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Development and validation of an analytical method for the determination of Fenaminophos residues in tobacco

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Abstract : A simple, quick, sensitive, accurate and precise method using gas chromatography has been developed for the determination of fenamiphos, fenamiphos sulfoxide and fenamiphos sulfone. The detection was carried out using a nitrogen-phosphorus detector. The column temperature was maintained at 230 °C while the temperature of the injection port and detector were maintained at 270 °C and 300 °C respectively. The method was validated by assessing parameters linearity, reproducibility, accuracy, recovery and repeatability. The percentage recovery for fenamiphos and its metabolites was more than 80 % with RSD values less than 2 %.
Key Words : Fenamiphos, Gas chromatography, Validation, Recovery.

1. Introduction

Fenamiphos is an organophosphate insecticide registered nationally for control of nematodes and insects in agricultural and commercial areas [1]. It is a systemic and contact insecticide used primarily for the control of the major genera of nematodes. It is absorbed by the roots of treated plants and translocated to the leaves [2]. Fenamiphos (Ethyl 3-methyl-4-(methylthio) phenyl (1-methylethyl) Phosphoramidate) is an active compound in Nemacur [3-7] (Figure 1). It is also used on a variety of plants including tobacco, turf, bananas, pineapples, citrus and other fruit vine, some vegetables and grains [1]. Pesticides are used to protect crops from insects, pests, weeds, moulds, diseases and by stopping food crops being contaminated by fungi while they are growing. Pesticides form a very wide and complex subject which includes issues such as residues in food, human health and safety, the effects on wild life and environmental and manufactures interests [8]. Many of these pesticides can cause moderate to severe damage to human health for example, some pesticides often cause respiratory problems and at times death of affected people. Fenamiphos is listed as one of the highly hazardous pesticide in the Pesticide Action Network International list (PAN) [9]. Since it is a highly hazardous pesticide there is great need to regulate its quantity in tobacco. Fenamiphos residues should not exceed the maximum residual limit (MRL) of 0.5ppm [10-11].

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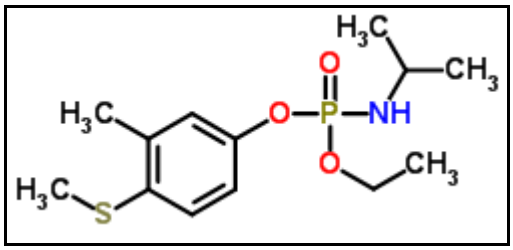

Figure 1: Chemical Structure of Fenamiphos

Fenamiphos is not easily biodegradable and its metabolites are neurotoxic. The main degradation products are fenamiphos-sulfoxide and fenamiphos-sulfone. Fenamiphos metabolism also occurs in the environment and in various matrices through different routes involving chemical reactions including hydrolysis, oxidation and reduction as well as microbial activity [12-14].

2. Experimental

2.1. Chemicals and Reagents

All chemicals and reagents were of analytical grade. Fenamiphos reference standard, fenamiphos sulfone and fenamiphos sulfoxide were purchased from Sigma Aldrich.

2.2. Instrumentation

A Perkin Elmer Auto System (GC NPD) Gas Chromatography with a Nitrogen – Phosphorus Detector was used for all GC measurements.

2.3. Preparation of Samples

The sample matrices were tobacco leaves obtained from TRB (Tobacco Research Board) fields.

2.3.1. Preparation of Standard Samples

Fenamiphos standard (0.01 g) was dissolved in 100 ml of 10 % acetone in hexane to obtain a 1 x 10^{-4} g/ml concentration. A similar procedure was repeated for the fenamiphos sulfone and fenamiphos sulfoxide standards.

2.3.2 Extraction procedure

Samples were socked in 30 ml double distilled water over night. A 50:50 volume mixture of dichloromethane and acetone was used to extract the analyte from the sample matrix. Samples were extracted by macerating three times for three minutes and the solution was filtered over anhydrous sodium sulphate and evaporated to dryness.

2.4. Analytical Method Validation

The parameters for method validation have been defined in different working groups of National and international committees. The parameters, as defined by the ICH and by other organizations and researchers include specificity/ selectivity, linearity, accuracy, precision and reproducibility, accuracy and recovery.

2.4.1 Linearity

Linearity is a method's ability to obtain test results that are directly proportional to the sample concentration over a given range [15]. Six tobacco samples were used to determine the linearity of the method by spiking at different levels of 0.70 ppm, 0.60 ppm, 0.55 ppm, 0.50 ppm, 0.45 ppm and 0.40 ppm of Fenamiphos, fenamiphos sulfone and fenamiphos sulfoxide. The samples were prepared and analysed using the developed method on GC NPD.

2.4.2 Accuracy and recovery

Accuracy is closeness in agreement of the accepted true value or a reference value to the actual result obtained. Recovery is the proportion of analyte remaining at the point of the final determination, following its addition (usually to a blank sample) immediately prior to extraction and is expressed as a percentage. The accuracy and recovery was tested by spiking six 5 g weights of tobacco samples at three different levels in duplicates. The spiking levels were 1 ppm, 0.5 ppm and 0.3 ppm. The samples were left for a day before carrying sample preparation and analysis done on GC NPD using the developed method.

2.4.3 Repeatability

The precision of measurement of an analyte usually obtained from recovery or analysis of reference materials. The repeatability of the method was determined by analysis of seven samples containing 0.2 ppm of fenamiphos, fenamiphos sulfone and fenamiphos sulfoxide.

2.4.4 Reproducibility

Internal reproducibility refers to procedures of an experiment being done is in a single laboratory. The precision of measurement of an analyte usually by means of recovery or analysis of reference materials, obtained using the same method by different analysts, the lab technician analysed the samples using the developed method.

2.4.5 Specificity

Specificity is obtained by choosing optimal columns and setting chromatographic conditions, such as mobile phase composition, column temperature and detector wavelength. This defines how well a method is able to discriminate between the analytes of interest and other components present in the matrix. This parameter was determined by analysis of the chromatograms.

3. Results and discussion

3.1 Linearity

To determine the linearity of the method six tobacco samples were used and spiked at different levels which span between 80 -140 % of the maximum residual level (MRL). A linear regression was obtained for each component. The equation with a line of best fit for fenamiphos was plotted on a scatter diagram of the expected and observed concentrations. Regression analysis showed a good linear relationship (r^2 >0.99) for all components and this showed that the method has a good response with respect to concentration (Figures 2-4).

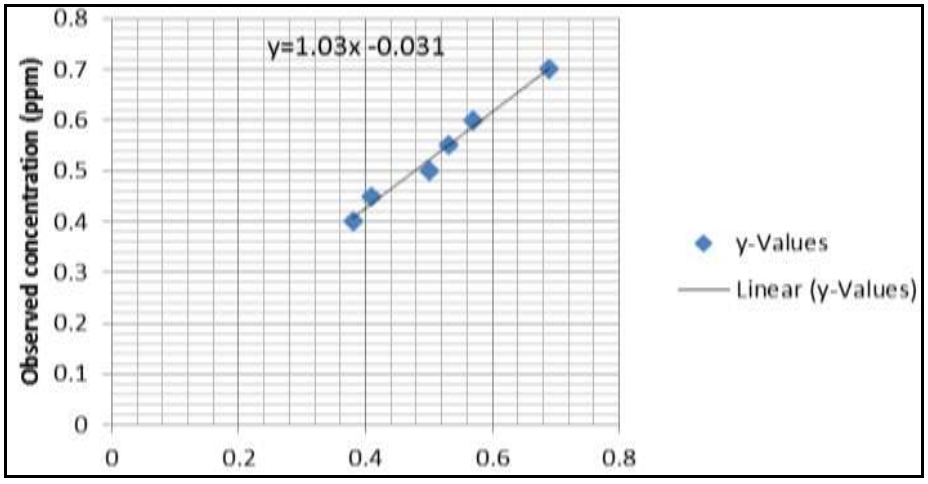

Figure 2: Scatter diagram of expected concentration vs. the observed concentration for Fenamiphos

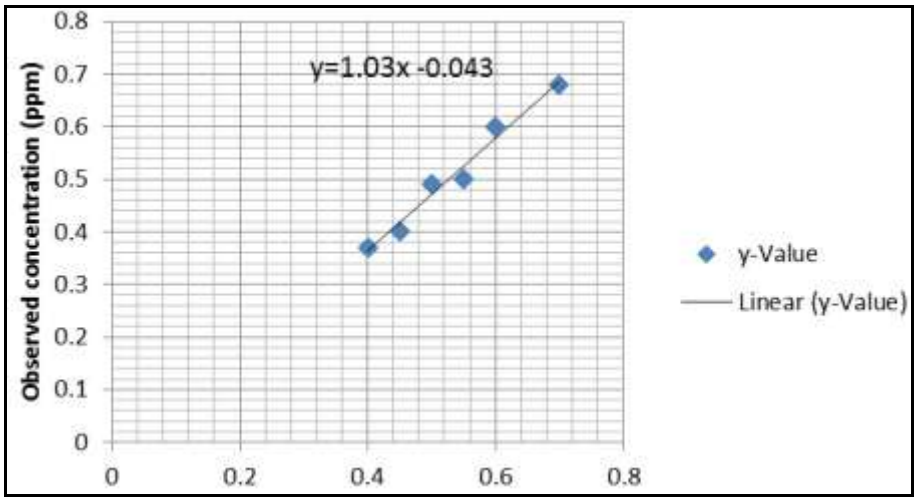

Figure 3: Scatter diagram of expected concentration vs. observed concentration for fenamiphos sulfoxide

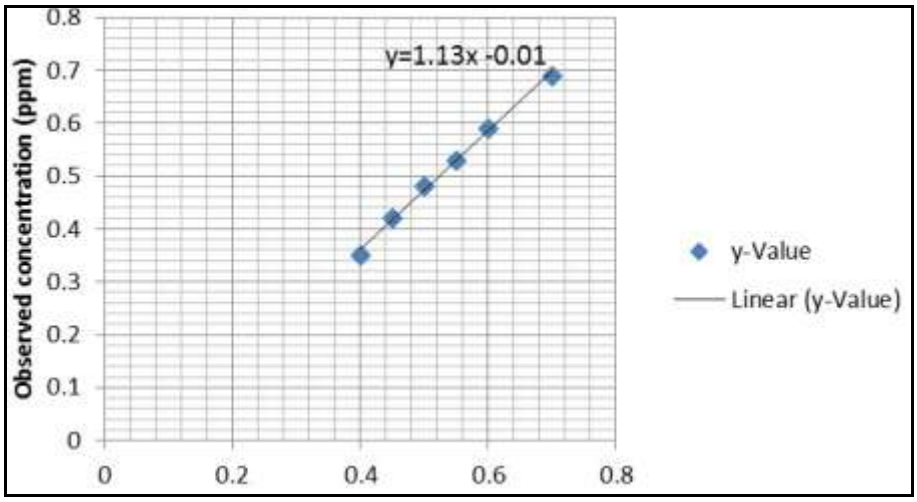

Figure 4: Scatter diagram of expected concentration vs. observed concentration for fenamiphos sulfone

3.2 Accuracy

The accuracy of the method was determined as the extent of recoveries from three quality control samples which had been spiked at different levels of known quantities of fenamiphos, fenamiphos sulfoxide and fenamiphos sulfone standard. The spiking and analysis was done in duplicate so as to increase the integrity of the results. Fenamiphos, fenamiphos sulfoxide and fenamiphos sulfone have 87.9 %, 85.2 % and 89.1 % average recoveries from three spiking levels (Tables 1-3), thus confirming the method to be accurate.

Table 1: Recoveries of three duplicate QC spiked samples at three different levels for fenamiphos

Sample	Expected [conc] in ppm	Obtained [conc] in ppm	% Recovery
QC1A	0.3	0.23	76.6
QC1B	0.3	0.21	70
QC2A	0.5	0.46	92
QC2B	0.5	0.48	96
QC3A	1.0	0.96	96
QC3B	1.0	0.97	97

Table 2: Recoveries of three dublicate QC spiked samples at three different levels for fenamiphos sulfoxide

Sample	Expected [conc] in ppm	Obtained [conc] in ppm	%Recovery
QC1A	0.3	0.21	70
QC1B	0.3	0.233	77.6
QC2A	0.5	0.438	87.6
QC2B	0.5	0.44	88
QC3A	1.0	0.95	95
QC3B	1.0	0.93	93

Table 3: Recoveries of three dublicate QC spiked samples at three different levels for fenamiphos sulfone

Sample	Expected [conc] in ppm	Obtained [conc] in ppm	% Recovery
QC1A	0.3	0.223	74.3
QC1B	0.3	0.26	86.6
QC2A	0.5	0.47	94
QC2B	0.5	0.45	90
QC3A	1.0	0.93	93
QC3B	1.0	0.97	97

3.3 Repeatability

Seven analytes of one sample extracted separately using the developed method and spiked at 2ppm gave average % recoveries for fenamiphos, fenamiphos sulfoxide and fenamiphos sulfone as 91.3%, 80.7% and 82.1% respectively (Table 4)

Table 4: % Recoveries of fenamiphos, fenamiphos sulfoxide and fenamiphos sulfone from seven samples

Sample	[conc]	% recovery fenamiphos	[conc]	% recovery fenamiphos sulfoxide	[conc]	% recovery fenamiphos sulfone
R1	1.73	86.5	1.85	92.5	1.65	82.5
R2	1.80	90	1.50	75	1.55	77.5
R3	1.90	95	1.45	72.5	1.73	86.5
R4	1.94	97	1.89	94.5	1.55	77.5
R5	1.84	92	1.55	77.5	1.65	82.5
R6	1.78	89	1.48	74	1.73	86.5
R7	1.91	95.5	1.58	79	1.63	81.5

3.4 Reproducibility

The reproducibility of the method gave very good % recoveries above 88% (Table 5)

Table 5: % Recovery from three QC samples for fenamiphos, fenamiphos sulfoxide and fenamiphos sulfone spiked at 2 ppm

Sample	[conc]	% recovery fenamiphos	[conc]	% recovery fenamiphos sulfoxide	[conc]	% recovery fenamiphos sulfone
QC2	1.85	92.5	1.78	89	1.87	93.5
QC3	1.90	95	1.88	94	1.85	92.5
QC4	1.87	93.5	1.92	96	1.84	92

3.5 Specificity

The method is specific to the analytes of interest as it was able to determine and discriminate between the analyte and the co- extractives that cause possible interferences during analysis preventing problems of having elevated or suppressed detector signals during quantification.

Conclusion

A GC-NPD method for the analysis of fenamiphos and its metabolites was developed. Parameters such as reproducibility, accuracy, recovery and linearity were performed and yielded good results with average recoveries above 85%. The developed method is simple quick and produces reliable results and can be used to determine and quantify fenamiphos residues in tobacco.

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