Colorimetric Method for the Determination of Captafol (Difolatan) in Commercial Formulations and Residues on Grains and Apples

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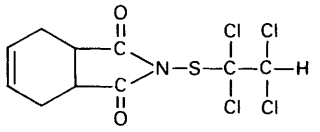
A simple and rapid colorimetric method for the microdetermination of captafol (difolatan), based on its reaction with a dithiocarbamate, has been developed. The bright yellow colour which develops instantaneously on mixing the fungicide with the reagent is stable for at least 12 h. The method has been successfully adapted to the determination of captafol in its formulated products and residues on grains and apples.

Keywords: *Captafol (difolatan) determination; dithiocarbamate; colorimetry; spectrophotometric titration; formulation and residue analysis*

Captafol, *N*-(1,1,2,2-tetrachloroethylthio)-1,2,3,6-tetrahydrophthalimide, is a protective, non-systemic fungicide used for the control of many fungal diseases of fruits, vegetables, ornamental plants and field and plantation crops.



Although safe on most crops, phytotoxicity has been reported for grapes, apples and roses under certain weather conditions.[1] Some people allergic to the fungicide suffer skin rash and irritation in the form of erythema, dermatitis of the eyelids and local oedema. Hence, convenient and reliable methods for the determination of captafol both in formulations and residues are desirable. The observations that (*i*) captafol reacts with a dithiocarbamate (R_2NCSS^-) in acetonitrile, instantaneously, to form a bright yellow solution showing maximum absorbance at 420 nm, and (*ii*) the dithiocarbamate required for the purpose can be rapidly and quantitatively generated *in situ* in acetonitrile by simply adding a drop of carbon disulphide to an amine, prompted the use of the captafol–dithiocarbamate reaction

$$2R_2NH + CS_2 \rightarrow R_2NCSS^-(NH_2R_2)^+$$
(I)

as the basis of a colorimetric method for the microdetermination of the agrochemical and its subsequent adaptation to the determination of captafol in formulations and residues. That carbon disulphide smoothly and quantitatively transforms an amine into an alkylammonium alkyl dithiocarbamate (**I**) has been established in previous studies.[2–4]

The proposed method consists in adding a drop of carbon disulphide to the fungicide in acetonitrile, followed by the addition of dibutylamine. The yellow colour which develops instantaneously is measured at 420 nm. The method has been successfully adapted to the determination of difolatan in its formulated products and residues on grains, apples and apple leaves. In the residue analysis, the residue on grains is extracted with acetonitrile and the extract mixed with carbon disulphide and an amine; the yellow colour is measured at 420 nm. For the apple fruits and leaves, the residue is extracted with acetonitrile and then chromatographed (for clean-up) on a silica-gel column. After concentration of the eluate by evaporation, the residue is diluted with acetonitrile, mixed with carbon disulphide and dibutylamine and the resulting colour measured at 420 nm.

Experimental

Reagents

Acetonitrile (*Merck*). This was distilled twice from phosphorus pentoxide (5 g l^{-1}).

Dimethylformamide (*BDH*). Dimethylformamide was purified by storing over analytical-reagent grade anhydrous sodium carbonate for 2 d. The solvent was decanted off and distilled, and the fraction distilling at 148.5–149.5 °C was collected in coloured bottles.

Dibutylamine (*Fluka*). This was distilled before use.

Eluting solvent. A mixture of equal volumes of hexane and diethyl ether.

Hexane. Light petroleum (boiling range 60–80 °C) and diethyl ether (Glaxo) were purified by previously reported procedures.[5]

Silica gel (60–120 *mesh, Sisco Research*). This was heated at 500 °C for 4 h, cooled and stored at 120 °C.

Captafol (*difolatan*). A standard of high purity was supplied by the US Environmental Protection Agency.

All other chemicals used in the investigation were of guaranteed-reagent grade.

Apparatus

A Bausch and Lomb spectrophotometer (Spectronic-20) with 1 cm matched glass cells was used for all absorption measurements. Column chromatography was performed with a glass column (30 × 1.3 cm i.d.).

Direct Colorimetric Procedure

Preparation of calibration graph for pure difolatan

Difolatan standard solution was prepared by dissolving 5 mg of the pure compound in dimethylformamide and diluting it to 5 ml with the same solvent. This solution contains 1 mg ml^{-1} of difolatan. Each aliquot of this solution was diluted to 10 ml prior to absorbance measurements.

Aliquots (0.01–1.0 ml) of standard solutions of pure difolatan in dimethylformamide were placed in 10 ml calibrated flasks and diluted to 6 ml with acetonitrile. Each solution was mixed with one drop (≈50 μl) of carbon disulphide and dibutylamine (1 ml, ≈0.004 mol dm^{-3} in acetonitrile) and finally the volume was made up to the mark with acetonitrile. The absorbance of the bright yellow solution obtained was measured at 420 nm against a reagent blank (the spectrum is illustrated in Fig. 1). The calibration graph was constructed by plotting the absorbance values against the concentration of difolatan used. Beer's law is obeyed in the range 1–60 μg ml^{-1} of difolatan.

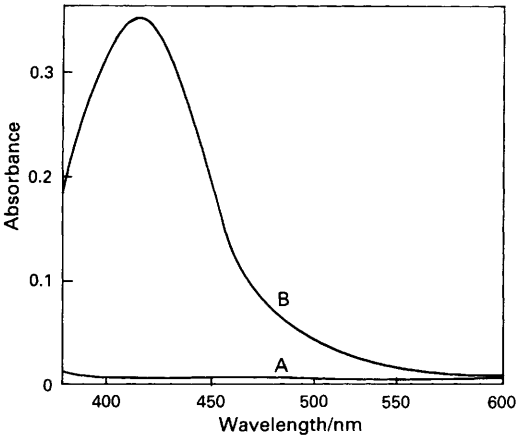


Fig. 1 Absorption spectrum of B, difolatan measured against A, a reagent blank

Table 1 Assay of a commercial fungicide formulation containing 80% of the active ingredient by a direct colorimetric procedure

Active ingredient taken/ µg	Active ingredient found* (%)	
	Proposed method	Comparison method†
2.0	79.2 ± 0.4	78.8 ± 0.5
4.0	79.2 ± 0.4	79.4 ± 0.5
10.0	78.9 ± 0.4	78.8 ± 0.6
20.0	79.4 ± 0.5	79.4 ± 0.6
30.0	78.6 ± 0.5	78.6 ± 0.8
50.0	78.5 ± 0.7	78.6 ± 0.6

* Values are the mean of five determinations ± SD.
† See reference 6.

Formulation analysis

A single large sample of the formulation containing 80% of the active ingredient was weighed, shaken manually at ≈24 °C with dimethylformamide and filtered through Whatman No. 40 filter-paper. The residue was washed 2–3 times with dimethylformamide. The filtrate and washings were diluted to a known volume with the same solvent. Suitable aliquots of this solution were placed in 10 ml calibrated flasks and processed as described above. The results of the analyses are given in Table 1. The analysis of the formulation by an independent method[6] gave comparable results (Table 1).

Captafol standard solution

This was prepared by dissolving 25 mg of captafol in 50 ml of dimethylformamide; 10 ml of this solution were diluted to 100 ml with acetonitrile. This solution then contains 50 µg ml^{-1} of captafol.

Recovery experiments

Aliquots of the standard solution of captafol were added to 5 g portions of grain (maize and rice). The samples were mixed thoroughly and extracted 4–5 times, each time using 3 ml of acetonitrile in 25 ml calibrated flasks. The combined extracts were then mixed with one drop (≈50 µl) of carbon disulphide and 3 ml of dibutylamine (≈0.004 mol dm^{-3} in acetonitrile) and the volume finally made up to 25 ml with acetonitrile. The colour which developed instantaneously was measured at 420 nm against a reagent blank. The results are given in Table 2.

For apples and apple leaves, samples were comminuted with a scalpel and 20 g were weighed into glass containers and sprayed with various amounts of standard difolatan solution (in acetonitrile). The samples were mixed thoroughly and then

Table 2 Recovery of difolatan from fortified samples of rice and maize

Active ingredient added/ µg	Recovery* (%)	
	Rice	Maize
5.0	98.6 ± 0.8	98.8 ± 0.7
10.0	96.1 ± 0.7	98.2 ± 0.6
20.0	98.3 ± 0.5	97.7 ± 0.6
40.0	94.3 ± 0.7	96.4 ± 0.5
60.0	94.1 ± 0.6	95.6 ± 0.5

* Values are the mean of three determinations ± SD.

Table 3 Recovery of difolatan from fortified samples of apples and apple leaves

Active ingredient added/ µg	Recovery* (%)	
	Apples	Apple leaves
4.0	97.8 ± 0.7	93.7 ± 0.6
8.0	94.4 ± 0.6	86.8 ± 0.6
16.0	96.5 ± 0.6	98.2 ± 0.7
32.0	98.8 ± 0.7	94.1 ± 0.5
64.0	94.7 ± 0.4	93.4 ± 0.7

* Values are the mean of five determinations ± SD.

Table 4 Results of residue analysis of treated grain samples

Strength of the spray used/ $g l^{-1}$	Residue found (ppm)	
	Rice	Maize
8.0	27.55	34.20
4.0	15.30	26.60
2.0	7.25	18.2
1.0	5.25	17.7

blended mechanically in the presence of acetonitrile in the same container and filtered through a Büchner funnel fitted with a glass sinter. The residue of each sample was washed 4–5 times with a sufficient amount of acetonitrile and the combined extracts were cleaned up on a silica-gel column using hexane–diethyl ether (1 + 1) as eluting solvent. Anhydrous sodium sulphate was placed at the top of the column to prevent the ingress of atmospheric moisture. The eluate was concentrated by evaporation. To the residue were added 15–20 ml of acetonitrile, one drop (≈50 µl) of carbon disulphide and 3 ml of dibutylamine (≈0.004 mol dm^{-3} in acetonitrile) and finally the volume was made up to 25 ml with acetonitrile. The analysis was concluded by measuring the absorbance of the yellow colour obtained at 420 nm against a reagent blank. The results of the analyses are given in Table 3.

Residue analysis

Grains (rice and maize) were sprayed with fungicide formulation (aqueous dispersion) at a concentration of 1–8 g l^{-1} at a rate of 100 ml kg^{-1} of grain. Sprayed samples were dried in the sun and from these lots, samples of 15–20 g were taken for residue analysis and processed as described under Recovery experiments. The results are given in Table 4.

For apples and apple leaves, a 0.15% spray solution was used. The apple plants bearing fruits and leaves were sprayed with the recommended dose of an aqueous dispersion of difolatan. After an interval of 7 d, the leaves and fruits were removed for residue analysis. The residue analysis was carried out as described under Recovery experiments. The results are given in Table 5.

Spectrophotometric Titrimetric Procedure

Determination of pure difolatan

Aliquots of solutions of pure difolatan in dimethylformamide were mixed with one drop of carbon disulphide and diluted to

5 ml with acetonitrile. The resulting solutions were titrated spectrophotometrically at 420 nm at room temperature (about 24 °C) with standard dibutylamine solution in acetonitrile. Dilution correction was applied and titration curves were plotted. A typical titration curve is depicted in Fig. 2. The end-point was obtained by extrapolation of the two straight lines. The results are given in Table 6.

Formulation analysis

Suitable aliquots of an extract of fungicide formulation in dimethylformamide were mixed with one drop of carbon disulphide and the volume was made up to 5 ml with acetonitrile and titrated at room temperature against standard dibutylamine (in acetonitrile) at 420 nm. The results are given in Table 7.

Results and Discussion

The proposed colorimetric method for the determination of difolatan involving a dithiocarbamate (amine–carbon disulphide reagent) is sensitive and can be employed for the determination of as little as 1 μg ml^{-1} of difolatan. The colour development is instantaneous and the colour is stable for 12 h. The method was applied to a commercial formulation containing 80% of the active ingredient. The recoveries (Table 1) were in the range 98.1–99.2% with relative standard deviations (RSDs) in the range 0.36–0.72%. Recoveries of difolatan from fortified grain samples (maize and rice) and apples and apple leaves were good, ranging from 93.4 to 98.8% with RSDs in the range 0.42–0.77% (Tables 2 and 3). The results of residue analyses of treated samples are given in Tables 4 and 5. The results presented in Table 6 indicate that difolatan can be determined in the range 3.5–45 μg by spectrophotometric titration with a maximum RSD of 0.68%. When applied to the determination of the difolatan content in a commercial formulation, the recoveries were in the range 98.2–99.4% with maximum RSDs in the range 0.30–0.61% (Table 7). The spectrophotometric titrimetric method has an advantage over the direct colorimetric method in that no calibration graph is required and this makes the method rapid.

The proposed methods are based on the reductive cleavage of the > N–S– linkage of difolatan with a dithiocarbamate (generated *in situ* in acetonitrile). The results obtained show that each molecule of difolatan reacts with 4 mol of the reagent. The most plausible reaction mechanism is as follows:

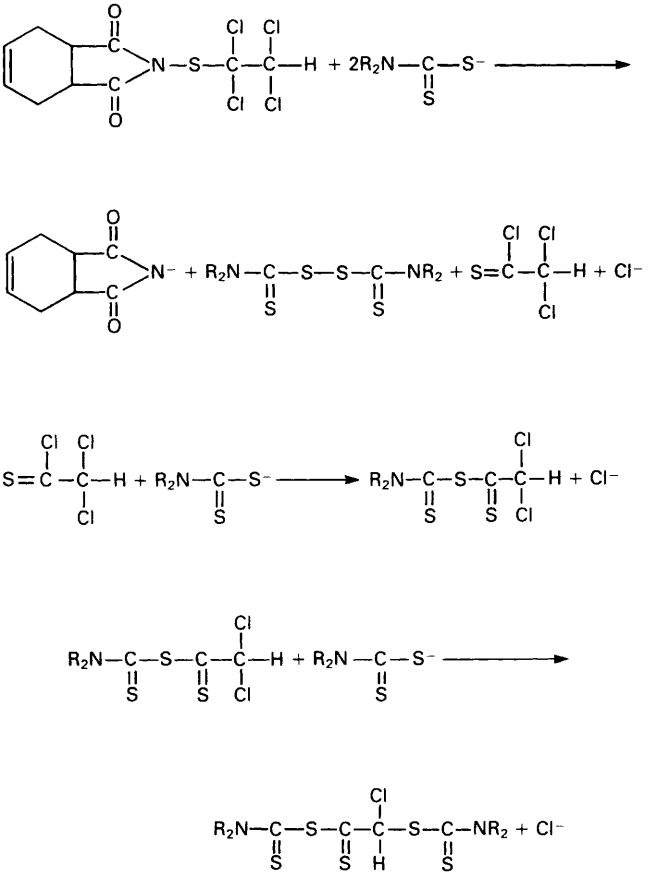


Table 5 Results of residue analysis of treated apple plants bearing fruit and leaves, sprayed with a 0.15% aqueous dispersion of difolatan (80 wettable powder)

	Residue found* (ppm)	
Time interval/d	Apples	Apple leaves
7	1.23	7.2
15	0.92	3.4
22	0.45	2.0
30	0.15	0.8

* Mean of three determinations.

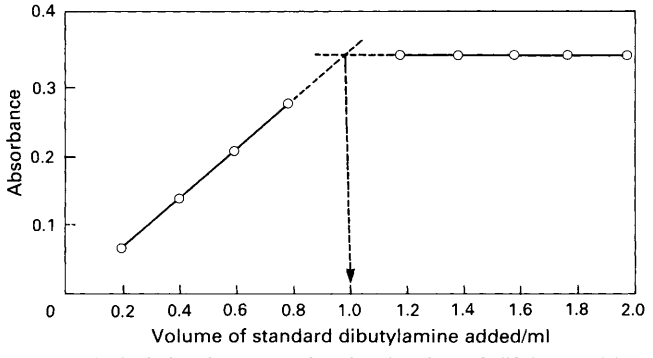


Fig. 2 Typical titration curve for the titration of difolatan with a standard solution of dibutylamine

Table 6 Spectrophotometric titrimetric determination of difolatan

	Difolatan	
	Amount taken/ μg	Amount found*/ μg
	3.5	3.53 ± 0.020
	7.5	7.45 ± 0.051
	15.0	14.88 ± 0.048
	22.5	22.62 ± 0.072
	37.5	37.68 ± 0.050
	45.0	44.62 ± 0.066

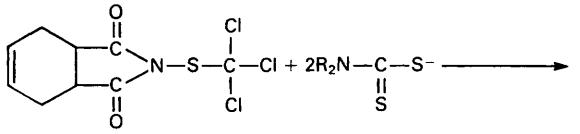
* Values are the mean of five determinations ± SD.

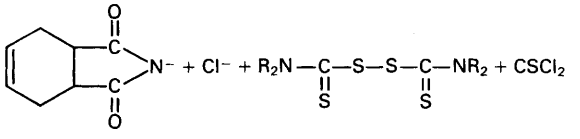
Table 7 Assay of a commercial fungicide formulation containing 80% of the active ingredient by a spectrophotometric titrimetric procedure

	Active ingredient found* (%)	
Active ingredient taken/ μg	Proposed method	Comparison method†
3.5	79.0 ± 0.3	78.4 ± 0.5
7.0	78.9 ± 0.4	78.9 ± 0.6
15.0	79.0 ± 0.3	78.9 ± 0.8
22.0	79.5 ± 0.6	79.6 ± 0.7
30.0	78.6 ± 0.6	78.5 ± 0.5
45.0	79.4 ± 0.5	79.4 ± 0.7

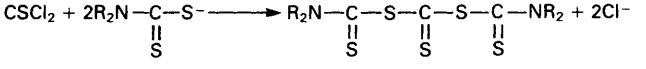
* Values are the mean of five determinations ± SD.
† See reference 6.

It is worth mentioning here that when captan or folpet is reacted with a dithiocarbamate in a similar fashion,[7,8] the yellow colour which appears on mixing the reagent (dithiocarbamate) fades immediately. The reaction mechanism for captan[8] is given as:

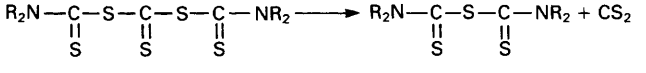




(II)



(III)



(IV)

The fading of the colour is due to the liberation of carbon disulphide from compound (**III**) resulting in the formation of the monosulphide (**IV**). This, however, does not occur with difolatan and consequently the yellow colour persists. That carbon disulphide is liberated in the reaction between captan or folpet and dithiocarbamate but not in the reaction between difolatan and dithiocarbamate has been established separately in this laboratory.

Attempts to use nabam (disodium ethylenebisdithiocarbamate) or similar dithiocarbamates in place of the proposed reagent were not satisfactory principally because of the extreme slowness of the reaction.

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