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ABSTRACT

An innovative method is given for the estimation of certain organophosphorus insecticides in the serum of occupational persons. The compounds are extracted with a mixture of acetone and diethyl ether (1:1 v/v) in acidic medium and the extraction residue is analyzed by gas chromatography with nitrogen phosphorus detection method. The method for percentage recovery for the six different organophosphorus insecticides was 84.3% for phorate, 76.3% for dimethoate, 81.3% for Malathion. Blood Serum samples of nine workers had taken and with some organophosphorus insecticide, specifically Malathion was found.

KEYWORDS: Malathion, organophosphorus pesticides, gas chromatography
**INTRODUCTION**

In the past, mainly when the heavy exposure to malathion occurs and toxicity observed, the treatment detections are mainly based on the symptoms. For some insecticides, the metabolites are detected in the urine and after oral ingestion, the compounds can be identified by the analysis of the gastric lavage. However, in order to manage patients with acute, sub acute, and chronic poisoning from these insecticides, it would be of interest to know the fate of the organophosphorus cholinesterase inhibitor and its relationship to the clinical situation. To solve this problem, serum blood concentrations of the insecticides would have to be determined. Several methods have been described in literature for the determination of organophosphorus insecticide levels in blood, e.g., gas chromatography without sample extraction, gas chromatography with sample extraction using an electron capture detector, a halogen phosphorus detector, and a nitrogen detector. The use of extraction followed by reversed phase high performance liquid chromatography has also been reported. These type of techniques sometimes give inaccurate results and due to that the proper treatment of the toxic conditions cannot be done.

**MATERIALS AND METHODS**

Chemicals
Highly pure organophosphorus insecticides were obtained from sigma company (US). HPLC Solvent grade ν-hexane, An. Sodium sulphate, Acetone, diethyl ether and 5N HCl were obtained from Merck chemicals.

Standard Solutions
Working solutions were prepared with the use of ν-hexane of the standard stock solutions of these organophosphate compounds in the range of 0.15 to 5.0 μg/mL. Thirty millilitres of standard stock solutions of the organophosphorus insecticides like phorate, dimethoate, Malathion, chlorpyriphos and Diazinon were prepared at a concentration of 0.5 mg/mL in HPLC Grade acetone. These solutions were stored at −15 °C, these were stable for at least three months. After the calibration of the instruments were done, the standard solutions were injected and the retention time was noted. After this the samples were injected into GC and retention time was noted.

Gas Chromatography
Analyses were done by Perkin –Elmer Gas Chromatography Instrument , fitted with a Capillary column having 2 mm ID and 120 cm long, PyrexR glass, packed with 1.5% OV-17/1.95 % QF-1 on Gas Chrom Q, 85/120 mesh or equivalent. The carrier gas here used was high pure nitrogen at a flow rate of 50 mL/min. The gases used were hydrogen and air at flow rates of 7 mL/min and 90 mL/min. The initial oven temperature was set at 160°C for 2 mins, after that increased at the rate of 7°C/min to 280°C. The injection port and detector temperatures were 270°C and 290°C respectively. Each and every compound, were eluted within 20 min. A small amount was taken in a 15 mL glass stoppered t.t. to which was added 6 mL of a mixture of acetone and diethyl ether. After that the mixture was agitated. This procedure is followed three times more and then acidified by HCl. Now residues were dissolved in 0.5 mL of ν-hexane and after proper homogenization it was injected to GC.
RESULTS AND DISCUSSION

Table : Retention times (RT) and Relative Retention Time (RRT) of the Samples for analysis of Pesticides

<table>
<thead>
<tr>
<th>Pesticides</th>
<th>RT (Min)</th>
<th>RRT</th>
</tr>
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<tbody>
<tr>
<td>Phorate</td>
<td>5.52 ± 0.170</td>
<td>0.490</td>
</tr>
<tr>
<td>Malathion</td>
<td>9.16 ± 0.010</td>
<td>0.810</td>
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</table>

Here after obtaining the results, it was found that when we compare it to other techniques, Gas chromatography gives more accurate results. Not only has it given results at lower concentrations but within a very short span of time. In order to these it was found that a higher number of samples can be analyzed with a very precise analysis with this method.

The Analysis results of the extracted samples from blood are given in Table. Here we have used hydrochloric acid for acidification, which gives better extraction and which lead to the good analysis and results. Here the quantitative analysis can also be done with the use of Calibration curves.

CONCLUSION

The Gas chromatography technique proved to be a very good technique using this method for the analysis of the organophosphorus pesticides, as it gives very good results at very low concentration with high precision. Here we have used acidification, which gives better extraction and which lead to the good analysis and results. The quantitative analysis can also be done with the use of Calibration curves.

REFERENCES