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Analytical method development of mixed pesticide Mesotrione 4% + S -Metolachlor 40% + Benoxacor 2% SE by HPLC analytical technique

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ABSTRACT

A simple reverse phase liquid chromatographic method has been developed and subsequently validated for the Mesotrione + S -Metolachlor + Benoxacor combination pesticide molecule. These molecules mixtures have been used as an effective weedicide, microbial control effectively in the presence of a chemical. The combination products Mesotrione + S -Metolachlor + Benoxacor is being used as a pesticide to manage at a time soil, crop plants, and weed management effectively. Such an important application of these combination products has fairly few analytical methods. These Mesotrione 4% + S -Metolachlor 40% + Benoxacor 2% molecules were separated through a mobile phase consisting of the mixture of acetonitrile and water in the ratio of 80:20 (v/v). The Column used in this separation is Shimadzu BDS C18; 250 mm length; 4.6 mm diameter with 5µ particle size dimensioned column; Flow rate: 1.0 ml/min; Detector: UV-Vis. Absorption (λ) at 230 nm of Shimadzu HPLC (model: LC-2030). The LC solution software was used for the analytical method, data integrations, and calculations in this analysis. There are two molecules were analyzed for separation and quantification. The results of the study showed that the proposed HPLC method is simple, rapid, precise and accurate, which is useful for the identification and quantifications of these molecules interims of validation parameters viz., separation, system suitability, System Precision and linearity in a simple HPLC analysis.

Keywords— *Mesotrione, S –Metolachlor, Benoxacor HPLC analysis, Validated method, SANCO 3030/99 Rev.4, ICH guideline*

1. INTRODUCTION

The aim of this analytical Method Development (AMD) is that to develop a simple, cost-effective, reproducible and less time consumed analytical method. Hence this analytical method was developed with an HPLC instrument. This HPLC method will be providing a solution to quantification of these three molecules (Mesotrione + S -Metolachlor + Benoxacor) within a single injection.

The analytical method is important to tool to determine the quality in terms of purity. The Genotoxicity molecule limitation is very stringent and hence it is very important to determine accurately to prevent the RNA and DNA damages. Hence the analytical method is very important in any analysis in terms of environmental, pharmaceutical, pesticides, chemical and other industries too for a good analysis sample preparation if the important base to achieve the good analytical method

Sample preparation is based upon the property of solubility of the molecule in a particular solvent. The mixed pesticide Mesotrione 4% + S -Metolachlor 40% + Benoxacor 2% were different in chemicals. Acetonitrile solvent was selected to dissolve these three molecules based upon its solubility nature. Any formulation contains filler, placebo, coloring agent, binding agent, dispersing agent and another matrix as required by the manufacturer. Hence separation of three molecules was challenging by HPLC analysis.

The Mesotrione is a herbicide and belonging to the Benzoylcyclohexanedione chemical class. This molecule Mesotrione alone is being used as a microbial controller in the soil management activity. The molecule Metolachlor also an herbicide and belonging to chloro acetamide chemical classes with an S isomer (optical isomer – L- form). This Metolachlor molecule is being used as a grass killer (weedicide) in the cultivated crop production. The molecule Benoxacor is a lab chemical being used as an industrial raw material and a pesticide too. In the pesticide industry, this molecule alone will not be used as a pesticide. The role of this molecule is that to manage the effects of herbicide as a selective manner with respect to the weed and the crop plants with the S

isomer of metolachlor and E & Z form of the benoxacor. Since this Benoxacor molecule has the E & Z enantiomer the selectivity processes will be effectively achieved, in the combination of this pesticide application. The combination products Mesotrione + S -Metolachlor + Benoxacor is being used as a pesticide to manage at a time soil, crop plants, and weed management effectively. Such an important application of these combination products does not have an analytical method.

The developed analytical method is completely validated as per the guideline SANCO 3030/99 Rev.4. The validation parameters specificity (selectivity), linearity, accuracy (recovery) and precision (repeatability) were bets its limits by a simple HPLC method.

2. MATERIALS AND METHOD

2.1 Reference standard used

The first reference standard name is Mesotrione, supplier Sigma – Aldrich, batch number is SZBE062XV, purity is 99.9%. The second reference standard name is Benoxacor, supplier Sigma – Aldrich, batch number is BCBT8607, purity is 98.4% and the third reference standard name is S-metolachlor, supplier Sigma – Aldrich, batch number is SZBD352XV, purity is 98.2%

2.2 Reagents and chemicals used

HPLC grade of Acetonitrile, make Rankem and triple distilled water was used in this analysis. Grade-A glass was used for preparations of all standard and sample in this analysis.

2.3 Instrument

A HPLC, Make, Shimadzu, model, LC-2030 with Prominence i series, detector, Uv-Vis. and coupled with autosampler. For the peak processes and data, collection analysis was used the LC solution (HPLC) software. From the pump A mobile phase acetonitrile (80 %) and from the pump B mobile phase water (20%) ratio was programmed prior to entering the HPLC column (Shimadzu BDS C18; 250mm length; 4.6 mm diameter with 5µ particle size dimensioned column) were used. A volume of 20 µl volume of sample and standard were used for each HPLC injections. The detection wavelength was 230nm used to detect all the three molecules. The Retention Time (RT) of these three molecules was achieved at about 3.8 min. for Mesotrione, about 6.4 min. for Benoxacor and about 5 min. for S-metolachlor. At the 40°C, the column oven temperature was maintained for the entire analytical method. The total run time was 20 min. for each HPLC run.

3. ANALYTICAL METHOD VALIDATION

3.1 Specificity

3.1.1 Preparation of standard stock solutions: An amount of 10.01 mg of Mesotrione reference standard with purity 99.9%, 10.17 mg of Benoxacor reference standard with purity 98.4% and 10.19 mg of S-metolachlor reference standard with purity 98.2% was weighed accurately in to a clean and dry 10 mL volumetric flask separately weighed and dissolved in mobile phase and made up to the mark with the mobile phase. This was equivalent to 1000 mg/L, 1000.73 mg/L and 1000.66 mg/L. From this, each 2.5ml solution was added in 25 ml volumetric flask and diluted with mobile phase. This solution was equivalent to 100 mg/L and analyzed to determine specificity.

3.1.2 Preparation of Sample Solution: An amount of 10.0 mg of the test substance was weighed accurately into a clean and dry 100 mL volumetric flask, dissolved with mobile phase and made up to the mark with the mobile phase. This was equivalent to 100 mg/L. This prepared solution was used for determination of Specificity. The specificity of HPLC method for Mesotrione, Benoxacor and S-Metolachlor were determined by injecting the Standard and Sample solutions along with blank (mobile phase) and observed that there was no interference found with the main peak of interest. Hence, this method was considered to be specific for the analysis of Mesotrione, Benoxacor, and S-metolachlor.

3.2 Linearity

3.2.1 Preparation of Standard Stock Solution and working standard: An amount of 10.0 mg of the standard was weighed into a 10 ml standard flask and this concentration (1000 mg/L) was used to prepare further dilutions to get the 0.1, 1, 10, 30, 60 and 90 mg/L separately. The dilution details are presented in table 1.

Table 1: Dilutions (Mesotrione 4% + S -Metolachlor 40% + Benoxacor 2% reference standard)

Std. Code	Stock Conc. (mg/L)	Dilution Vol. (ml)	Final Vol. (ml)	Final Conc. (mg/L)
Stock	1000	2.5	25	100
STD-1	100	0.01	10	0.1
STD-2	100	0.1	10	1
STD-3	100	1.0	10	10
STD-4	100	3.0	10	30
STD-5	100	6.0	10	60
STD-6	100	9.0	10	90

These standard solutions were injected into HPLC and a linear curve was plotted for the Concentration of standard versus observed peak area and the correlation coefficient was determined respectively. The linearity of Benoxacor, mesotrione, and S-metolachlor were given in table 2, 3 and 4 and the linearity curve for the Benoxacor, mesotrione, and S-metolachlor were given in figure 1, 2 and 3 respectively.

Table 2: Linearity of Benoxacor reference standard

Std. Code	Conc. (mg/L)	Replication	Ref. Std. Area	Mean Std. Area
Std-1	0.1	R1	4767	4995
		R2	5222	
Std-2	1	R1	40185	40494
		R2	40803	
Std-3	10	R1	392041	392043
		R2	392044	
Std-4	30	R1	1012182	1012151
		R2	1012119	
Std-5	60	R1	2246685	2246480
		R2	2246275	
Std-6	90	R1	3387102	3387294
		R2	3387485	
			Intercept	-15341.4463
			Slope	37548.4232
			Correlation Coefficient	0.999

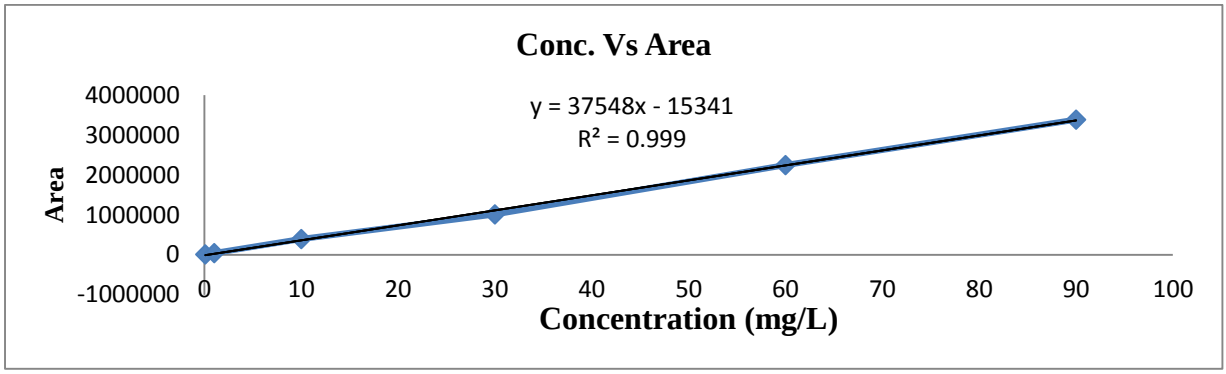

Fig. 1: Linearity curve for Benoxacor

Table 3: Linearity of Mesotrione reference standard

Std. Code	Conc. (mg/L)	Replication	Ref. Std. Area	Mean Std. Area
Std-1	0.1	R1	5107	5143
		R2	5179	
Std-2	1	R1	61793	62274
		R2	62755	
Std-3	10	R1	674425	673792
		R2	673158	
Std-4	30	R1	1753628	1753747
		R2	1753865	
Std-5	60	R1	3997208	3996480
		R2	3995752	
Std-6	90	R1	6059029	6061456
		R2	6063882	
			Intercept	-50245.0595
			Slope	67265.1013
			Correlation Coefficient	0.999

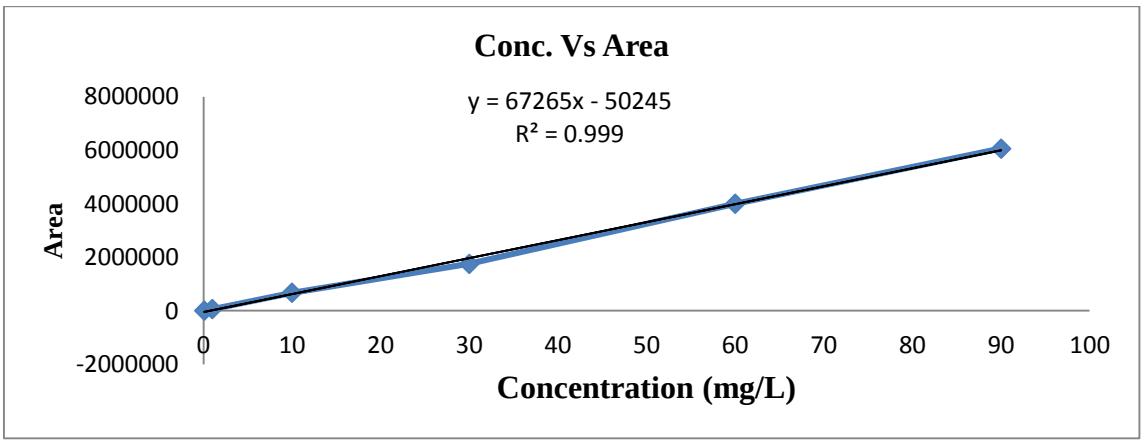

Fig. 2: Linearity curve for Mesotrione

Table 4: Linearity of S-Metolachlor reference standard

Std. Code	Conc. (mg/L)	Replication	Ref. Std. Area	Mean Std. Area
Std-1	0.1	R1	19519	19564
		R2	19609	
Std-2	1	R1	188178	187855
		R2	187531	
Std-3	10	R1	1864002	1864368
		R2	1864734	
Std-4	30	R1	4733059	4733397
		R2	4733735	
Std-5	60	R1	9936930	9937326
		R2	9937721	
Std-6	90	R1	14294464	14303832
		R2	14313199	
		Intercept		91497.9115
		Slope		159588.4512
		Correlation Coefficient		0.9995

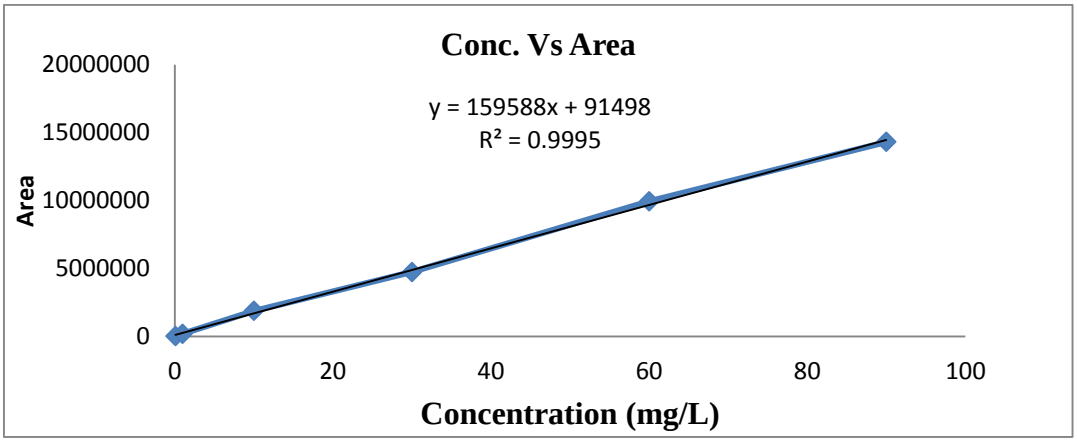

Fig. 3: Linearity curve for S-Metolachlor

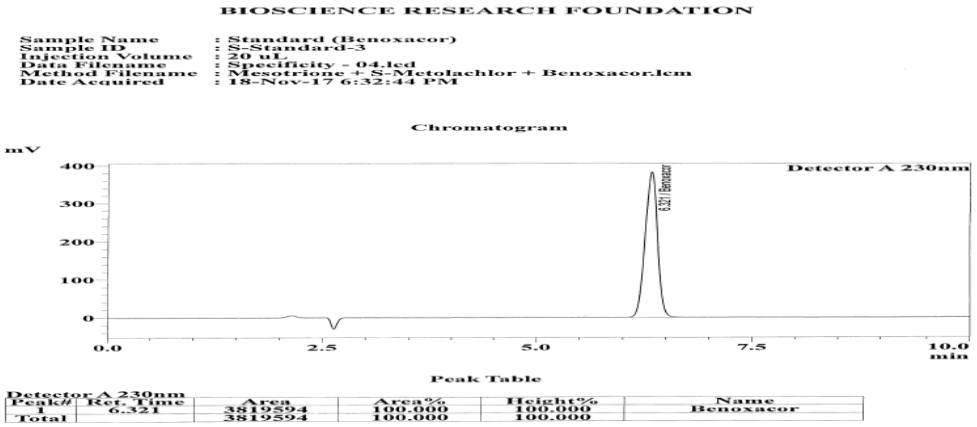

Fig. 4: A typical HPLC chromatogram for specificity for Benoxacor

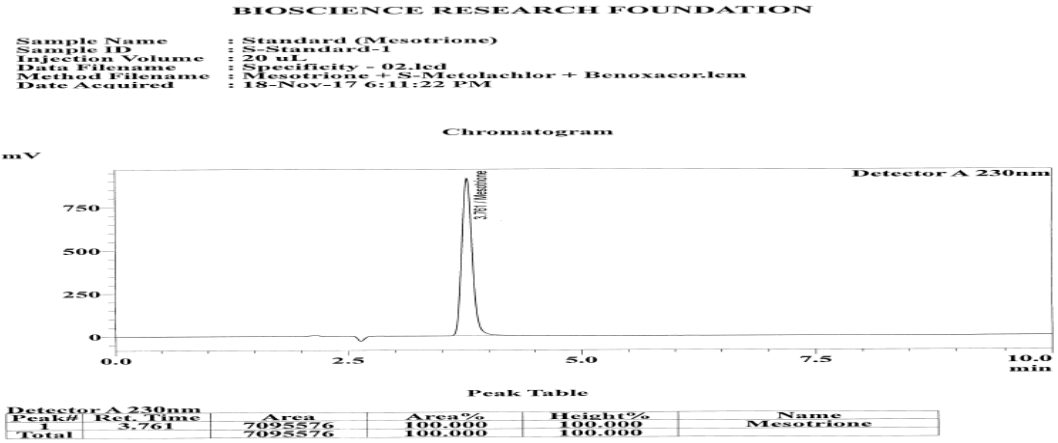

Fig. 5: A typical HPLC chromatogram for specificity for Mesotrione

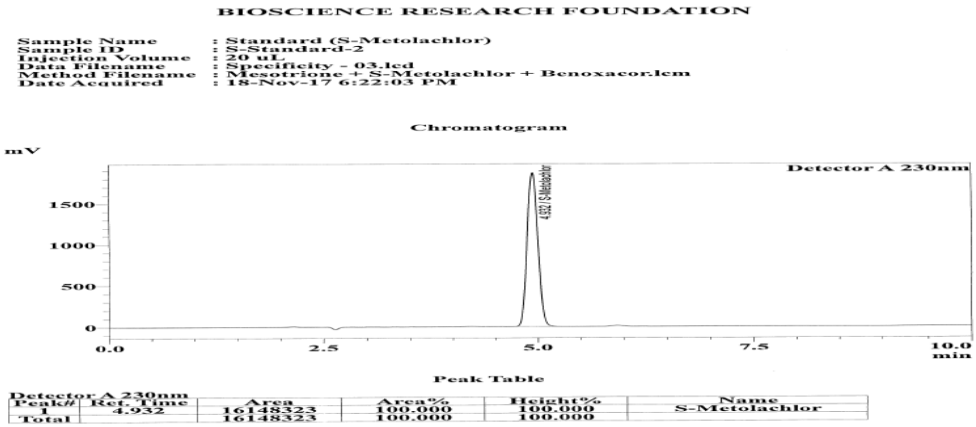

Fig. 6: A typical HPLC chromatogram for specificity for S-Metolachlor

4. PRECISION

4.1 Preparation of Standard Solution

A mixture of three standard 10 mg/L was prepared and used for the precision determination.

4.2 Preparation of Sample Solution

An amount of 6.20, 6.21, 6.19, 6.18 and 6.17 mg of the test substance was weighed in clean and dry 100 ml volumetric flask separately, dissolved the contents with mobile phase and made up to the mark with the mobile phase. This solutions were equivalent to 62.0, 62.1, 61.9, 61.8 and 61.9 mg/L. The prepared solutions were injected into HPLC and % RSD was calculated and the results are presented in table 5 to table 7.

Table 5: Precision (Benoxacor)

Sample ID	Std. Conc. (mg/L)	Std./Sample Area	Average Std. Area	Sample Conc. (mg/L)	Purity (P) %	A.I. Content (%)
Std -R1	10	391762	391650.5		98.4	-
Replication-1		50275		62.0		2.04
Replication-2		50205		62.1		2.03
Replication-3		50165		61.9		2.04
Replication-4		50129		61.8		2.04
Replication-5		50846		61.7		2.07
Std - R2		391539				-
					MEAN	2.04
					SD	0.016
					% RSD	0.773

Table 6: Precision (Mesotrione)

Sample ID	Std. Conc. (mg/L)	Std./Sample Area	Average Std. Area	Sample Conc. (mg/L)	Purity (P)%	A.I. Content (%)
Std -R1	10	674086	674122.0		99.9	-
Replication-1		176101		62.0		4.21
Replication-2		177550		62.1		4.24
Replication-3		175137		61.9		4.19
Replication-4		178634		61.8		4.28
Replication-5		177755		61.7		4.27
Std - R2		674158				-
					MEAN	4.24
					SD	0.038
					% RSD	0.908

Table 7: Precision (S-Metolachlor)

Sample ID	Std. Conc. (mg/L)	Std./Sample Area	Average Std. Area	Sample Conc. (mg/L)	Purity (P)%	A.I. Content (%)
Std -R1	10	1864888	1865212.5		98.2	-
Replication-1		4734107		62.0		40.20
Replication-2		4737238		62.1		40.16
Replication-3		4735753		61.9		40.28
Replication-4		4735959		61.8		40.35
Replication-5		4736140		61.7		40.41
Std - R2		1865537				-
					MEAN	40.28
					SD	0.103
					% RSD	0.255

The % RSD is within limit according to the modified Horwitz equation (Acceptable Limit <1.3% RSD for 100% active content as per SANCO/3029/99 Rev.4)

Formula:

$$\text{A. I. Content (\%)} = \frac{\text{Sample Area x Std. Conc. (mg/L)}}{\text{Average Std. Area x Sample Conc. (mg/L)}} \times \text{Purity (P) \%}$$

Example Calculation: P1 (S-METOLACHLOR)

$$\text{A. I. Content (\%)} = \frac{4734107 \text{ x } 10}{1865212.5 \text{ x } 62.0} \times 98.2 = 40.20\%$$

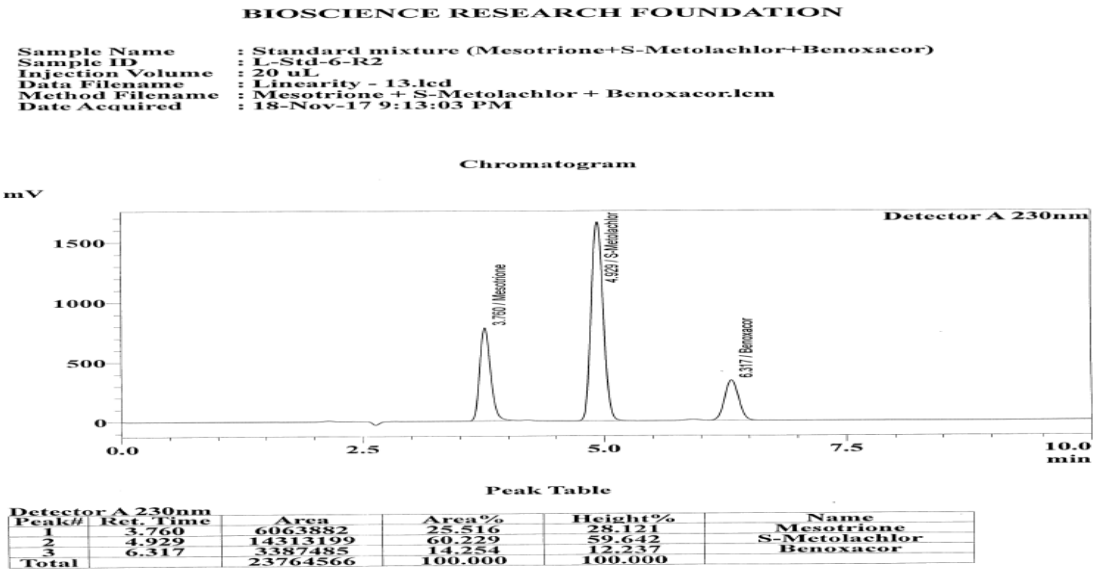

Fig. 7: A typical HPLC Chromatogram for linearity

5. ACCURACY (% RECOVERY)

The recovery processes and the recovery determination was validated with two fortification level (26 mg/L and 58 mg/L) of processes.

5.1 Preparation of Standard Solution

The standard solution prepared for linearity (10 mg/L) was used as a standard in percent recovery determination.

5.2 Preparation of Fortification Level 1– 26.0 mg/L (Benoxacor, Mesotrione & S- Metolachlor)

An amount of 2.60 mg of Mesotrione reference standard with purity 99.9%, 2.64 mg of Benoxacor reference standard with purity 98.4% and 2.60 mg of S- Metolachlor reference standard with purity 98.2% were weighed accurately in to a clean and dry 100 mL volumetric flask contains 50 ml of distilled water, sonicated and made up to the mark with the distilled water. This solution was equivalent to 26.0 mg/L.

5.3 Preparation of Fortification Level 1 – 58.0 mg/L (Benoxacor, Mesotrione & S- Metolachlor)

An amount of 5.89 mg of Mesotrione reference standard with purity 99.9%, 5.89 mg of Benoxacor reference standard with purity 98.4% and 5.91 mg of S- Metolachlor reference standard with purity 98.2% were weighed accurately in to a clean and dry 100 mL volumetric flask contains 50 ml of distilled water, sonicated and made up to the mark with the distilled water. This solution was equivalent to 58.0 mg/L. These prepared solutions were used for % recovery determination.

The above preparations were analyzed under HPLC and checked for recovery (%). The results are presented in following table 8 to table 10.

Table 8: Accuracy (Level-1 & 2 Recovery %) of Benoxacor

Fortification Code	Std. Conc. (mg/L)	Std. / Sample area	Mean Std. Area	Recovery Conc. (mg/L)	Fortified Conc. (mg/L)	Recovery (%)	Avg. Recovery (%)
Std-R1	10	390951	391117.5	-	26.0	-	99.65
Level 1-R1		1012938		25.8986		99.61	
Level 1-R2		1013844		25.9217		99.70	
Level 1-R3		1012541		25.8884		99.57	
Level 1-R4		1013776		25.9200		99.69	
Level 1-R5		1013569		25.9147		99.67	
Level 2-R1		2257748		57.7256	58.0	99.53	99.42
Level 2-R2		2256610		57.6965		99.48	
Level 2-R3		2252178		57.5832		99.28	
Level 2-R4		2254700		57.6476		99.39	
Level 2-R5		2255070		57.6571		99.41	
Std. - R2		391284		-		-	

Table 9: Accuracy (Level-1 and 2 Recovery %) of Mesotrione

Fortification Code	Std. Conc. (mg/L)	Std. / Sample area	Mean Std. Area	Recovery Conc. (mg/L)	Fortified Conc. (mg/L)	Recovery (%)	Avg. Recovery (%)
Std-R1	10	673255	673376	-	26.0	-	-
Level 1-R1		1752738		26.03		100.11	100.12
Level 1-R2		1753378		26.04		100.15	
Level 1-R3		1753031		26.03		100.13	
Level 1-R4		1752448		26.02		100.10	
Level 1-R5		1752451		26.02		100.10	
Level 2-R1		4013269		59.60	58.0	102.76	102.66
Level 2-R2		4012395		59.59		102.73	
Level 2-R3		4008517		59.53		102.64	
Level 2-R4		4006754		59.50		102.59	
Level 2-R5		4007260		59.51		102.60	
Std. - R2		673497		-		-	

Table 10: Accuracy (Level-1 and 2 Recovery %) of S –Metolachlor

Fortification Code	Std. Conc. (mg/L)	Std. / Sample area	Mean Std. Area	Recovery Conc. (mg/L)	Fortified Conc. (mg/L)	Recovery (%)	Avg. Recovery (%)
Std-R1	10	1866018	1866558.5	-	26.0	-	-
Level 1-R1		4737863		25.38		97.63	97.66
Level 1-R2		4738175		25.38		97.63	
Level 1-R3		4740292		25.40		97.68	
Level 1-R4		4739406		25.39		97.66	
Level 1-R5		4741115		25.40		97.69	
Level 2-R1		9979577		53.47	58.0	92.18	92.10
Level 2-R2		9973412		53.43		92.12	
Level 2-R3		9962154		53.37		92.02	
Level 2-R4		9968278		53.40		92.08	
Level 2-R5		9972907		53.43		92.12	
Std. - R2		1867099		-		-	

Example Calculation: **RECOVERY (MESOTRIONE) T2R5**

$$\text{Recovery Conc.}\left(\frac{\text{mg}}{\text{L}}\right) = \frac{\text{Std. Conc. (mg/L)} \times \text{Sample area}}{\text{Mean Std. Area}} = \frac{10 \times 4007260}{673376} = 59.51$$

$$\text{Recovery (\%)} = \frac{\text{Recovery Conc. (mg/L)}}{\text{Fortified Conc. (mg/L)}} \times 100 = \frac{59.51}{58.0} \times 100 = 102.60\%$$

6. LIMIT OF DETECTION (LOD) & LIMIT OF QUANTIFICATION (LOQ)

From the Linearity Standard Solution concentration of 10 mg/L was used in these LOD & LOQ determinations. From this solution, 1 mg/L solution was prepared and further diluted to get the 0.03 & 0.1 mg/L concentration solutions were prepared and the results are presented in the following Table 11 to Table 13 for LOD & LOQ of the three pesticides (Benoxacor + Mesotrione + S– Metolachlor).

Table 11: Limit of Detection (LOD) and Limit of Quantification (LOQ) of Mesotrione

Sample Code	Std. Conc. (mg/L)	Std./Sample Area	Average Std. Area	A. I. Content (mg/L)
Std.-1	10	672240	672975.5	-
Replication-R1		1306		0.019
Replication-R2		971		0.014
Replication-R3		1248		0.019
Std.-2		673711		-
			MEAN	0.0175
			SD	0.00266
			LOD	0.03

Sample ID	Std. Conc. (mg/L)	Std./ Sample Area	Average Std. Area	A. I. Content (mg/L)
STD-1	10	672240	672975.5	-
R1		4993		0.074
R2		4634		0.069
R3		4710		0.070
STD-2		673711		-
			MEAN	0.071
			SD	0.00281
			LOQ	0.10

Table 12: Limit of Detection (LOD) and Limit of Quantification (LOQ) of Benoxacor

Sample Code	Std. Conc. (mg/L)	Std./Sample Area	Average Std. Area	A. I. Content (mg/L)
Std.-1	10	392060	391630.5	-
Replication-R1		917		0.0234
Replication-R2		928		0.0237
Replication-R3		903		0.0231
Std.-2		391201		-
			MEAN	0.0234
			SD	0.00032
			LOD	0.02

Sample ID	Std. Conc. (mg/L)	Std./ Sample Area	Average Std. Area	A. I. Content (mg/L)
STD-1	10	392060	391630.5	-
R1		5061		0.129
R2		5018		0.128
R3		5074		0.130
STD-2		391201		-
			MEAN	0.129
			SD	0.00075
			LOQ	0.14

Table 13: Limit of Detection (lod) and Limit of Quantification (loq) of S-Metolachlor

Sample ID	Std. Conc. (mg/L)	Std./ Sample Area	Average Std. Area	A. I. Content (mg/L)
Std.-1	10	1867383	1866863.5	-
Replication-R1		5028		0.0269
Replication-R2		5023		0.0269
Replication-R3		5055		0.0271
Std.-2		1866344		-
			MEAN	0.0270
			SD	0.00009
			LOD	0.03

Sample ID	Std. Conc. (mg/L)	Std./ Sample Area	Average Std. Area	A. I. Content (mg/L)
STD-1	10	1867383	1866863.5	-
R1		19257		0.103
R2		19542		0.105
R3		19900		0.107
STD-2		1866344		-
			MEAN	0.105
			SD	0.00173
			LOQ	0.12

Example Calculation: (LOD& LOQ)

Limit of Detection (S-METOLACHLOR) R1

$$\text{A. I Content } \left(\frac{\text{mg}}{\text{L}}\right) = \frac{\text{Std. Conc. (mg/L)} \times \text{Sample Area}}{\text{Average Std. Area}}$$

$$= \frac{10 \times 5028}{1866863.5} = 0.0269 \text{ mg/L}$$

$$\text{LOD= Mean Value + (3 × SD)} = 0.0270 + (3 \times 0.00009) = 0.03$$

Limit of Quantification (S-METOLACHLOR) R1

$$\text{A. I Content } \left(\frac{\text{mg}}{\text{L}}\right) = \frac{\text{Std. Conc. (mg/L)} \times \text{Sample Area}}{\text{Average Std. Area}}$$

$$= \frac{10 \times 19257}{1866863.5} = 0.103 \text{ mg/L}$$

$$\text{LOQ= Mean Value + (10 × SD)} = 0.105 + (10 \times 0.00173) = 0.12$$

7. ACTIVE CONTENT ANALYSIS OF MESOTRIONE 4% + S -METOLACHLOR 40% + BENOXACOR 2% FORMULATION

7.1 Preparation of Standard solution

An amount of 10 mg of the standard was dissolved in 100 ml of mobile phase and diluted to get 10 mg/L was used as a standard in concentration analysis.

7.2 Preparation of Sample Solutions

The received test solutions (200 mg/mL) was prepared and diluted appropriately and injected into HPLC to calculate the actual content in the taken formulated pesticide molecule.

$$\text{Mesotrione 4\% + S − Metolachlor 40\% + Benoxacor 2\% } \left(\frac{\text{mg}}{\text{L}}\right) = \frac{A \times B \times DF}{C}$$

Where,

A- Concentration of standard (ppm)

B- Area of the sample solution

C- Area of standard solution

DF- Dilution Factor

The analyzed results were satisfactory for the determination and quantifications of the given formulation sample by the validated analytical method.

8. CONCLUSION

8.1 Specificity: The blank, standard and the sample peaks did not interfere with each other, hence the specificity was achieved as per the guideline SANCO 3030/99 Rev.4 requirement.

8.2 Linearity: The Linearity correlation coefficient is achieved NLT 0.99 as per (SANCO 3030/99 Rev.4

8.3 System Precision: The system precision is achieved as the % RDS for 5 replicates observed as 1% for Mesotrione, + S -Metolachlor and Benoxacor, hence the minimum requirement of the (SANCO 3030/99 Rev.4 was NMT 15% RSD was achieved

8.4 System Recovery: The system recovery > 99.42 % for Benoxacor; > 100.12 % for S -Metolachlor and > 92.10 % for Mesotrione, hence the minimum requirement of the (SANCO 3030/99 Rev.4.

8.5 Details of the Laboratory work were carried out.

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