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Modified Popoff's Reaction for Indirect Spectrophotometric Determination of Cypermethrin

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ABSTRACT

A new and highly sensitive spectrophotometric method is developed for the determination of parts per million levels of widely used Cypermethrin insecticide. The method is based on hydrolysis of Cypermethrin (Popoff's Reaction), which further react with potassium iodide-iodate solution under acidic conditions to liberate iodine and the liberated iodine selectively oxidizes leucomalachite green to malachite green dye. The absorption maxima of the dye formed is measured at 610 nm in acidic medium. Beer's law is obeyed over the concentration range of 2.0 to 16µg in a final solution volume of 25 ml (0.12-0.68ppm). The molar absorptivity and Sandell's sensitivity were found to be $3.3x10^5$ *L* $mole^{-1}cm^{-1}$ *and 0.054 µg* cm^{-2} *respectively. The standard deviation and relative standard deviation were found to be ± 0.001 and 0.22% respectively. The method is simple sensitive and free from interferences of other pesticides and diverse ions. Other pyrethroid insecticides do not interfere in the proposed method. The method has been satisfactorily applied to the determination of Cypermethrin in various environmental and biological samples. Statistical treatment of the experimental results indicates that the method is precise and accurate.*

Keywords: Popoff's reaction, leucomalachite green, pyrethroid insecticide.

INTRODUCTION

Pyrethroid insecticides are used to control a number of insect species on economic crops. The pyrethroid insecticides containing a nitrile group, viz., Cypermethrin ($C_{22}H_{19}Cl_2NO_3$) has been identified as highly effective contact insecticides. Cypermethrin are effective pest control chemical and have low mammalian toxicity. Owing of its availability, insecticides are misused in homicidal suicidal poisoning cases. Consequently, characterization of these insecticides is necessary in forensic toxicology. Pyrethroid insecticides including Cypermethrin, have been used in agricultural, veterinary and home formulation for more than 40 years and account for approximately one-fourth of the world wide insecticide market. [1-6]. Cypermethrin (Ambush, Atroban, Biothrin) or (RS) cyano – 3 – phenoxybenzyl (1 RS, 3RS; 1RS, 3RS) (2, 2 – dichlorovinyl) 2, 2 – dimethylcyclopropane carboxylate ion is a digestive and contact insecticide effective against a wide range of pests, particularly leaf and fruit eating lepidoptera and coleoptera in cotton, fruit, vegetables, wines, tobacco and other crops. It is also used for crack, crevice, and spot

treatment to control insect pests in stores, warehouses, industrial buildings, houses, apartment buildings, greenhouses, laboratories, and on ships, railcars, buses, trucks, and aircraft. It may also be used in non-food areas in schools, nursing homes, hospitals, restaurants, hotels, in food processing plants, and as a barrier treatment insect repellent for horses[7-9] .Technical Cypermethrin is a mixture of eight different isomers, each of which may have its own chemical and biological properties[10-12]. Cypermethrin concentrations were found in remarkable amount in pond water, soil and clay. The overdoses of pesticides make the residue problem in vegetables like cauliflower and brinjal, which might pollute our food and environment[13-17]. The toxic symptoms of exposure include numbness, tingling and itching, burning sensation, loss of bladder control, in coordination, and possible death. High-dose ingestion include nausea, prolonged vomiting, stomach pains, and diarrhea which progresses to coma. Poisoning in humans include facial burning and tingling (called paraesthesia), dizziness, headache and anorexia [18-23]. Cypermethrin is very highly toxic to fish, water insects aquatic invertebrates, bees and low toxic to birds. It causes genetic damage and chromosome abnormalities in mice and rabbits[24-30].

The techniques used for the determination of pesticides including Cypermethrin, are generally spectrophotometry, thin layer chromatography (TLC), high performance liquid chromatography (HPLC), high performance liquid chromatography-mass spectrophotometry (HPLC-MS), gas chromatography (GC). GC-MS, Electron capture method, coupled column liquid chromatography, capillary GC-MS and FT-IR. Multi residue analysis of pesticides in fruits and vegetables like pepper, tomato, cabbage, cauliflower, radish, wheat, pulses and medicinal plants have been done by above methods[31-49].

The aim of the present work is to develop a rapid, accurate and simple analytical method for the determination of widely used Cypermethrin insecticide at trace levels. In this method, alkaline hydrolysis of Cypermethrin to phenoxy benzene (**Popoff's Reaction**) and cyanide ion is done, which further react with potassium iodide-iodate solution under acidic conditions to liberate iodine and the liberated iodine selectively oxidizes leucomalachite green to malachite green dye. The absorption maxima of the dye formed is measured at 610 nm in acidic medium. Beer's law is obeyed over the concentration range of 2.0 to 16µg in a final solution volume of 25 ml (0.12-0.68ppm). The molar absorptivity and Sandell's sensitivity were found to be $3.3x10^5$ $mole^{-1}cm^{-1}$ and 0.054 µg cm^{-2} respectively. The standard deviation and relative standard deviation were found to be ± 0.001 and 0.22% respectively. The method has been applied to the determination of Cypermethrin in various samples of water, vegetables, fruits, foliages and biological samples.

MATERIALS AND METHODS

Apparatus: A Systronics UV-VIS spectrophotometric (model 104) with matched silica cells was used for all spectral measurements. Systronics pH meter (model 331) was used for pH measurements. A Remi C-854/4 clinical centrifuge force of 1850 g with fixed swing out rotors was used for centrifugation.

Reagents: All the reagents were of AnalR Grade and double distilled demineralized water was used throughout the study.

Cypermethrin **(**Syngenta Crop Protection Private Limited, India**):** A stock solution of 1 mg ml^{-1} was prepared in ethanol. Working standard solutions were prepared by appropriate dilution of the stock standard solution with water.

LeucoMalachiteGreen **(**Sigma-Aldrich, S. Germany**):** 0.05% solution was prepared by dissolving 25 mg of LMG in 100 ml of water and 1.5 ml of 85% of phosphoric acid in a 500 ml of volumetric flask and by shaking gently until the dye dissolved (phosphoric acid was added to dissolve the dye completely and to keep the solution stable for longer time). The content of the flask were then diluted to 500 ml with water[50].

Acetate Buffer:- was prepared by dissolving 13.6 g (1 M) sodium acetate trihydrate in 80ml of water, solution pH was adjusted to 4.5 with acetic acid, and the mixture was diluted to 100 ml with water [51].

Hydrochloric acid: 3% aqueous solution was used. Sodium hydroxide: 20% m/v aqueous solution was used.

Potassium iodide: 0.1% aqueous solution was used.

Procedure: An aliquot of sample solution containing 5 to 18 µg of Cypermethrin was taken in a 25 ml graduated tube and 2 ml of 20% sodium hydroxide was added to it. Now the solution was kept for 10 min at room temperature for complete hydrolysis. After that, 1 ml of 0.1% potassium iodide was added in acidic medium, to liberate iodine and then 1 ml leucomalachite green reagent was added and shaken thoroughly. This mixture was kept 15 min. for full color development. The green dye was produced. The solution was then diluted to the mark with water and absorbance of formed dye was measured at 610 nm against distilled water as reference. The concentration of Cypermethrin content was established from the calibration graph.

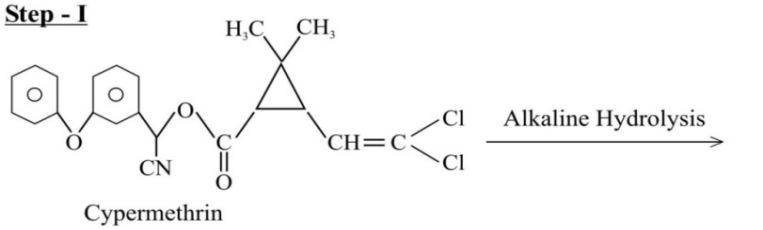
RESULTS AND DISCUSSION

Formation of color derivative: (Color Reaction of Cypermethrin)The color reaction may involve the following steps:-

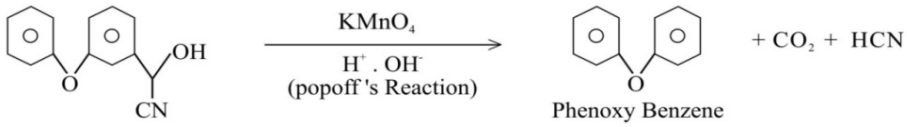
1. Hydrolysis of Cypermethrin to 3(phenoxy) 2-hydroxy ethane nitrile and permethrinic acid .
2. Further Hydrolysis of 3(phenoxy) 2-hydroxy ethane nitrile to phenoxy benzene, CO_2 and cyanide ion (**Popoff's reaction**).
3. Reaction of CN^- and KIO_3 Liberates Iodine.
4. Librated Iodine selectively oxidized Leucomalachite green in to Malachite green(610nm).

Proposed Reaction Mechanism :-

Step - I



Step - II



Step - III

Step - IV

Figure 1 : Reaction Mechanism

Hydrolysis of cypermethrin to cyanide ion was studied at different temperatures and alkalinity. It was observed that acidic conditions were required for the hydrolysis. Maximum hydrolysis was observed with 20% sodium hydroxide at temperature range of 35-45°C as it gave maximum absorbance values, good stability and quantitative results . It was observed that 1 ml of Leucomalachite green reagent was sufficient for complete color reaction.

Absorption Spectra The reactions of cypermethrin with potassium iodide potassium iodate mixture solution in acidic medium, liberated iodine[50,52]. The liberated iodine selectively oxidizes the leuco base dye (Scheme I). The green color of the dye was developed in an acetate buffer (pH 4.0-4.8) on heating in a water bath (~40°C) for 5 min. A time period of 5 min. was required for complete color development after dilution to 25 ml. The dye showed maximum absorbance at 620 nm, and the reagent blank had negligible absorbance at this wavelength (Figure 2).

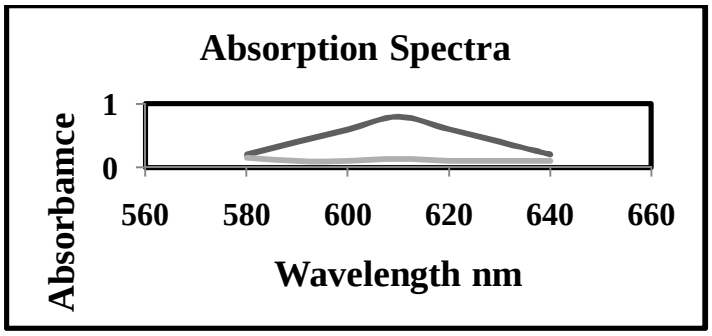


Figure 2: Absorption Spectra between Cypermethrin and leuco malachite green.

Effect of Time and Temperature: Hydrolysis of Cypermethrin to cyanide ion was studied at different temperatures. The maximum hydrolysis was observed with 20% sodium hydroxide at temperature range of 30 - 35^{o}C as it gave maximum absorbance values, good stability and quantitative results [53,54]. (Figure 3).

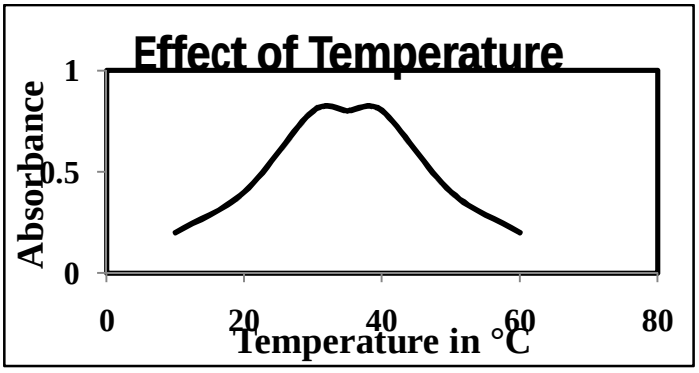


Figure 3: Effect of Temperature on absorbance of cypermethrin.

Effect of pH: The effect of pH on the color reaction was studied and it was found that constant absorbance values were obtained at pH range of 4.5 – 5.5 was required to stabilize the color. The colored species remain stable for more than 7 days under optimum conditions. (Figure 4)

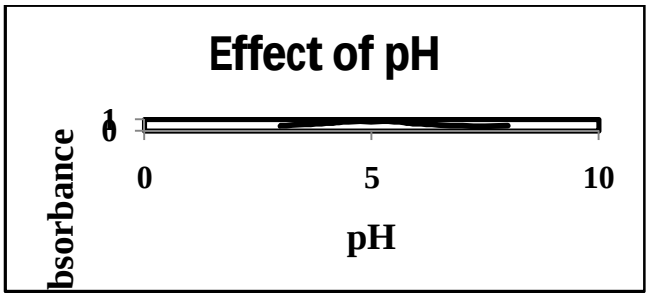


Figure 4 : Effect of pH on absorbance.

Effect of the Reagents Concentration: Effect of Leuco Malachite Green (LMG):-It was observed that 1 ml of leuco malachite green was sufficient for complete color reaction (Fig -5).

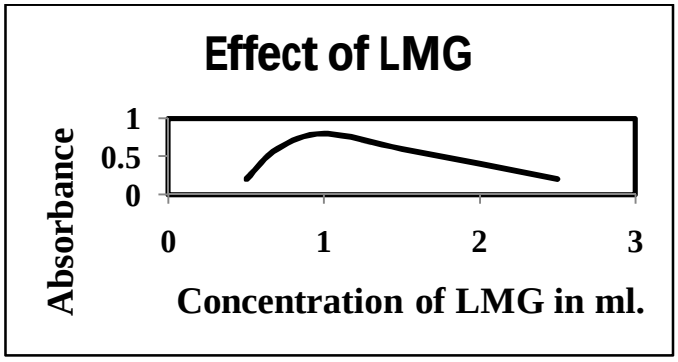


Figure 5: Effect of the concentration of LMG on absorbance

Effect of the concentration of Cypermethrin: - The constant and maximum absorbance of the yellow colored compound formed is found at the concentration range of 5.0 µg in a final solution volume of 25 ml. (Figure-6).

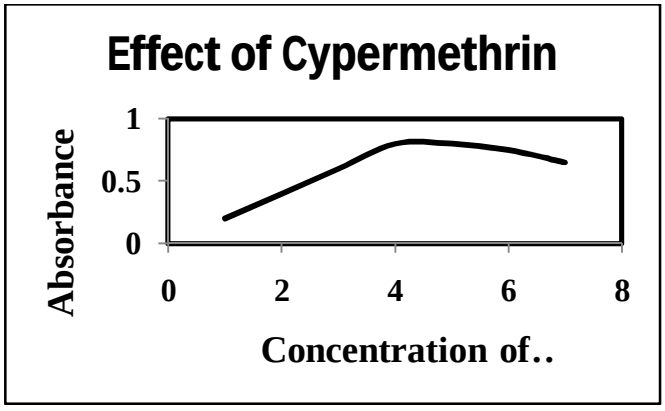


Figure 6: Effect of the concentration of Cypermethrin on absorbance.

Effect of Sodium Hydroxide: - It was observed that maximum hydrolysis was observed with 1.0 mol l^{-1} sodium hydroxide at temperature range of 35-45^{o}C as it gave maximum absorbance values, good stability and quantitative results. (Figure-7).

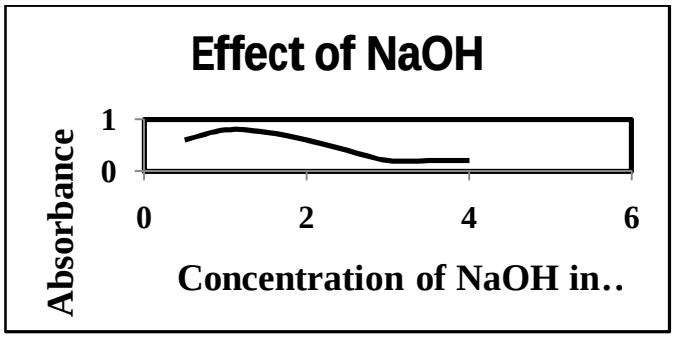


Figure 7 : Effect of the concentration of NaOH on absorbance .

Effect of the Foreign Species: The effect of common foreign species and pesticides was studied to assess the validity of the method. Known amounts of foreign species and pesticide were added to the standard solution contained 10µg of cypermethrin prior to hydrolysis and the solution was analysed by the proposed method .The method was found to be free from interference of most of the foreign species and pesticides. (Table -1).

Table 1: Effect of foreign species for cypermethrin. (10 µg cypermethrin in 25ml).

Foreign species	Tolerance limit*	Foreign species	Tolerance limit*
Benzene	4500	Al^{3+}, Mg^{2+}, Co^{2+}	2000
Phenol, Ethanol	2500	Zn^{2+}, Cu^{2+} , Mn^{2+}	1500
Benzaldehyde	2200	Fe^{3+} Fe^{2+}, Sb^{3+}	1300
Toluene, Xylene	1300	Ni^{2+}, Pb^{2+}, Ca^{2+}	850
Aniline,Formaldehyde	900	Br^-, CO_3^{2-}, Cl^-	400
Parathion, Malathion, Cresol	500 450	NO_2^-	150
Fenvalerate, Deltamethrin	2**		

* = The amount causing an error of ±2% in absorbance value.

*** *** = Tolerance limit without its removal from the sample**.**

APPLICATIONS

The proposed method has been applied satisfactorily to the determination of cypermethrin in various samples of polluted water which was collected from the specific locations of river, vegetable and fruits, which are collected from market and analysed with the addition of detectable amount of cypermethrin , and in biological fluids.

Determination of cypermethrin in polluted water: Kharun River (Raipur) water sample, which received runoff water from agricultural field, was collected in amber glass bottles. A volume of 1 mL of 0.1M Na_2 S_2O_3 per liter of

water sample was added on site to suppress the interferences of chloride, humic acid, fulvic acids[55]. These samples were filtrated through a Whatman No. 40 filter paper. Aliquots of water samples were taken to 25-ml graduated tube and sodium hydroxide was added. They were kept in a hot water bath analysed as described above. (Table-2)

Table 2: **De**termination of Cypermethrin in river water samples**.**

Sample No.	Cypermethrin originally found (µg) x*	Cypermethrin added (µg) y	Total Cypermethrin found (µg)z	Difference (µg) z-x	Recovery % z-x × 100/y
1	1.35	4.0	5.25	3.95	98.75
2	1.37	8.0	9.29	7.92	99.00
3	1.33	12.0	12.96	11.66	97.16
4	1.30	16.0	16.85	15.55	97.18
5	1.34	20.0	21.07	19.73	98.66
6	1.36	24.0	25.14	23.78	99.08
Avarage					**98.30**

*=Water sample 25 ml, 1ml aliquot of sample was analyzed, after treatment as described in procedure.

Determination of Cypermethrin in vegetable and fruits: Various sample of vegetable, fruits and foliages each of 50gm, were taken collected from agricultural field, Cypermethrin had been sprayed as an insecticide. The samples were macerated with two 20 ml portions of ethanol-demineralized water (1+1), filtered though a thin cotton cloth and filtrate was centrifuged at 1850g for 10 min.[56] Filtrate were diluted in 50ml flask by ethanol. In a 25 ml graduated tube 1ml.of this solution, 1.0 ml of 1.0 mol l^{-1} sodium hydroxide and 1ml. of leucomalachite green were added. Then shaken thoroughly and kept at 0–5^0 C for 15 min for full color development. (Table-3)

Table 3: Determination of Cypermethrin in vegetable and fruits

Sample	Cypermethrin originally* found (µg) x	Cypermethrin added (µg) y	Total Cypermethrin found (µg)z	Difference (µg) z-x	Recovery % z-x × 100/y
Tomato**	2.35 3.33	4.0 8.0	6.24 11.08	3.89 7.75	97.22 96.87
Apple**	1.37 2.98	4.0 8.0	5.18 10.03	3.81 7.73	95.22 96.87
Grapes**	2.12 3.24	4.0 8.0	6.06 11.17	3.94 7.93	98.50 99.12
Cauliflower**	1.33 3.87	4.0 8.0	5.19 11.56	3.86 7.69	96.50 96.12

Brinjal**	2.34 3.75	4.0 8.0	6.32 11.66	3.98 7.91	99.50 98.85

* = Mean of three replicate analyses.

** = Sample 25 gm (sample taken from a field where Cypermethrin had been sprayed)

Recovery of cypermethrin in biological samples: As the presence of Cypermethrin in blood, urine and cystein has been reported in detectable concentration[56-58]. The method has been applied for the determination of Cypermethrin in biological samples. Synthetic samples were, collected from local pathology laboratory and prepared by adding known amounts of Cypermethrin to these samples and then analysed after deproteination with trichloroacetic acid as described above[59-61]. (Table 4).

Table 4: Recovery of Cypermethrin in biological samples.

Samples	Amount added* (µg)	Amount found * (µg)	Recovery %
Blood**			
A	4.0	3.89	97.22
B	8.0	7.76	97.00
Urine**			
A	4.0	3.89	97.25
B	8.0	7.81	97.62
Cystein**			
A	4.0	3.92	98.00
B	8.0	7.86	98.25

* = Mean of three replicate analyses.

** = Amount of biological sample =1ml.

CONCLUSIONS

The proposed method reports the use of leuco malachite green for the first time as a new reagent for the spectrophotometric determination of Cypermethrin. It offers a sensitivity, selectivity, simplicity and cost-effectiveness of the method. The method involves no extraction steps, thereby the use of organic solvents, which are generally toxic in nature are avoided. The stability of formed malachite green dye is an added advantage of the method. The sensitivity in terms of molar absorptivity and precision in terms of relative standard deviation of the present method indicated it to be very reliable for the determination of Cypermethrin in various samples. This method is good alternative to some reported costly instrumental method. The results summarized in Tables 1,2, 3,and 4 clearly showed that the developed method worked satisfactorily.

REFERENCES

[1] " Pesticide Residues in Food 1981, Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Expert Group on Pesticide Residues," FAO Plant Production and Protection Paper, Rome, **1982** No. 37.
[2] B.C. Fine, "*Pyrethrum Post"*, **1963**, 7, 18.
[3] A.W. Farnham," *Pestic. Sci"*, **1973**, 4, 513**.**
[4] M.Elliott,N.F. Janes, and C.Potter, "*Annu. Rev. Entomol*.", **1978**, 23, 443.
[5] M.Elliott, "*Pestic. Sci*.", **1980**, 11, 119.
[6] T.J. Shafer,D.A. Meyer and K.M. Kroften , "*Environmental Health Persp.*",**2005**,113,123-136.
[7] B.P. Vitthal,T.S. Murlidhar,V.P. Sudhakar , "*Analyst"*,**1992**, 117, 75**.**
[8] M. Abdullah,O. Sarnthoy,S. Jiwajinda,J. *Kasetsart, "Nat. Sci."*,**2001**, 35, 115.

[9] Y. Ding ,A.W. Catherine ,S. Muralidhara ,V.B. James ,G.B. Michael , "*J. Chromatogr.*",**2004**, 810, 221.
[10] World Health Organization. Safe Use of Pesticides in Public Health, Geneva, Switzerland, **1967**, **2**, 29**.**
[11] World Health Organization *Environmental Health Criteria*. Cypermethrin, **2004.**
[12] Bhadekar Rama, Pote Swanandi,Tale Vidya, Nirichan Bipinraj, *"American Journal of Analytical Chemistry"*, **2011**,2, 1-15**.**
[13] D.C.G. Muir,G.P. Rawn, B.E.Townsend and W.L.Lockhart , "*Environ. Toxicol. Chem.*" **1985**, 4, 51-61**.**
[14] N.P.Agnihotri, "*J. Entomol. Res.*" **1986**, 10, 2, 147-51.
[15] H.Kidd, and D.R. James, "*The Agrochemicals Handbook"*, III[rd] Edition. Royal Society of Chemistry Information Services, Cambridge, UK, **1991**, 2-13**.**
[16] World Health Organization. World health report 2002 reducing risk, promoting healthy life, Geneva, WHO, **2002.**
[17] Subhash Chandra, Anil N. Mahindrakar and L.P.Shinde, "*International Journal of ChemTech Research"*, **2010**, Vol.2 (2), 908-911.
[18] Geneva, United Nations Environmental Programe, the international Labour organization, and the world Health Organization, **1989**. 82.
[19] F.He, "*Arch. Toxico."***1989**, 63, 54**.**
[20] *Extension Toxicology Network (EXTOXNET) Cypermethrin*. Cooperative Extension offices of Cornell University, Michigan State University, Oregon State University, and University of California, Davis, **1993**.
[21] D.E.Ray, Pesticides derived from plants and other organisms, "*In Handbook of Pesticide Toxicology*", Academic Press, New York, 2, **1991.**
[22] U.S. Environmental Protection Agency. *Pesticide Fact Sheet Number 199, Cypermethrin. Office of Pesticides and Toxic Substances,* Washington, 2, **1989.**
[23] P.C. Baker and P. Bottomlely, "*Analyst"*, **1980**,105, 515.
[24] S.P.Bradbury and J.S. Coats, "*Environ. Toxicol. Chem"*, **1989**, 8,373-380**.**
[25] G.D.Waller, "*Econ. Entomol*."**1988**, 81, 4, 1022-6.
[26] M. KamalShah , A. Khan, F. Rizvi, M. Siddiqui , "*PakistaniVat.Journal"*, **2007**,27(4),171-175**.**
[27] United States Environmental Protection Agency. *Cypermethrin Pesticide Fact Sheet.* Washington, D.C. **1989.**
[28] R.N.Das and Parajuli Sudip**,** *"Internet Journal of Medical Update"*, **2006**, 1, 2**.**
[29] **R.**Baselt, "*Disposition of Toxic Drugs and Chemicals in Man"*, 8th edition, Biomedical Publications, Foster City, CA, **2008**, 389-391**.**
[30] J.X.Hu, Y.F.Li, J.Li,C. Pan,Z. He,H.Y. Dong,L.C. Xu, " *Journal of applied toxicology"* , **2011**,12,1769 .
[31] H.S.Rathore and T. Begum, "*J.Chromatogr"*, **1993,** 643, 321- 329.
[32] V.B.Patil and M.S.Shingare, "*Analyst"*, **1994**,119, 415-416**.**
[33] A.Antonio, J.M. Maria ,P. Rosa ,I.M. Maria , S.Adolfo , G.Miguel , M.Angel ,"*J. Agric. Food Chem."*, **2001**,49, 4, 1707-1712**.**
[34] Shehali Islam, *"American J. of Environmental Science"*, **2009**, 5(3), 325-329**.**
[35] D. Debayle, G. Dessalces, M.Florence, G. Loustalot, 2008, 391(3), 1011- 1020**.**
[36] D.N. Thanh , E.Y. Ji , M.L. Dae, H,L, Gae ,"*Food Chemistry"*, **2008**,110(1), 207-213**.**
[37] M.E. Leon – Gonzolez,M. Plaza – Arroyo,L.V. Perez – Arribos , L.M.Polo – Diez , *"Anal.Bio. anal.Chem"*, **2005,**382, 527**.**
[38] P. Parrille Vazquez, M.D.Gil Garcia,D. Barranco Martinez and M.Martinez Galera, "*Anal.Bio. anal.Chem"*, **2005**,381, 1217**.**
[39] P. Serodio and J.M.F. Nagueira, "*Anal.Bio. anal.Chem"*, **2005**,382, 1141.
[40] A.Colume, J. Diework and B.Lendl "*Intern.J.Environ.Anal.Chem"*, **2004**, 84, 835**.**
[41] A.Edelmann,J.Diework, K.C.Schuster and B. Lendl , "*J. Agric. Food.Chem"*, **2001**,49, 113.
[43] F.Jose, H.Pilar, M.M.Carmen , M.Marta ,F. Pilar ,"*Food Chemistry"*, 2007,105(2), 711-719**.**
[44] Ash Lal, Guanhaut Tan, Meeken Chai,"*Analytical Science"*,**2008,** 24(2), 231**.**
[45] P.C.Abhilash,V. Singh, N.Singh,"*Food Chemistry"*, **2009**,113(1), 267-271**.**
[46] A. Colume, S.Cardenas, M. Gallego and M.Valcarcel , " *J.Chromatogr. A"*, **2001**,912, 83**.**
[47] Beena Kumari, *"ARPN Journal of Agricultural and Biological Science"*, **2008**, 3(4), 6-51.
[48] H.Guan,Tan and T. Me Soo, "*Malaysian J. of Chemistry"*, **2001**, 3(1), 1-12**.**
[49] D.Elosa Caldas, Maria Hosana conceicao, C.Maria Clara Miranda, K.R.Luiz cesar de Souza and Joaq, "*J. Agric. Food Chem."*, **2001**, 49(10), 4521-4525**.**
[50] E.K. Janghel, J.K.Rai, M.K.Rai, V.K.Gupta,"*J. Braz. Chem.Soc."*,**2007**, 18,3**.**
[51] Kishore K.Tiwari, "*Journal of the Chinese Chemical Society"*, **2010**, *57*, 105-110.

[52] H.D.Revanasiddappa, B.P.Dayananda,T.N.K. Kumar, " *Environ. Chem. Lett"*.**2007,** *5*, 151-155**.**
[53**]** E.Y.Backeet,K.M. Emara, H.F.Askal,G.A. Saleh , *"Analyst"***1991**,116, 861.
[54] V.B. Patil, M.V. Sevalkar andS.V.Padalikar , *"Analyst"*, **1992**,117, 75.
[55] R.S. Dhundhel & M. K. Rai , "Asian Journal of Biochemical and Pharmaceutical Research", **2011**,3(1),381-390.
[56] B. Krishna Priya, P. Subrahmaniyam, K.Dakshyani and P. Chiranjeevi, "*E-Journal of Chemistry*", **2007**,4(4), 480-486.
[57] V.Agrawal, and V.K.Gupta, "*Fresenius J. Anal.Chem,*" **1993**,345,720**.**
[58] S. Upadhyay and V.K. Gupta, "*Analyst"*, **1984,**109, 1619.
[59] J.Raju and V.K. Gupta, "*Microchem J.*", **1989**, 39, 25.
[60] W.N.Aldridge, "*Analyst"*, **1994**, 69, 262.
[61] J.V.Das and V.K. Gupta , "*Chem. Anal.(*Warsaw)", **1994**,39, 693.