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INTERLABORATORY COMPARISON OF UNKNOWN SAMPLES OF INSECTICIDES ON GAS CHROMATOGRAPH USING CAPILLARY COLUMNS

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ABSTRACT

The unknown samples S1, and S2 were analysed on Gas Chromatograph (GC) equipped with Electron Capture Detector (ECD) and Nitrogen Phosphorous Detector (NPD) using the capillary column. The sample S1 was found to contain α-endosulfan (14.8 ± 02.3 mg) and β-endosulfan (17.0 ± 13.2 mg). S2 was found to contain primicarb (13.8 ± 07.8 mg) and azin phosmethyl (32.6 ± 03.5 mg). The result of all four pesticides were found to be satisfactory except primicartb, hanging little bit for residue analysis, when compared with actual amount i.e. α-endosulfan (11.0 mg), β-endosulfan (16.9 mg), primicarb (24.0 mg) and azin phosmethyl (32.0 mg) furnished by the Danish Institute of Plant and soil science, Denmark.

Keywords: Insecticides, Endosulfan, Azinphosmethyl , Primicarb, Gas chromatograph, Capillary column,

INTRODUCTION

After the realization of importance and side effect of pesticides on human health, ecosystem and on environment research activities started in Pakistan in order to determine the occurrence and concentration of insecticides in various types of food (Tahir *et al*., 2004; Anwar *et al*., 2004 and 2005). Because of large variation of the data in different commodities the authority concerned in Pakistan realized the question about the reliability of the measurement. Many laboratories perform analysis with out appropriate in house validations and evaluation of the performance of the applied methods. Consequently Ecotoxicology Research Institute, started a primary inter calibration scheme for its Chemical laboratory for the improvement of analytical data with Danish Institute of Plant and soil science, Department of Weed Control and pesticide Ecology, Denmark. The laboratory was allowed to apply its own analytical method for the analysis of test samples. Performance indictors were attributed to the result of analysis demonstrating the analytical capabilities of the laboratory. Interlaboratory comparisons are reported internationally to assess Solid Phase Extraction (SPE) disk (Markell, *et al*., 1991) and recently Anwar *et al*. (2006) has compared the SPE with Liquid-Liquid Extraction (LLE) technique. There is great need for the establishment and validation of method for extraction and cleanup due to the change in instrument specially form packed gas columns to capillary and wild bore column (Luke, 1996). The objective of the present paper is to compare the out come of interlaboratory comparison studies on the analysis of unknown sample of insecticides.

MATERIALS AND METHODS

A set of test material of unknown samples in vial labeled as S1 and S2 and vials labeled A, B, C and D for four different standards of insecticides received by air mail at Chemical Analysis laboratory, Ecotoxicological Research Institute, National Agricultural Research Centre, (NARC), Islamabad form the Danish Institute of Plant and soil science, Department of Weed Control and pesticide Ecology, Denmark. Stock solutions of standard insecticides were prepared in 100 ml of ethylacetate. Serial dilution of α-endosulfan was prepared in the range of 0.19-1.1 μg/ml followed by β-endosufan (0.2-1.2), primicarb (1.7-10.3) and azinphosmethyl (0.86-5.14). Retention time for each standard insecticide was determined on GC. Then calibration curve of each standard insecticide was prepared by using a computer software Turbocrome® and then the limit of detection was calculated by computer software SuperCal-5. On the basis of retention time and the peak are the component of sample were identified and quantified respectively. The unknown samples S1 and S2 were analyzed on GC using the capillary column with the following parameters. After analysis the final result were fax to the Denmark for comparison with the actual results.

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OPERATING CONDITION OF GC-PEKIN ELMER

Sample-S1 and Standard (α and β-endosulfan)

Detector: Electron Capture Detector, Temp. 300 °C
Column: 25 methyl silicone, I. D 0.53 mm, 2.0 μm film thickness
Carrier: N_2 (30-32 ml/min)
Oven: 60 °C (0.5 min), 30 °C/min to 180 °C (0 min), 4 °C/min to 280 °C (17 min).
Injection: 1 μl split less

Sample-S2 and Standard (azinphosmethyl)

Detector: Electron Capture Detector, Temp. 300 °C
Column: 25 methyl silicone, I. D 0.53 mm, 2.0 μm film thickness
Carrier: N_2 (30-32 ml/min)
Oven: 80 °C (0 min), 45 °C/min to 280 °C (5 min).
Injection: 1 μl split less

Sample-S2 and Standard (primicarb)

Detector: Nitrogen Phosphorus Detector, Temp. 300 °C
Column: 6M methyl 10% phenyl silicone, I. D 0.32 mm, 0.5 μm film thickness
Flow rates: N_2 (2.5 ml/min)
H_2 (2.0 ml/min)
Air (100 ml/min)
Oven: 80 °C (0 min), 45 °C/min to 280 °C (5 min).
Injection: 1 μl split less

RESULTS AND DISCUSSION

The results of analysis are summarized in Table 2. The sample S1 was found to contain α-endosulfan (14.8 ± 02.3 mg)_and β-endosulfan (17.0 ± 13.2 mg). S2 was found to contain primicarb (13.8 ± 07.8 mg) and azinphosmethyl (32.6 ± 3.5 mg). The over all evaluation of the results demonstrated satisfactory performance of the laboratories from a general point of view. However, primicarb did not responded on ECD and high value of 95% confidence interval was observed due to great variation in response of NPD and found to be deviated 57.5% from the actual result i.e. 24.0 mg. However, the possibility for degradation of pesticides could not be ignored during transportation (Moller *et al*., 2000). Whereas, tailing effects (Fig. 1) was observed for azinphosmethyl on the same detector. Similarly 95% confidence interval was also calculated high for β-endosufan when analysed on ECD due to the larger amount injected resulted in moving up higher on the calibration curve and away from Limit of Detection (LOD) and would have smaller 95% limits if injected in low doses and this is only for the apparatus. Due to the varying analytical detection techniques, columns and temperature programs, the variation in analytical results between laboratories is not surprising (Senseman *et al.*, 2003). A more precise determination of the critical steps of the analysis protocols was not possible because of the large variability of the methodological details. As long as this does not change, it will not be possible to draw more conclusive results. The only way to prove this is the determination of the reproducibility standards deviation for one particular analysis method from an interlaboratory validation study (Klaffke *et al*., 2005). Such studies are needed to be planned in the laboratories of Pakistan dealing with the pesticides analysis to ovoid the chromatogram showing erratic values during analysis the inter calibration is required for methods in which detector response produce by natural compounds in the unclean assay solution and response produce by pesticides at level less than 0.1 ppm.

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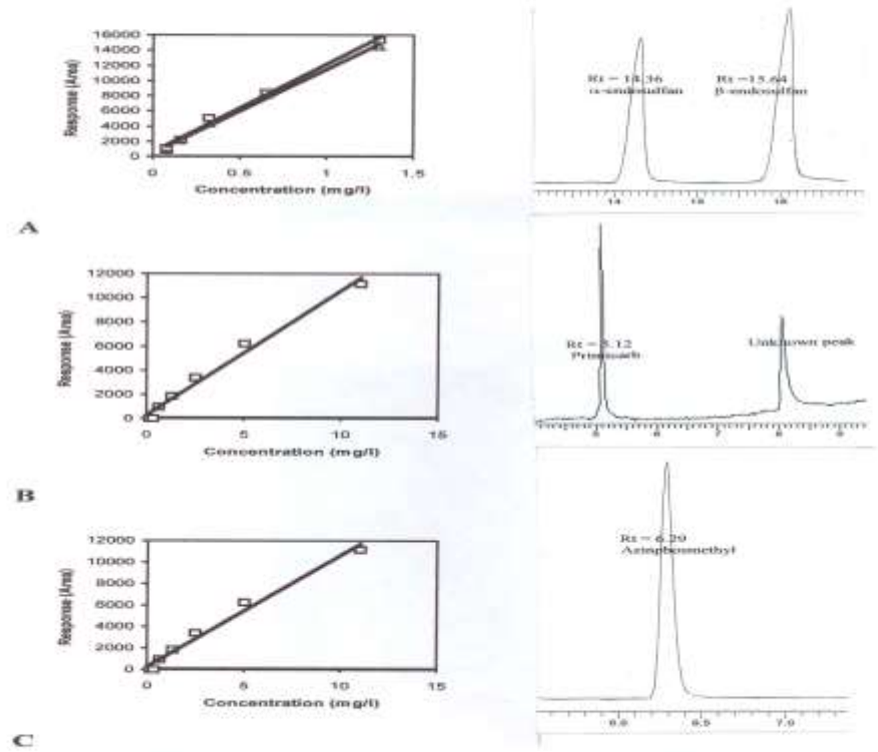

Fig. 1 Response of standards insecticides on detectors with samples chromatograms (A) α and β endosulfan on ECD (B) primicarb on NPD and (C) azinphosmethly on ECD.

Table 2. Identification and determination of actual components and amount in an unknown samples on Gas chromatograph (GC) using capillary column.

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1	α-endosulfan	0.18	Y=718+1201X, r = 0.99	14.8 ± 02.3	11.0
2	β-endosulfan	0.15	Y=462+1128X, r = 0.99	17.0 ± 13.2	16.9
3	Primicarb	0.89	Y=543+981X, r = 0.99	13.8 ± 07.8	24.0
4	Azinphosmethly	0.47	Y=114+1179X, r = 0.99	32.6 ± 03.5	32.1

[*] LOD is the Limit of Detection of Insecticides calculated by Super Cal-5 computer software.

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