Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2016, 8(4):241-248

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Dissipation kinetics of kresoxim-methyl fungicide in different p^{H} waters under sun light

Tentu Nageswara Rao[1]*, S. N. V. S. Murthy[2], K. Parameswar[3], N. Krishna Rao[1] and Karri Apparao[1]

[1]*Department of Chemistry, Krishna University, Machilipatnam, Andhra Pradesh, India*
[2]*Department of Chemistry, DLR PG College, G. Mamidada, Andhra Pradesh, India*
[3]*Department of Pharmacy, Princeton college of Pharmacy, Korremulla, Telangana, India*

ABSTRACT

Studied the dissipation behavior of Kresoxim-methyl in acidic, neutral and basic water of pH 4.0, 7.0 and 9.0, an experiment was conducted by spiking in three different pH Waters having pH 4.0, 7.0 and 9.0 to give the uniform concentrations of T0 – Untreated Control, T1 –Kresoxim-methyl 50% WG @ 1 mg/L of water and T2 –Kresoxim-methyl 50% WG @ 2 mg/L of water. The spiked samples have been kept under sunlight. The sampling occasions were 0, 1st, 3rd, 5th, 7th, 10th, 15th and 20th day for acidic water (pH 4.0), neutral (pH 7.0) and basic water (pH 9.0) Samples were collected on different occasions (0, 1, 3, 5, 7, 10 and 15). All of the samples had been analyzed until the residues have been below detectable level. The residues of Kresoxim-methyl were quantified using a validated HPLC-UV method. The DT50 (Half Life) of Kresoxim-methyl calculated by regression analysis from the dissipation data.

Key words: Kresoxim-methyl, Dissipation, DT50, Residues and aqueous buffer solutions.

INTRODUCTION

Fungicides are the predominant part of agriculture crop management for better yields. In this process several new molecules have been introduced for the potential control of pests and diseases. Fungicides can be divided into protectant and specific types [1]. Protectant is the older type and includes copper and sulfur based products. They form a protective film on the plant surface and inhibit the germination of fungal spores [2]. Specific type fungicides are so called because they act on one specific chemical reaction in the fungus. Strobilurin compounds, they inhibit the respiratory electron transport is fungus and thereby killing fungus [3-6]. They act as efficient inhibitors. One of the most commonly used strobilurin fungicides; kresoxim methyl is mainly used for the control of powdery mildew and scab in apples, pears, grapes, strawberries and vegetables [7-10]. It is one of the most frequently used fungicides in Indian viticulture, where application is done by foliar spray and also through drip irrigation [11-14]. The present research was aimed to investigate the fate of residues of kresoxim methyl in three different aqueous buffer solutions under sun light.

EXPERIMENTAL SECTION

Reference analytical standards of kresoxim methyl (purity 99%) were obtained from Sigma Aldrich. The test item kresoxim methyl 50% Wettable granules (WG) was purchased from local market. Acetonitrile, Water HPLC grade,

ortho phosphoric acid AR grade, Sodium hydroxide LR grade, Potassium chloride GR grade, Boric acid GR grade, Potassium biphthalate GR grade and Potassium phosphate AR grade were obtained from the Merck India limited. Distilled water was purified by using the milli-Q Plus apparatus (Millipore, Bedford, MA, USA).

Standard stock solution
Accurately 10.56 mg of Kresoxim-methyl reference standard, purity (99.0 %) was weighed into 20 mL volumetric flask. The content was dissolved in 10 mL of acetonitrile, sonicated and made up to the mark with the same solvent. The concentration was 522.72 mg/L solution. and stored in a freezer at -18°C. The stock standard solutions were used for up to 3 months. Suitable concentrations of working standards were prepared from the stock solutions by dilution using acetonitrile, immediately prior to sample preparation.

Sample stock solution
Accurately 49.88 mg of test item (purity 50.12%) of Kresoxim-methyl was taken into a 25 mL volumetric flask. The content was dissolved in 5 mL of acetonitrile, sonicated and made up to the mark with the acetonitrile. The concentration was 1000 mg/L solution. The stock sample solution was used for preparation of dose samples (T1 and T2) in different aqua's buffers.

Preparation of acidic water (pH 4.0)
About 8g of potassium dihydrogen phosphate was dissolved in distilled water and diluted to 1000 mL. The pH was adjusted to 4.0 with the same.

Preparation of neutral water (pH 7.0)
About 6.3 g of disodium hydrogen was phosphate and 5g of potassium dihydrogen phosphate was dissolved in distilled water and diluted to 1000 mL. The pH of the buffer was adjusted with disodium hydrogen ortho phosphate and potassium dihydrogen phosphate.

Preparation of basic water (pH 9.0)
12.5g of boric acid & 15g potassium chloride was dissolved in distilled water and diluted to 1000 mL. The pH was adjusted to 9.0 using 0.1 M sodium hydroxide solution.

APPLICATION DATA

Name of the buffers	Acidic, Neutral and Basic
Replications	Three
Method of fortification	T_0 (Untreated Control) T1 (1 mg/L Level) 1.0 mL of test item stock solution was fortified in to 1000 mL (1Liter) different buffer samples to get the uniform concentrations. T2 (2 mg/L Level) 2.0 mL of test item stock solution was fortified in to 1000 mL (1Liter) different buffer samples to get the uniform concentrations.

SAMPLING DATA

Samples Exposure	Under direct sunlight	
Sample preparation	During each sampling occasion, water samples were mixed thoroughly and sub sampled 20mL using a pipette.	
Occasion (Days)	p^H 4 and 7 (0, 1, 3, 5, 7, 10, 15, 20) and p^H 9 (0, 1, 3, 5, 7, 10, 15)	
Laboratory condition	**Temperature**	
	Minimum	Maximum
	20.2°C	25.4°C

CHROMATOGRAPHIC SEPARATION PARAMETERS

The HPLC-UV system used, consisted shimadzu high performance liquid chromatography with LC- 20AT pump and SPD-20A interfaced with LC solution software, equipped with a reversed phase C18 analytical column of 250 mm x 4.6 mm and particle size 5 µm (PhenomenexLuna-C18) Column oven temperature was maintained at 30°C. The injected sample volume was 20µL. Mobile Phases A and B was Acetonitrile and 0.1% ortho phosphoric acid (80:20 (v/v)). The flow- rate used was kept at 1.0 mL/min with a detector wavelength at 230 nm. The external standard method of Calibration was used for this analysis.

METHOD VALIDATION

Method validation ensures analysis credibility. In this study, the parameters accuracy, precision, linearity and Limits of Detection (LOD) and Quantification (LOQ) were considered [15]. The accuracy of the method was determined by recovery tests, using samples spiked at concentration levels of 0.05 and 0.5 µg/mL. Linearity was determined by different known concentrations (0.05, 0.1, 0.5, 1.0 and 2.0, 5.0 µg/mL) which were prepared by diluting the stock solution. The Limit of Detection (LOD, µg/mL) was determined as the lowest concentration giving a response of 3 times the baseline noise defined from the analysis of control sample. The Limit of Quantification (LOQ, µg/mL) was determined as the lowest concentration of a given fungicide giving a response of 10 times the baseline noise.

RESULTS AND DISCUSSION

Specificity

Specificity was confirmed by injecting the Mobile phase solvents i.e., Acetonitrile and 0.1% Orthophosphoric acid, HPLC water, sample solution standard solution and buffer controls (acidic, neutral, basic) There were no matrix peaks in the chromatograms to interfere with the analysis of fungicide residues shown in **Fig.1, Fig. 2 and Fig.3**. Furthermore, the retention time of Kresoxim-methyl was constant at 5.2 ± 0.2 min.

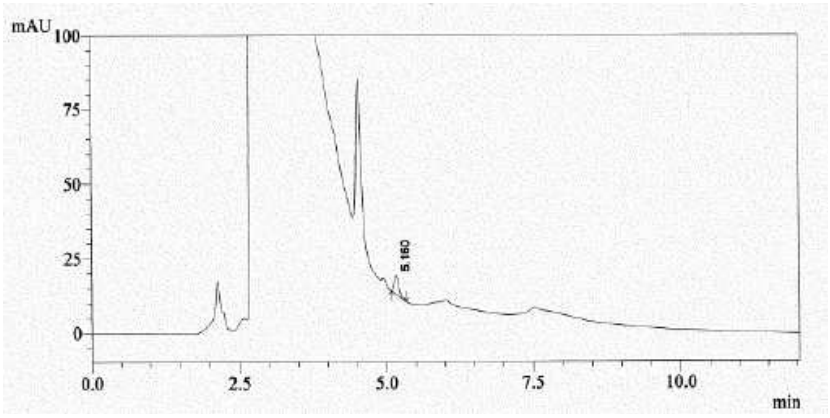

Fig.1. Representative chromatogram of kresoxim methyl test item in acidic water - day 0

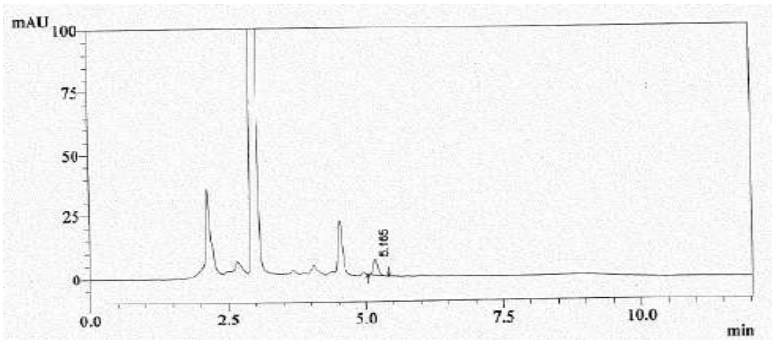

Fig.2. Representative Chromatogram of kresoxim methyl test item in neutral water - day 0

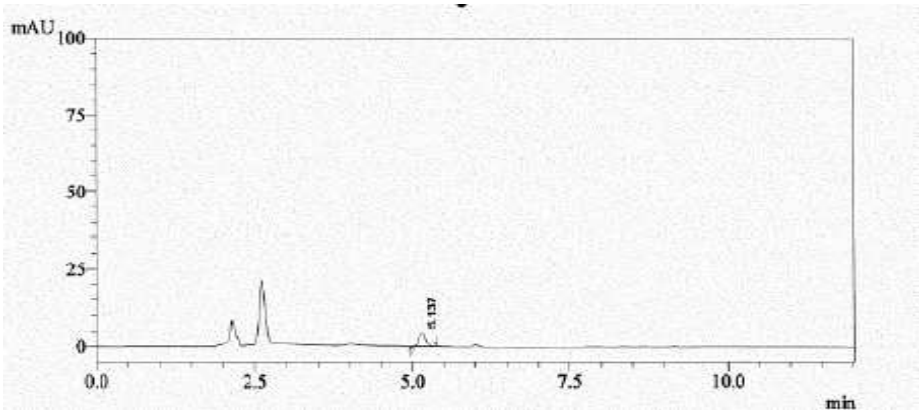

Fig.3. Representative Chromatogram of kresoxim methyl test item in basic water - day 0

Linearity

Different known concentrations of fungicides (0.05, 0.1, 0.5, 1.0, 2.0 and 5.0 µg/mL) were prepared into a different 10 mL volumetric flasks by diluting the stock solution. The serial dilution details were presented in **Table 1**. These standard solutions were directly injected into a HPLC. A calibration curve has been plotted for concentration of the standards injected versus area observed and the linearity of method was evaluated by analyzing six standard concentration solutions. The peak areas obtained from different concentrations of standards were used to calculate linear regression equation. This was Y=30799.81X + 9.11 with correlation coefficient of 0.9998 respectively. A calibration curve is showed in **(Figure IV).**

Table 1. Serial dilutions for linearity standard solutions

Stock solution concentration (µg/mL)	Volume taken from stock solution (mL)	Final make up volume (mL)	Obtained concentration (µg/mL)
522.72	1.910	10	100
100	0.500	10	5
100	0.200	10	2
100	0.100	10	1
5	1.000	10	0.5
5	0.200	10	0.1
1	0.500	10	0.05

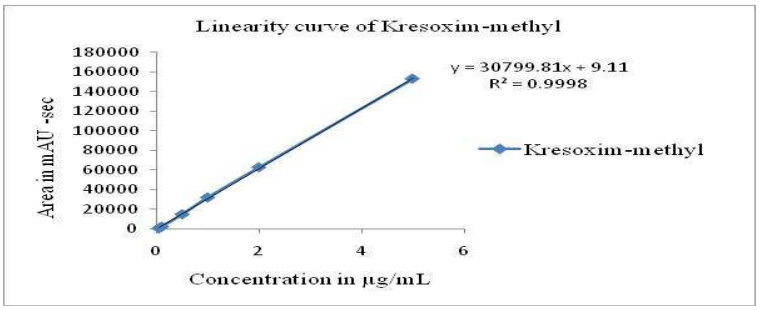

Fig.4. Representative Calibration curve of kresoxim methyl standard

Accuracy and Precision

The analytical method was validated for the recovery of the test item at two concentration levels with acidic, neutral and basic water.

Preparation of Test item stock solution:

Accurately 5.19 mg of test item (purity 50.12%) of Kresoxim-methyl was taken into a 50 mL volumetric flask. The content was dissolved in 5 mL of acetonitrile, sonicated and made up to the mark with the same solvent. This concentration was 52.0 mg/L solution.

Fortified test item concentration – 1 mg/L
0.481 mL of test item stock solution was taken into a 25 mL volumetric flask and made up to the mark with acetonitrile.

Preparation of 0.05 mg/L Fortification Level
0.5 mL aliquot of 1.0 mg/L test item solution was fortified into each of the 10mL of buffer solutions (acidic, neutral, basic). This was done in 6 replications.

Preparation of 0.5 mg/L Fortification Level
5.0 mL aliquot of 1.0 mg/L test item solution was fortified into each of the 10mL of buffer solutions (acidic, neutral, basic). This was done in 6 replications.

The samples were assayed for accuracy and repeatability in HPLC. Accuracy was calculated as %recovery and repeatability as %RSD and the results are mentioned in **Table 2**.

Table 2. Recoveries of the Kresoxim-methyl from aqueous buffer solutions samples (n=6)

Fortification Concentration in µg/mL	Replication	Recovery (%)		
		Acidic water	Neutral water	Basic water
0.05	R1	85	84	85
	R2	86	84	83
	R3	89	86	84
	R4	88	88	85
	R5	87	85	86
	R6	85	86	84
	Mean	**86.67**	**85.50**	**84.50**
	RSD	**1.88**	**1.77**	**1.24**
0.5	R1	92	91	90
	R2	91	90	92
	R3	95	93	90
	R4	94	91	91
	R5	93	91	90
	R6	94	92	92
	Mean	**93.17**	**91.33**	**90.83**
	RSD	**1.58**	**1.13**	**1.08**

Detection and Quantification Limits
The limit of quantification was determined to be 0.05 µg/mL. The quantitation limit was defined as the lowest fortification level evaluated at which acceptable average recoveries (85-93%, RSD<2%) were achieved. This quantification limit also reflects the fortification level at which an analyte peak is consistently generated at approximately 10 times the baseline noise in the chromatogram. The limit of detection was determined to be 0.02 µg/mL at a level of approximately three times the back ground of control injection around the retention time of the peak of interest.

Dissipation details
Acidic water
The initial concentration of Kresoxim-methyl in acidic water (day 0) was 0.984mg/L and 1.965mg/L in T1and T2 dosages respectively, which on day 1 dissipated to 0.948mg/L and 1.944mg/L. The day 3 samples showed the residues 0.741mg/L (T1) and 1.683mg/L (T2), day 5 samples showed 0.548mg/L (T1) and 1.040mg/L (T2), day 7 samples showed 0.395mg/L (T1) and 0.756mg/L in (T2), day 10 samples showed 0.313mg/L (T1) and 0.628mg/L (T2), day 15 samples showed 0.112 mg/L (T1) and 0.244 mg/L (T2). A complete dissipation of residues to below detectable level (BDL) was observed on day 20 in both the tested dosages (T1) and (T2).

Neutral water
The initial concentration of Kresoxim-methyl in neutral water (day 0) was 0.946 mg/L and 1.964 mg/L in T1and T2 dosages respectively, which on day 1 dissipated to 0.939 mg/L and 1.873 mg/L. The day 3 samples showed the residues 0.826 mg/L (T1) and 1.651 mg/L (T2), day 5 samples showed 0.440 mg/L (T1) and 0.876 mg/L (T2), day 7 samples showed 0.379 mg/L (T1) and 0.801 mg/L in (T2), day 10 samples showed 0.285 mg/L (T1) and 0.556 mg/L

(T2), day 15 samples showed 0.097 mg/L (T1) and 0.199 mg/L (T2). A complete dissipation of residues to below detectable level (BDL) was observed on day 20 in both the tested dosages (T1) and (T2).

Basic water

The initial concentration of Kresoxim-methyl in basic water (day 0) was 0.942 mg/L and 1.903 mg/L in T1and T2 dosages respectively, which on day 1 dissipated to 0.880 mg/L and 1.828 mg/L. The day 3 samples showed the residues 0.838 mg/L (T1) and 1.672 mg/L (T2), day 5 samples showed 0.670 mg/L (T1) and 1.342 mg/L (T2), day 7 samples showed 0.391 mg/L (T1) and 0.790 mg/L in (T2), day 10 samples showed 0.092 mg/L (T1) and 0.199 mg/L (T2).

The dissipation curve plotted between concentration of the analyte and sampling occasions is presented in **Fig.5, Fig.6 and Fig. 7**. DT50 value was calculated using the following formula

$$DT50 = \ln 2/(k)$$

Where,

'k' is slope of the curve obtained from the dissipation data.

During the dissipation kinetics study it was observed that the compound degraded to below detectable level within 4.91 days in acidic water (pH 4) and 4.55 days in neutral water (pH 7). The complete dissipation of residues in basic water (pH 9) was observed by 3.28 days.

The analysis changed into carryout in laboratory circumstance at 25°C and shown the half life values around 4.91 days in acidic water. In neutral water the half-life was around 4.55 days. Further the degradation of fungicide in basic water was rapid; the half-life value was around 3.28 days.

The calculated DT 50 (Time required to degrade 50% of residues) values of Kresoxim-methyl in different pH waters (Acidic (pH -4), Neutral (pH -7) and Basic (pH -9)) under the influence of sunlight presented in **Table 3, 4 and 5.** The rate constant value was calculated by linear regression equation from the first order rate equation.

$$K = \ln a/a\text{-}x/dt$$

Where, dt is the time interval between t_1 and t_2 and a, x are the concentration of pesticides at times t_1 and t_2 respectively. A plot of concentration of the residues and rate with the R^2 indicates first order kinetics in dissipation of the fungicide. The DT50 (Half Life) of Kresoxim-methyl calculated by regression analysis from the dissipation data.

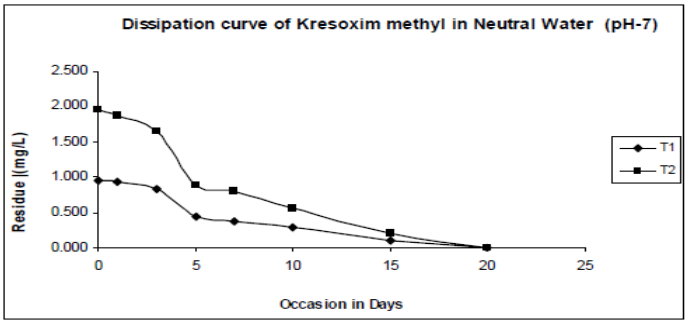

Fig.5. Dissipation curve of kresoxim methyl in acidic water

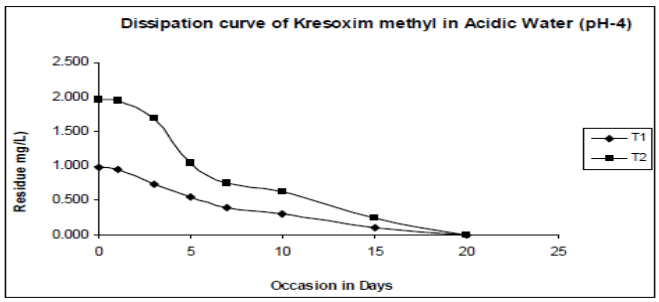

Fig.6. Dissipation curve of kresoxim methyl in neutral water

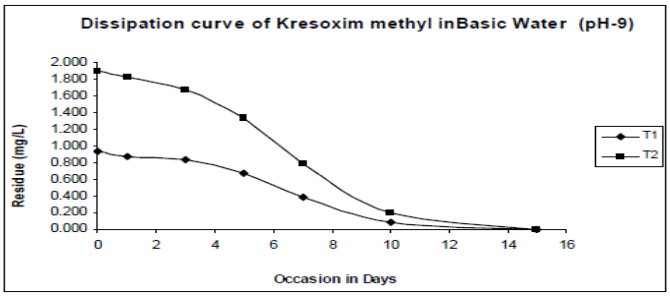

Fig.7. Dissipation curve of kresoxim methyl in basic water

Table 3. Regression Analysis – Acidic water (pH -4) for of Kresoxim-methyl

Parameters	Dosages	
	T1	T2
Regression équation	Y = 0.038– 0.062* X	Y = 0.343–0.061* X
Half-life (Days)	4.85	4.91
Correlation co-efficient	0.991	0.989

Table 4. Regression Analysis – neutral water (pH -7) for of Kresoxim-methyl

Parameters	Dosages	
	T1	T2
Regression equation	Y = 0.035– 0.066* X	Y = 0.341 – 0.066 * X
Half-life (Days)	4.54	4.55
Correlation co-efficient	0.984	0.987

Table 5. Regression Analysis – basic water (pH -9) for of Kresoxim-methyl

Parameters	Dosages	
	T1	T2
Regression equation	Y = -0.109– 0.094* X	Y = 0.412–0.092* X
Half-life (Days)	3.22	3.28
Correlation co-efficient	0.910	0.917

CONCLUSION

The Dissipation kinetics of Kresoxim-methyl in different pH waters under direct sunlight become very fast whilst as compared with dissipation facts acquired at 25°C. In basic water degradation changed into faster while compared to acidic and impartial water. Dissipation of Kresoxim-methyl in different pH waters followed first order kinetics and this paper describes a fast, simple sensitive analytical method based on HPLC-UV to determine the kresoxim methyl residues in three different types of buffers. The mobile phase Acetonitrile and 0.1% ortho phosphoric acid confirmed excellent separation and decision and the analysis time required for the chromatographic determination of three different type of buffers is very short (around 15 min for a chromatographic run).

Satisfactory validation parameters such as linearity, recovery, precision and LOQ and DT 50 values were established by following South African National Civic Organization (SANCO) and Environmental Protection Agency (EPA) guidelines [16]. Hence, the proposed analytical procedure and dissipation information would be valuable for regulatory monitoring authority, residue labs and research scholars to determine the kresoxim methyl residues in different commodities (crop, water and soil samples).

Acknowledgements
The authors are thankful to the Dr. B. Gowtham Prasad, SVV University, for providing necessary facility to conduct the Laboratory experiment.

REFERENCES

[1] Dave W Bartlett; Dave W Bartlett; John M Clough; Jeremy R Godwin; Alison A Hall; Mick Hamer; Bob Parr-Dobrzanski, *Pest Management Science.,* **2002,** 58, 649- 662.
[2] Balba H, *Journal of Environmental Science and Health, Part B.*,**2007,** 42, 441-451.
[3] Rupali P; Sabale; et al, *Environmental monitoring and assessment.,* **2015,** 187, 436.
[4] Nageswara Rao T; et al, *Indian Journal of scientific Research.,* **2012**, 3(1): 113-118.
[5] Nageswara Rao T; Sreenivasulu D; Patrudu B; Sreenivasula Reddy EG, *Scholars Academic Journal of Biosciences*., **2013**, 1(3), 80-84.
[6] Tentu Nageswara Rao; Murthy. SNVS; Seshamma. S; and Sreenivasulu. D, *International journal of Chem Tech Research.*, **2015**, 8 (3), 1149-1155.
[7] Tang; et al, *Journal of food science.,* **2013**, 77(5), 105-109.
[8] Zuin V.G; et al, *Braz.Chem.Soc.,* **2003,** 14, 304-309.
[9] Steven J. Lehotay, *Journal of AOAC International.*, **2000**, 83 (3), 5.
[10] Li JZ; WuX; Hu JY, *J Environ Sci Health.,* **2006,** 41(4), 427-436.
[11] Fernandez. M; et al, *Chromatographia* ., **2001**, 54(5), 302-308.
[12] P Cabras; A Agioni; VL Garu; FM Oirisi; V Brandolini; must and wine, *Journal of AOAC International*., **1998**, 81(6), 1185-1189.
[13] Alberto Navalo n; Avismelsi Prieto; Lilia Araujo; Jose Luis Vilchez, *Journal of Chromatography A***., 2002,** 975, 355-360.
[14] Vijay Tularam Gajbhive; Suman Gupta; Irani Mukherjee; Shashi Bala Singh; Neera Singh; Prem Dureja; Yogesh Kumar, *Bulletin of Environmental Contamination and Toxicology*., **2011,**86(1), 90-94.
[15] Jyot, Gagan; Arora; Parshotam Kumar, *Bulletin of environmental contamination and toxicology.,* **2010**, 84(3), 305-310.
[16] SANCO Guidelines, *Document NO. SANCO/10684/2009.*, **2009.**