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Analytical method validation of Cypermethrin and Quinalphos Emulsion Concentrate (EC) Formulation by Reverse Phase High Performance Liquid Chromatography (R-HPLC)

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ABSTRACT

The Quinalphos and Cypermethrin molecules are being used alone and a combination as an effective insecticide successfully in the agriculture and domestic area of application. Both the molecules (Quinalphos and Cypermethrin) being used large quantity in the environmental application and the residue of these insecticide has to be controlled by applying a required quantity with in dissipation time of these molecules. These molecules are also toxic to humans and animals hence the lowest concentration also has to be detected for monitoring and controlling purpose. A simple HPLC analytical method; in a single run both the molecule was detected at 0.4mg/L concentration. With the Apollo Silica 5µ (250 mm × 4.6 mm) HPLC column; acetonitrile and water as a mobile phase with ration of 80:20 (volume/volume) at 1.5 ml/min. flow rate. The detection wavelength is 316 nm for Quinalphos and 278nm for Cypermethrin were detected by the PDA detector of Shimadzu LC2030 model HPLC. The results of the analysis deliver 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— *Quinalphos and Cypermethrin, HPLC analysis, Validated method, SANCO 3030/99 Rev.4, ICH Guideline*

1. INTRODUCTION

Quinalphos is a chemical; it is consisting of organo phosphorothionate, diethyl and quinoxaline residual systems. The molecule Quinalphos is a derivative of phosphoric acid; where three active acidic protons of phosphoric acids were substituted by different residual systems; two hydrogen were replaced by two ethyl groups and the third hydrogen was replaced by quinoxaline system. The phosphorothioate and the quinoxaline systems were together produced a reddish brown color of the Quinalphos molecule. These two type of substituted system in the phosphoric acid being used as an effective insecticide in the plant production domain. Cypermethrin is a well-known insecticide in the plant production kingdom in the recent years because of its unique nature of controlling the insecticide even in the domestic application also. Basically Cypermethrin is a pyrethrin derivative and this controls the insecticide through its central nerve system of the insects. The Cypermethrin consist of many functional systems viz; chloride, keto, cynide, phenoxy, alkene, tricyclic alkane and ester system in its structural arrangement. The neurotoxic affect in the CNS of insecticide by producing the various metabolic products. These Quinalphos and Cypermethrin are considers as a very effective insecticide and wide application in the plant production area. The application should not retain residue in the environmental like water, soil and air; hence the combine pesticide Quinalphos and Cypermethrin has to be analyzed completely. The developed analytical method is so cost effective, easy and reproducible in terms of qualitative and quantitative analysis.

2. MATERIALS AND METHOD

2.1 Reagents and chemicals used

All the analytical grade solvents and water were used in this analytical method development. All the class A glass wear used in this research analytical method development.

2.2 Instrument

In this experiment used HPLC was periodically calibrated and maintained to develop this analytical method development for chloro triazine compounds (Quinalphos and Cypermethrin). The HPLC make Shimadzu, Model LC 2030; Detector UV-Vis.; Absorption at 220 nm; Column used, Qualisil BDS C18 (250 x 4.6, 5µ); mobile phase used Acetonitrile and Water; ratio of 80:20 (v/v) with flow rate 1 ml/min. With this HPLC condition the chloro triazine molecules Quinalphos and Cypermethrin was eluted at 3.4 minutes and 4.0 minutes respectively.

2.3 Preparation of Mobile phase

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

3. ANALYTICAL METHOD VALIDATION

3.1 Specificity

3.1.1 Preparation of standard stock solutions: An amount of 10.09 mg Quinalphos reference standard with purity 99.1% and 10.05 mg Cypermethrin reference standard with purity 99.5% were weighed accurately into a clean and dry 10 mL volumetric flask separately, dissolved with mobile phase and made upto the mark with mobile phase. This solution was equivalent to 1000 mg/L respectively. From this, an aliquot of each 1ml solution was mixed 10 mL volumetric flask, diluted with mobile phase. This solution was equivalent to 100 mg/L and analyzed to determine specificity.

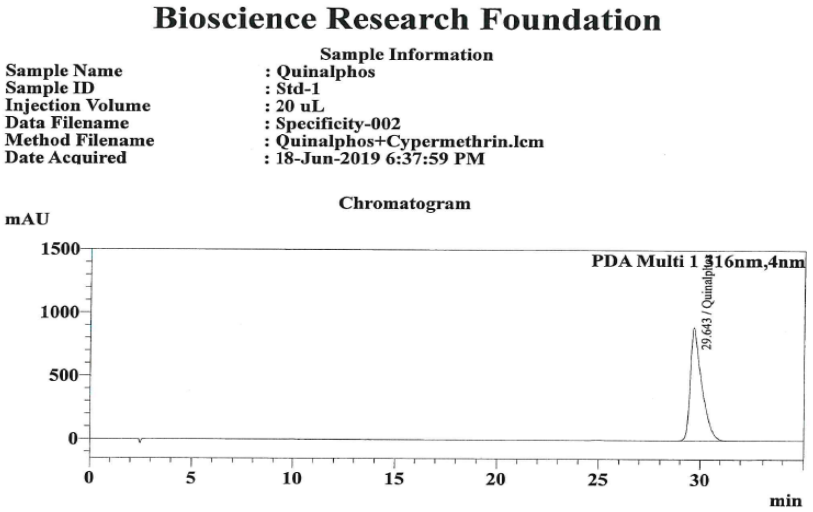

Fig. 1: Typical Chromatogram for Quinalphos

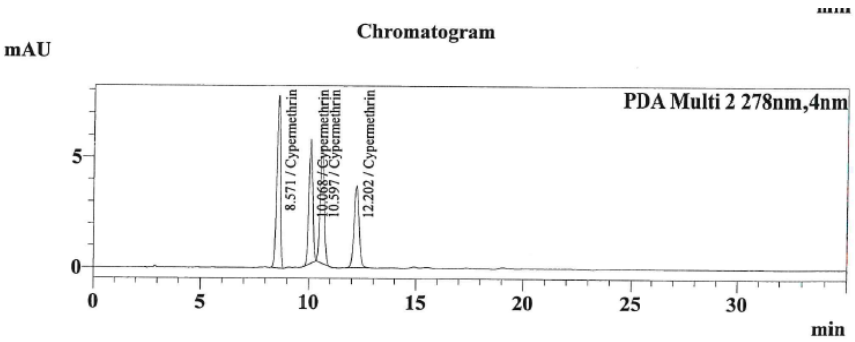

Fig. 2: Typical Chromatogram for Cypermethrin

3.1.2 Preparation of Sample Solution: An amount of 10.0 mg test substance was weighed accurately into a clean and dry 100 mL volumetric flask and dissolved in mobile phase and made upto the mark with the mobile phase. This solution was equivalent to 100 mg/L and used for determination of Specificity. The specificity of HPLC method for Quinalphos and Cypermethrin 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 the test substance

3.2 Linearity

3.2.1 Preparation of Standard Stock Solution and working standard: The standard solution, (100 mg/L) was prepared from the standard stock solution (1000 mg/L). The serial dilutions were made to prepare further concentrations such as 0.5, 10, 20, 30, 40 and 50 mg/L separately. The dilution details are presented in table 1. 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 1.

Table 1:Linearity of Quinalphos and Cypermethrin Reference Standard

Code	Replication	Std.Conc (quinalphos)	Std. area (quinalphos)	Mean Std. Area (quinalphos)	Std.Conc (cypermethrin)	Std. area (cypermethrin)	Mean Std. Area (cypermethrin)
STD-1	R1	0.5	4208	4199	3	1088	1077
	R2		4102			1064	
	R3		4287			1080	
STD-2	R1	10	85324	85548	25	9722	9753
	R2		85798			9778	
	R3		85523			9758	
STD-3	R1	20	181027	180958	40	15039	15055

	R2		181053			15084	
	R3		180795			15043	
STD-4	R1	30	267253	267482	55	20624	20637
	R2		268306			20607	
	R3		266888			20681	
STD-5	R1	40	348362	348436	70	25877	25862
	R2		348381			25843	
	R3		348564			25867	
STD-6	R1	50	447716	447786	85	32262	31498
	R2		447780			31104	
	R3		447861			31128	

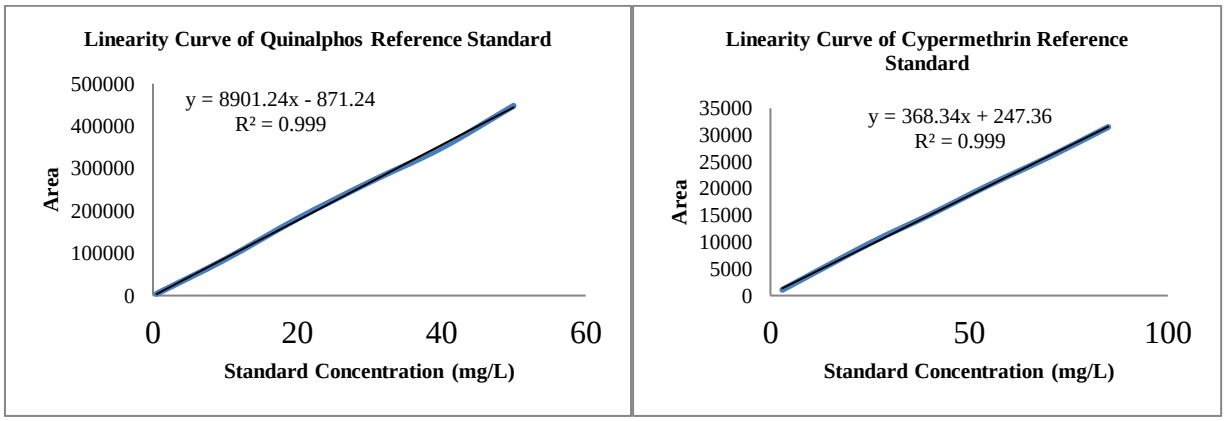

Fig. 3: Linearity Curve for Quinalphos and Cypermethrin

4. PRECISION

4.1 Preparation of Standard Solution

The Linearity standard solution (Standard – 4) 30 mg/L was prepared and used for the precision determination.

4.2 Preparation of Sample Solution

An amount of 14.56, 14.57, 14.62, 14.58 and 14.63 mg of Quinalphos 20% + Cypermethrin 3% EC was weighed into five different 10 mL volumetric flasks, the contents were dissolved and made upto the mark with the mobile phase. The concentrations of these solutions were equivalent to 1456, 1457, 1462, 1458 and 1463 mg/L respectively. An aliquot of 1 mL sample solution (1456, 1457, 1462, 1458 and 1463 mg/L) was taken into five different 10 mL volumetric flasks and diluted with mobile phase. The concentrations of these solutions were equivalent to 145.6, 145.7, 146.2, 145.8 and 146.3 mg/L respectively. These prepared solutions were injected into HPLC. The results are presented in table 2, 3.

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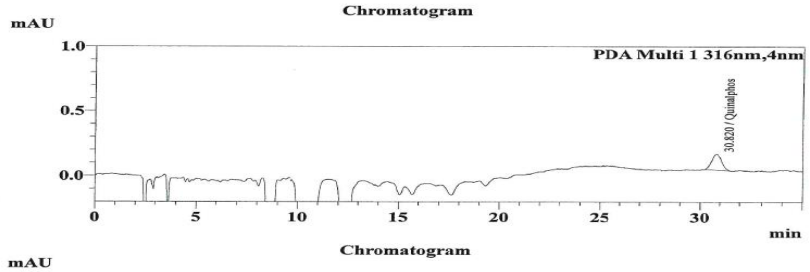
Sample Information

Sample Name	: Quinalphos + Cypermethrin
Sample ID	: L-Std-1
Injection Volume	: 20 uL
Data Filename	: Linearity-002
Method Filename	: Quinalphos+Cypermethrin.lcm
Date Acquired	: 18-Jun-2019 9:00:10 PM



Peak Table

PDA Ch1 316nm

Peak#	Ret. Time	Area	Area%	Height	Height%	Name
1	30.820	4199	100.000	125	100.000	Quinalphos
Total		4199	100.000	125	100.000	

Peak Table

PDA Ch2 278nm

Peak#	Ret. Time	Area	Area%	Height	Height%	Name
1	8.639	1880	30.536	167	34.758	Cypermethrin
2	10.175	1524	24.759	121	25.212	Cypermethrin
3	10.703	1482	24.077	112	23.311	Cypermethrin
4	12.335	1270	20.628	81	16.719	Cypermethrin
Total		6156	100.000	482	100.000	

Table 2: Precision (Quinalphos)

(Code) Sample /Standard	Standard Concentration (S)/Sample Concentration (W) (mg/L)	Standard Area /Sample Area (H_w)	Average Standard Area (H_s)	Purity of Calibration Solution (%)	Quinalphos Content (% w/w)	Density of Test Substance (g/ml)	Quinalphos Content (% w/v)	Mean Quinalphos Content (% w/v)
Std-R1	30	268355	269082	100	-	0.9661	-	-
S1R1	145.6	271163			20.764		20.060	20.061
S1R2		271184			20.765		20.061	
S2R1	145.7	271099			20.745		20.041	20.041
S2R2		271081			20.743		20.040	
S3R1	146.2	272766			20.801		20.096	20.095
S3R2		272738			20.799		20.094	
S4R1	145.8	271837			20.787		20.082	20.087
S4R2		271974			20.797		20.092	
S5R1	146.3	272411			20.760		20.056	20.055
S5R2		272379			20.757		20.053	
Std-R2	30	269809			-	-	-	-

Table 3: Precision (Cypermethrin)

(Code) Sample /Standard	Standard Concentration (S)/Sample Concentration (W) (mg/L)	Standard Area /Sample Area (H_w)	Average Standard Area (H_s)	Purity of Calibration Solution (%)	Cypermethrin Content (% w/w)	Density of Test Substance (g/ml)	Cypermethrin Content (% w/v)	Mean Cypermethrin Content (% w/v)
Std-R1	55	21423	21144	100	-	0.9661	-	-
S1R1	145.6	1778			3.177		3.069	3.073
S1R2		1783			3.185		3.077	
S2R1	145.7	1788			3.192		3.084	3.087
S2R2		1792			3.199		3.091	
S3R1	146.2	1794			3.192		3.084	3.088
S3R2		1799			3.201		3.092	
S4R1	145.8	1789			3.192		3.084	3.089
S4R2		1795			3.203		3.094	
S5R1	146.3	1797			3.195		3.087	3.088
S5R2		1798			3.197		3.089	
Std-R2	55	20864			-	-	-	-

Formula for Active content Calculation

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

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

5. ACCURACY (% RECOVERY)

The recovery processes and the recovery determination was validated with three fortification levels of processes.

5.1 Preparation of Standard Solution

The standard solution prepared for linearity (30 mg/L of Quinalphos and 55 mg/L of Cypermethrin) was used as standard in percent recovery determination.

5.2 Preparation of Blank Sample Solution

An amount of 32.5 mg of Quinalphos 20% + Cypermethrin 3% EC was weighed into 50 mL volumetric flasks, the contents were dissolved and made up to the mark with the mobile phase. The concentrations of these solutions were equivalent to 650 mg/L.

5.3 Preparation of Standard for Fortification

5.3.1 Preparation of Standard (Stock-H) Solution (Quinalphos): An aliquot of 1 ml Standard (Stock-A) solution (1000.34 mg/L) was taken into 10 ml volumetric flask, diluted with mobile phase and made upto the mark with the mobile phase. The prepared solution was equivalent to 100.03 mg/L.

5.3.2 Preparation of Standard (Stock-I) Solution (Cypermethrin): An aliquot of 1 ml Standard (Stock-C) solution (1000.07 mg/L) was taken into 10 ml volumetric flask, diluted with mobile phase and made upto the mark with the mobile phase. The prepared solution was equivalent to 100 mg/L.

5.3.3 Fortification Level –T1 (0.5 mg/L and 3 mg/L): An aliquot of 0.5 mL and 1.2 mL Linearity (Std-2) solution (10 mg/L of Quinalphos and 25 mg/L Cypermethrin) and was transferred into a 10 mL volumetric flask, diluted with blank sample solution and made upto the mark with blank sample solution. This solution was equivalent to 0.5 mg/L and 3 mg/L respectively.

5.3.4 Fortification Level –T2 (20 mg/L and 31 mg/L): An aliquot of 2 mL standard (Stock-H) solution (100.03 mg/L) and 3.1 mL standard (Stock-I) solution (100 mg/L) was transferred into a 10 mL volumetric flask, diluted with blank sample solution and made upto the mark with blank sample solution. This solution was equivalent to 20 mg/L and 31 mg/L respectively.

5.3.5 Fortification Level –T3 (30 mg/L and 44 mg/L): An aliquot of 3 mL standard (Stock-H) solution (100.03 mg/L) and 4.4 mL standard (Stock-I) solution (100 mg/L) was transferred into a 10 mL volumetric flask, diluted with blank sample solution and made upto the mark with blank sample solution. This solution was equivalent to 30 mg/L and 44 mg/L respectively. The above preparations were analyzed under HPLC. The results are presented in table 4, 5.

Formula

$$Fortified\ Area = Detected\ Area - \ Blank\ Sample\ Average\ Area$$

$$\text{Recovered Concentration } \left(\frac{\text{mg}}{\text{L}}\right) = \frac{\text{Standard Concentration (mg/L)}}{\text{Standard Average Area}} \times \text{ Fortified Area}$$

$$\text{Recovery } \ (\%) = \frac{\text{Recovered Concentration (mg/L)}}{\text{Fortified Concentration (mg/L)}} \times \ \ 100$$

The above preparations were analyzed under HPLC and checked for recovery (%). The results are presented in following table 4 and 5.

Table 4: Recovery – (Quinalphos; level 1and 2)

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	318737	318851.0	-	29.00	-	-
T1R1		925036		29.0115		100.04	99.96
T1R2		921780		28.9094		99.69	
T1R3		924487		28.9943		99.98	
T1R4		925028		29.0113		100.04	
T1R5		925279		29.0192		100.07	
T2R1		1506822		47.2579	48.0	98.45	98.50
T2R2		1504947		47.1991		98.33	
T2R3		1507640		47.2835		98.51	
T2R4		1510372		47.3692		98.69	
T2R5		1508068		47.2970		98.54	
Std-R2		318965		-		-	

Table 5: Recovery – (Cypermethrin; level 1and 2)

Code	Detected Area / Blank /samp/std	Blk/Sam./std Area /	Std. Conc.	Fortified Area	Recovered Conc.(mg/L)	Fortified Concentration (mg/L)	Recovery (%)	Average Recovery (%)	SD	RSD
R-Std-R1	20861	20782	55	-	-	-	-	-	-	-
R-T1R1	9258		-	1144	3.029	3.0	100.953	100.835	0.135	0.134
R-T1R2	9257			1143	3.026		100.864			
R-T1R3	9255			1141	3.021		100.688			
R-T2R1	19680			11566	30.611	31	98.746	98.464	0.257	0.261
R-T2R2	19640			11526	30.505		98.405			
R-T2R3	19621			11507	30.455		98.242			
R-T3R1	24841			16727	44.270	44	100.614	100.380	0.208	0.207
R-T3R2	24775			16661	44.096		100.217			
R-T3R3	24790			16676	44.135		100.308			
R-Std-R2	20702		55	-	-	-	**Ave.**	99.89	-	-

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

From the Linearity Standard Solution concentration of 30 mg/L was used in these LOD and LOQ determinations. From this solution 1 mg/L solution was prepared and further diluted to get the 0.01 and 0.1 mg/L concentration solutions were prepared. The dilution details were given in the table 6, and the results are presented in following table 6, 7, 8.

Table 6: Dilutions (LOD and LOQ) for LOD – Quinalphos and Cypermethrin

Stock Concentration (mg/L)	Dilution Volume (ml)	Final Volume (ml)	Final Concentration (mg/L)
1.0	1	10	0.2
0.1	1	10	1.5

Formula:

$$LOD = Average + (3\ x\ Standard\ Deviation)$$

$$LOQ = Average + (10\ x\ Standard\ Deviation)$$

Table 7: Limit of Detection (LOD) and Limit of Quantification (LOQ) Of Quinalphos

Sample ID	Std. Conc. (mg/L)	Std./ Sample Area	Average Std. Area	A. I. Content (mg/L)
STD-1	30	7046894	6990767	-
R1		951		0.004
R2		634		0.003
R3		895		0.004
STD-2		6934639		-
			MEAN	0.0035
			SD	0.00073
			LOD	0.01

Sample ID	Std. Conc. (mg/L)	Std./ Sample Area	Average Std. Area	A. I. Content (mg/L)
STD-1	30	7046894	6990767	-
R1		27180		0.117
R2		24161		0.104
R3		23974		0.103
STD-2		6934639		-
			MEAN	0.108
			SD	0.00772
			LOQ	0.18

Table 8: Limit of Detection (LOD) And Limit of Quantification (LOQ) Of Cypermethrin Example Calculation: (LODand LOQ)

Sample ID	Std. Conc. (mg/L)	Std./ Sample Area	Average Std. Area	A. I. Content (mg/L)
STD-1	30	5700139	5735571	-
R1		1362		0.0071
R2		1292		0.0068
R3		1354		0.0071
STD-2		5771003		-
			MEAN	0.0070
			SD	0.00020
			LOD	0.01

Sample ID	Std. Conc. (mg/L)	Std./ Sample Area	Average Std. Area	A. I. Content (mg/L)
STD-1	30	5700139	5735571	-
R1		19976		0.104
R2		19851		0.104
R3		19949		0.104
STD-2		5771003		-
			MEAN	0.104
			SD	0.00034
			LOQ	0.11

Limit of Detection (Cypermethrin) R1

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

$$= \frac{30 \times 1362}{5735571} = 0.0071$$

$$LOD = Mean\ Value + (3 \times SD)$$

$$= 0.0070 + (3 \times 0.0002) = 0.01$$

Limit of Quantification (Cypermethrin) R1

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

$$= \frac{30 \times 19976}{5735571} = 0.104\ \text{mg/L}$$

$$LOD = Mean\ Value + (10 \times SD)$$

$$= 0.104 + (10 \times 0.00034) = 0.11$$

7. ACTIVE CONTENT ANALYSIS OF QUINALPHOS AND CYPERMETHRIN

7.1 Preparation of Standard solution

An amount of 15 mg of the standard was dissolved in 100 ml of mobile phase and diluted to get 30 mg/L was used as standard in concentration analysis.

7.2 Preparation of Sample Solutions

The formulation sample (10 mg/L) was prepared and dissolved by sonication and diluted appropriately and injected into HPLC.

$$\frac{\text{Cypermethrin}}{\text{Quinalphos}}\left(\frac{\text{mg}}{\text{L}}\right) = \frac{\text{Concentration of standard (mg/L) x Area of sample solution x Dilution Factor}}{\text{Area of standard solution}}$$

8. CONCLUSION

8.1 Specificity

The blank, standard and the sample peaks were not co-eluted each other. The Chloro triazine based compounds Quinalphos and Cypermethrin was separated well with this simple HPLC (Reverse Phase) method. Hence the specificity was achieved as per the guideline SANCO 3030/99 Rev.4 requirement.

8.2 Linearity

The Linearity correlation co-efficient 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 Quinalphos and Cypermethrin, 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, 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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