Reverse Phase High Performance Liquid Chromatography (R-HPLC) Validation of an Analytical Method for the Formulation of Cypermethrin and Quinalphos Emulsion Concentrate (EC)

Dr. P. Srinivasulu, P. Haritha, I. Praveena
Professor[1], Assistant.Professor[2,3]
CSE DEPARTMENT
Swarnandhra College of Engineering & Technology, Narsapuram-534275.

ABSTRACT
The insecticides quinolphos and cypermethrin have been extensively used in pest management for both agricultural and residential purposes, either alone or in combination. Insecticides like pyrethroid and cypermethrin are used in large amounts in environmental applications, thus it's important to control their residue by giving the right quantity throughout their dissipation period. It is critical to detect even trace levels of these substances for efficient management and monitoring since they are just as dangerous to humans and other animals. Both compounds were detected in a single run at a concentration of 0.4 mg/L using an easy HPLC analytical method. With a flow rate of 1.5 ml/min, the mobile phase is composed of acetonitrile and water in a volume/volume ratio of 80:20. The Apollo Silica 5 (250 mm × 4.6 mm) HPLC column is used. Quinolphos and cyclomethrin were both detected at 316 and 278 nm, respectively, using the PDA detector of the Shimadzu LC2030 model HPLC. The proposed RP-HPLC technique is perfect for detecting and quantifying these compounds since it is simple to implement, fast to run, accurate, and exact, as shown by the results obtained from a basic HPLC analysis using the validation criteria of linearity, system appropriateness, system precision, and separation.

Keywords—Analysis of Quinalphos and Cypermethrin by High-Performance Liquid Chromatography (HPLC) in Compliance with SANCO 3030/99 Rev.4 and ICH Guidelines

INTRODUCTION

Quinoxaline residual systems, organophosphorothionate, and diethyl are the building blocks of quinalphos. In quinoxaline, the third hydrogen is replaced by a quinoxaline system, and in quialphos, two ethyl groups replace the acidic protons. The compound is a derivative of phosphoric acid. When the phosphorothioate and quinoxaline systems were mixed, quiralphos took on a reddish-brown tint. As a pesticide, phosphoric acid makes effective use of these two kinds of replacement systems in the plant production domain. The pyrethroid insecticide has lately been famous in the plant production sector for its innovative pest management features, which have seen extensive use in both public and private spheres. The insect's central nervous system regulates the insecticide cypermethrin, a form of pyrethrin. Chloride, keto, cynide, phenoxy, alkene, tricyclic alkane, and ester systems are all present in cyclomethrin's structural arrangement. When used on insects, pesticides release a series of toxic metabolic byproducts that may reach the brain and spinal cord. Due to their efficacy, quinolphos and cypermethrin are extensively used as pesticides in the area of plant production. To guarantee that no residue is left in the environment (air, water, and soil) following application of the combination pesticide Quinalphos and Cypermethrin, a comprehensive examination of the substance is required. In addition to being easy to implement, cheap, and reproducible, the proposed method of analysis excels in both quantitative and qualitative data.

MATERIALSANDMETHOD

Reagentsandchemicalsused

All the analytical grade solvents and water were used in this analytical method development. All the class A glasswear used inthisresearchanalyticalmethoddevelopment.

Instrument

In this experiment used HPLC was periodically calibrated and maintained to develop this analytical method development forchlorotriazine 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)withflowrate1ml/min.WiththisHPLCconditionthechlorotriazinemoleculesQuinalphosandCypermethrinwaselutedat3.4minutes and4.0minutesrespectively.

Preparationof Mobilephase

Anvolumeof80%Acetonitrileand20%weremixedwell, sonicatedandusedforanalysis.

ANALYTICALMETHODVALIDATION

Specificity

Preparationofstandardstocksolutions:Anamountof10.09mgQuinalphosreferencestandardwithpurity99.1%and
10.05 mg Cypermethrin reference standard with purity 99.5% were weighed accurately into a clean and dry 10 mL volumetricflask separately, dissolved with mobile phase and made upto the mark with mobile phase. This solution was equivalent to 1000mg/L respectively. From this, an aliquot of each 1ml solution was mixed 10 mL volumetric flask, diluted with mobile phase. Thissolutionwas equivalentto100mg/Land analyzedtodeterminespecificity.

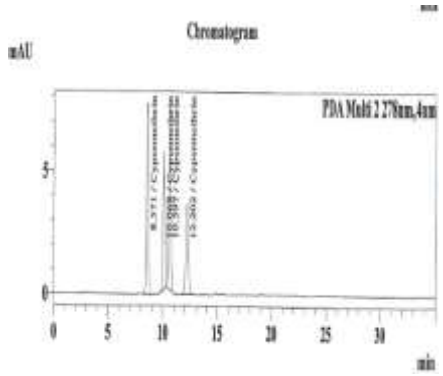

Fig.1:TypicalChromatogramforQuinalphos

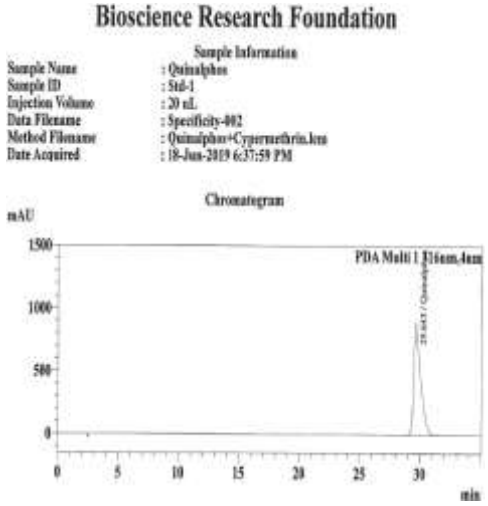

Fig.2:TypicalChromatogramforCypermethrin

PreparationofSampleSolution:A 100 mL volumetric flask that was clean and dry was used to correctly measure out 10.0 mg of test material. Then, the substance was dissolved in mobile phase until it reached the mark. The Specificity was determined using this solution, which had a concentration of 100 mg/L. When testing the HPLC technique for Quinalphos and Cypermethrin, it was noticed that there was no interference with the primary peak of interest when injecting the Standard and Sample solutions together with the blank (mobile phase). Accordingly, the test substance's analysis was thought to be well covered by this procedure.

Linearity

Preparation of Standard Stock Solutionand working standard: Starting with a standard stock solution of 1000 mg/L, the standard solution was diluted to 100 mg/L. Separate concentrations of0.5,10,20,30,40, and 50 mg/L were prepared by means of the serial dilutions. Table 1 displays the specifics of the dilution. A linear curve was drawn for the concentration of the standard against observed peak area and the correlation coefficient was obtained, respectively, after injecting the prepared standard solutions into an HPLC system using an auto sampler. Theresults arepresentedintable 1.

Table 1:Linearity ofQuinalphosandCypermethrinReference Standard

Code	Replication	Std.Conc(quinalphos)	Std. area(quinalphos)	Mean Std. Area(quinalphos)	Std.Conc(cypermethrin)	Std. area(cypermethrin)	MeanStd.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	81027	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	

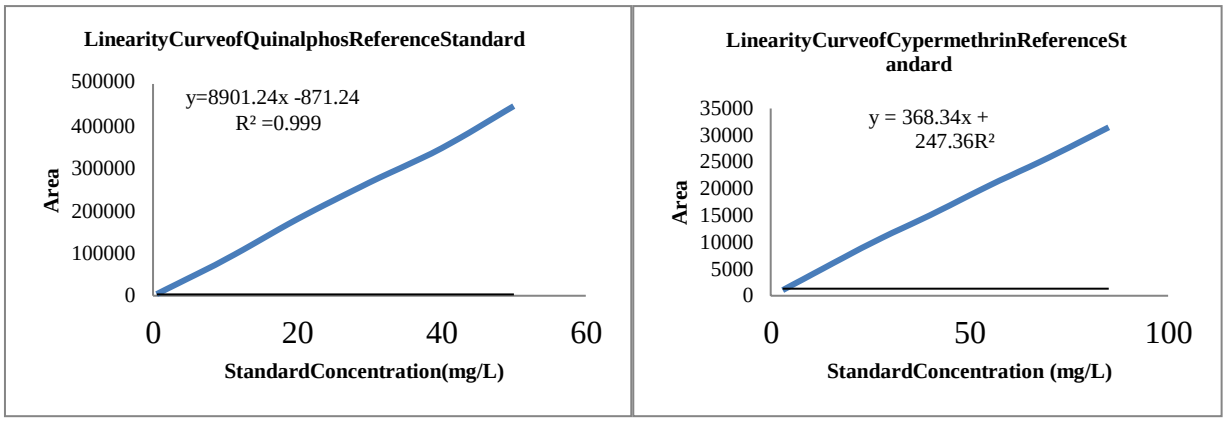

Fig.3:LinearityCurveforQuinalphosandCypermethrin

1. PRECISION

1.1 PreparationofStandardSolution

TheLinearity standardsolution(Standard – 4)30mg/Lwasprepared andused fortheprecisiondetermination.

1.2 Preparationof SampleSolution

An amountof14.56, 14.57, 14.62, 14.58 and 14.63 mgof Quinalphos 20%+ Cypermethrin 3% EC was weighed into fivedifferent 10 mL volumetric flasks, the contents were dissolved and made upto the mark with the mobile phase. The concentrationsof these solutions were equivalent to 1456, 1457, 1462, 1458 and 1463 mg/L respectively. An aliquot of 1 mL sample solution(1456,1457,1462,1458and1463mg/L)wastakenintofivedifferent10mLvolumetricflasksanddilutedwithmobilephase.The concentrations of these solutions were equivalent to 145.6, 145.7, 146.2, 145.8 and 146.3 mg/L respectively. These preparedsolutionswereinjectedintoHPLC.Theresults arepresentedintable2, 3.

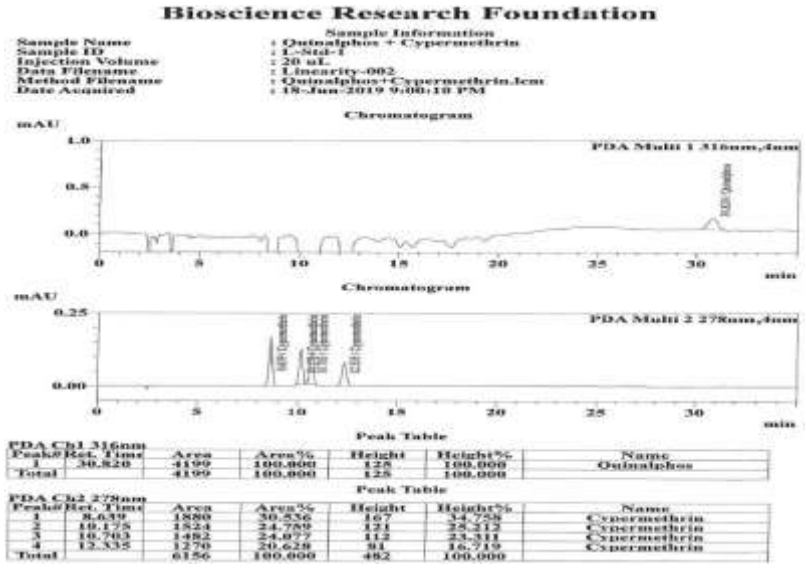

Table2:Precision (Quinalphos)

(Code)Sample/Standard	Standard Concentration(S)/Sample Concentration	Standard Area /SampleArea(H_w)	Average Standard Area(H_s)	Purity of CalibrationSolut	Quinalphos Content(%w/	Density of Test Substance(g/ml	Quinalphos Content(%w/	Mean Quinalphos Content(%w/v)
Std-R1	30	268355	269082	100	-	-	-	-
S1R1	145.6	271163			20.764	0.9661	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			-	-	-	-

Table3:Precision (Cypermethrin)

(Code)Sample/Standard	StandardConcentration(S)/SampleConcentration (W)(mg/L)	Standard Area /Sample Area(H_w)	AverageStandard Area(H_s)	Purity ofCalibration Solution(%)	Cypermethrin Content(% w/w)	Density of TestSubstance(g	Cypermethrin Content(% w/v)	MeanCypermethrinContent (%w/v)
Std-R1	55	21423	21144	100	-	-	-	-
S1R1	145.6	1778			3.177	0.9661	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			-	-	-	-

FormulaforActivecontentCalculation

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

The%RSDiswithinlimitaccordingtothemodifiedHorwitzequation(AcceptableLimit<1.3RSDfor100%activecontentasperSANCO/3030/99Rev.4)

2. ACCURACY(%RECOVERY)

Therecoveryprocessesandtherecoverydeterminationwasvalidatedwiththree fortificationlevelsof processes.

2.1 PreparationofStandardSolution

Thestandardsolutionpreparedforlinearity(30mg/LofQuinalphosand55mg/LofCypermethrin)wasusedasstandardinpercentrecoverydetermination.

2.2 Preparationof BlankSampleSolution

Anamountof32.5mgofQuinalphos20%+Cypermethrin3%ECwasweighedinto50mLvolumetricflasks,thecontentsweredissolvedand madeup tothemark withthemobilephase.Theconcentrationsofthese solutionswereequivalent to650 mg/L.

2.3 Preparationof StandardforFortification

2.3.1 Preparation of Standard (Stock-H) Solution (Quinalphos): An aliquot of 1 ml Standard (Stock-A) solution (1000.34mg/L) was taken into 10 ml volumetric flask, diluted with mobile phase and made upto the mark with the mobile phase. Theprepared solutionwas equivalentto100.03mg/L.

2.3.2 Preparation of Standard (Stock-I) Solution (Cypermethrin): An aliquot of 1 ml Standard (Stock-C) solution (1000.07mg/L) was taken into 10 ml volumetric flask, diluted with mobile phase and made upto the mark with the mobile phase. Theprepared solutionwas equivalentto100mg/L.

2.3.3 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 ofQuinalphosand25mg/LCypermethrin)andwastransferredintoa10mLvolumetricflask,dilutedwithblanksamplesolutionand madeuptothemark withblank sample solution.Thissolutionwas equivalentto0.5mg/Land3mg/Lrespectively.

2.3.4 Fortification Level–T2 (20 mg/L and 31 mg/L): An aliquot of 2 mL standard (Stock-H) solution (100.03 mg/L) and 3.1mL standard (Stock-I) solution (100 mg/L) was transferred into a 10 mL volumetric flask, diluted with blank sample solution andmadeuptothemark withblank samplesolution.This solutionwasequivalentto20mg/Land31mg/Lrespectively.

2.3.5 Fortification Level–T3 (30 mg/L and 44 mg/L): An aliquot of 3 mL standard (Stock-H) solution (100.03 mg/L) and 4.4mL standard (Stock-I) solution (100 mg/L) was transferred into a 10 mL volumetric flask, diluted with blank sample solution andmade upto the mark with blank sample solution. This solution was equivalent to 30 mg/L and 44 mg/L respectively. The abovepreparationswereanalyzedunderHPLC.Theresultsarepresentedintable4, 5.

Formula

$$FortifiedArea = DetectedArea - BlankSampleAverageArea$$

$$\text{coveredConcentration}(_{L}) = \frac{\text{mgRe}}{\text{StandardAverageArea}} \times \text{FortifiedArea}$$

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

Theabovepreparationswereanalyzedunder HPLCandcheckedforrecovery(%).Theresultsarepresentedinfollowingtable4and5.

Table4:Recovery–(Quinalphos;level1and2)

Fortification Level	Std. Conc.(mg/L)	Std. /Samplearea	MeanStd. 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;level1and2)

Code	DetectedArea/Blank /samp/std	Blk/Sam./stdArea /	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	-	-

3. LIMITOFDETECTION(LOD)ANDLIMITOFQUANTIFICATION(LOQ)

From the Linearity Standard Solution concentration of 30 mg/L was used in these LOD and LOQ determinations. From thissolution 1 mg/L solution was prepared and further diluted to get the 0.01 and 0.1 mg/L concentration solutions were prepared. Thedilution details were giveninthetable6,and theresultsarepresentedinfollowingtable6,7,8.

Table 6:Dilutions(LODandLOQ)forLOD–Quinalphos andCypermethrin

StockConcentration(mg/L)	DilutionVolume(ml)	FinalVolume(ml)	FinalConcentration(mg/L)
1.0	1	10	0.2
0.1	1	10	1.5

Formula:

$$LOD=Average+(3xStandard\ Deviation)$$

$$LOQ=Average+(10xStandardDeviation)$$

Table 7:LimitofDetection(LOD) andLimitofQuantification(LOQ)OfQuinalphos

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: (LODandLOQ)

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

LimitofDetection(Cypermethrin)R1

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

$$=\frac{30\times1362}{5735571}=0.0071$$

$$LOD=MeanValue+(3\times SD)$$

$$=0.0070+(3\times0.0002)=0.01$$

LimitofQuantification(Cypermethrin)R1

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

$$=\frac{30\times19976}{5735571}=0.104\text{mg/L}$$

$$LOD=MeanValue+(10\times SD)$$

$$=0.104+(10\times0.00034)=0.11$$

4. ACTIVECONTENTANALYSISOFQUINALPHOSANDCYPERMETHRIN

4.1 PreparationofStandardsolution

Anamountof15mgofthestandardwasdissolvedin100mlofmobilephaseanddilutedtoget30 mg/Lwasusedasstandardinconcentrationanalysis.

4.2 Preparationof SampleSolutions

Theformulationsample (10mg/L)waspreparedanddissolvedbysonicationanddilutedappropriatelyandinjectedintoHPLC.

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

5. CONCLUSION

5.1 Specificity

The blank, standard and the sample peaks were not co-eluted each other. The Chlorotriazine based compounds Quinalphos andCypermethrin was separated well with this simple HPLC (Reverse Phase) method. Hence the specificity was achieved as per theguideline SANCO3030/99Rev.4requirement.

5.2 Linearity

TheLinearity correlation co-efficientisachievedNLT0.99asper(SANCO 3030/99Rev.4)

5.3 SystemPrecision

Thesystemprecisionisachievedasthe%RDSfor5replicatesobservedas0.1%forQuinalphosandCypermethrin,hencetheminimumrequirementofthe(SANCO3030/99Rev.4 wasNMT15%RSDwas achieved

5.4 SystemRecovery

The systemrecovery 92%to101%wereachievedfor,hence theminimumrequirementofthe(SANCO 3030/99Rev.4).

5.5 Detailsofthe Laboratoryworkwerecarried out

BioscienceResearchFoundation,SengaduvillageandPost,ViaManavalanagar,Kandamangalam–602002,KanchipuramDistrict,Tamilnadu,IndiaPh:+914427658293/94/95/96,Email:brfchennai@gmail.com

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thetertiaryWith contributions from Ines Domingues, Joanne Perez Moureiro, and Marta MonteiroAmadeu Mario V. M. Soares and Susana E. To reduce chlorpyrifos's toxicity to developing zebrafish, quinalphos and cypermethrin work together. The rerioDanio Springer

Page numbers 4671–4680 of Volume 20, Issue 4, Research in Environmental Science and Pollution, 2013 (DOI: 10.1007/s11356-012-1443-6).

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[6][5]The cited paper is "Simple regression models for the qualitative prediction of Quinalphos and Cypermethrin leaching" (14:2, 58-62, DOI: 10.1080/02571862.1997.10635082) written by C. F. Reinhardt and K. J. Hugo and published in the South African Journal of Plant and Soil.

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