifuge at 5000 rpm for 5 minutes and take 1 ml organic layer into vials & inject 1µl on GC/MS/MS.

**VALIDATIONOF HPLC METHOD**

**Specificity**

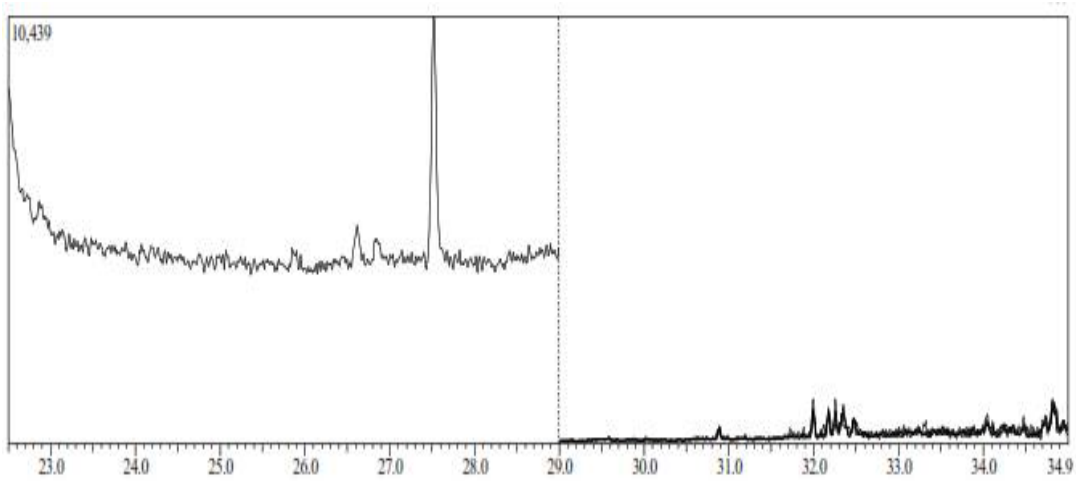
The specificity is defined as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present such as residual, degradation product and matrix components.

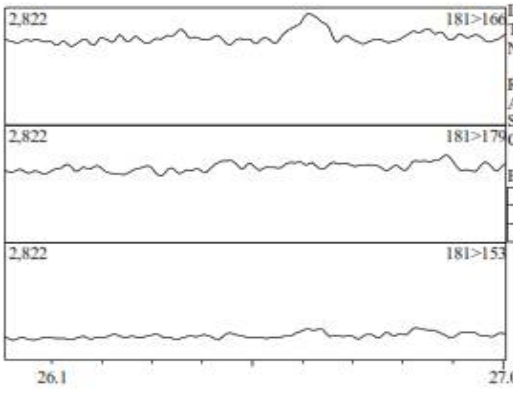
Inject the Blank (as Diluent), Standard solution and analyse the blank sample and three replicates of spiked matrix at the level of quantification limit. Check the interference at the retention time of analyte.

There should not be any interference in blank (as Diluent) and blank sample at the retention time of analyte. If any peak is present at the retention time of analyte that response should not be more than 20% of the response at the quantification limit (LOQ).

There is no interference observed between the responses of blank (as diluent), blank sample, and spiked samples of pesticides in Rice. Hence, the method is very selective and specific for the estimation of pesticide residues in Rice.

**Chromatograms of study**




Quantitation
ID#:1 Mass:181.10>166.10
Type:Target
Name:Bifenthrin

R.T:0.000
Area:0
SI:0
Conc: N.D.(Peak)

Event:1:MRM

| # | m/z | Intensity | Ratio | Setting | Ref.Band |
|---|---|---|---|---|---|
| 1 | 181.10 > 179.10 | 0 | * 0.00 | 18.05 | 30 |
| 2 | 181.10 > 153.10 | 0 | * 0.00 | 5.55 | 30 |

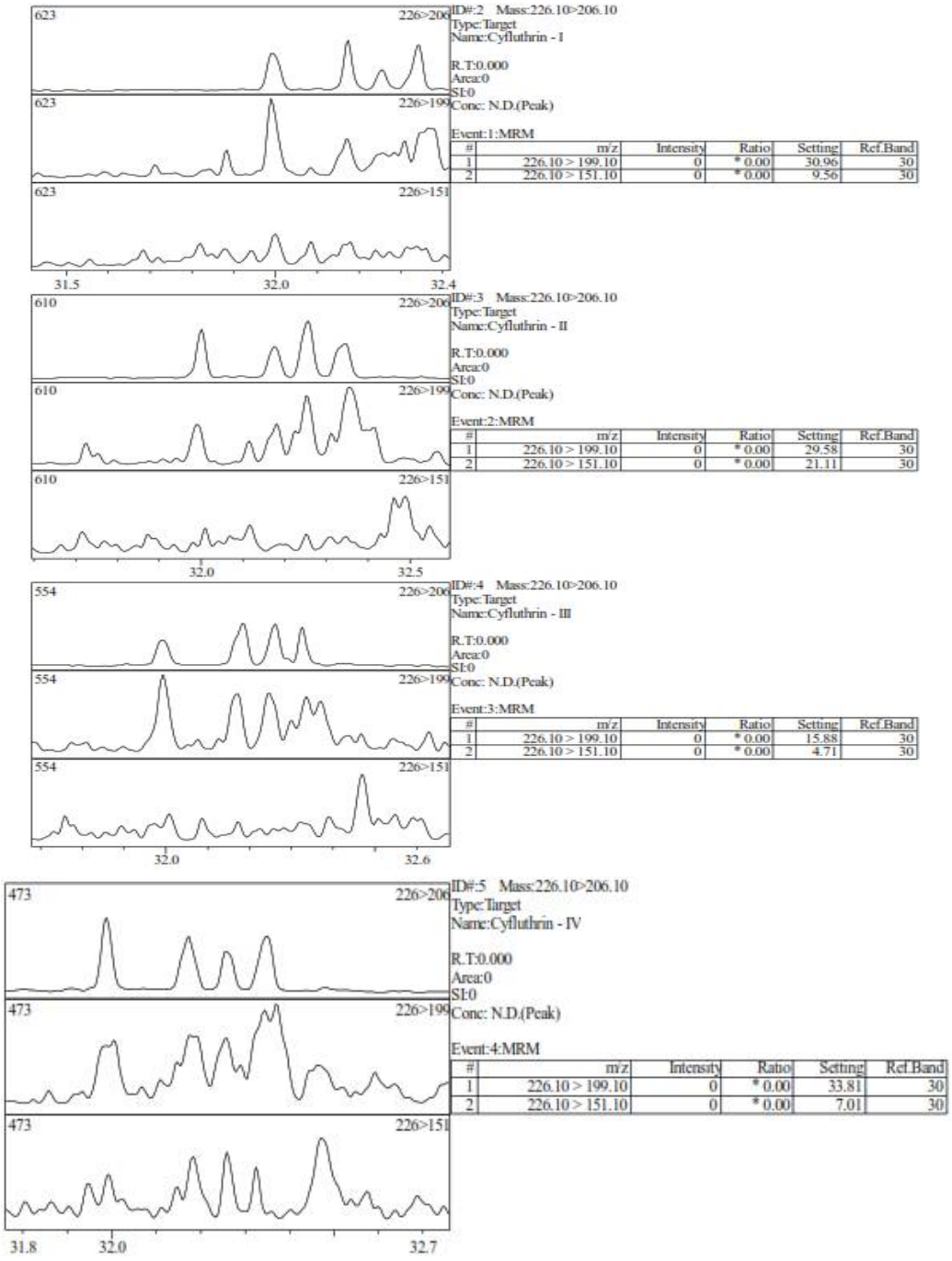
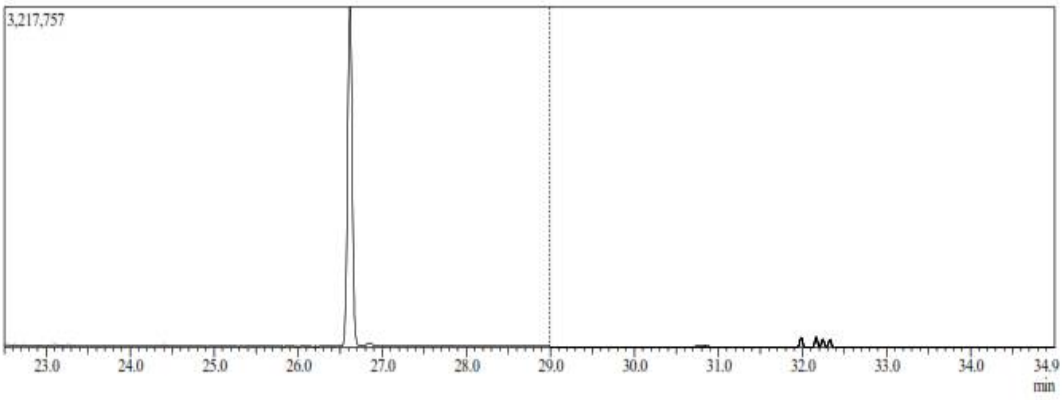
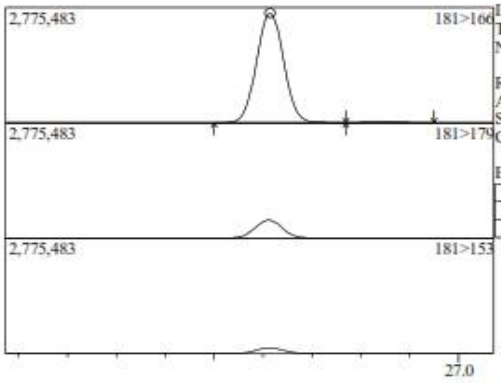



**Fig No. 1: Blank Chromatogram**




Quantitative Result Table

| ID# | Name | R.Time | m/z | Area | Height | Conc. | Conc.Unit |
|---|---|---|---|---|---|---|---|
| 1 | Bifenthrin | 26.615 | 181.10 > 166.10 | 10181815 | 2609450 | 99.523 | ppb |
| 2 | Cyfluthrin - I | 31.985 | 226.10 > 206.10 | 133315 | 52556 | 99.367 | ppb |
| 3 | Cyfluthrin - II | 32.164 | 226.10 > 206.10 | 127285 | 51987 | 100.296 | ppb |
| 4 | Cyfluthrin - III | 32.242 | 226.10 > 206.10 | 84389 | 37928 | 99.684 | ppb |
| 5 | Cyfluthrin - IV | 32.328 | 226.10 > 206.10 | 88485 | 38232 | 99.113 | ppb |

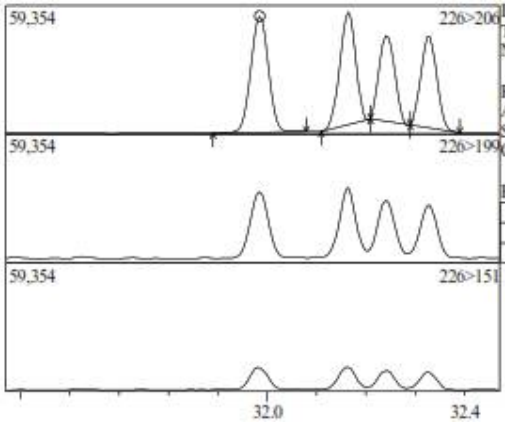



Quantitation
ID#:1 Mass:181.10>166.10
Type:Target
Name:Bifenthrin

R.T:26.615
Area:10181815
SI:44
Conc:99.52ppb

Event:1:MRM

| # | m/z | Intensity | Ratio | Setting | Ref.Band |
|---|---|---|---|---|---|
| 1 | 181.10 > 179.10 | 436764 | 16.22 | 18.05 | 30 |
| 2 | 181.10 > 153.10 | 139496 | 5.18 | 5.55 | 30 |

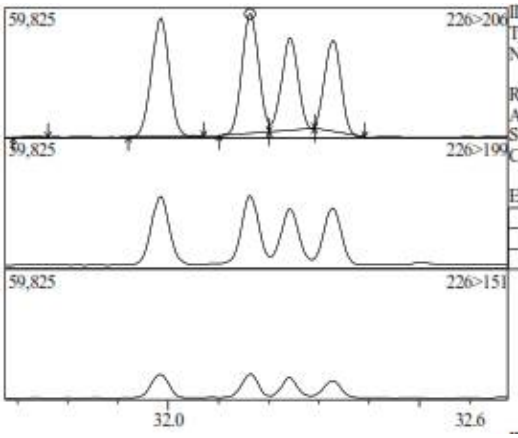



ID#:2 Mass:226.10>206.10
Type:Target
Name:Cyfluthrin - I

R.T:31.985
Area:133315
SI:53
Conc:99.37ppb

Event:1:MRM

| # | m/z | Intensity | Ratio | Setting | Ref.Band |
|---|---|---|---|---|---|
| 1 | 226.10 > 199.10 | 33128 | 58.09 | 30.96 | 30 |
| 2 | 226.10 > 151.10 | 10814 | 18.96 | 9.56 | 30 |

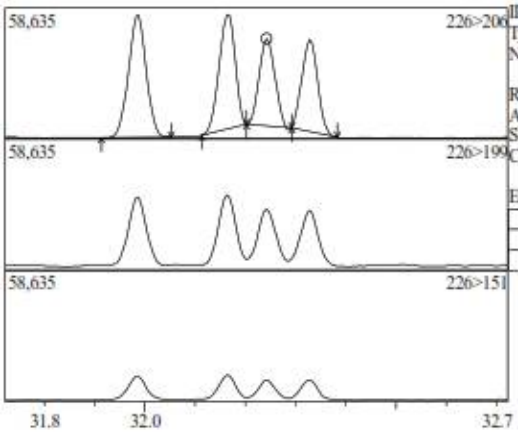



ID#:3 Mass:226.10>206.10
Type:Target
Name:Cyfluthrin - II

R.T:32.164
Area:127285
SI:51
Conc:100.30ppb

Event:2:MRM

| # | m/z | Intensity | Ratio | Setting | Ref.Band |
|---|---|---|---|---|---|
| 1 | 226.10 > 199.10 | 32745 | 56.01 | 29.58 | 30 |
| 2 | 226.10 > 151.10 | 11730 | 20.07 | 21.11 | 30 |

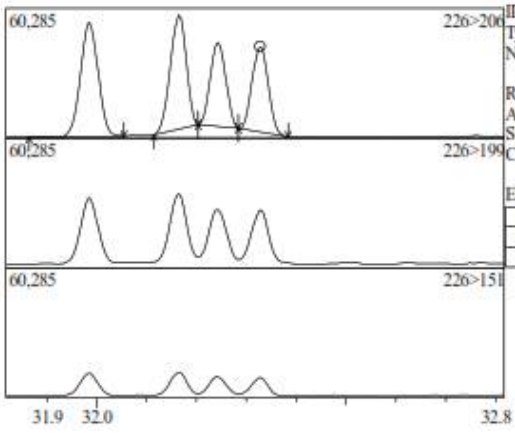



ID#:4 Mass:226.10>206.10
Type:Target
Name:Cyfluthrin - III

R.T:32.242
Area:84389
SI:52
Conc:99.68ppb

Event:3:MRM

| # | m/z | Intensity | Ratio | Setting | Ref.Band |
|---|---|---|---|---|---|
| 1 | 226.10 > 199.10 | 25224 | * 56.94 | 15.88 | 30 |
| 2 | 226.10 > 151.10 | 9047 | 20.42 | 4.71 | 30 |




ID#:5 Mass:226.10>206.10
Type:Target
Name:Cyfluthrin - IV

R.T:32.328
Area:88485
SI:51
Conc:99.11ppb

Event:4:MRM

| # | m/z | Intensity | Ratio | Setting | Ref.Band |
|---|---|---|---|---|---|
| 1 | 226.10 > 199.10 | 26280 | 60.22 | 33.81 | 30 |
| 2 | 226.10 > 151.10 | 9184 | 21.05 | 7.01 | 30 |

**Fig No.2: Standard Chromatogram**

## Linearity

A linear relationship should be evaluated across the range of the analytical procedure. It may be demonstrated directly on the analyte by dilution of a standard stock solution using the proposed procedure. Linearity should be evaluated by visual inspection of a plot of signals as a function of analyte concentration or content. If there is a linear relationship, test results should be evaluated by appropriate statistical methods by calculation of a regression line. The correlation coefficient, y-intercept, slope of the regression line should be calculated.

The total number of six concentration levels considered and inject the single injection of each concentration level to define a calibration graph. The acceptable value of the correlation coefficient (r2) should be more than 0.99 for correlation coefficient.

The accuracy of the standard solution at each level should be within 80% to 120%.

**Table I (Solvent linearity)**

| Sr. No. | Compounds Name | Range (ppb) | Correlation coefficient (r2) |
|---|---|---|---|
| 1 | Bifenthrin | 2.5 ppb - 100.0ppb | 0.999 |
| 2 | Cyfluthrin (I) | 2.5 ppb - 100.0ppb | 0.999 |
| | Cyfluthrin (II) | | 0.997 |
| | Cyfluthrin (III) | | 0.999 |
| | Cyfluthrin (IV) | | 0.997 |
| | Cyfluthrin | | 0.998 |

**Table II (Matrix linearity)**

| Sr. No. | Compounds Name | Range (ppb) | Correlation coefficient (r2) |
|---|---|---|---|
| 1 | Bifenthrin | 2.5 ppb - 100.0ppb | 0.999 |
| 2 | Cyfluthrin (I) | 2.5 ppb - 100.0ppb | 0.999 |
| | Cyfluthrin (II) | | 0.999 |
| | Cyfluthrin (III) | | 0.999 |
| | Cyfluthrin (IV) | | 0.999 |
| | Cyfluthrin | | 0.999 |

Correlation coefficient for the linearity curve of Pesticide Residues in Rice found >0.99. For details, refer Table - I & Table-II.

The area response increases with the increase in the concentration of the analyte.

Graph of concentration versus intensities is linear.

## Limit of detection (LOD)

It is the smallest amount or concentration of an analyte that can be estimated with acceptable reliability. The detection limit is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected.

The limit of detection is determined by establishing the signal to noise ratio. Inject the blank and standard solutions at lower concentration and calculate the signal to noise ratio. A signal-to-noise ratio between 3:1 estimating the detection limit.

The detection limit for Pesticide Residues in Rice found 0.0025mg/kg. For details, refer Table – III.

**Table III**

| Sr. No. | Compounds Name | Detection Limit (LOD) (mg/kg) |
|---|---|---|
| 1 | Bifenthrin | 0.0025 |
| 2 | Cyfluthrin | 0.0025 |

## Limit of quantitation (LOQ)

The Quantitation limit is generally determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision.

The limit of quantification is determined by establishing the signal to noise ratio. Inject the blank sample and the spiked sample at LOQ level in six replicates and calculate signal to noise ratio and the % RSD at LOQ level.

A signal-to-noise ratio between 10:1 estimating the quantification limit.

The quantification limit for Pesticide Residues in Rice found 0.005mg/kg. For details, refer Table – IV.

**Table IV**

| Sr. No. | Compounds Name | Quantitation Limit (LOQ) (mg/kg) |
|---|---|---|
| 1 | Bifenthrin | 0.005 |
| 2 | Cyfluthrin | 0.005 |

## Recovery

Recovery means the percentage of the true concentration of a substance recovered during the analytical procedure.

Recovery assessed using a minimum of 6 determinations over a minimum of 3 concentration levels (3 concentrations/6 replicates each of the total analytical procedure).

Acceptable limits for a recovery result during validation should be within the range of 70% - 120%.However the lower recovery may be acceptable if the results are consistent (i.e. good precision).

The percentage of average recovery for Pesticide Residues in Rice found >70%. For details, refer Table –V. The % Recovery at 0.005 mg/kg, 0.010 mg/kg and 0.020 mg/kg found >80%. For details, refer Table – VI.

| Bifenthrin | | | | | | | |
|---|---|---|---|---|---|---|---|
| Conc.(ppb) | Replicates | Recovery found | Sample wt. (gm) | Dilution (ml) | % Recovery Observed | | |
| 5 | 1 | 5.06 | 10.0523 | 10 | 100.63 | Avg | 99.60 |
| 5 | 2 | 4.88 | 10.0631 | 10 | 97.01 | | |
| 5 | 3 | 5.05 | 10.1032 | 10 | 100.05 | Stdev | 2.02 |
| 5 | 4 | 5.19 | 10.2595 | 10 | 101.25 | | |
| 5 | 5 | 5.08 | 10.4516 | 10 | 97.13 | Rsd % | 2.03 |
| 5 | 6 | 5.12 | 10.0813 | 10 | 101.51 | | |
| 10 | 1 | 10.77 | 10.0051 | 10 | 107.68 | Avg | 105.18 |
| 10 | 2 | 10.68 | 10.1432 | 10 | 105.32 | | |
| 10 | 3 | 10.35 | 10.0371 | 10 | 103.10 | Stdev | 1.51 |
| 10 | 4 | 10.64 | 10.1027 | 10 | 105.30 | | |
| 10 | 5 | 10.60 | 10.0596 | 10 | 105.37 | Rsd % | 1.43 |
| 10 | 6 | 10.48 | 10.0464 | 10 | 104.32 | | |
| 20.0 | 1 | 20.23 | 10.0468 | 10 | 100.68 | Avg | 100.91 |
| 20.0 | 2 | 20.48 | 10.0813 | 10 | 101.56 | | |
| 20.0 | 3 | 20.89 | 10.1681 | 10 | 102.71 | Stdev | 2.00 |
| 20.0 | 4 | 20.78 | 10.0927 | 10 | 102.93 | | |
| 20.0 | 5 | 20.34 | 10.1653 | 10 | 100.06 | Rsd % | 1.98 |
| 20.0 | 6 | 19.67 | 10.0864 | 10 | 97.53 | | |
| | | | | Avg | 101.90 | | |
| | | | | Stdev | 3.01 | | |
| | | | | Rsd % | 2.95 | | |

| Cyfluthrin | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Conc.(ppb) | Replicates | % Rec. Cyf (I) | % Rec. Cyf (II) | % Rec. Cyf (III) | % Rec. Cyf (IV) | % Rec. Cyfluthrin | | |
| 5 | 1 | 100.73 | 93.67 | 82.69 | 72.28 | 87.34 | Avg | 92.96 |
| 5 | 2 | 105.04 | 95.22 | 94.42 | 84.55 | 94.81 | | |
| 5 | 3 | 91.00 | 100.56 | 101.16 | 81.52 | 93.56 | Stdev | 2.81 |
| 5 | 4 | 79.44 | 119.40 | 110.06 | 70.18 | 94.77 | | |
| 5 | 5 | 77.96 | 108.21 | 79.18 | 109.13 | 93.62 | Rsd % | 3.03 |
| 5 | 6 | 80.03 | 94.25 | 94.17 | 106.26 | 93.68 | | |
| 10 | 1 | 109.52 | 109.22 | 100.83 | 100.80 | 105.09 | Avg | 104.59 |
| 10 | 2 | 105.59 | 115.04 | 104.03 | 89.87 | 103.63 | | |
| 10 | 3 | 100.06 | 117.42 | 103.45 | 97.50 | 104.61 | Stdev | 0.60 |
| 10 | 4 | 105.36 | 109.80 | 110.25 | 92.37 | 104.44 | | |
| 10 | 5 | 103.81 | 114.59 | 97.98 | 101.25 | 104.41 | Rsd % | 0.58 |
| 10 | 6 | 106.80 | 108.43 | 103.98 | 102.26 | 105.37 | | |
| 20.0 | 1 | 101.92 | 107.11 | 105.04 | 99.38 | 103.37 | Avg | 102.78 |
| 20.0 | 2 | 104.83 | 107.66 | 105.25 | 99.36 | 104.28 | | |
| 20.0 | 3 | 101.91 | 106.01 | 110.10 | 107.34 | 106.34 | Stdev | 2.37 |
| 20.0 | 4 | 104.84 | 100.15 | 103.46 | 99.62 | 102.02 | | |
| 20.0 | 5 | 99.83 | 105.59 | 98.18 | 95.76 | 99.84 | Rsd % | 2.31 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 20.0 | 6 | 96.75 | 102.02 | 105.31 | 99.39 | 100.87 | | |
| | | | | | Avg | 100.11 | | |
| | | | | | Stdev | 5.633 | | |
| | | | | | Rsd % | 5.626 | | |

**Table V**

| Sr. No. | Compounds Name | Average %Recovery |
|---|---|---|
| 1 | Bifenthrin | 101.90 |
| 2 | Cyfluthrin | 100.11 |

**Table VI**

| Sr.No. | Compounds | %Recovery at each Level | | |
|---|---|---|---|---|
| | | 5 µg/kg | 10 µg/kg | 20 µg/kg |
| 1 | Bifenthrin | 99.60 | 105.18 | 100.91 |
| 2 | Cyfluthrin | 92.96 | 104.59 | 102.78 |

## Precision

The precision determined under equal conditions with same homogeneous spiked sample (six different sample preparation) as per recommended test method and %RSD of the results obtained shall be calculated.

The repeatability is established by estimating the six replicates of spiked sample and calculates the %RSD.

The %RSD for the analysis of spiked sample should not be more than 20%.

The % RSD at three concentration levels ( 5 µg/kg, 10 µg/kg, and 20 µg/kg) found <20%. Global %RSD found <20%. For details, refer Table – VII and Table – VIII.

| Bifenthrin | | | | | | | |
|---|---|---|---|---|---|---|---|
| Conc.(ppb) | Replicates | Recovery found | Sample wt. (gm) | Dilution (ml) | % Recovery Observed | | |
| 5 | 1 | 5.05 | 10.0523 | 10 | 100.55 | Avg | 99.98 |
| 5 | 2 | 5.00 | 10.0631 | 10 | 99.29 | | |
| 5 | 3 | 5.12 | 10.1032 | 10 | 101.43 | Stdev | 2.38 |
| 5 | 4 | 5.27 | 10.2595 | 10 | 102.77 | | |
| 5 | 5 | 5.01 | 10.4516 | 10 | 95.77 | Rsd % | 2.38 |
| 5 | 6 | 5.04 | 10.0813 | 10 | 100.05 | | |
| 10 | 1 | 10.48 | 10.0051 | 10 | 104.70 | Avg | 103.80 |
| 10 | 2 | 10.50 | 10.1432 | 10 | 103.50 | | |
| 10 | 3 | 10.43 | 10.0371 | 10 | 103.86 | Stdev | 0.69 |
| 10 | 4 | 10.47 | 10.1027 | 10 | 103.60 | | |
| 10 | 5 | 10.34 | 10.0596 | 10 | 102.76 | Rsd % | 0.66 |
| 10 | 6 | 10.49 | 10.0464 | 10 | 104.38 | | |
| 20.0 | 1 | 19.25 | 10.0468 | 10 | 95.82 | Avg | 97.74 |
| 20.0 | 2 | 19.68 | 10.0813 | 10 | 97.63 | | |
| 20.0 | 3 | 20.17 | 10.1681 | 10 | 99.19 | Stdev | 2.07 |
| 20.0 | 4 | 20.01 | 10.0927 | 10 | 99.13 | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 20.0 | 5 | 20.31 | 10.1653 | 10 | 99.91 | Rsd % | 2.12 |
| 20.0 | 6 | 19.12 | 10.0864 | 10 | 94.76 | | |
| | | | | Avg | 100.51 | | |
| | | | | Stdev | 3.11 | | |
| | | | | Rsd % | 3.10 | | |

| **Cyfluthrin** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Conc.(ppb) | Replicates | % Rec. Cyf (I) | % Rec. Cyf (II) | % Rec. Cyf (III) | % Rec. Cyf (IV) | % Rec. Cyfluthrin | | |
| 5 | 1 | 105.79 | 89.03 | 80.54 | 89.05 | 91.10 | Avg | 97.52 |
| 5 | 2 | 106.98 | 100.19 | 74.59 | 105.91 | 96.92 | | |
| 5 | 3 | 109.98 | 111.63 | 76.21 | 97.71 | 98.88 | Stdev | 4.77 |
| 5 | 4 | 105.83 | 102.93 | 78.11 | 108.76 | 98.91 | | |
| 5 | 5 | 100.35 | 103.08 | 80.85 | 92.48 | 94.19 | Rsd % | 4.89 |
| 5 | 6 | 110.34 | 116.65 | 86.73 | 106.63 | 105.09 | | |
| 10 | 1 | 107.07 | 92.28 | 95.55 | 105.89 | 100.20 | Avg | 100.89 |
| 10 | 2 | 109.67 | 102.57 | 99.45 | 97.18 | 102.22 | | |
| 10 | 3 | 108.16 | 100.31 | 84.48 | 93.65 | 96.65 | Stdev | 3.46 |
| 10 | 4 | 108.27 | 105.35 | 98.31 | 89.66 | 100.40 | | |
| 10 | 5 | 106.86 | 94.53 | 103.83 | 90.75 | 98.99 | Rsd % | 3.43 |
| 10 | 6 | 102.15 | 112.05 | 100.15 | 113.15 | 106.87 | | |
| 20.0 | 1 | 103.21 | 91.31 | 99.11 | 92.65 | 96.57 | Avg | 96.66 |
| 20.0 | 2 | 97.38 | 89.67 | 99.27 | 88.14 | 93.62 | | |
| 20.0 | 3 | 106.29 | 91.60 | 101.51 | 96.35 | 98.94 | Stdev | 2.12 |
| 20.0 | 4 | 100.41 | 92.77 | 94.80 | 98.08 | 96.51 | | |
| 20.0 | 5 | 99.64 | 97.17 | 92.72 | 106.85 | 99.10 | Rsd % | 2.19 |
| 20.0 | 6 | 98.23 | 90.94 | 95.72 | 95.99 | 95.22 | | |
| | | | | | **Avg** | 98.35 | | |
| | | | | | Stdev | 3.881 | | |
| | | | | | **Rsd %** | 3.946 | | |

**Table VII**

| Sr. No. | Compounds Name | Average %Recovery | %RSD |
|---|---|---|---|
| 1 | Bifenthrin | 100.51 | 3.10 |
| 2 | Cyfluthrin | 98.35 | 3.95 |

**Table VIII**

| Sr.No. | Compounds | | %RSD | |
|---|---|---|---|---|
| | | 5 µg/kg | 10 µg/kg | 20 µg/kg |
| 1 | Bifenthrin | 2.38 | 0.66 | 2.12 |
| 2 | Cyfluthrin | 4.89 | 3.43 | 2.19 |

## DISCUSSION

A chromatographic method involves demonstrating specificity, which is the ability of the method to accurately measure the analyte response in the presence of all potential sample components. The chromatographic and mass spectroscopy parameters were fixed and GC-MS/MS system was studied for suitability of residual analysis. The developed method was performed for linearity, precision, Accuracy, specificity, LOD and LOQ.

## CONCLUSION

A simple and sensitive method for the determination pyrethroid residues by using GC-MS/MS was developed, validated and applied for the analysis of rice samples. The samples were extracted with water and acetonitrile mixture and little matrix effect on MS detection was eliminated by following dispersive SPE clean up. The method was validated to ensure the feasibility of the method for its application in routine analysis. The LOQs achieved through this method were lower than the MRLs established by the FSSAI and EU legislations.

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