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Analytical method development of Flonicamid by R – HPLC

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ABSTRACT

A simple reverse phase liquid chromatographic method has been developed and subsequently validated for the Flonicamid are a fungicide molecule; which is applicable for the treatment of the vegetable and fruits fungal decease. These Flonicamid molecules were separated through a mobile phase consisting of the mixture of acetonitrile, methanol, and water in the ration of 10:30:60. All these solvents are HPLC grade. Column: Qualis BDS C18 (250 x 4, 5µ); 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 RP-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— *Flonicamid, HPLC analysis, Validated Method, SANCO 3030/99 Rev.4, ICH Guideline*

1. INTRODUCTION

A pyridine based organic molecule Flonicamid was used as an effective pesticide to control the sucking insects in the plant growth regulation. All the young part of the plants was affected by a small type of sucking insect. Heavy damages in the plant production had been faced due to this infection. The small molecule Flonicamid consists of Cyanide, secondary amine, amide, pyridine, and tri-fluoro functional groups together. All these functional groups were together effectively acting against the small sucking insect. Any molecule has to be analyzed for its better understanding about its content and better handling usage in various forms of the product.

Maximum research journals have analyzed this molecule Flonicamid by Mass detector only for identification and its metabolic degradation in terms of residue. Since the molecule Flonicamid has many chromophores, it is better to an analysis by a most widely available, simple and common analytical technique HPLC with UV-Vis. detector. In this research article, the Flonicamid molecule was analyzed for is identification and quantification by a simple mobile phase ration of Acetonitrile and Water at the ratio of 80 and 20 v/v. with a BDS C18 silica column, at 260nm for an analysis time of 10 min. The response peak of Flonicamid was recorded at about 4 min. time. Hence this analytical method most beneficial in terms of analysis time cost-effective and more authenticate for the usage in Research and development and any quality control of production section.

This analytical method was validated as per 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 Reagents and chemicals used: Acetonitrile (Rankem, HPLC grade) and Distilled water (HPLC grade) were used in this analysis. All the glassware (standard flask / volumetric flasks) of class A grade were used in this analysis.

2.2 Instrument: A HPLC, Make, Shimadzu, model, LC-2030 with Prominence I series, detector, Uv-Vis. and coupled with the 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. The injection volume was 10 µl and the analysis was performed at 25°C temperature (ambient).

3. ANALYTICAL METHOD VALIDATION

3.1 Specificity

3.1.1 Preparation of standard stock solutions: An amount of 10.08 mg of Flonicamid reference standard with purity 99.23 % was weighed accurately into a clean and dry 10 mL volumetric flask and dissolved in mobile phase and made up to the mark with

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the mobile phase. This solution was equivalent to 1000.24 mg/L. From this, an aliquot of 2.5 mL solution was diluted in 25 mL of mobile phase. This solution was equivalent to 100 mg/L.

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 and dissolved in mobile phase and made up to the mark with the mobile phase. This solution was equivalent to 100 mg/L and used for determination of Specificity. The specificity of the HPLC method for Flonicamid was 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 specificity for the analysis of Flonicamid.

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 (Flonicamid reference standard)

Std. Code	Stock Concentration (mg/L)	Dilution Volume (ml)	Final Volume (ml)	Final Concentration (mg/L)
STD-6	100	9.0	10	90
STD-5	100	6.0	10	60
STD-4	100	3.0	10	30
STD-3	100	1.0	10	10
STD-2	10	1.0	10	1.0
STD-1	1.0	1.0	10	0.1
Stock	1000.24	2.5	25	100

The prepared standard solutions were injected by an autosampler into the HPLC system and a linear curve was plotted for the concentration of standard versus observed peak area and the correlation coefficient was determined respectively. The results are presented in table 2.

Table 2: Linearity of Flonicamid reference standard

Std. Code	Concentration (mg/L)	Replication	Std. Area	Mean Std. Area
Std-1	0.1	R1	3855	3783.0
		R2	3711	
Std-2	1	R1	34280	34354
		R2	34427	
Std-3	10	R1	323087	319266
		R2	315445	
Std-4	30	R1	868883	869539
		R2	870195	
Std-5	60	R1	1751624	1742475
		R2	1733326	
Std-6	90	R1	2531731	2531716
		R2	2531700	
			Intercept	18795.0832
			Slope	28196.5542
			Correlation Coefficient	0.9998

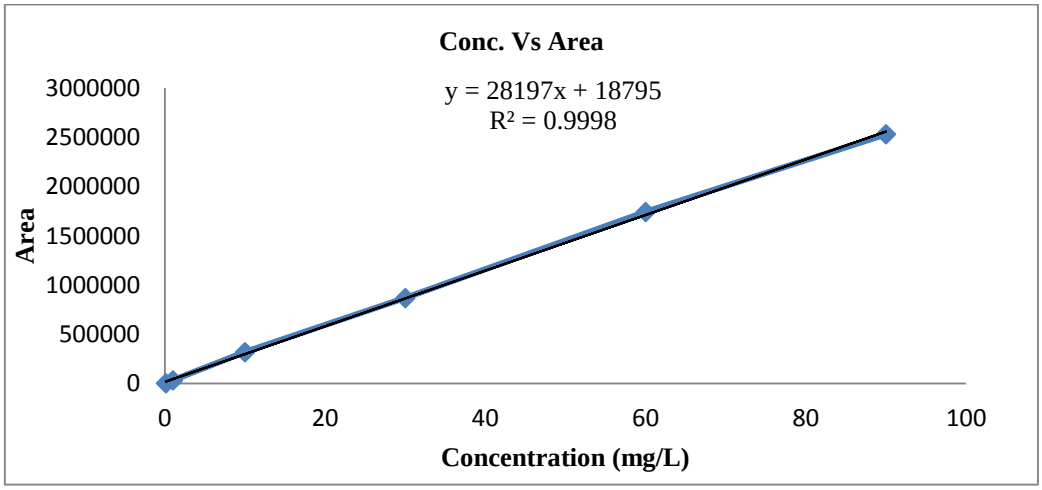

Fig. 1: Linearity curve for Flonicamid

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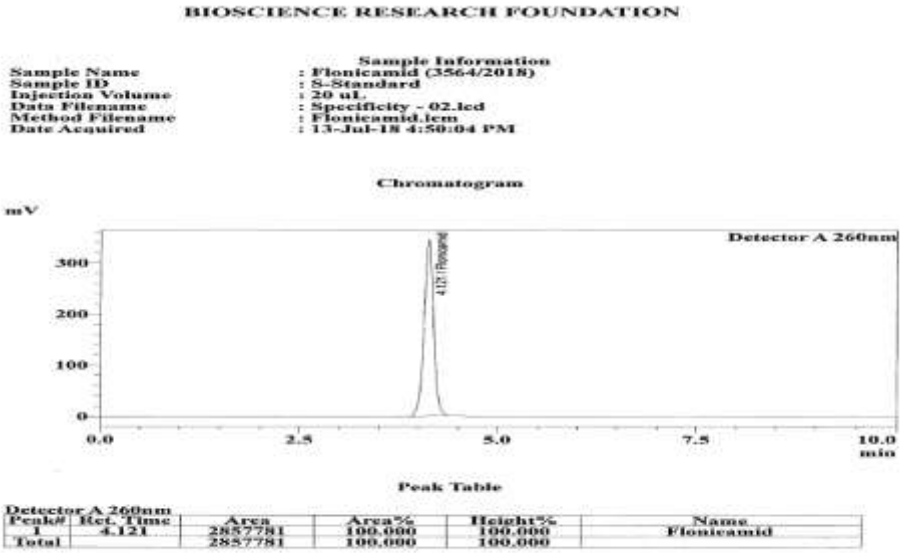

Fig. 2: A typical HPLC chromatogram for specificity

4. PRECISION

4.1 Preparation of Standard Solution

The Linearity standard solution 10 mg/L was prepared and used for the precision determination.

4.2 Preparation of Sample Solution

An amount of 2.784, 2.785, 2.786, 2.788 and 2.789 mg of Flonicamid Technical was weighed in clean and dry 1000 ml volumetric flask separately, dissolved the contents with mobile phase and made up to the mark with the mobile phase. This solutions are equivalent to 27.84, 27.85, 27.86, 27.88 and 27.89 mg/L. The prepared solutions were injected into HPLC and % RSD was calculated and the results are presented in table 3.

Table 3: Precision (Flonicamid)

Sample ID	Std. Conc. (mg/L)	Std. / Sample Area	Average Std. Area	Sample Conc. (mg/L)	Purity (P) %	A.I. Content (%)
Std -R1	10	315315	315424	-	99.23	-
P1		871737		27.84		98.51
P2		872126		27.85		98.51
P3		872420		27.86		98.51
P4		873105		27.88		98.52
P5		873486		27.89		98.53
Std - R2		315533		-		-
					MEAN	98.52
					SD	0.008
					% RSD	0.008

Formula:

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

The % RSD is within limit according to the modified Horwitz equation (Acceptable Limit <1.3% RSD for 100% active analyte as per SANCO/3030/99 Rev.4)

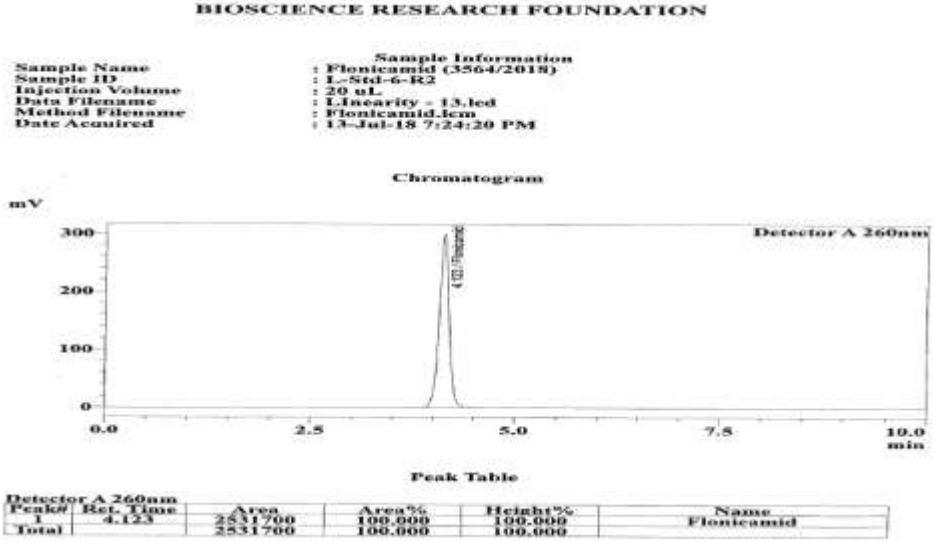

Fig. 3: A typical HPLC chromatogram for linearity

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5. ACCURACY (% RECOVERY)

The recovery processes and the recovery determination was validated with two fortification level 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 (28 mg/L)

An amount of 2.82 mg of Flonicamid reference standard with purity 99.23 % was weighed accurately into a clean and dry 100 mL volumetric flask and dissolved in mobile phase and made up to the mark with the mobile phase. This solution was equivalent to 28.0 mg/L.

5.3 Preparation of Fortification Level 2 (56 mg/L)

An amount of 5.64 mg of Flonicamid reference standard with purity 99.23 % was weighed accurately into a clean and dry 100 mL volumetric flask and dissolved in mobile phase and made up to the mark with the mobile phase. This solution was equivalent to 56.0 mg/L.

The above preparations were analyzed under HPLC and checked for recovery (%). The results are presented in the following table 4.

Table 4: Accuracy (Level-1 & 2 recovery %) of Flonicamid

Fortification Level	Std. Conc. (mg/L)	Std./Sample area	Mean Std. Area	Recovery Conc. (mg/L)	Fortified Conc. (mg/L)	Recovery (%)	Avg. Recovery (%)
Std-R1	10.0	312663	312927	-	28.0	-	-
T1R1		872853		27.89		99.62	99.78
T1R2		875446		27.98		99.91	
T1R3		874253		27.94		99.78	
T1R4		874497		27.95		99.81	
T1R5		874436		27.94		99.80	
T2R1		1737634		55.53	56.0	99.16	99.12
T2R2		1733675		55.40		98.93	
T2R3		1738112		55.54		99.19	
T2R4		1737477		55.52		99.15	
T2R5		1737776		55.53		99.17	
Std - R2		313191		-		-	

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 0.02 & 0.1 mg/L concentration solutions were prepared. The dilution details were given in the Table No. 5, and the results are presented in following Table 6 and 7.

Table 5: Dilutions (LOD & LOQ)

Stock concentration (mg/L)	Dilution Volume (ml)	Final Volume (ml)	Final Concentration (mg/L)
1.0	0.2	10	0.02
1.0	1.0	10	0.1

Formula:

LOD = Average + (3 x Standard Deviation)

LOQ = Average + (10 x Standard Deviation)

Table 6: Limit of Detection (LOD) Flonicamid

Sample ID	Std. Conc. (mg/L)	Std./ Sample Area	Average Std. Area	A. I. Content (mg/L)
STD-1	10	316335	317723.0	-
R1		751		0.024
R2		740		0.023
R3		746		0.023
STD-2		319111		-
			MEAN	0.0235
			SD	0.00017
			LOD	0.024

Table 7: Limit OF Quantification (LOQ)

Sample ID	Std. Conc. (mg/L)	Std./ Sample Area	Average Std. Area	A. I. Content (mg/L)
STD-1	10	316335	317723.0	-
R1		3644		0.115
R2		3650		0.115
R3		3824		0.120
STD-2		319111		-
			MEAN	0.117
			SD	0.00322
			LOQ	0.15

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Example Calculation: (LOD& LOQ)

Formula:

LOD = Average + (3 x Standard Deviation)

LOQ = Average + (10 x Standard Deviation)

Limit of Detection -R1

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

$$= \frac{10 \times 751}{317723} = 0.024 \text{ mg/L}$$

LOD = Mean Value + (3 × SD)

= 0.024 + (3 × 0.00017) = 0.024

Limit of Quantification -R1

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

$$= \frac{10 \times 3644}{317723} = 0.115 \text{ mg/L}$$

LOQ = Mean Value + (10 × SD)

= 0.117 + (10 × 0.00322) = 0.15

7. ACTIVE CONTENT ANALYSIS OF FLONICAMID

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) from the Toxicology Department were shaken vigorously, diluted appropriately and injected into HPLC.

$$\text{Flonicamid (mg/L)} = \frac{An \times B \times DF}{C}$$

Where,

A - Concentration of standard (ppm)

B - Area of the sample solution

C - Area of standard solution

DF - Dilution Factor

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 0.1% for flonicamid, hence the minimum requirement of the (SANCO 3030/99 Rev.4 was NMT 15% RSD was achieved

8.4 System Recovery: The system recovery 92% to 101 % were achieved for flonicamid, 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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