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Research Paper

Development and Validation of HPLC Method for Determination of Triazophos Pesticide in Water

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Abstract: While studying the fate of pesticide in different environmental segments such as soil and water, its accurate quantification is required. Therefore, development of suitable analytical method for determination of organophosphorus pesticide residues in water is an important area of research. This study deals with the development and validation of HPLC method for determination of triazophos residues in water. The various HPLC parameters were optimized to obtain the clear and sharp chromatograms. The developed method was validated for accuracy, precision, linearity and robustness following the standard procedures. The results were found to be quite accurate and precise with standard deviation below 5%. The recovery varied from 92.1 to 108.5%. The limit of detection and limit of quantification were found to be 0.2449 and 0.8163 mg/L. The method was robust with respect to small change in mobile phase ratio and flow rate. But increase in flow rate decreased the retention time proportionately. The developed method can be used successfully without separation of pesticide from aqueous phase.

Keywords: accuracy, HPLC, method, triazophos, validation.

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Introduction

Organophosphorus pesticides (OPPs) are an alternative to more persistent and highly toxic organochlorine pesticides. There has been a very fast growth to the use of OPPs pesticides mainly due to their low persistence in the environment as compared to the more persistent organochlorine pesticides [1]. Moreover, many of the organochlorine pesticides have been banned for use in agriculture. But due to the continuous and indiscriminate use especially in the developing countries, the residues of these highly toxic OPPs have been reported in soil as well as aqueous media. The transportation of these pesticides to different environmental segments takes place mainly through adsorption by soil, leaching to the water bodies, volatilization, and uptake by the plants etc. [2, 3]. During the laboratory study on sorption behaviour, degradation and persistence, the determination of the concentration of OPPs in water is the most important step in the process. Generally this requires the lengthy and complicated procedure of separation of OPPs from water, dissolution in organic solvent such as acetone, methanol etc. and then analysis of

the sample. Earlier spectrophotometer methods were used for analysis. But nowadays more sophisticated methods such as HPLC and GC are employed for accurate and precise quantification of the pesticides. The HPLC methods have been developed successfully for organophosphorus pesticides [4,5].

The triazophos pesticide is being regularly used for control of different insects and pests in number of fruits and vegetables such as brinjal, cauliflower and citrus etc. Triazophos (*O,O*-diethyl *O*-1-phenyl-1H-1,2,4-triazol-3-yl phosphorothioate) is a moderately toxic and broad spectrum, nonsystemic pesticide [6,7]. The structure of the pesticide is shown in Fig 1. The development of reliable and reproducible methods for environmental analysis of pesticides residues is an important field of research. Use of HPLC technique is quite an accurate way of determination of pesticide residues in different environment segments. Basically the HPLC system does the separation of pesticide in the stationary column with the help of mobile phase and then detects and quantifies it with the use of a suitable

detector and system software. The different components of HPLC and their sequence of operation are shown in Fig. 2. The present study deals with the development of HPLC method for determination of triazophos contents in water. This method is quite easy and simple to use. An attempt has been made to validate the developed method for their precision, accuracy, reproducibility and robustness.

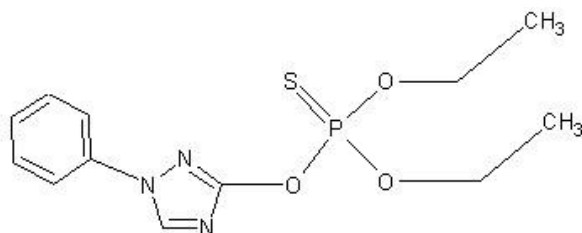


Figure 1: Structures of triazophos

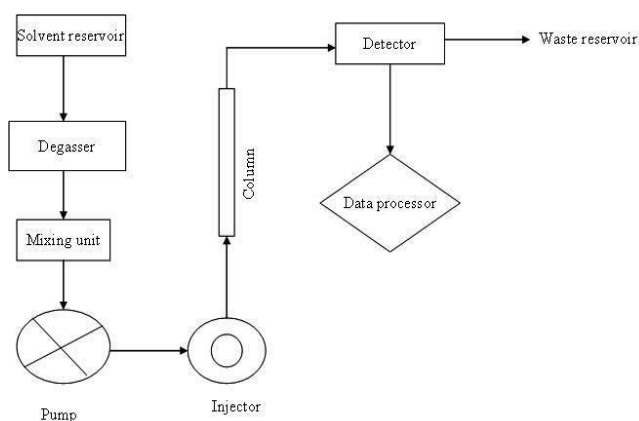


Figure 2: Components of HPLC system

Material and Methods

Instrumentation

The HPLC (Shimadzu Corporation, Japan) equipped with UV detector was used for analysis. This Chromatographic system consisted of Shimadzu-LC 20AT pump, stationary phase C18 Phenomenex Luna 5 μ m 100A 250 X 4.6 mm analytical column, SPD-20A (UV-VIS) detector and autosampler (SIL-20A HT).

Chemicals and reagents

The organophosphorus pesticide standards of triazophos (>99 % purity) was purchased from Sigma-Aldrich (India), Bangalore. The chemicals used for the mobile phase – acetonitrile and water were of HPLC grade and purchased from SD Fine-Chem Limited (India). Only milli-Q millipore water was used for making standard solutions of the pesticides.

Method development

Preparation of standard solution

Stock solutions of triazophos were prepared by dissolving 20 mg of each pesticide in Millipore water so that a concentration of 20 mg/L can be obtained. From

these stock solutions, working standards of 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 mg/L concentrations were prepared by diluting the stock solution with Millipore water. The solutions were stored under refrigerated conditions at 2-3°C and were analyzed within 24 hours.

Selection of mobile phase

Many authors have used different ratios of acetonitrile and water as mobile phases for analysis of the above said pesticide on HPLC system. Different ratios of these phases were tried at different flow rates to get the clear and sharp peak for the pesticides. The best conditions were obtained at the acetonitrile : water ratio of 80 :20 at the flow rate of 1 ml/min. These conditions were used for the analysis of the pesticide on HPLC. Both the mobile phases were filtered through 0.45 μ m filter and sonicated for about 10 minutes before being used

Sample preparation

The stock solution of the pesticides was brought to room temperature after being taken out of the refrigerator. About 2 ml of the sample was passed through 0.2 μ m syringe filter and was put into the sample vial. The vial was loaded into the auto sampler of HPLC from which the sample of set volume was injected into the column of HPLC system.

HPLC conditions

The various HPLC conditions such as UV detector wave length, components of mobile phase and their ratio, flow rate and injection volume etc. were optimized for the analysis of triazophos pesticide. These values are presented in Table 1.

Table 1: The conditions for analysis of triazophos pesticide on HPLC system

HPLC parameters	Conditions
<i>Type of column</i>	
UV-Vis detector wave length	270 nm
Oven temperature	30°C
Mobile phase acetonitrile: water	80:20
Flow rate of mobile phase	1 ml/min
Injection volume	10 μ l

Validation of methods

The validation of any developed method ensures credibility of analysis. It demonstrates the scientific soundness of the measurement or characterization. It is required to varying extents throughout the regulatory submission process. In the present study, following parameters were studied for validation

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found^[8]. For determination of accuracy of the developed method, three different levels

of pesticides concentration, namely low (80%), intermediate (100%) and high (120%) were prepared from the standard stock solutions of 10 mg/L of the pesticide and analyzed under the developed HPLC conditions. Accuracy was assessed as percentage relative standard deviation (%RSD) and mean percent recovery. The %RSD was calculated as follows

$$\%RSD = \frac{\sigma}{x} * 100 \quad (1)$$

Where σ is the standard deviation and x is the average value of the detector response.

Precision (reproducibility)

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions^[8]. The developed method should be such that it produces the same results, when analysis of the same sample is performed repeatedly over a period of time. It must ensure reproducibility. Inter day and intraday analysis were performed for finding out the variation in retention time and recovery of the samples of same concentration. Precision was determined in terms of %RSD (relative standard deviation) of the peak area.

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample^[8]. Linearity of any analytical method is its ability to obtain the results that are defined by a definite mathematical relationship proportional to the concentration of analyte in samples within the given range. The linear calibration curves were prepared with different concentration levels varying from 2 mg/L to 20 mg/L. The linearity of curve plotted between concentration level and detector response was evaluated by the linear regression using the least square method. The Microsoft Excel software was used for this purpose.

Limit of detection and limit of quantification

The limit of detection (LOD) of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The LOD was obtained as per the ICH (International Conference for Harmonisation) guidelines^[8] using the following formula

$$LOD = 3.3\sigma / S \quad (2)$$

Where σ is the standard deviation of the detector responses in the linearity range and S is the slope of the calibration curve

The limit of quantification (LOQ) of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The LOQ was obtained as per the ICH guidelines using the following formula

$$LOQ = 10\sigma / S \quad (3)$$

Where σ is the standard deviation of the detector responses in the linearity range and S is the slope of the calibration curve

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. It was determined by the change in chromatographic parameters such as flow rate of mobile phase and mobile phase ratio. The flow rate of mobile phase was changed from 0.9% to 1.1% for triazophos. The ratios of the mobile phase were changed from 70:30 to 90:10 (acetonitrile: water). While conducting the analysis with change in one parameter, other chromatographic conditions were set as per the developed method. During the analysis with change in chromatographic conditions, the change in retention time and peak area were noted.

Results and Discussion

The present study attempts to develop the HPLC method for determination of the concentration of triazophos in water. The Figure 3 shows the typical chromatogram of triazophos. The retention time was found to be 6.15 ± 0.1 minutes. This chromatogram was obtained under the HPLC conditions mentioned in Table 1.

The developed methods was validated for linearity, range, accuracy, inter day and intra day precision, LOD, LOQ and robustness as per the ICH guidelines.

The linearity was determined by analyzing five standards of different concentrations for the pesticide. The calibration plot of concentration of pesticide (mg/L) versus peak area for triazophos is shown in Figure 4. Within the concentration range of 2 to 20 mg/L, the curve was found to be linear with coefficients of determination 0.998. As per the ICH guidelines, a R^2 value of more than 0.998 is sufficient for an acceptable fit of data to the regression line^[8,9]. The regression equation is given below

$$y = 7945x \quad (4)$$

Where

$$\begin{aligned} y &= \text{Peak area, } \mu\text{v min} \\ x &= \text{concentration of pesticide (mg/L)} \end{aligned}$$

The LOD and LOQ were found to be 0.2449 and 0.8163 mg/L showing the sensitivity of the equipment.

Accuracy

The accuracy was measured as per the ICH guidelines by taking three different levels of pesticide concentration, namely low (80%), intermediate (100%) and high (120%), prepared from the standard stock solutions of 10 mg/L. The results in terms of standard deviation and

recovery range are given in Table 2. As clear from the Table, the recovery range for triazophos existed within range of 92.1 and 108.5%. Similarly the standard deviation

remained below 5%. Both these parameters indicate that the developed method is quite acceptable in terms of its accuracy for measurement.

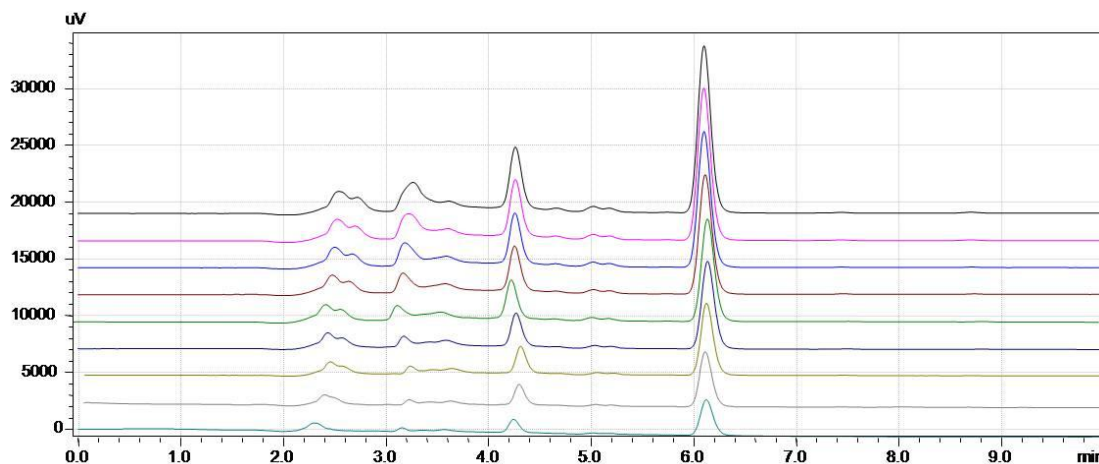


Figure 3: Chromatograms of triazophos obtained at different concentrations

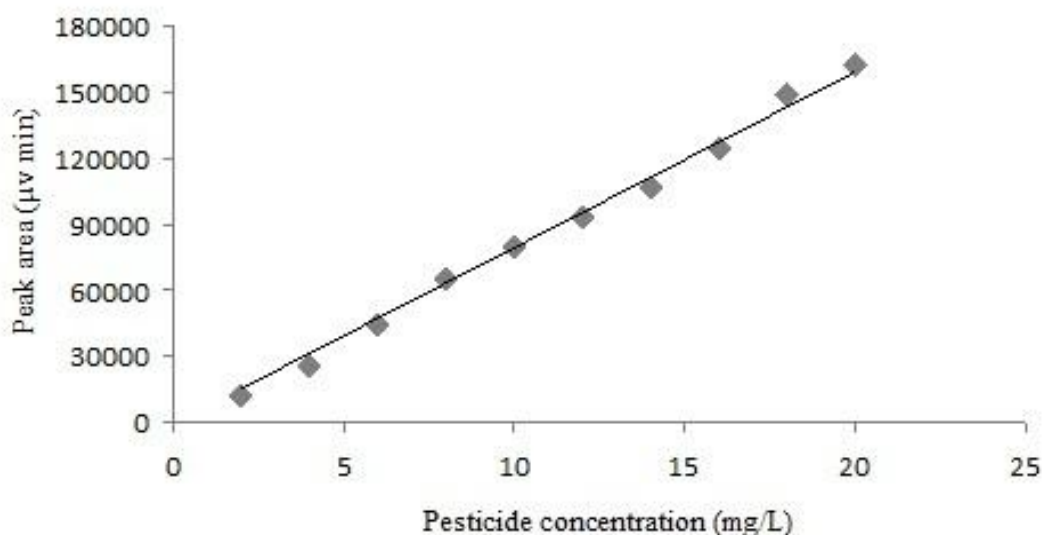


Figure 4 : Calibration curve for triazophos

Table 2: Accuracy in determination of pesticide concentration in water

Pesticide	Concentration level (mg/L)	Recovery range (%)	Standard deviation
Triazophos	8	94.3-105.3	3.54
	10	92.1-107.2	4.42
	12	93.1-108.5	3.38

Precision (reproducibility)

The precision of any developed method depends on how the results can be reproduced over a period of time. For judging the precision or reproducibility of the developed method, the concentrations of 2, 5, 10, 15 and 20 mg/l of the pesticide were used for analysis on HPLC. Five

replications of each concentration were made on the same day as well as on three different days for assessing the intra day and inter day reproducibility of the results. The findings are shown in the Table 3.

Table 3: Precision in determination of pesticide concentration in water

Pesticide concentration (mg/L)	% RSD	
	Intra day	Inter day
2	0.870	0.892
5	0.244	0.932
10	0.426	0.654
15	0.241	1.432
20	0.132	0.987

As clear from the Table, the intra day %RSD values are below 0.5 indicating a high reproducibility of the developed method. The inter day % RSD values was found to be around 1.0% or higher. So the reproducibility was lost up to some extent when the analysis was done over a period of few days.

Robustness

The robustness is related with the change in results obtained with the slight variation in the optimum values of the parameters of the developed method. It measures the reliability of the method. Here the robustness of the methods was checked by varying in the ratio of the mobile phase and its flow rate. Results were measured in terms of percent change in detector response due to change in method parameters. The results are shown in Table 4. It is clear that by the deviation of nearly 10 % from the optimized mobile phase ratio and its flow rate, the %RSD was not more than 2.66 % for mobile phase flow rate and not more than 2.24% for mobile phase ratio. But the increase in flow rate reduced the retention time proportionately.

Table 4: Robustness study of the developed method for triazophos

Mobile phase flow rate (ml/min)	%RSD
1.1	2.661
0.9	2.067
Mobile phase ratio (acetonitrile: water)	
70:30	2.243
90:10	2.122

Conclusion

The development of analytical method for determination of pesticide residues in water is an important field of research. The present study is an attempt to develop and validate of HPLC method for determination of triazophos concentration in water. The various parameters such as selection and composition of mobile phase, its flow rate, detector wave length and injection volume etc. were optimized. The method was validated for accuracy, linearity, precision and robustness. The method was quite

accurate with %RSD below 1%. The LOD and LOQ were 0.2449 and 0.8163 mg/L. The results could be reproduced over a period of time, although the inter day precision was less than that of intra day precision. The calibration curves were found to be linear over a range of 2 to 20 mg/L. The method was found to be robust with standard deviation remaining below 5.0 for nearly 10% variation in optimized mobile phase ratio and its flow rate. Overall, it can be said that the developed method is very simple and easy to use without going in for separation of pesticide from the aqueous phase.

References

- Kamath V. and Rajini P.S., Altered glucose homeostasis and oxidative impairment in pancreas of rats subjected to dimethoate intoxication, *Toxicol.*, **231**, 137-146 (2007)
- Durovic R., Jelena G.U. and Dordevic T., Effects of organic matter and clay content in soil on pesticide adsorption processes, *Pestic Phytomed.*, **24**, 51-57 (2009)
- Rani S. and Sud D., Time and temperature dependent sorption behaviour of dimethoate pesticide in various Indian soils, *Int. Agrophys.*, **28**, 479-490 (2014)
- Aleksandra N.K., Meri M., Jean J.A. and Zoran Z., Determination of dimethoate, 2,4-dichlorophenoxy acetic acid, mecoprop and linuron pesticides in environmental waters in republic of Macedonia by high performance liquid chromatography, *Maced J Chem Chem Eng.*, **27**, 25-33 (2008)
- Pawan K.C. and Gurupadayya B.M., Analytical method development and validation of dimethoate pesticide using HPLC method, *Biochem Anal Biochem.*, **2**, 127 (2013)
- Sud D. and Kaur P., Heterogenous photocatalytic degradation of selected organophosphate pesticides: A review, *Crit. Rev. Env. Sci. Tec.*, **42**, 2365-2407 (2012)
- Tomlin CDS (Ed.). The pesticide manual (15th Ed.). Hampshire, England: BCPC, (2009)
- ICH Harmonised tripartite guideline, Validation of analytical procedures: text and methodology Q2(R1) http://www.ich.org/fileadmin/Public/Web_Site/ICH_products/Guidelines/Quality/Q2_R1/Step4/Q2_R1_Guideline.pdf.2005. (assessed November 19, 2014)
- Chitturi S.R., Bharathi C.H., Reddy A.V. R., Reddy K. C., Sharma H.K., Handa V.K., Dandala R. and Bindu V.M., Impurity profile study of lopinavir and validation of HPLC method for the determination of related substances in lopinavir drug substance, *J. Pharmaceut. Biomed. Anal.*, **48**, 1430-1440 (2008)