CHAPTER FIVE

A New Reagent System For The Determination of Captan Pesticide
A New Reagent System For The Determination Of Captan (N-trichloro methyl thio tetra hydro phthalimide) Pesticide.

SUMMARY

A Spectro photometric method for the determination of Captan, a widely used pesticide at ppm level is described. The method is a modified form of Fujimura reaction i.e. discharging the colour obtained by adding pyridine and alkali, with acetic acid followed by the addition of sulphanilic-formic acid reagent resulting in the formation of an orange-yellow coloured polymethine dye having absorption maxima at 500nm. The sensitivity and stability after this modification is much more than the conventional Fujimura method. Beer's law is obeyed in the range of 10-60 μg (1-6 ppm) of Captan per 10ml of solution. The method is found to be free from the interference of various other pesticides and inorganic ions, under the condition employed. The method has been successfully applied for the determination of Captan in fruits and grains.

INTRODUCTION

Captan (N-trichloromethylthio tetra hydro phthalimide) is a general fungicide for treatment of soil and seed borne diseases including apple scab, grape mildews, corn seed infections and many fruit, vegetable and ornamental plant diseases(1). Captan is non-volatile and stable but has a pungent odour. It is persistent and is compatible with most pesticides. Captan is used as a seed dresser against damping off and other soil and root diseases.

Captan controls apple and pear scab, grey mould on strawberries and lettuces, as well as blight on potatoes and tomatoes. Captan decreases fruit damage and increases yield of apples and pears (2). It is used as a spray on farms, orchards and private gardens to control black spot on rose and stem rot of tomatoes(3).

Captan is marketed as a 50 percent wettable powder and is also available as a 75 percent seed dressing. It is used at the rate of 1.2 to 2.4 kilograms of active ingredient per hectare. As a seed disinfectant it is used both as dust and slurry method (4).

Captan is non-phytotoxic. The acute oral LD₅₀ (for rats) is 9,000 mg/kg. It can cause skin irritation (5). The threshold limit value of Captan is 5mg/m³ (6). Captan is of low mammalian toxicity and presents a few hazards to man so long as the powder is kept away from the skin. In common with many other pesticides it is highly toxic to fish (2). Captan has been identified as a potential carcinogen by IARC and NIOSH. Russian studies showed evidence of genetic risks (3). Owens and Black (7, 8) showed that Captan inhibits many oxidative enzymes and considered that it's basic toxic action was an interaction of the intact captan molecule with free Sulphydryl groups.

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\begin{array}{c}
\text{Structure of Captan (N-Trichloro methyl thio} \\
\text{tetra hydro phthalimide)}
\end{array}
\]
Various methods have been reported for the determination of Captan. Various techniques employed for the determination are column chromatography (9, 10), thin layer chromatography (11-13), high pressure liquid chromatography (14-18), capillary gas chromatography (19), titrimetry (20), gas-liquid chromatography (21, 22), gas chromatography (23-25), magnetic particle based immunoassay (26), enzyme immunoassay (27) etc.

Spectrophotometric methods reported for the determination of Captan use reagents like resorcinol (28), pyridine and tetra ethyl ammonium hydroxide (29).

In the present communication, a modified Fujiwara reaction reported for polyhalogenated compounds (30, 31), has been developed for determination of Captan. The method is based on the reaction of Captan with pyridine and alkali which on mild heating forms an orange coloured chromophore. The orange colour is discharged by addition of acetic acid followed by the addition of sulphanilic-formic acid reagent to obtain a yellow-orange coloured dye with a $\lambda_{max}$ at 500 nm. This modification increases the stability and sensitivity of the Fujiwara reaction (32, 33). The method (32) uses benzidine-formic acid reagent which is highly carcinogenic and so in the present method it has been replaced by sulphanilic-formic acid reagent which is non-toxic and cheap.

**EXPERIMENTAL**

**Apparatus -**

A Carl Zeiss spectrophotometer with 1cm matched silica cell was used for all spectral measurements.

**Reagents -**

**Captan :**

Northern Minerals Limited.

A stock solution containing 1mg/ml Captan was prepared in 50% acetone and 50% methanol.
**Sulphanilic acid : formic acid reagent** -

A 1% solution of sulphanilic acid in 50% formic acid was used.

**Sodium hydroxide :**

A 5M aqueous solution was used.

**Hydrochloric acid :**

6M solution.

**Acetic acid (glacial).**

**Pyridine :**

All chemicals used were of analytical reagents grade or the best available quality.

**PROCEDURE:**

Aliquots containing 10-60μg (1-6 ppm) of Captan were transferred to a 25ml volumetric tube. To it 1 ml of pyridine and 1 ml of 5M Sodium hydroxide solution were added and thoroughly shaken. The contents were kept in water at 70°C for 2 min. and shaken time to time. The orange coloured solution so obtained was cooled in ice-cold water and then decolourised with 2 ml glacial acetic acid. To this solution 2 ml of sulphanilic acid-formic acid reagent and 1 ml of 6 M hydrochloric acid were added and the contents were allowed to stand for 5 minutes. The volume was made upto 10 ml with water and orange-yellow coloured polymethine dye having an absorption maxima at 500 nm was measured against a colourless reagent blank.

**RESULTS AND DISCUSSION:**

**Spectral Characteristics -**

The yellow-orange coloured dye formed showed maximum absorbance at 500 nm. The reagent blank showed no absorbance in this range (Fig. 5.1).

**Reagent Concentration:**

One ml of pyridine and 1ml of 5 M Sodium hydroxide solution were required.
for maximum colour intensity. Excess of sodium hydroxide made the solution slightly cloudy due to undissolved sodium hydroxide. 2 ml of glacial acetic acid was sufficient for decolorisation of the orange colour. An excess amount however does not effect the reaction. A minimum of 2ml of sulphanilic acid in 50% formic acid was required for maximum colour intensity during regeneration of colour. An excess of sulphanilic-formic acid reagent decreased the colour intensity of dye (Fig. 5.2, 5.3, 5.4).

**Time and Temperature**

It has been observed that heating the reaction mixture for about 2 minutes at ~ 70°C gave optimum absorbance values. Lower absorbance values were obtained on prolonged heating. The yellow-orange dye was found to be stable for ~ 15 minutes and thereafter showed gradual decrease in intensity with time (Fig.5.5).

Effect of pH - It was found that the maximum absorbance and stability of the dye formed was in the pH range 4-6. At higher or lower pH the absorbance values decreased.

**Beer’s law, Sandell’s sensitivity, Molar absorptivity**

The colour system was found to obey Beer’s law in the range of 1 to 6 ppm of captan. Molar absorptivity and Sandell’s sensitivity were calculated and found to be $4.2 \times 10^3$ litre mol$^{-1}$ cm$^{-1}$ ($\pm$ 100) and 0.0071 $\mu$g/cm$^2$ respectively (Fig.5.6).

**Reproducibility:**

The reproducibility of the colour reaction was checked by replicate analyses of 10$\mu$g/10ml of captan sample analysed over a period of seven days. The standard deviation and relative standard deviation were found to be $\pm$ 0.003 and 2.08% respectively (Table-1).

**Effect of foreign species:**

The effect of various foreign species was studied by adding known amounts of foreign species to 10$\mu$g of captan prior to analysis. It was found that the method is free from the interference of most of the inorganic ions and other pesticides. The results are tabulated in Table-2.
APPLICATIONS OF THE METHOD:

The method has been used for the determination of captan in fruits and grains. Since the samples analysed were found to be free from captan, fruits like grapes and wheat and paddy grains were weighed (50gms) and then spiked with known amounts of captan. After 2-3 hours captan was extracted in a solvent containing 50% acetone and 50% methanol and determined by the proposed method. The recoveries are shown in Table-3.

CONCLUSION:

The method proposed for captain is simple, sensitive, free from interference and selective. The dye formed is stable for ~ 15 minutes.
FIG. 5-1 ABSORPTION SPECTRA OF THE DYE.
A. CONCENTRATION OF CAPTAN = 40μg/10 ml.
B. CONCENTRATION OF CAPTAN = 30μg/10 ml.
C. REAGENT BLANK.
Fig. 5.2: Effect of amount of pyridine on colour development.
Concentration of Captan = 30 µg/10 ml.

Fig. 5.3: Effect of amount of NaOH on colour development.
Concentration of Captan = 30 µg/10 ml.
FIG. 5. EFFECT OF AMOUNT OF SULPHANILIC-FORMIC ACID REAGENT ON FINAL ABSORBANCE
CONCENTRATION OF CAPTAN = 30 μg/10 ml.

FIG. 6. EFFECT OF HEATING TIME ON FINAL ABSORBANCE
CONCENTRATION OF CAPTAN = 30 μg/10 ml.
FIG. 56 CALIBRATION FOR THE DETERMINATION OF CAPTAN.
Table -1.
Reproducibility of the Method.
Concentration of Capton = 10 μg / 10ml.

<table>
<thead>
<tr>
<th>No. of days</th>
<th>Absorbance max* 500 nm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>.145</td>
</tr>
<tr>
<td>2</td>
<td>.140</td>
</tr>
<tr>
<td>3</td>
<td>.145</td>
</tr>
<tr>
<td>4</td>
<td>.145</td>
</tr>
<tr>
<td>5</td>
<td>.140</td>
</tr>
<tr>
<td>6</td>
<td>.145</td>
</tr>
<tr>
<td>7</td>
<td>.145</td>
</tr>
</tbody>
</table>

Mean = .143
Standard deviation = (±) 0.003
Relative standard deviation = 2.08%

* Mean of three repetitive analyses.

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Table - 2.
Effect of Foreign Species.
Concentration of capton = 10 μg / 10ml (1ppm).

<table>
<thead>
<tr>
<th>Foreign Species</th>
<th>Tolerance limit * (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D.D.T.</td>
<td>1000</td>
</tr>
<tr>
<td>Carbaryl, Propoxur, 2,4-D, 2,4,5-T.</td>
<td>500</td>
</tr>
<tr>
<td>Malathion, Parathion, Paraquat</td>
<td>150</td>
</tr>
<tr>
<td>B.H.C.</td>
<td>100</td>
</tr>
<tr>
<td>Ca²⁺, Cd²⁺</td>
<td>1000</td>
</tr>
<tr>
<td>Cu²⁺, Ni²⁺</td>
<td>800</td>
</tr>
<tr>
<td>NO₃⁻</td>
<td>500</td>
</tr>
<tr>
<td>Sn²⁺, Hg²⁺</td>
<td>400</td>
</tr>
<tr>
<td>PO₄³⁻</td>
<td>150</td>
</tr>
</tbody>
</table>

* Causing an error of (±) 2% in absorbance value.


Table - 3.

Recovery of Capton from Grapes, Paddy and Wheat:

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Sample**</th>
<th>Captan added (µg)</th>
<th>Captan* found (µg)</th>
<th>Percentage Recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Grapes</td>
<td>10</td>
<td>9.2</td>
<td>92.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15</td>
<td>13.5</td>
<td>90.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>18.5</td>
<td>94.0</td>
</tr>
<tr>
<td>2.</td>
<td>Paddy</td>
<td>5</td>
<td>4.6</td>
<td>92.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>9.5</td>
<td>95.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15</td>
<td>19.2</td>
<td>96.0</td>
</tr>
<tr>
<td>3.</td>
<td>Wheat</td>
<td>10</td>
<td>9.2</td>
<td>92.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15</td>
<td>14.2</td>
<td>96.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>18.6</td>
<td>93.0</td>
</tr>
</tbody>
</table>

* Mean of three repetitive analyses.

** Volume of extract - 10ml.
REFERENCES:


31- F. Feigl, "Spot Tests in Organic Analysis", "Elsevier Scientific Publishing Company, Amsterdam,