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Pesticide Residue Analysis of Lettuce by Gas Chromatography: Estimation of Pesticides using Analyte Protectants

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Abstract—*In pesticide residue analysis the detection of pesticide residues was performed by Gas Chromatography and it was confirmed by the use of standards of respective pesticide. Especially in urban and semi urban areas the demand of Lettuce is high and it is suitable for farmers to grow it in big scale. It is a winter crop. For head growth, the night air temperature should be 3-12 °C and day temperature should be 17-28 °C. [1]*

The feature of pesticides and matrix was kept into consideration while using different chemicals especially solvents for the extraction of pesticides from the matrix. The addition of analyte protectants mask the active sites in GC column and hence protect the pesticides from degradative interaction which improves their recovery estimation. The main factors which were considered in the search for a good analyte protecting agent (or combination of protectants) included: (i) hydrogen bonding ability; (ii) volatility; and (iii) practical aspects. [2]

A mixture of 3-ethoxy-1, 2- propanediol (a diol compound), d-sorbitol (a sugar alcohol) and l-gulonic acid γ -lactone (a sugar derivative) was proposed by Mastovska et al. [3]; this mixture was chosen in this study because it covers the entire volatility range of GC-amenable pesticides [4]. The recovery of pesticides was recorded 87-103% with standard deviation ranging from 0.18 to 1.36.

Keywords*: Gas Chromatography, Analyte protectants.*

1. INTRODUCTION

Multi residue analysis (MRA) is widely used to detect the level of pesticides in the food materials. Various pesticides are applied on the fruits and vegetables in order to get higher productivity. In the present work a methodology was used for the extraction of mixture of pesticides. The previously assayed analyte protectants mixture of 3-ethoxy-1, 2- propanediol (a diol compound), d-sorbitol (a sugar alcohol) and l-gulonic acid γ -lactone (a sugar derivative) was proposed by Mastovska et al. *[3]*, were used to minimize the effect of active sites generated in the GC column. A wide range of analytes with different matrix effect were detected in a very short and safe protocol. The analytes Phorate, Chlorothalonil, Malathion, Chlorpyrifos, Captan, Methyl Parathion, Endosulfan, Quinalphos, Ethion were detected in lettuce with this protocol. The extraction was done with the Acetonitrile, Disodium hydrogen citrate sesquehydrate, Trisodium citrate dihydrate, $MgSO_4$ and NaCl. Clean-up was performed with c-florisil, acetonitrile and magnesium sulphate.

The extract was dissolved in acetonitrile mixed with analyte protectants and injected into Gas Chromatography with the help of Hamilton`s micro injection syringe for the analysis of the recovery of each pesticide.

2. EXPERIMENTAL

2.1-Chemicals and reagents

Phorate; 95.6% (Dr. Ehrenstorfer), Chlorothalonil; 98.5% solid powder (Dr. Ehrenstorfer), Malathion; 97.6% (Dr. Ehrenstorfer), Chlorpyrifos, Captan; 98.5% solid powder (Dr. Ehrenstorfer), Methyl Parathion; 95.6% White solid (Dr. Ehrenstorfer), Endosulfan cream solid 96.6% (Dr. Ehrenstorfer), Quinalphos; 95.8% (Dr. Ehrenstorfer), Ethion 97.7% (Dr. Ehrenstorfer), Acetonitrile (GR, Merck), Acetone (GR, Merck), Toulene (GR, Merck), Sodium sulphate (Na_2SO_4), Magnesium Sulphate ($MgSO_4$), Sodium chloride (NaCl), cflorisil (60-100 mesh, CDH), disodium hydrogen citrate sesquehydrate, trisodium citrate dihydrate, dry ice, glass wool and distilled water.

2.2-Extraction and clean-up process

The pesticide treated matrix (Lettuce) were placed in the bowl of chopper and the chopping was carried out for 6-7 minutes. The matrix was stirred constantly in between the subsequent intervals of chopping. Extraction of sample was done by acetonitrile, disodium hydrogen citrate sesquehydrate, trisodium citrate dihydrate. Then $MgSO_4$ and NaCl were added in each analytical portion. Each sample was vortexed for one minute and centrifuged for 5 minute. The extracted supernatant was dried over rotary evaporator. Clean-up was done by activated c-florisil which was packed in chromatographic column using solvent acetonitrile and a layer of 2 cm magnesium sulphate was added over it. Then the rate of column flow was adjusted at 1 ml per minute. The dried up extract was dissolved in acetonitrile and eluted through the packed column. Eluate was dried over rotary evaporator. The dried up extract was redissolved with 5 ml of acetonitrile and was collected in a glass vial. Mixture of analyte protectants was added in each sample. Further 2 µl of this sample was injected into Gas Chromatography with the help of Hamilton`s micro injection syringe for the analysis of the recovery of each pesticide.

2.3-Pesticide residue determination

Gas chromatography is a chemical analysis instrument for the quantative analysis of samples. In the pesticide residue analysis of the fruits and vegetables this basic technique was applied for the identification and quantification of pesticides.

In this present study GC- Shimadzu 17AAF was used to analyze multi pesticide residues from fruits and vegetable samples. While starting the analysis of pesticides by this instrument we standardized and calibrated it for each pesticide. Then the mixture of pesticides spiked to the samples were separated and programmed on ECD detector. The electron capture detector (ECD) is used in detecting electron-absorbing components in the output stream of a gas chromatograph. The ECD uses a radioactive Beta particle (electrons) emitter- a typical source contains a metal foil holding 10 millicuries of Nickel-63. The electrons formed are attracted to a positively charged anode, generating a steady current.

3. RESULT AND DISCUSSION

The recoveries of each pesticide from the matrix were estimated for each replicate of sub sampled analytical portions. The recovery was calculated from the regression equations obtained from solvent (S), solvent with analyte protectants (SAP), matrix match calibration (MMC) and matrix match calibration with analyte protectants (MMCAP) for all the samples. The recovery of analytes with different calibration curves have been shown in figure-1 and the table-1.

The recovery of pesticides varied in all the four above mentioned calibration batches. Phorate was recovered around 98% with analyte protectants calibration and 106-115% with calibration without analyte protectants. The above results show that matrix effects have been controlled by analyte protectants.

The recovery of chlorothalonil with analyte protectants calibration was 111-103% and 140-113% with calibration without analyte protectants. The recovery of methyl parathion was 91% with analyte protectants calibration and 169-104% with calibration without analyte protectants. Matrix diminishing effect reduced the recovery of malathion to 57% but with analyte protectant the recovery raised to 91%. While the recovery of chlorpyrifos was 126% with solvent calibration without analyte protectants and 105% with solvent calibration with analyte protectants. By the use of analyte protectants the rececery with MMC was 99%. Here analyte protectants nullified the matrix effect.

The matrix resulted in enhancement effect on captan. Its recovery with MMCAP was 90% but with MMC it was much higher because of matrix enhancement effect.

The lettuce matrix resulted both in diminishing and enhancement effect on the recovery of different pesticides. Due to the diminishing effect of matrix on the quinalphos, its recovery was 56% with MMC without AP. Its improved recovery was recorded with MMCAP.

The recovery of endosulfan alpha with MMCAP was 102%. Its recovery enhanced more or less up to 150% showing the matrix enhancement effect. The reproducibility of endosulfan recovery with MMCAP was 0.79 which was within the acceptable limit. 93% of Endosulfan beta was recovered with MMCAP and the reproducibility was 0.66.

Ethion recovery was 87% with MMCAP curve and the reproducibility was 0.44%, well below the acceptable limit.

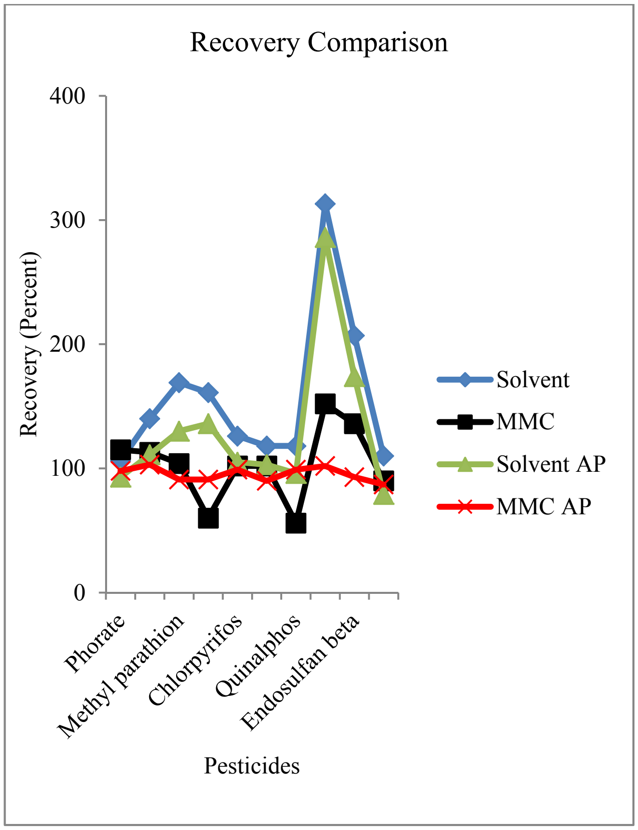

Fig. 1: Comparison of average recoveries of pesticides from Lettuce samples.

4. MATRIX MATCH CALIBRATION (AP)

Table 1: Inter-batch study of recoveries of pesticides from Lettuce.

S. No.	Pesticides	Average Recovery (%)	Ks	SD	CV
1	**Phorate**	98	0.001	0.78	0.008
2	**Chlorothalonil**	103	0.004	1.36	0.013
3	**Methyl parathion**	91	0.000	0.18	0.002
4	**Malathion**	91	0.000	0.43	0.005
5	**Chlorpyrifos**	99	0.002	1.31	0.013
6	**Captan**	90	0.000	1.01	0.011
7	**Quinalphos**	99	0.005	0.95	0.010
8	**Endosulfan alpha**	102	0.000	0.79	0.008
9	**Endosulfan beta**	93	0.000	0.66	0.007
10	**Ethion**	87	0.000	0.44	0.005

5. CONCLUSION-

Four types of calibration curves were constructed to calculate the recovery of pesticides. These were standard in solvent (Solvent), standard in solvent with analyte protectants (Solvent AP), standard in matrix (MMC) and standard in matrix with analyte protectants (MMC AP) to nullify the effect of matrix. The residues were analyzed and confirmed by GC. Calibration curve of matrix with analyte protectants (MMCAP) estimated the pesticide recoveries around 87-103% lettuce. Lettuce sample represents green leafy vegetables with high water and chlorophyll contents that interfered during the analysis of residues of pesticides and caused major matrix effects both diminishing and enhancement effects.

Analyte protectants are compounds that strongly interact with active sites in GC system and decreases degradation and/or adsorption of co-injected analytes. Hence the precision of recovery enhanced.

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