ON SPOT DETECTION OF PROFENOS AND CYPERMETHRIN BY FLUORESCENCE DECAY IN NIMBDIN AS SENSING METHOD

Aaeen Alchi¹, Dr. Kapil Kumar² and Dr. Himanshu A. Pandya³
1. Research Scholar Department of Biochemistry and Forensic Science, Gujarat University.
2. Associate Professor Department of Biochemistry and Forensic Science, Gujarat University.
3. Professor & Head, Department of Biochemistry and Forensic Science, Gujarat University.

Abstract

The principle hours of a crime scene investigation are usually of critical prominence for the police/forensic analysts to get more information and insights about the identity of potential suspects; and to obtain relevant facts and data. The development of portable methods that allow forensic analysis at scene of crime with good sensitivity and precision is extremely desirable. In this sense, the detection of toxic substances is of great importance to strategic and humanitarian demining, forensic criminal investigation, as well as remediation of the victim at the crime scene. In cases of poisoning a simple change in colour or intensity may indicate alertness for critical decision. Pesticides are major poisonous substances encountered in suicide as well as in accidental cases. Profenos and Cypermethrin are two such OP pesticides reported in several poisoning cases. In the present study, to detect these pesticides, Nimbdin (Azadirecta indica), is used as sensing media. When sample is diluted and added into this extract, its fluorescence intensity decay. The decay of fluorescence is measured by fluorescence spectrophotometry, fluorometric graph shows the decay of fluorescence is decreased when volume of sample is added in it.

Introduction:

Acute pesticide self-poisoning is a prime public health problem in many developing countries, killing thousands of people each year (Hayes, Westhuizen, M., & Gelfand, 1978) (Jeyaratnam, 1990) (Bardin, Eeden, Moolman, Foden, & Joubert, 1994) (Gunnell, Eddleston, Phillips, & Konradsen, 2007) (Mew, et al., 2017). The World Health Organization (WHO) acknowledges pesticide poisoning to be one of the three most common means of suicide worldwide (World Health Organization, 2014). Organophosphorus (OP) insecticide poisoning is a principally severe problem, accounting for around 2/3 of demises (M., 2000)(Futagami, et al., 1997) within the developing world, in Sri Lanka for the last 40 years (Eddleston, 2000) (Knipe, et al., 2014) and poisoning due to suicidal attempt accounts for at least 40–60% of all cases in some African countries (Bardin, Westhuizen, & Gelfand, Organophosphate poisoning : grading the severity and comparing treatment between atropine and glycopyrolate, 1990).

The rapid identification of the unpremeditated insecticides would provide very favorable information to clinicians for making treatment decisions in suicide attempt tragedies. (Futagami, et al., 1997)
Great efforts have been dedicated to emerging highly sensitive methods for the determination of OP pesticide remains in foods and water. Numerous instrumental methods have been described for the detection of pesticides generally analyzed by spectrophotometry (Caldas, et al., 2001) (Janghel, Rai, Rai, & Gupta, 2007), thin layer chromatography (TLC) (V.B. & M.S., 1994) and GC-MS (D.N., E.y., M.L., & L., 2008), liquid chromatography-mass spectrometry (C., Fernadez, Pico, & Font, 2004) gas chromatography (R.C., F.J., E.R, Hernandez, & M., 2001) (Leoni, Iori, Palmieri, & Agric, 1991) (Sherma, 1995) (Lacorte & Barcelo, 1994), high-performance liquid chromatography and mass spectroscopy (Hall, Mourer, & Shibamoto, 1997). These methods are of high efficiency and allow refinement among diverse types of Organophosphorus compounds, but call for tedious sample pretreatments, highly experienced engineers and sophisticated instruments. Moreover, they are time-consuming, and not appropriate for field investigation of multiple samples. Therefore, several rapid, relatively inexpensive, sensitive screening analytical techniques which need almost no sample pretreatments were developed for the identification and quantification of such pesticides. (Liu, Yuan, Yue, Zheng, & Tang, 2008)

The aim of the study was to develop a highly sensitive, eco-friendly, non-hazardous and rapid method using green route for the detection of Cypermethrin and Profenos on the spot in crime scene investigation.

Materials and Method:-
Sample preparation:
The pesticide samples were purchased from local market of Ahmedabad sold on the brand name of Profess super EC liquid which is composed, which is claimed to possess 40% Profenos and 4% Cypermethrin whereas all chemicals used are of HPLC grade purchased from Sigma Aldrich.

Stock solution (100 µL) of samples was prepared by dissolving 10 µL of pesticide in 100 mL methanol. The standard solution were found stable for months without alteration when kept in amber colour bottle when stored at 25°C. From this stock solution 10 ppm concentration was taken for further analysis.

Extraction of Nimbdin from Azadirecta indica:
Healthy plant leaves of Azadirecta indica were collected from the Botanical garden, Gujarat University, Ahmedabad India, in the month of September. The washed leaves were completely dried and coarsely powdered. Successive extraction was done by taking 50 g of leaves in 250 ml methanol kept for three days with periodic shaking. Then, the extract was filtered by Whatmann No. 1 and the filtrate was subjected to rotary evaporation and subsequently concentrated under reduced pressure (in vacuum at 40°C). The final extract was evaporated to dryness and stored at 4°C in an air tight bottle.

Instrumentation:-
The absorption spectra, intensity verses wavelength were acquired on UV-Visible spectrophotometer Shimadzu 1800, Japan. The fluorescence spectra were recorded by Fluoromax-4 Spectro-flurometer, Horiba Japan. UFLC analysis were performed using a Shimadzu Ultra-Fast High performance LC system.

Results and Discussion:-
Characterization by UV visible spectrophotometer:
The pesticide samples were analyzed on a calibrated UV Visible spectrophotometer Shimadzu 1800 at full wavelength range of 800 to 200nm and absorbance range of 0.0 to 3.0 units using matched pair quartz sample cells of 1 cm path length. The identification of the sample on the basis of specific wavelength (λmax) values at which maximum absorbance obtained was 670nm with the baseline of methanol. To support the results obtained from UV Visible studies, titrations were carried out by absorption spectroscopy. The absorption spectral studies carried out for the titration of ‘Profenos and Cypermethrin’ with the ‘Nimbdin’, the absorbance spectra was obtained at 660 to 670 nm.
Characterization by UFLC:

UFLC analysis were performed using a Shimadzu Ultra-Fast High performance LC system (Shimadzu, US). UFLC separation was achieved using Shim-pack VP-ODS (150mm × 4.6mm i.d., 5µm), maintained at 25 °C. The Shimadzu operating pressure was at 6000 psi using a binary gradient pump (2 LC- 10At) to deliver the solvent mixtures into the C18 column. Isocratic elution and a run time of 15 minutes. The mobile phase was a mixture of water and methanol (60: 40), flow rate- 1.2 ml/min. mobile phase was degasses automatically upon setting the parameters. The injection volume was set at 10 µL. Determination was performed using UV- Visible detector (SPD-20A/20AV/M20A)

The calibrated UFLC Shimadzu DGU – 20A5R was used for detection of extract of nimbdin, sample and reaction of both extract and sample. The small glass vial used for the loading the sample and extract.
Characterization by Fluorometer
The fluorescence spectra were recorded with Horiba Fluoromax-4 Spectro-fluorometer, in standard configuration with a 150 W xenon lamp and a single photon multiplier tube (PMT) detector. The gratings used have 1200 grooves mm⁻¹, having range of 200 - 600 nm with increment of 1nm and band pass of 5 nm has dispersion of 4.25nm/mm, and are blazed at 330n (excitation) and 500 n (emission). Samples were contained in a 5x5 mm quartz cuvette, maintained at 10 ± 0.5 °C. The slits width was set to 1.5 nm and integration time to 0.5 seconds to optimize the signal to noise ratio. The wavelength selected are optimal for excitation in the UV-Visible, and for emission in high UV to near IR. The fluorescence decay spectra of the pesticide sample at various concentrations were obtained at λmax 670 at pH=7.0.

Firstly the nimbdin extract was put in the fluorometer for the reference. Then the sample was added for observing the decay of fluorescence. We took multiple samples for obtaining the spectra, graph is decreasing after adding sample in extract up to 10 ppm.

For Nimbdin fluorescence decay by pesticide, the solution were gently poured into the cuvette and the emission spectrum (unquenched nimbdin extract) is recorded. Upon every pesticide sample addition, the solution containing the pesticide is mixed with the nimbdin by manual inversion, avoiding vigorous shaking to minimize bubbles formation. The sample is then left to equilibrate at 25 °C in a thermostatted cell holder for 2 minutes, the cuvette is extracted, mixed again by manual inversion and repositioned in the cell holder. Measurements are taken after 50-60 seconds, and the mixing procedure is repeated before each spectrum collection.

Once proper instrumental parameters were defined in order to yield a good signal intensity in single wavelength experiments using a standard 5x5 mm cuvette, reproducible steady- state emission spectra were collected. No correction was applied for inner filters effects induced by nimbdin, that in our experimental settings has an absorbance well below 0.5 OD even at the higher concentration exploited.
Calibration curves were constructed by plotting the ratio of Nimbdin, λ max to that of standard Nimbdin against pesticide sample of various concentration expectable linear relationship were found over the concentration ranges 0.10 to 10 mg/ml. Nimbdin in methanol, since distribution of ‘Profenos & Cypermethrin’ was within +/- 5%, no weight factor was applied. The limit of detection (LOD) was up to 10 ppm in methanol. No interfering substances were observed in fluorescence graph at intensity of fluorescence graph of Nimbdin and sample had relative decrease in fluorescence without peak tailing. The precision and accuracy of calibration standard concentration for Nimbdin were within expectable limits as defined in ICH guidelines. The plot demonstrates the luminescence intensity changes of ‘Nimbdin’ in the presence of different concentrations of “Profenos and Cypermethrin”. To evaluate the sensitivity of Nimbdin based calorimetric assay, we evaluate different concentrations of “Profenos and Cypermethrin” detected up to 10 ppm.

Conclusion:-
In most of the poisoning cases due to Profenos & Cypermethrin forensic exhibits are sent to laboratories for analysis purpose. Time lapsed from seizure to laboratory analysis can delay the investigation procedures. This delay becomes more fatal and harmful in cases where the victim is still alive but the nature of toxic substance is not known. Analytical techniques used by laboratories majorly includes chromatographic techniques such as TLC, HPLC, GCMS, and HPTLC etc. Though these techniques are highly sensitive and give better results but are time consuming and requires a tedious sample preparation, analysis only possible in laboratorial conditions. In the developed technique for detection of Profenos and Cypermethrin as reaction of nimbdin itself gives distinguishable results in the form of fluorescent decay and requires UV light source for confirmation of reaction with a detection limit of 10ppm. Because of the simplicity and sensitivity of this developed method it can be performed right on the crime scene, eliminating a separate trip to the forensic laboratory ensuring more prevalent and timely testing. In cases of suicide, homicide, and accidental deaths due to pesticides, the rapid detection can narrow down search for antidotes and can provide appropriate and well-timed medical assistance.

Acknowledgements:-
Financial support of Maulana Azad National Fellowship for minority students, University Grant Commission, India, is greatly acknowledged.

References:-
1. Bardin, P., Eeden, S. V., Moolman, J., Foden, A., & Joubert, J. (1994). Organophosphate and Carbamate poisoning. Arch Intern Med, 154, 1433-1441.
2. Bardin, P., Westhuizen, N. V., & Gelfand. (1990). Organophosphate poisoning : grading the severity and comparing treatment between atropine and glycopyrolate. Crit Care Med, 54, 956-960.
3. C., B., Fernadez, M., Pico, Y., & Font. (2004). Comparision of solid-phase microextraction and stir bar sorptive extraction for determining six organophosphorous insecticides in honey by liquid chromatography-mass spectrometry. j. of Chromatography, 1030, 77-85.
4. Caldas, E. D., conceicao, M. H., Miranda, M. C., cesar, L., Souza, K. D., & Joaq. (2001). Determination of dithiocarbamate fungicide residue in food by spectrophotometric method using a vertical disulfide reaction system. J. Agric. Food Chem., 49(10), 4521-4525.
5. D.N., T., E.y., J., M.L., D., & L., G. H. (2008). A multiresidue method for determination of 107 pesticides in cabbage and raddish using QuECHERS sample preparation method and gas chromatography mass spectrometry . Food Chemistry, 110(1), 207-213.
6. Eddleston, M. (2000). Patterns and problems of deliberate self- poisoning in developing world. Q. J. Med, 93, 715-31.
7. Futagami, Koujiro, Narazaki, Chie, Kataoka, Yasufumi, . . . Ryozo. (1997). Application of hih-performance thin-layer chromatography for the detection of organophosphorus insecticides in human serum after acute poisoning. Journal of Chromatography B, 369-373.
8. Gunnell, D., Eddleston, M., Phillips, M., & Konradsen, F. (2007). The global distribution of fatal pesticide, self poisoning; systematic review. BMC Public Health, 7, 357.
9. Hall, G., Mourer, C., & Shibamoto, T. (1997). Development and Validation of an analytical method for naled and dichlorovos in air. J. Agric. Food Chem., 45, 145-148.
10. Hayes, M., Westhuizen, N. V., M., & Gelfand. (1978). Organophosphate poisoning in Rhodesia. S. Afr Med J, 54, 230-234.
11. Janghel, E. K., Rai, J., Rai, M., & Gupta, V. (2007). New sensitive spectrophotometric determination of cypermethrin insecticide in environmental and biological samples. J. Braz. Chem. Soc., 18(3), 590-594.
12. Jeyaratnam, J. (1990). Acute pesticide poisonin: A major global health proble. World Health Stat Q, 43, 139-44.
13. Knipe, D., Metcalfe, C., Fernando, R., Pearson, M., Konradsen, F., & Eddleston, M. (2014). Suicide in Sri Lanka 1975-2012: age period and cohort analysis of police and hospital data. BMC Public Health, 14, 839.
14. Lacorte, S., & Barcelo, D. (1994). Rapid degradation of fenithrothion in estuarine waters. Environ. Sci. Technol., 28, 1159-1163.
15. Leoni, O., Iori, R., Palmieri, & Agric, J. (1991). Immobilisation of myrosinase on membrane for determining the glucosinate content of cruciferous material. Food Chem., 39, 2322-2326.
16. Liu, S., Yuan, L., Yue, X., Zheng, Z., & Tang, Z. (2008). Recent Advances in Nanosensors for Organophosphate pesticide detection. Advanced Powder Technology, 19, 419-441.
17. M., E. (2000). Patterns and Problems of deliberate self poisoning in developing world. Q J Med, 93, 715-31.
18. Mew, E., Padmanathan, P., Konradsen, Eddleston, M., Chang, S., & Phillips, M. (2017). The local burden of fatal self-poisoning with pesticides. J Affect Discord., 219, 93-104.
19. R.C., M., F.J., R., E.R, G., Hernandez, E., & M., P. F. (2001). Determination of herbicides and metabolites by solid-phase extraction and liquid chromatography: Evaluation of pollution due to herbicides in surface and ground waters. J. of Chromatography, 950, 157-166.
20. Sherma, J. (1995). Pesticides. Anal. Chem., 67, R1-R20.
21. V.B., P., & M.S., S. (1994). Thin layer chromatographic spray reagent for the screening of biological material for the presence of carbyl. Analyst, 119, 415-416.
22. World Health Organization. (2014). Preventing Suicide. A global imperative,. Geneva: WHO.