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Analytical method development of a formulation of transfluthrin, piperonyl butoxide, and cyfluthrin by GC-FID analytical technique

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ABSTRACT

A simple validated Gas Chromatography-Flame Ionization (GC-FID) method has been created for a mixture of insecticide and a chemical separation and quantitative measurement of Transfluthrin, Piperonyl butoxide, and Cyfluthrin formulation. Because it is time-saving, dependable, cost-effective, and repeatable, this GC approach is required. Developing a simple and cost-effective approach is difficult; yet, a GC analytical method validates one's capacity to meet regulatory / SANCO standards. In the current scenario, this pesticide mixture is necessary to manage all pesticides with a single dose application. Insecticides like Transfluthrin, Piperonyl butoxide, and Cyfluthrin are used to efficiently manage insects in crop plants. There are limited separation and determination analytical methods for such an important application of combined pesticides. The simple analytical instrument GC with Flam Ionisation Detector (GC/FID) used to detect 0.01ppm LOQ level, this approach analyses Transfluthrin, Piperonyl butoxide, and Cyfluthrin mixtures. A Rxi-5ms capillary column was used to isolate these three compounds. A 30m capillary column with a 0.25mm ID and 0.50m length was loaded into the Shimadzu GC (model: GC-2010). A pre-installed, confirmed GC solution software equipped Shimadzu instrument was used for separation and quantification, as well as data processing and computations. These three chemicals were identified and quantified separately. In a single study, the simple GC method provides a quick way to determine the total amount of these three compounds.

Keywords: *Transfluthrin, Piperonyl Butoxide and Cyfluthrin Gc-Fid Analysis, Validated Method, Sante/2020/12830 And Ich Guideline.*

1. INTRODUCTION

The goal of this GC/FID Analytical Method Development (AMD) is to provide an analytical process that is simple, cost-effective, repeatable, and time-saving. As a result, an analytical method based on a GC system and a low-cost simple flame ionization detector was created. Within a single injection, our GC method will isolate and properly determine these Transfluthrin, Piperonyl butoxide, and Cyfluthrin molecules from their formulation.

Analytical procedures are critical for identifying and determining the proper application of chemical substances that we encounter in our daily lives. Because all biological and chemical processes occur in a symbiotic manner throughout the life cycle, with the proper proportions and combinations, the next objective is reached. In terms of active content and impurity determinations, the gas chromatography analytical method is significant equipment for determining halogenated, volatile organic substances such as pesticides, drug molecules, industrial chemicals, residual solvents, and more. As a result, the analytical method is critical in any analysis in the environmental, pharmaceutical, insecticide, chemical, and other industries. Sample preparation is an important framework for achieving a successful analytical method.

The developed analytical method has been thoroughly validated in accordance with the SANTE/2020/12830/2021 Rev.1 guideline. A simple GC method was used to test the validation parameters specificity (selectivity), linearity, LOD & LOQ, accuracy (recovery), precision (repeatability) and comply with Horwitz equation for RSD percentage.

2. MATERIALS AND METHOD

Reference standard

The reference standards *viz.;* transfluthrin, from Dr.Ehrenstorfer (batch number: G164132, purity: 98.47%; β-cyfluthrin, from Sigma – Aldrich (batch nuber: BCBV1615, purity: 99.30% and piperonylbutoxide from Sigma – Aldrich (batch number: BCBX0805, purity 98.60%.

Reagents and chemicals used
GC grade of acetone from Finar, batch number: 268461102JR with purity 99.90% has been used for the entire analysis.

Glassware
A calibrated (class A) pipette and volumetric flasks (glassware) were used in the entire analysis.

Instrument
In this study, a calibrated Gas Chromatography (FID –detector / detector response, column oven, injection volume injection, temperature, and detector temperature) made by Shimadzu, model Nexis, GC-2030 with auto sampler (AOC 20Si) was utilized. The analysis for the peak processes is done with GC solution software. The instrument is in good working order. Flame Ionization Detector (FID); GC column: SH-Rxi-5ms (30 mm 0.25 mm); Injection Temperature: 200°C; Column Oven Temperature: 250°C, Solvent Acetone; Injection volume: 1.0l; nitrogen flow rate: 34 ml/min Transfluthrin has a retention time of 8.1 minutes, piperonyl butoxide has a retention time of 13.4 minutes, and cyflthrin isomers have a retention time of cyflthrin isomers of cyflthrin isomers of cyflthrin isomers of cyflthrin isomers of c Cyfluthrin isomer 1 has a retention time of 21.0 minutes; Cyfluthrin isomer 2 has a retention time of 21.5 minutes; Cyfluthrin isomer 3 has a retention time of 21.7 minutes; Cyfluthrin isomer 4 has a retention time of 21.9 minutes; and the overall GC run time is 25 minutes.

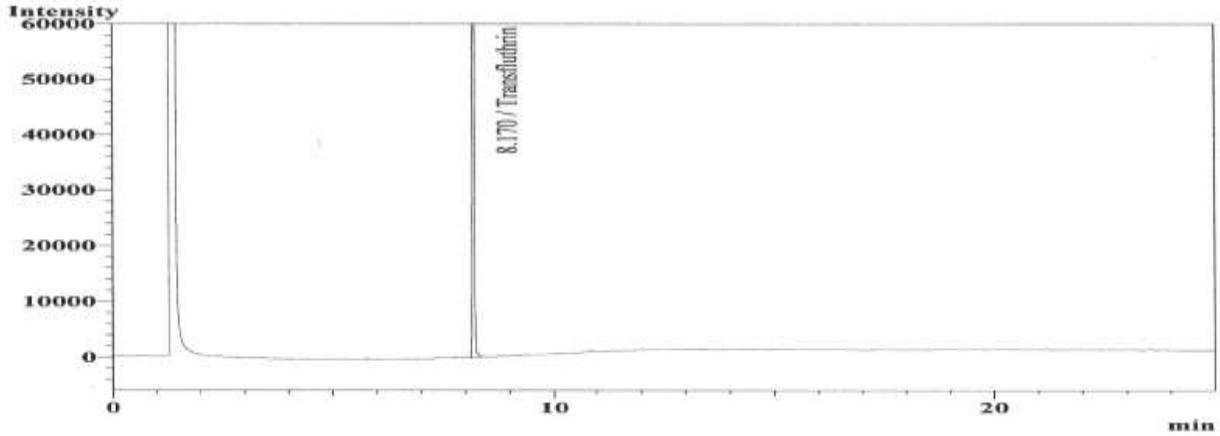
3. ANALYTICAL METHOD VALIDATION

Specificity

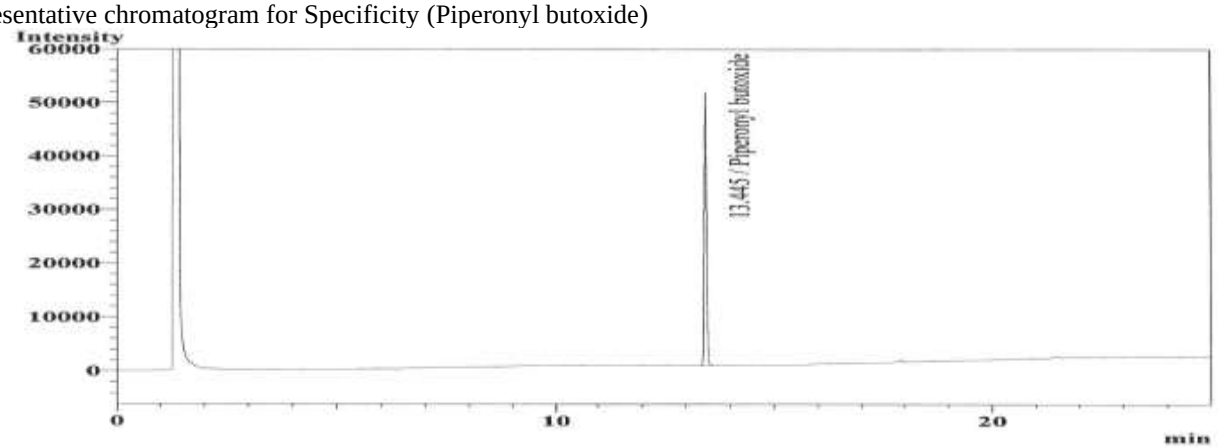
Preparation of standard stock solutions
An amount of 10.16 mg, 10.15 mg and 10.08mg of Transfluthrin, Piperonyl butoxide and β-Cyfluthrin reference standards with purity 98.47%, 98.6%, and 99.3% were weighed transferred into clean and empty dry 10 mL volumetric flasks separately, dissolved and made up to the mark with acetone. From these solutions prepared 100 mg/L concentration solutions prepared and analyzed to determine specificity.

Preparation of Sample Solution
The Specificity of GC method for Transfluthrin, Piperonyl butoxide and Cyfluthrin formulation mixture sample was determined by injecting (10mg/L) the Standard and Sample solutions along with blank (Acetone) and observed that there was no interference found with main peak of interest. Hence, this method was considered to be specific for the analysis of the test substance.

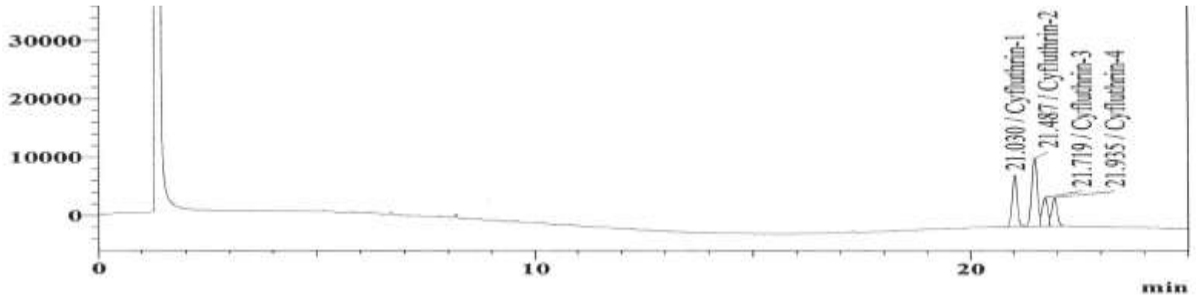
Representative chromatogram for Specificity (Transfluthrin)



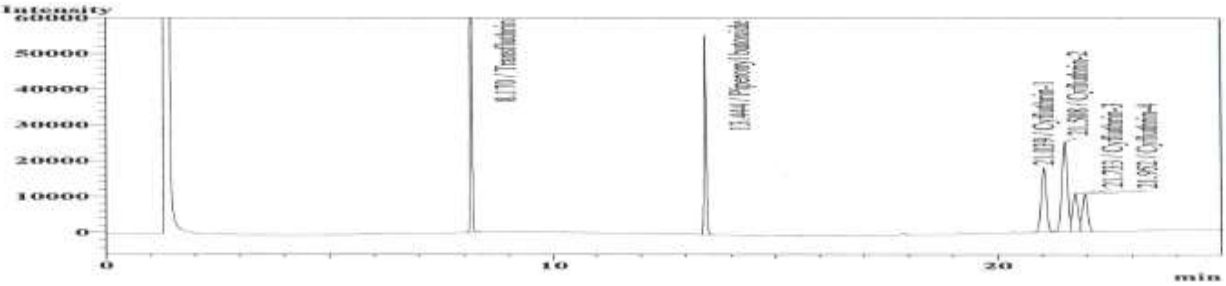
Representative chromatogram for Specificity (Piperonyl butoxide)



Representative chromatogram for Specificity (β-Cyfluthrin)



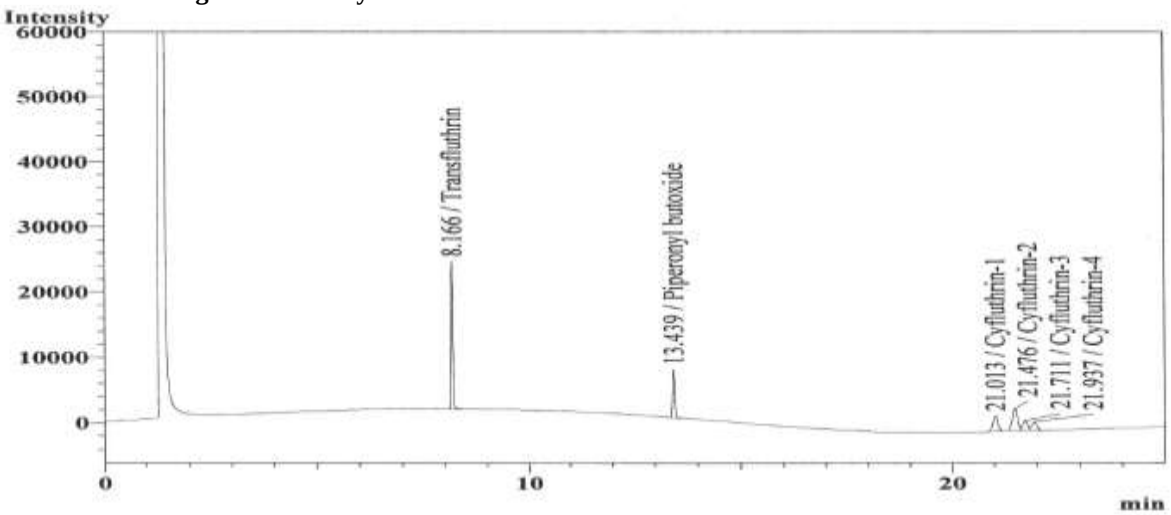
Representative chromatogram for Specificity (Transfluthrin, Piperonyl butoxide and β-Cyfluthrin)



Preparation of calibration / linearity Solutions

The standard solution (100 mg/L) was used for the determination of Linearity, from the 100ml/L standard prepared further concentrations such as 1, 5, 10, 15, 20 and 25 mg/L. Analysed by GC-FID, The results are presented in table and the respective chromatograms.

Representative chromatogram Linearity



Peak Table - Channel 1

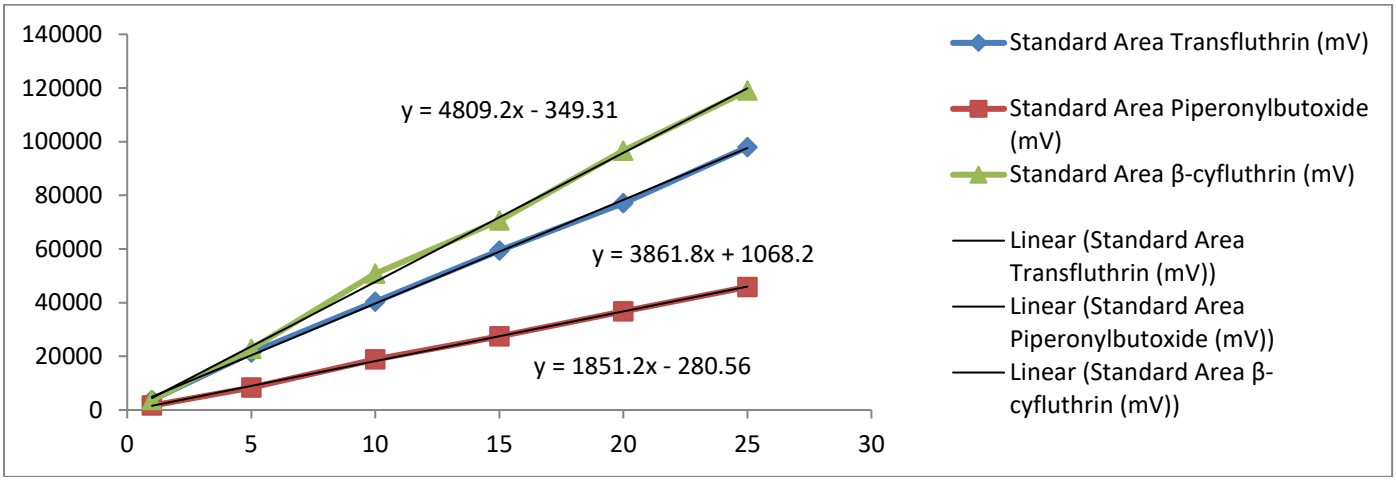
Peak#	Ret.Time	Area	Area%	Height	Height%	Name
1	8.166	59430	37.7324	22493	58.5664	Transfluthrin
2	13.439	27492	17.4545	7420	19.3191	Piperonyl butoxide
3	21.013	19623	12.4589	2347	6.1122	Cyfluthrin-1
4	21.476	27796	17.6480	3338	8.6909	Cyfluthrin-2
5	21.711	12115	7.6921	1474	3.8373	Cyfluthrin-3
6	21.937	11048	7.0141	1334	3.4741	Cyfluthrin-4
Total		157504	100.0000	38406	100.0000	

Linearity of Transfluthrin, Piperonylbutoxide and β-Cyfluthrin Reference Standards

Std. Code	Concentration (mg/L)	Stdard Area Transfluthrin (mV)	Standard area Piperonylbutoxide (mV)	Standard area β-cyfluthrin (mV)
STD-1	1	3748	1631	3678
STD-2	5	21370	8410	22765
STD-3	10	40379	18892	50787
STD-4	15	59430	27492	70582
STD-5	20	77056	36729	96600
STD-6	25	97921	45857	118995
Intercept		1068.2	-280.6	-349.3
Slope		3861.8	1851.2	4809.2

Correlation Coefficient	0.9996	0.9997	0.9993

Figure: Linearity Curve of Transfluthrin, Piperonylbutoxide and B-Cyfluthrin Reference Standard



The linearity of the method was established by auto injecting six different concentrations of Transfluthrin, Piperonyl butoxide, and -Cyfluthrin reference standard by GC and plotting the respective Concentration (mg/L) against their respective peak areas, with the correlation coefficient (r) of 0.9996 for Transfluthrin, 0.9997 for Piperonyl butoxide, and 0.9993 for -Cyfluthrin, and the correlation.

Precision: Preparation of Standard Solution and sample solution for precision
The 10mg/L standard solution was used for the precision determination. An amount of 6.45 and 10.5 mg Test substance were weighed accurately into a clean and dry 100 mL volumetric flask, dissolved the contents with Acetone and made up to the mark with Acetone. These solutions were equivalent to 64.50 and 105.0 mg/L. These prepared solutions were injected into GC and % RSD was calculated and the results are presented in the table.

Precision for Transfluthrin

Sample ID	Std. Conc. (mg/L)	Std. / Sample Area (mV)	Average Std. Area (mV)	Sample Conc. (mg/L)	Purity of Calibration solution (P) %	A.I. Content (%)
Std-R1	10	38722	39042	-	100	-
P1R1		5237		64.500		2.08
P1R2		5183				2.06
P1R3		5160				2.05
P1R4		5132				2.04
P1R5		5168				2.05
P2R1		8102		105.000		1.98
P2R2		7698				1.88
P2R3		8580				2.09
P2R4		8510				2.08
P2R5		8638				2.11
Std-R2		39362		-		-
MEAN						

The % RSD is within limit according to the modified Horwitz equation (Acceptable Limit <2.41% RSD for 2.04% active analyte as per SANCO/3030/99 Rev.4)

Precision for Piperonylbutoxide

Sample ID	Std. Conc. (mg/L)	Std. / Sample Area (mV)	Average Std. Area (mV)	Sample Conc. (mg/L)	Purity of Calibration solution (P) %	A.I. Content (%)
Std-R1	10	17473	17897	-	100	-
P1R1		9394		64.500		8.14
P1R2		9368				8.12
P1R3		9376				8.12
P1R4		9373				8.12

P1R5		9368				8.12
P2R1		15317		105.000		8.15
P2R2		15220				8.10
P2R3		15349				8.17
P2R4		15358				8.17
P2R5		15335				8.16
Std-R2		18321		-		-
MEAN						

The % RSD is within limit according to the modified Horwitz equation (Acceptable Limit <1.95% RSD for 8.14% active content as per SANCO/3030/99 Rev.4)

Precision for β-cyfluthrin

Sample ID	Std. Conc. (mg/L)	Std. / Sample Area (mV)	Average Std. Area (mV)	Sample Conc. (mg/L)	Purity of Calibration solution (P) %	A.I. Content (%)
Std-R1	10	46402	45885	-	100	-
P1R1		23593		64.500		7.97
P1R2		23659				7.99
P1R3		23623				7.98
P1R4		23813				8.05
P1R5		23695				8.01
P2R1		38726		105.000		8.04
P2R2		38396				7.97
P2R3		38588				8.01
P2R4		38690				8.03
P2R5		38292				7.95
Std-R2		45367		-		-

The RSD (%) is within limit according to the modified Horwitz equation (Acceptable Limit <1.96% RSD for 8.00% active analyte as per SANCO/3030/99 Rev.4)

Recovery : The analytical method was validated in terms of recovery of the standard at two fortification levels.

Preparation of Standard Solution: The 10mg/L standard solution was used for the accuracy determination.

Preparation of Fortification Level 1 (1 mg/L): An aliquot 0.1 ml Specificity standard solution (100 mg/L) was transferred into a clean and dry 10 mL volumetric flask, fortified in distilled water and made upto the mark with distilled water. This solution was equivalent to 1 mg/L.

Preparation of Fortification Level 2 (10 mg/L): An aliquot 1 ml Specificity standard solution (100 mg/L) was transferred into a clean and dry 10 mL volumetric flask, fortified in distilled water and made upto the mark with distilled water. This solution was equivalent to 10 mg/L.

The above two fortified concentrations were concentrated using rotary vacuum evaporator near to dryness and diluted appropriately with Acetone and injected into GC for recovery analysis and these results are presented in following table.

Recovery/Accuracy (Level-1 & 2) For Transfluthrin

Fortification Level	Std. Conc. (mg/L)	Std. / Sample Area (mV)	Mean Std. Area (mV)	Detected Conc. (mg/L)	Fortified Conc. (mg/L)	Recovery (%)
Std-R1	10	38922	39940	-	1.0	-
F1R1		3996		1.001		100.05
F1R2		3974		0.995		99.50
F1R3		4010		1.004		100.40
F1R4		4034		1.010		101.00
F1R5		4020		1.007		100.65
F2R1		39999		10.015	10	100.15

F2R2		40183		10.061		100.61
F2R3		40053		10.028		100.28
F2R4		39829		9.972		99.72
F2R5		40418		10.120		101.20
Std-R2		40958		-		-

Recovery/Accuracy (level-1 & 2) for Piperonyl butoxide

Fortification Level	Std. Conc. (mg/L)	Std. / Sample Area (mV)	Mean Std. Area (mV)	Detected Conc. (mg/L)	Fortified Conc. (mg/L)	Recovery (%)
Std-R1	10	17727	18237	-	1.0	-
F1R1		1797		0.985		98.54
F1R2		1788		0.980		98.04
F1R3		1807		0.991		99.08
F1R4		1809		0.992		99.19
F1R5		1811		0.993		99.30
F2R1		18140		9.947	10	99.47
F2R2		18100		9.925		99.25
F2R3		18111		9.931		99.31
F2R4		18129		9.941		99.41
F2R5		18125		9.939		99.39
Std-R2		18747		-		-

Recovery/Accuracy (Level-1 & 2) for B-Cyfluthrin

Fortification Level	Std. Conc. (mg/L)	Std. / Sample Area (mV)	Mean Std. Area (mV)	Detected Conc. (mg/L)	Fortified Conc. (mg/L)	Recovery (%)
Std-R1	10	45254	47526	-	1.0	-
F1R1		4770		1.004		100.37
F1R2		4753		1.000		100.01
F1R3		4775		1.005		100.47
F1R4		4797		1.009		100.94
F1R5		4711		0.991		99.13
F2R1		47518		9.998	10	99.98
F2R2		48470		10.199		101.99
SF2R3		48255		10.153		101.53
F2R4		47577		10.011		100.11
F2R5		47694		10.035		100.35
Std-R2		49797		-		-

Limit of Detection (LOD) & Limit of Quantification (LOQ)

The lowest concentration of linearity standard solution-1 signal to noise ratio used for limit of detection (LOD) and limit of quantification (LOQ) determination.

Formula:

$$\text{LOQ (mg/L)} = \frac{10 \times C_{std.}}{S/N} \qquad \text{LOD (mg/L)} = \frac{3 \times C_{std.}}{S/N}$$

Where:

C std. = Concentration of linearity standard solution & S/N = Signal to noise ratio.

Calculation results for Transfluthrin; LOD 0.304 & LOQ 1.01 mg/L; For Piperonyl butoxide LOD 0.301 & LOQ 1.01 mg/L and for β-Cyfluthrin LOD 0.301 & LOQ 1.00 mg/L.

4. FORMULATION SAMPLE ANALYSIS

Preparation of Standard and sample solutions

The standard solution 5 mg/L was used for the determination of active content verification analysis. The formulation saple also the same concentration was prepared and analysed b GC instrument. For the active content determination the following formula was used and found that the labled active content are matching with the determined results of this formulation sample.

$$\text{Detected Concentration (mg/L)} = \frac{A \times B \times DF}{C}$$

Where,

A - Concentration of standard (mg/L); B - Area of sample solution; C - Area of standard solution; DF - Dilution Factor

5. CONCLUSION

Specificity: The blank, standard and the sample peaks were not interfered each other, hence the specificity were achieved as per the guideline SANCO 3030/99 Rev.4 requirement.

Linearity: The Linearity correlation co-efficient is achieved NLT 0.99 as per (SANCO 3030/99 Rev.4

System Precision: The system precision is achieved as the % RDS for all replicates observed as 1% for Transfluthrin, Piperonyl butoxide and Cyfluthrin, hence the minimum requirement of the (SANCO 3030/99 Rev.4 was NMT 15% RSD was achieved

System Recovery: The system recovery 100 % for all the three Transfluthrin, Piperonyl butoxide and Cyfluthrin, hence the minimum requirement of the (SANCO 3030/99 Rev.4. complies

LOD & LOQ determination: The LOD and LOQ was determined as 0.304 and 1.01 mg/L respectively for all the three molecules per the Signal to Noise ratio (S/N) calculation method

Horwitz Equation The % RSD is within limit according to the modified Horwitz equation (Acceptable Limit <2.41% RSD for 2.04% active analyte as per SANCO/3030/99 Rev.4

Details of the Laboratory work were carried out.

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