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ANALYTICAL METHOD DEVELOPMENT OF ABAMECTIN + IMIDACLOPRIDBY R-HPLC

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ICH Guideline.

ABSTRACT

Abamectin is a natural product being used as an insecticide, which has the power of killing the worms, it is also being used as a plant parasitic nematodes. Imidacloprid is a chemical which has the property of neurotoxin to insects. by affecting the Central Nerve System (CNS) of insect and this causes the growth of the insect. The concentrations of these two molecules as a pesticide consider as an important combination against insecticide in the pest management programs. A simple HPLC chromatographic method has been developed and subsequently validated for the combination pesticide (Abamectin + Imidacloprid) separation and quantification. TheseAbamectin + Imidaclopridmoleculeswere separatedthrough a mobile phase consisting of the mixture of acetonitrile and water (fortified with 0.1% of Ortho Phosphoric Acid) in the ratio of 80:20 v/v. The separation was achieved through theQualisil BDS C18 (250 x 4, 5µ) column with the flow rate as 1.0 ml/min with the detection at 230 nm. These method parameters were loaded in the Shimadzu HPLC (model: LC-2030). The LC solution Shimadzu software was used for all the calculations in this analytical method validation analysis. 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 moleculesinterims of validation parameters viz., separation, system suitability, System Precision and linearity in a simple HPLC analysis.

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INTRODUCTION

The natural product Abamectin is being used as an insecticide, which has the power of killing the (antiphrastic to worms) (helminthes), it is also used to the plant parasitic nematodes. This molecule also used to kill the ticks & mites all these processes had been identified without any significant side effect. Abamectin benzoate salt derivative also being used as an insecticide widely in the pest management programs. Abamectin molecular structure has four axial methyl groups in the closed cyclic system of this molecule. This molecule has much activity to will pest hence it is important to analysed to understand the concentration level. Imidacloprid is a chemical class compound has the property of neurotoxin property in the insect kingdom. This molecule interfere the Central Nerve System (CNS) of insect and this causes the growth of the insect. A part from this molecule prevents the transformation of signal from brain to other parts of body and vice versa.

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The treatment against Insecticide were effective because of toxic nature of these combination product; whereas less toxic to the mammalians. The concentrations of these two molecules as a pesticide consider as an important combination against insecticide in the pest management programs. Therefore it is important to understand the active content of these molecules with a single analysis.

MATERIALS AND METHODS

Reagents and chemicals used: All the analytical grade solvents and water were used in this analytical method development. All the class A glass weare used in this resear analytical method development.

Instrument: A calibrated chromatography HPLC instrument was used to develop this analytical method development for Imidacloprid and Abamectin (B1b & B1a). The instrument parameters were given as:

Name of the instrument : High Performance Liquid Chromatography (HPLC)
Calibration Method : External Standard Method
Make : Shimadzu
Model : LC 2030
Detector : UV-Visible
Wavelength (λ) : 230 nm
Column Temperature : 40ºC
Column : Qualisil BDS C18 (250 x 4.6 mm, 5µ)
Mobile Phase : Acetonitrile: Water (0.1% OPA); ration of 80:20 (v/v)
Flow rate : 1.0 ml/min
Injection volume : 20 µl
Retention time (Approximately) : Imidacloprid – 2.9 minutes
: Abamectin B1b – 9.6 minutes
: Abamectin B1a – 11.4 minutes

Preparation of Mobile phase

An volume of 80% Acetonitrile and 20% of 0.1% Ortho Phosphoric acid were mixed well, sonicated and used for analysis.

A typical Chromatogram for Specificity

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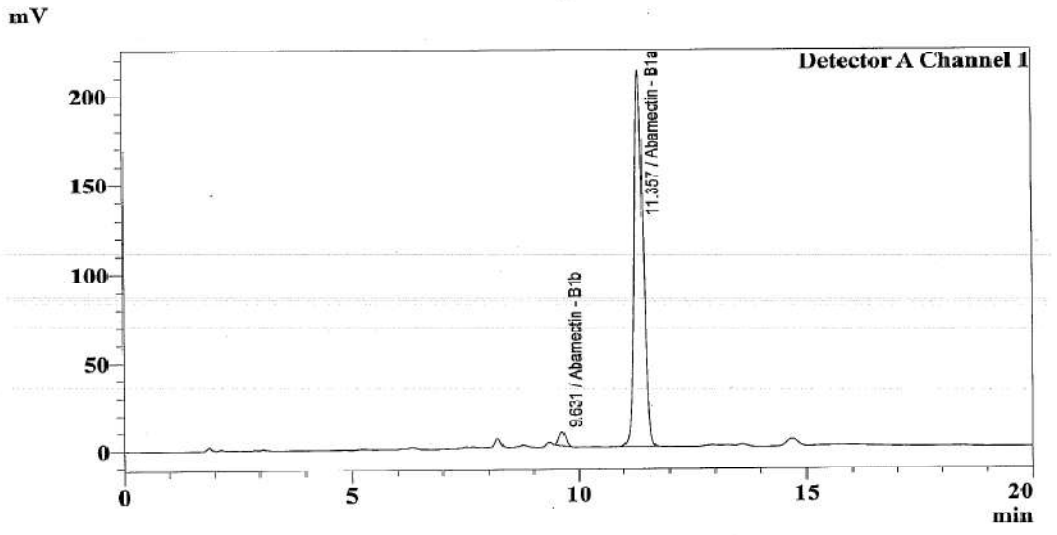
Sample Name : Abamectin
Sample ID : Std-1
Injection Volume : 20 uL
Data Filename : 002.lcd
Method Filename : Abamectin + Imidacloprid.lcm
Date Acquired : 15-Dec-17 11:13:14 AM

Chromatogram



Peak Table

Detector A Channel 1 230nm

Peak#	Ret. Time	Area	Area%	Height%	Name
1	9.631	82227	2.773	3.577	Abamectin - B1b
2	11.357	2882605	97.227	96.423	Abamectin - B1a
Total		2964832	100.000	100.000	

Preparation of 0.1% Ortho Phosphoric acid

An volume of 0.1% of Ortho Phosphoric acid and 99.9% Distilled water were mixed well, sonicated and used for analysis.

Analytical method validation

Specificity

Preparation of standard stock solutions: An amount of 10.11 mg of Imidacloprid reference standard with purity 99.0% and 10.38 mg of Abamectin reference standard with purity 96.4% was weighed accurately in to a clean and dry 10 mL volumetric flask separately and dissolved in mobile phase and made up to the mark with the mobile phase. This was equivalent to 1000.89 mg/L and 1000.63 mg/L. From this each 2.5ml solution was added in 25 ml volumetric flak and diluted with mobile phase. This solution was equivalent to 100 mg/L and analyzed to determine specificity.

Preparation of Sample Solution

An amount of 10.0 mg of 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 was equivalent to 100 mg/L. This prepared solution was used for determination of Specificity. The specificity of HPLC method for Imidacloprid 16% and Abamectin 4% SC was determined by injecting the Standard andSample solutions along with blank (mobile phase) and observed that there was

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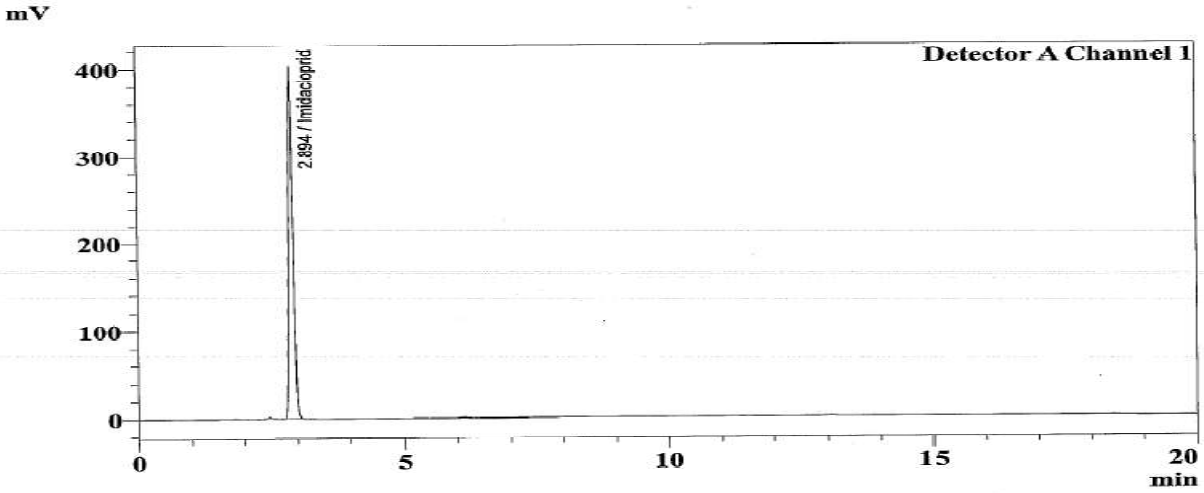
Sample Name : Abamectin + Imidacloprid
Sample ID : Std-2
Injection Volume : 20 uL
Data Filename : 003.lcd
Method Filename : Abamectin + Imidacloprid.lcm
Date Acquired : 15-Dec-17 11:34:57 AM

Chromatogram



Peak Table

Detector A Channel 1 230nm

Peak#	Ret. Time	Area	Area%	Height%	Name
1	2.894	2327562	100.000	100.000	Imidacloprid
Total		2327562	100.000	100.000	

no interference found with the main peak of interest. Hence, this method was considered to be specific for the analysis of Imidacloprid and Abamectin.

Linearity

Preparation of Standard Stock Solution and working standard

An amount of 10.0 mg of standard was weighed in to a 100 ml standard flask and this concentration (100 mg/L) was used toprepared further dilutions to get the 0.15, 1, 5, 10, 15 and 20 mg/L separately. The dilution details are presented in table No.1

Table 1. Dilutions (abamectin + imidacloprid reference standard)

Standard Code	Stock Dose (mg/L)	Dilution Volume (ml)	Final Volume (ml)	Final Concentration (mg/L)
Stock	1000	2.5	25	100
1	100	0.015	10	0.15
2	100	0.1	10	1
3	100	0.5	10	5
4	100	1.0	10	10
5	100	1.5	10	15
6	100	2.0	10	20

The prepared standard solutions were injected by an auto sampler into 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 No. 2 and 3.

Table 2. Linearity of abamectin reference standard

Std. Code	Concentration (mg/L)	Replication	Ref. Std. Area	Mean Std. Area
1	0.15	Replication 1	1877	2185
		Replication 2	2493	
2	1	Replication 1	19114	19094
		Replication 2	19074	
3	5	Replication 1	80263	80341
		Replication 2	80418	
4	10	Replication 1	157200	156688
		Replication 2	156175	
5	15	Replication 1	240955	242684
		Replication 2	244412	
6	20	Replication 1	310734	310049
		Replication 2	309363	
			Intercept	2139.8127
			Slope	15605.0855
			Correlation Coefficient	1.000

Precision

Preparation of Standard Solution: The Linearity standard solution 5 mg/L was prepared and used for the precision determination.

Preparation of Sample Solution: An amount of 5.95, 5.80, 5.81, 5.90 and 6.0mg of Abamectin + Imidacloprid Technical were weighed in clean and dry 1000 ml volumetric flask separately, dissolved the contents with mobile phase and made upto the mark with the mobile phase.

Table 2. Linearity of imidacloprid reference standard

Std. Code	Concentration (mg/L)	Replication	Ref. Std. Area	Mean Std. Area
1	0.15	Replication 1	1374	1378
		Replication 2	1382	
2	1	Replication 1	13116	13073
		Replication 2	13030	
3	5	Replication 1	51961	51906
		Replication 2	51851	
4	10	Replication 1	105992	106053
		Replication 2	106114	
5	15	Replication 1	159737	159528
		Replication 2	159319	
6	20	Replication 1	214689	214291
		Replication 2	213892	
			Intercept	218.2907
			Slope	10653.3481
			Correlation Coefficient	1.000

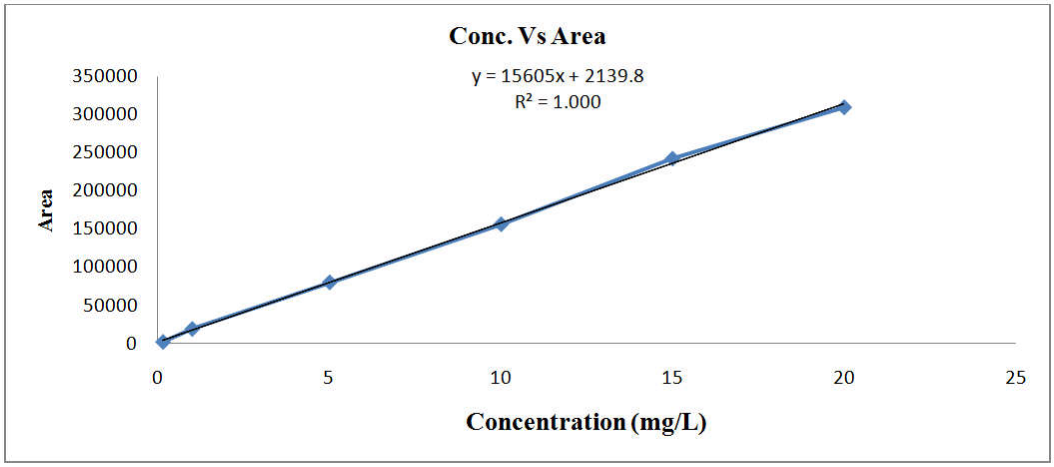


Figure 1. Linearity curve for abamectin

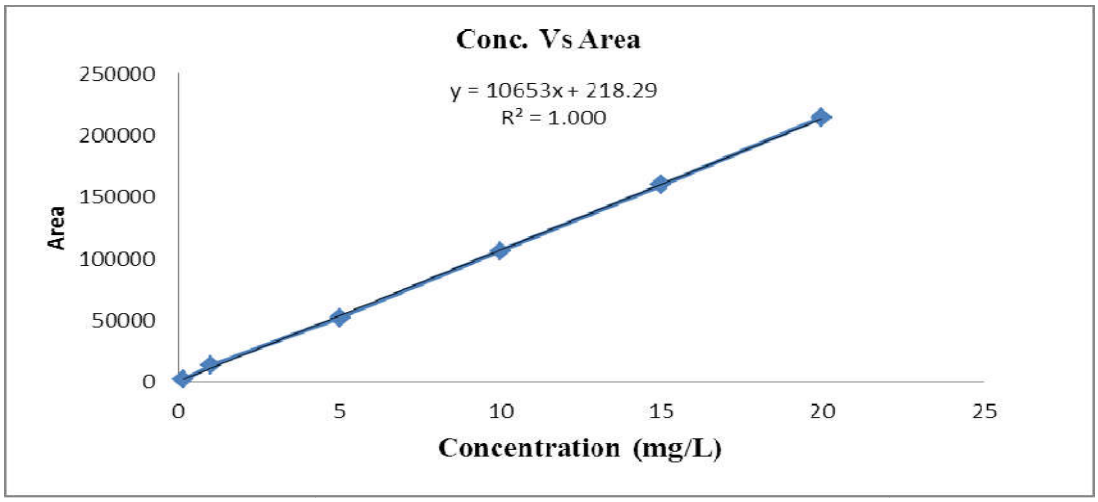


Figure 2. Linearity Curve For Imidacloprid

Table 4. Precision (abamectin)

Sample ID	Std. Conc. (mg/L)	Std. / Sample Area	Average Std. Area	Sample Conc. (mg/L)	Purity (P) %	A.I. Content (%)
Std -R1	5	83716	83657.5		96.4	-
P1		47827		59.5		4.63
P2		46276		58.0		4.60
P3		45937		58.1		4.56
P4		45112		59.0		4.41
P5		47845		60.0		4.59
Std - R2		83599				-
					MEAN	4.56
					SD	0.089
					% RSD	1.948

Table 5. Precision (Imidacloprid)

Sample ID	Std. Conc. (mg/L)	Std. / Sample Area	Average Std. Area	Sample Conc. (mg/L)	Purity (P) %	A.I. Content (%)
Std -R1	5	51988	51873.0		99	-
P1		102168		59.5		16.39
P2		103191		58.0		16.98
P3		102435		58.1		16.82
P4		102277		59.0		16.54
P5		104318		60.0		16.59
Std - R2		51758				-
					MEAN	16.66
					SD	0.235
					% RSD	1.413

Table 5. Accuracy (level-1 & 2 recovery%) of abamectin

Fortification Level	Std. Conc. (mg/L)	Std. / Sample area	Mean Std. Area	Recovery Conc. (mg/L)	Fortified Conc (mg/L)	Recovery (%)	Avg. Recovery (%)	SD	RSD (%)
Std-R1	5.0	84107	84053.5	-	0.15	-	-	0.95	0.94
L1R1		2545		0.151		100.93	100.84		
L1R2		2560		0.152		101.52			
L1R3		2502		0.149		99.22			
L1R4		2560		0.152		101.52			
L1R5		2547		0.152		101.01			
L2R1		25365		1.51	1.50	100.59	100.41	0.29	0.29
L2R2		25222		1.50		100.02			
L2R3		25260		1.50		100.17			
L2R4		25369		1.51		100.61			
L2R5		25382		1.51		100.66			
Std - R2		84000		-		-			

Table 6. Accuracy (level-1 & 2 recovery %) of imidacloprid

Fortification Level	Std. Conc. (mg/L)	Std. / Sample area	Mean Std. Area	Recovery Conc. (mg/L)	Fortified Conc (mg/L)	Recovery (%)	Avg. Recovery (%)	SD	RSD (%)
Std-R1	5.0	51947	51983.0	-	0.15	-	-	0.49	0.494
L1R1		1538		0.148		98.62	98.72		
L1R2		1535		0.148		98.43			
L1R3		1553		0.149		99.58			
L1R4		1535		0.148		98.43			
L1R5		1537		0.148		98.56			
L2R1		15533		1.494	1.50	99.60	99.70	0.15	0.145
L2R2		15576		1.498		99.88			
L2R3		15571		1.498		99.85			
L2R4		15532		1.494		99.60			
L2R5		15532		1.494		99.60			
Std - R2		52019		-		-	-		

This solutions are equivalent to 59.5, 58.0, 58.1, 59.0 and 60.0mg/L. The prepared solutions were injected into HPLC and % RSD was calculated and the results are presented in TABLE 4.

Formula:

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

The % RSD is within limit according to the modified Horwitz equation (Acceptable Limit <1.413 RSD for 100% active analyte as per SANCO/3030/99 Rev.4)

Accuracy (% Recovery): The recovery processes and the recovery determination was validated with two fortification level of processes.

Preparation of Standard Solution: The standard solution prepared for linearity (5 mg/L) was used as standard in percent recovery determination.

Preparation of Fortification Level 1 (0.15mg/L): An amount of 2.82 mg of Abamectin + Imidacloprid 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 0.15 mg/L.

Preparation of Fortification Level 2 (1.5 mg/L): An amount of 5.64 mg of Abamectin + Imidacloprid 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 1.5 mg/L. The above preparations were analyzed under HPLC and checked for recovery (%). The results are presented in following table No. 5& 6

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

$$\text{Recovery Conc. (mg/L)} = \frac{\text{Std. Conc. (mg/L)} \times \text{Sample area}}{\text{Mean Std. Area}} = \frac{5 \times 15532}{51983} = 1.494$$

Table 7. 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

Table 8. Limit of detection (lod) and limit of quantification (loq) of abamectin

Sample ID	Std. Conc. (mg/L)	Std./ Sample Area	Average Std. Area	A. I. Content (mg/L)	Sample ID	Std. Conc. (mg/L)	Std./ Sample Area	Average Std. Area	A. I. Content (mg/L)
STD-1	5	80233	82009.5	-	STD-1	5	80233	82009.5	-
R1		127		0.008	R1		1867		0.114
R2		118		0.007	R2		1900		0.116
R3		117		0.007	R3		1967		0.120
STD-2		83786		-	STD-2		83786		-
			MEAN	0.0074				MEAN	0.117
			SD	0.00034				SD	0.00311
			LOD	0.01				LOQ	0.15

Table 9. Limit of Detection (Lod) And Limit of Quantification (Loq) Of Imidacloprid

Sample ID	Std. Conc. (mg/L)	Std./ Sample Area	Average Std. Area	A. I. Content (mg/L)	Sample ID	Std. Conc. (mg/L)	Std./ Sample Area	Average Std. Area	A. I. Content (mg/L)
STD-1	5	51714	51685.0	-	STD-1	5	51714	51685.0	-
R1		108		0.0104	R1		1406		0.136
R2		111		0.0107	R2		1375		0.133
R3		113		0.0109	R3		1364		0.132
STD-2		51656		-	STD-2		51656		-
			MEAN	0.0107				MEAN	0.134
			SD	0.00024				SD	0.00211
			LOD	0.01				LOQ	0.15

$$\text{Recovery (\%)} = \frac{\text{Recovery Conc. (mg/L)}}{\text{Fortified Conc (mg/L)}} \times 100 = \frac{1.494}{1.50} \times 100 = 99.60\%$$

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

From the Linearity Standard Solution concentration of 10 mg/Lwas 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. 7, and the results are presented in following Table 87 & 9.

Formula:

LOD = Average + (3 x Standard Deviation)
LOQ = Average + (10 x Standard Deviation)

Example Calculation: (LOD& LOQ)

Formula:

LOD = Average + (3 x Standard Deviation).
LOQ = Average + (10 x Standard Deviation)

Example Calculation: (LOD& LOQ)

Limit Of Detection (ABAMECTIN) R1

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

$$= \frac{5 \times 127}{82009.5} = 0.008 \text{ mg/L}$$

LOD = Mean Value + (3 × SD)

= 0.0074 + (3 × 0.00034) = 0.01

Limit of Quantification (ABAMECTIN) R1

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

$$= \frac{5 \times 1867}{82009.5} = 0.114 \text{ mg/L}$$

LOQ = Mean Value + (10 × SD)

= 0.117 + (10 × 0.00311) = 0.15

A Typical Chromatogram for Sample analysis

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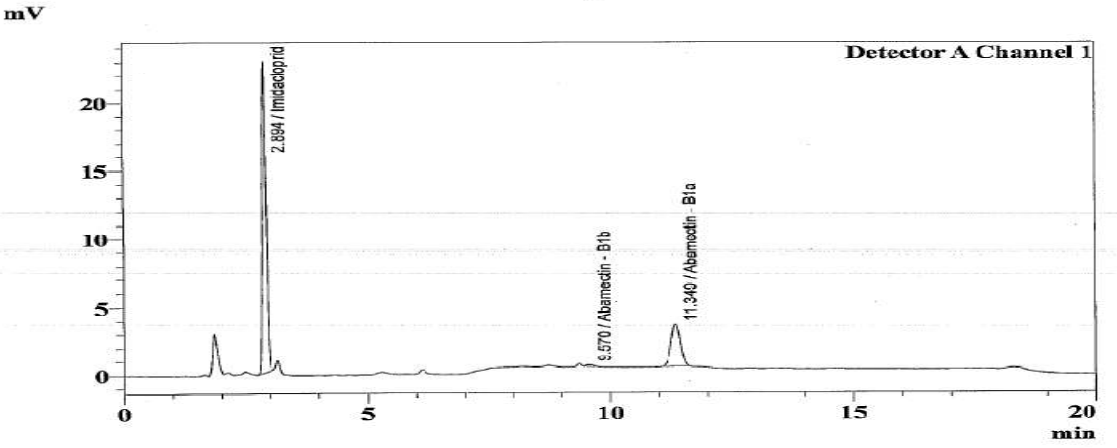
Sample Name : Abamectin 4% + Imidacloprid 16% SC
Sample ID : Sample - R1
Injection Volume : 20 uL
Data Filename : Precision - 02.lcd
Method Filename : Abamectin + Imidacloprid.lcm
Date Acquired : 15-Dec-17 6:27:33 PM

Chromatogram



Peak Table

Detector A Channel 1 230nm

Peak#	Ret. Time	Area	Area%	Height%	Name
1	2.894	102168	68.114	86.311	Imidacloprid
2	9.570	1213	0.809	0.454	Abamectin - B1b
3	11.349	46614	31.077	13.235	Abamectin - B1a
Total		149995	100.000	100.000	

Preparation of Standard solution: An amount of 5 mg of the standard was dissolved in 100 ml of mobile phase and diluted to get 10 mg/L was used as standard in concentration analysis.

Preparation of Sample Solutions: The received test solutions (30 mg/mL) was prepared and dissolved by sonication and diluted appropriately and injected into HPLC.

$$\text{Abamectin + Imidacloprid (mg/L)} = \frac{A \times B \times DF}{C}$$

Where,

A - Concentration of standard (ppm)
B - Area of sample solution
C - Area of standard solution
DF - Dilution Factor

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 5 replicates observed as 0.1% for Abamectin + Imidacloprid, hence the minimum requirement of the (SANCO 3030/99 Rev.4 was NMT 15% RSD was achieved

System Recovery: The system recovery 92% to 101 % were achieved for Abamectin + Imidacloprid, hence the minimum requirement of the (SANCO 3030/99 Rev.4).

Details of the Laboratory work were carried out: Bioscience Research Foundation, Sengadu village & Post, Via Manavalanagar, Kandamangalam – 602002, Kanchipuram District, Tamilnadu, India Ph: +91 44 27601082, Email: brfchennai@gmail.com

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