Xournals

Academic Journal of Forensic Sciences

ISSN: 2581-4273 | Volume 01 | Issue 01 | April-2018

Recent Developments in Extraction Methods of Pesticides from Biological Samples: A Review

Neha Jain[1]

Available online at: www.xournals.com

Received 23th January 2018 | Revised 10th February 2018 | Accepted 30th March 2018

Abstract:

Pesticides are the most commonly encountered toxic substances in almost every substrate which includes any environmental sample like water, soil, fruits, vegetables or any biological sample involving viscera, blood, urine and other body fluids. This occurrence of pesticides in the biological samples is because of their tendency of deposition and accumulation in the adipose tissues of the body. The determination of these pesticides from the biological samples (viscera and other body fluids) begins with their successful extraction and isolation from the matrix. The procedure of extraction and isolation depends on the nature of the matrix and also on the selection of the method that utilizes minimum amount of solvent and is capable of providing high yield. Various methods has been developed for this purpose and used to carry out the isolation of these pesticides from the viscera sample, blood and urine and other body fluids. Numerous studies has been conducted to find out the best method to achieve this like liquid liquid extraction, solid phase extraction, and the recent ones including accelerated solvent extraction and others. In this paper a various methods that have been used for the extraction of pesticides from the biological samples is reviewed for determining the best suitable method which has the tendency to provide the results free from the matrix contaminants and have maximum amount of recovery.

Key Words: *biological Evidences, determination of pesticides from biological samples*

Authors:

1. *Lok Nayak Jai Prakash Narain National Institute of Criminology and Forensic Science, INDIA*

Introduction

Forensic Toxicology is an important discipline of forensic science which deals with the detection and analysis of poisons. Detection of poisons utilizes the application of sequential procedure which involves isolation and extraction of the poisonous substance followed by their separation and analysis using certain sophisticated chromatographic and spectrophotometric techniques. These poisons be it any drug or its metabolite or any pesticide residue because of their tendency of getting deposited into the tissues needs to be isolated from different biological matrixes such as viscera, stomach contents, post mortem blood, serum, urine etc. through various methods (Lanjewar, 2014).

Pesticides are defined as those chemical substances used commonly in agricultural practices to prevent the plants and crops from pests, weeds and other plant diseases. These pesticides being toxic in nature stored in the body of living organisms in fatty tissues and their accumulation increases there over time (Das, 2014). There occurs numerous classes of pesticides based onto the species on which they act. For instance, those which fight against insects are insecticides, similarly those resists fungus are fungicides, and the class of pesticides which prevents the herbs from diseases are known as herbicides and so on. There is another classification which categorize these pesticides on the basis of their chemical nature and function which is organochlorines, organophosphates, carbamates and pyethroids.

These are categorized based on their chemical structure as Organophosphates are the substances that contains phosphate group like Malathion, Dichlorovos, Parathion, Monocrotophos and so on. Similarly Organochlorines are those compounds that contains Chlorine in their structure like DDT, BHC, Eldrin, Endosulfan etc., carbamates are those which are derivatives of Carbamic acid like Carbofuran, Carbryl etc., and Pyrethroids are the ones, which can either be obtained from natural plant source i.e. from *Chrysanthemum cinnarefolium* or these may be synthetic like Cypermethrin, Fenvalerate, Allethrin and so on.

Since, pesticides gets accumulated in the adipose tissues their determination from the matrix poses a serious challenge because of the interference of the large amount of other analytes and substances that can be co-extracted with these and thereby affects the results of the analysis (Tuzimski, 2012).

The protocol used for the preparation of sample is considered to be the most essential step in the detection of these pesticides so as to maintain the sensitivity of the system. Such extraction methods must be of the type that only the relevant or active component or sample molecule is extracted and the other co- extracts and matrix components gets filtered out (Well, 1988).

Nowadays, technology emerged with the development of large number of advanced and modern methods for the extraction of pesticides which are less time consuming and are efficient in providing the maximum % of recovery without the matrix error. Some of these methods includes Soxhlet extraction, Solid Phase Extraction (SPE), and Accelerated Solvent Extraction (ASE), QuEChERS method of extraction and Supercritical Fluid Extraction etc. (Das, 2014).

Liquid Liquid Extraction (LLE) – This is the most widely used technique for the extraction of pesticides from the any biological matrix or sample. The technique utilizes two or more immiscible solvents of different polarity that carries out the selective isolation of the pesticide from any solid or liquid mixture by bringing it in contact with a solvent phase. Process of isolation or separation of any sample (drug or pesticide or other) selectively from the reaction mixture in the form of solution or clear extract or liquid by extraction using another immiscible solvent is termed as solvent extraction (Srivastava and Yadav, 2014).

Steam Distillation – This is another method used for the pesticides extraction through which the steam volatile natural oils, fats, and other co extracts can be removed from the pesticide embedded sample (tissue) (Das, 2014).

Solid Phase Extraction (SPE) – Solid Phase Extraction is the method of extraction that utilizes a solid phase and a liquid phase to isolate different types of analytes from a solution. It is usually used to clean up a sample before using a chromatographic or other analytical method (Das, 2014). This method works on the same principle of liquid liquid extraction (LLE) involves partitioning of the solutes between two phases but difference lies in the fact that instead of two immiscible liquid phases as in LLE, SPE method of extraction involves partitioning between a liquid (sample matrix or solvent with analytes) and a solid (sorbent) phase. Solid Phase Extraction method involves firstly the adsorption of

the analytes onto the stationary phase which can be normal polar phase (silica or alumina) or it can be reverse phase i.e. the polar phase is made non polar by replacing the OH group of silica with the incorporation of C18 (carbon octadecylsilyl group). The adsorption causes the retention of the analytes onto the surface of stationary phase which is followed by its desorption or elution using favorable solvents. The extraction of the sample by this method begins with loading of the sample solution onto the SPE solid phase followed by washing of undesirable components from the matrix and collection of the analyte (Pesticide) into a solution form (Ferenc and Biziuk, 2006).

Supercritical Fluid Extraction- This is another extraction method that involves the sample extraction using supercritical fluid i.e. Carbon dioxide stored at critical temperature 31.3^0C. The process utilizes a thimble in which the sample is placed and the supercritical fluid is pumped through the thimble which leads to the extraction of the soluble compounds of the sample (pesticide) into the collection trap via a restricted nozzle. The fluid is then allowed to vent in to the collection trap which causes the escape of the solvent and this leads to the extraction of the product left in the collection trap. The efficiency of the method depends on the temperature and pressure (Das, 2014).

Accelerated Solvent Extraction (ASE) - Accelerated Solvent Extraction is an automated method for extracting poisonous substance from the biological matrix by the use of organic solvents at an elevated temperature and pressure. The sample is placed in the extraction tube after sectioning into thin pieces which is then placed in the assembly with all parameters set in. This method is advanced as it utilizes minimum amount of solvent and takes less amount of time for the extraction (Lanjewar *et al*, 2014).

QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method- QuEChERS is another method developed to overcome the problems associated with the conventional methods of extraction. The process involves the extraction of sample by simply shaking or through vortex mixing using 10 ml of acetonitrile (MeCN). This is followed by the addition of extraction salts (4 g of $MgSO_4$ and 2 g of NaCl) so as to carried out the partitioning of the analytes between the aqueous residue and the solvent. After the aforesaid treatment, it is allowed to centrifuge and the residual water is removed by dispersion Solid Phase Extraction (d- SPE) containing PSA adsorbent and anhydrous MgSO4 salt which are then allowed to mixed with the sample extract for further analysis. This method is beneficial as it requires minimum amount of time than the traditional method of Solid Phase Extraction and also removes residual water and other polar matrix components simultaneously (Deshpande and Srivastava, 2016).

So, pesticides can be extracted from the biological samples (tissues and body fluids) by a large number of methods but the best suitable method is the one which is capable of providing maximum amount of yield and results in highest % of recovery of these pesticides from such samples without the matrix interferences.

Review of Literature

Stalling, et al, (1979) - adopted the method of pesticide extraction using dichloromethane as the solvent from the biological samples but the results obtained are not much efficient because of the not much recovery and wastage of large amount of solvent.

Ballschmiter *et al*, (1981) - worked on the methodology of cold column extraction and doing liquid liquid extraction of the pesticide present in the tissue sample. The extraction is carried out using a 2:1 solvent of acetone and hexane in a gravity fed column that is filled with the pesticide containing dried adipose tissue.

Mes, (1984)- extracted the pesticides from the adipose tissues of visceral samples and other body fluids using conventional liquid liquid extraction by applying cold blending technique for the extracts using a mixture of organic polar solvents like methanol and dichloromethane in 1:1 ratio which gives successful results leads to more than 80% recovery. He also found that other organic solvents including benzene, benzene or acetone mixture does not provide efficient results.

Wells, (1988) - Soxhlet extraction method is used for the isolation of pesticides which involves heating and thereby reduces the polarity of the solvents used, but it leads to a disadvantage i.e. extraction of coextractants (fats) with the desired sample which gets precipitated on cooling and thereby decreases the yield.

Repetto *et al,* (2001) - has developed an analytical method for determination of pesticides from human blood using solid phase technique for the extraction of such samples. The pesticides namely endosulfan, lindane, parathion, ethyl-azinphos, diazinon, malathion, alachlor, tetradifon, fenthion and dicofol are extracted from the blood with the application of a reverse phase i.e. C 18 cartridges during the procedure of isolation. This method of extraction has replaced the conventional method (liquid liquid extraction) i.e. a mixture of hexane and benzene in 1:1 ratio, generally opted for isolation of such samples from body fluids. The extracts obtained are then analyzed with gas chromatography with NPD detector along with the use of two internal standards perthane and triphenylphosphate. The study reveals successful results with recovery range of 71.83 to 97.10%.

Ueyama *et al,* (2006) - carried out the analysis of various pesticides mainly of class organophosphates in the biological samples of human urine. Since organophosphates gets metabolized in the body and excreted in human urine in the form of Dialkylphosphates (DAPs). These metabolites of organophosphates namely dimethylphosphate (DMP), diethylphosphate (DEP), dimethylthiophosphate (DMTP), and diethylthiophosphate (DETP) are extracted from the sample of urine by the application of Solid Phase Extraction (SPE) technique along with derivatization using pentafluorobenzylbromide followed by the routine clean up procedure. The extracts obtained when later analyzed by Gas Chromatography- Mass Spectrometry (GC- MS), found that good results were obtained revealing that DMP can be detected approximately upto 0.3 l g/L, and remaining samples DEP, DMTP, and DETP upto 0.1 g/L.

Srivastava and Yadav, (2014) – isolated an organochlorine pesticide Endosulfan from the various biological samples (blood, urine, viscera) using conventional extraction method i.e. liquid liquid extraction and method of steam distillation. The blood is extracted for the pesticide isolation by treating 20ml of blood with 10% solution of sodium tungstate along with the sulphuric acid and shaken for 2 minutes to obtain the acidified clear sample which then extracted with hexane normally using solvent extraction technique and proceeded for further analysis. The same procedure of solvent extraction is applied to the urine sample after doing its refluxing on water bath but the pesticide extraction from viscera is carried out by its acidification using sulphuric acid followed by extraction using steam distillation method.

Lanjewar *et al,* (2014) - performed the pesticide residue analysis of major organophosphorus pesticides namely Malathion, Chlorpyrifos, Monocrotophos, Dimethoate and Dichlorovos, using accelerated solvent extraction method followed by analysis through Gas Chromatography employing NPD as detector. The samples of organophosphorus pesticides are extracted from 20 gm. of viscera mixed with diatomaceous earth material and cut down in small pieces so that a dry mass is obtained for its easy transfer into the extraction cell fitted with a cellulose disk at the bottom. The entire mass is then loaded onto the extraction cell, capped and placed in the tray slots of ASE. The collection vials are then placed at the lower level of the assembly of the method, parameters are set and samples are allowed to extract, dry using sodium sulfate and concentrate for further analysis using GC. Different pesticides are recovered at with different recovery rates differences in limits of detection. Like 0.05ug of Malathion is recovered with 99% recovery, 0.05ug Chlorpyriphos is recovered with 90% recovery, 0.5ug of Monocrotophos is recovered with 90% recovery, 0.05ug of Dimethoate is recovered with 72 % recovery and 0.05ug of Dichlorovos is recovered with 85% recovery when extraction is carried out using Accelerated Solvent Extractor (ASE- 200).

Chutke *et al,* (2016) - extracted and isolated fenvalerate, a pesticide of the class pyrethroids by ASE-200 by heating at 70°C and 80°C in three cycles using a mixture of solvents ethyl acetate and acetonitrile (8:2) by spiking the 20gm of viscera sample with 10mg of Fenvalerate and placed it in the extraction cell. The results of the study showed that at 70°C, 86% of the pesticide is recovered which is better in efficiency than the manual extraction procedure and the amount decreases to 62% because of the extraction of the fats and lipid components from the viscera, when the temperature is increased from 70-80°C. The extracts are than analyzed through Gas chromatography using FID detector and positive results with 86% recovery of the fenvalerate are obtained.

Deshpande and Srivastava, (2016) - has suggested a method for extraction of pesticides from the tissues using QuEChERS method (quick, easy, cheap, effective, rugged and safe) which involves the cutting of the biological tissue into small fine pieces

followed by its treatment with acidified acetonitrile solution along with the addition of 2ml of water in order to achieve the desired consistency and to remove the tissue dryness. The sample is placed in the extraction tube of SampliQ QuEChERS kit and allowed to sonicate for 5 minutes which is later treated with extraction salts (magnesium sulfate) which carries out the extraction. The sample tube is then shaken vigorously and centrifuged for about 4 minutes at 4000rpm to get a clear upper layer of supernatant which is again subjected to same treatment which involves centrifugation for 3 minutes in the SampliQ QuEChERS dispersive Solid Phase Extraction (SPE) tube to achieve a clear liquid extract. Deshpande and Srivastava has also extracted the pesticides from the body fluids (blood) using QuEChERS method by diluting 0.5ml of blood with distilled water in a SPE extraction tube containing 0.5 g pre-packed extraction preparation and a stainless steel bead. The spiking of the blood sample is done with the pesticide along with the addition of 2 ml acidified ACN (1% acetic acid/ACN), followed by vigorous shaking of the tube for a minute and allowed to centrifuge at 4000 rpm for 5 min. The supernatant obtained is then transfer to centrifuge tube after containing SPE sorbent, shaken vigorously and again centrifuged at 4000 rpm for 1 min so as to get a clear liquid extract. These extracts of both tissue and blood are then analyzed using high performance liquid chromatography. QuEChERS sample preparation reduces potential cross contamination of samples thereby facilitating rapid and efficient analyzes of a large number of samples with an ordinary chromatographic instrumentation.

Discussion and Conclusion

The method of extraction applied for the pesticides analysis depends on the type of solvent or solvent mixture used, chemical and physical properties of the insecticide, the type of substrate (matrix) from which it is isolated and the method of estimation to be employed.

Stalling, et al, (1979); Ballschmiter *et al*, (1981); Mes, (1984); Srivastava and Yadav, (2014) – has carried out the liquid liquid extraction and steam distillation of pesticide using various polar solvents but the results obtained are not satisfactorily as the percentage of recovery is not much.

Wells, (1988) - conducted the analysis of pesticides (organochlorines) using Soxhlet extraction method but the results of the study showed that other co extracts are also come in the extracts as contaminants.

Repetto *et al*, (2001); Ueyama *et al*, (2006) – performed the solid phase extraction of the large number of pesticides using reverse phase medium C18, and derivatization Solid phase extraction using pentafluorobenzylbromide. The extracts obtained after clean-up are analyzed with gas chromatography and Gas chromatography Mass spectrometry (GC-MS) which showed that the differences occurred in their limit of detection and also in the recovery rate.

Lanjewar *et al*, (2014) - used accelerated solvent extraction method for the organophosphorus pesticides isolation from the biological samples and found that results of the study are encouraging as the detection upto minimum amounts along with high recovery percentage because the extracts are free from the matrix contaminants.

Chutke *et al*, (2016) – applied accelerated solvent extraction method for the isolation of fenvalerate (pyrethroid) followed by its determination using gas chromatography and found that the pesticide even subjected to higher temperature and pressure does not gets decomposed with 86% recovery.

Deshpande and Srivastava, (2016) – used QuEChERS method for the extraction of the pesticides from the biological samples and leads to the conclusion that the samples obtained are free from the matrix contaminants because of the efficiency of the method applied.

Extraction of pesticides from the biological matrix like viscera, tissues or body fluids through liquid liquid extraction or solid phase extraction methods requires large volume of solvent and are more time consuming therefore development in these extraction procedure using accelerated solvent extraction or other methods are considered to be better than conventional liquid liquid extraction and solid phase extraction method methods because of its less requirement of solvents, easy preparation of sample, less time consumption and greater efficiency for isolation and separation. QuEChERS sample preparation method can also be utilized for extraction of pesticides as this method reduces potential of cross contamination of samples and carries the rapid and efficient analysis of a large number of samples which can later detected with chromatographic methods. So, according to this paper the extraction of pesticides from the biological

samples using Accelerated Solvent Extraction method and QuEChERS method are supposed to be the optimum methods as the results obtained are with maximum % recovery and also free from the interferences of matrix contaminants.

.

References:

Chutke, Nitin L., Santosh R. Kote, and Manoj R. Bhandarkar. "Accelerated Solvent Extraction Of Pyrethroid Insecticide, Fenvalerate From Biological Materials." *World Journal of Pharmacy and Pharmaceutical Sciences* 5.8 (2016): 1374-378. Web. 04 Apr. 2017.

Das, Shaon Kumar. "Recent Developments in Clean up Techniques of Pesticide Residue Analysis for Toxicology Study: A Critical Review." *Universal Journal of Agricultural Research* 2.6 (2014): 199-203. Web. 03 Apr. 2017.

Deshpande, Deepti S., and Ashwini K. Srivastava. "Method validation for simultaneous determination of pesticide residues in post-mortem samples by high performance liquid chromatography ultraviolet method." *European Journal of Forensic Sciences* 3.3 (2016): 1-6. Web. 6 Apr. 2017.

Lanjewar RB., Chutke NL., and Lanjewar MR. " Isolation of Organophosphorus Pesticides from Biological Material by Accelerated Solvent Extractor (ASE-200) and its Determination by Gas Chromatographic Method". *Int. Res. J. of Science & Engineering*. 2.2, (2014): 63-67.Web. 04 Apr. 2017.

Repetto, Rosario García, M. P. Giménez, and M. Repetto. "New method for determination of ten pesticides in human blood." *Journal of AOAC International* 82.2 (2001): 342-349. Web. 06 Apr. 2017.

Srivastava, Ankit, and V. K. Yadav. "Extraction & Identification of Endosulfan from Viscera, Urine & Blood By Using Different Solvent System." *US China Law Review* 11 (2014): 781-91. Web**.** 06 Apr. 2017.

Tuzimski, Tomasz. "Methods of Pesticide Residue Analysis." *Journal of AOA C International* 95.5 (2012): 1256-257. Web. 04 Apr. 2017.

Ueyama, J., Saito, I., Kamijima, M., Nakajima, T., Gotoh, M., Suzuki, T., Shibata, E., Kondo, T., Takagi, K., Miyamoto, K., Takamatsu, J., Hasegawa, T., Takagi, K. *"Simultaneous determination of urinary dialkylphosphate metabolites of organophosphorous pesticides using gas chromatography–mass spectrometry"*. Journal of Chromatography. B 832, (2006): 58–66. Web. 03 Apr. 2017.

Wells, D. E. "Extraction, Clean-Up and Group Separation Techniques in Organochlorine Trace Analysis." *Pure and Applied Chemistry Journal* 60.9 (1988): 1437-448. Web. 04 Apr. 2017.

Żwir-Ferenc, A. , and M. Biziuk. "TechSolid Phase Extraction Technique – Trends, Opportunities and Applications." *Polish Journal of Environmental Studies* 15.5 (2006): 677-90. Web. 04 Apr. 2017.