Bayer CropScience

Triadimefon
352

HPLC method
Method Extension

Report to CIPAC
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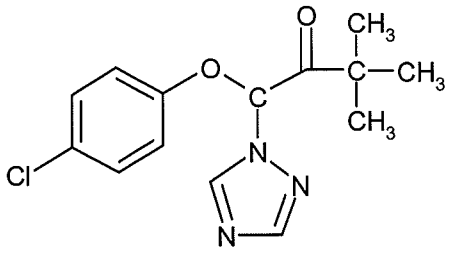
1. INTRODUCTION

1.1 Scope

So far the CIPAC method 352 for the determination of triadimefon covers only Technical (TC) and Wettable Powder (WP).
The validity of CIPAC method 352 for the formulation types Emulsion Concentrate (EC), Wettable Granule (WG) and Granule (GR) has been investigated.
Therefore, method details and validation data are provided in this report in order to demonstrate that the method is applicable to these formulation types.
For comparison reasons in addition to EC, WG and GR representative samples of a TK and WP were selected respectively and precision determined.

1.2 General information on the active substance



ISO common name: triadimefon

CAS index name: 2-butanone, 1-(4-chlorophenoxy)-3,3-dimethyl-1-(1*H*-1,2,4-triazol-1-yl)- (9Cl)

IUPAC Name: 1-(4-chlorophenoxy)-3,3-dimethyl-1-(1*H*-1,2,4-triazol-1-yl)butan-2-one

CAS-No.: 43121-43-3

Empirical formula: $C_{14}H_{16}ClN_3O_2$

RMM: 293.7 g/mol

Remark: Racemat

m.p.: 82.3 °C

Solubility at 20 °C: In water: 0.07 g/l, 2-propanol: 100 – 200 g/l; toluene: 400 – 600 g/l

Description: Form: white powder

Formulations: WP, EC, WG, GR

2. METHOD DESCRIPTION

Triadimefon
352/TC/(M)/-

1 Sampling. Take at least 100 g.

2 Identity tests.

2.1 Infrared spectroscopy.
(a) Technique: KBr disc
Prapare KBr disc using 1 mg of sample and 200 mg of KBr and also using a standard triadimefon. Scan the disc from 4000 to 400 cm^{-1} (2.50 to 25 µm). The spectrum produced from the sample disc should not differ significantly from that from the standard
Reference spectrum:

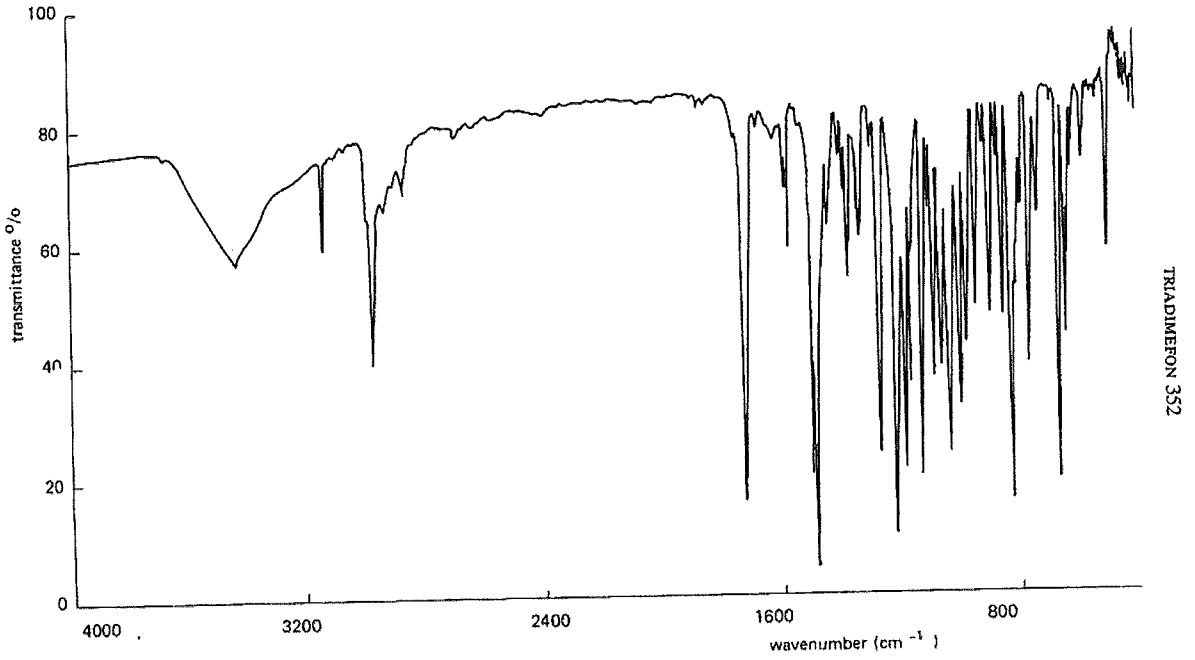
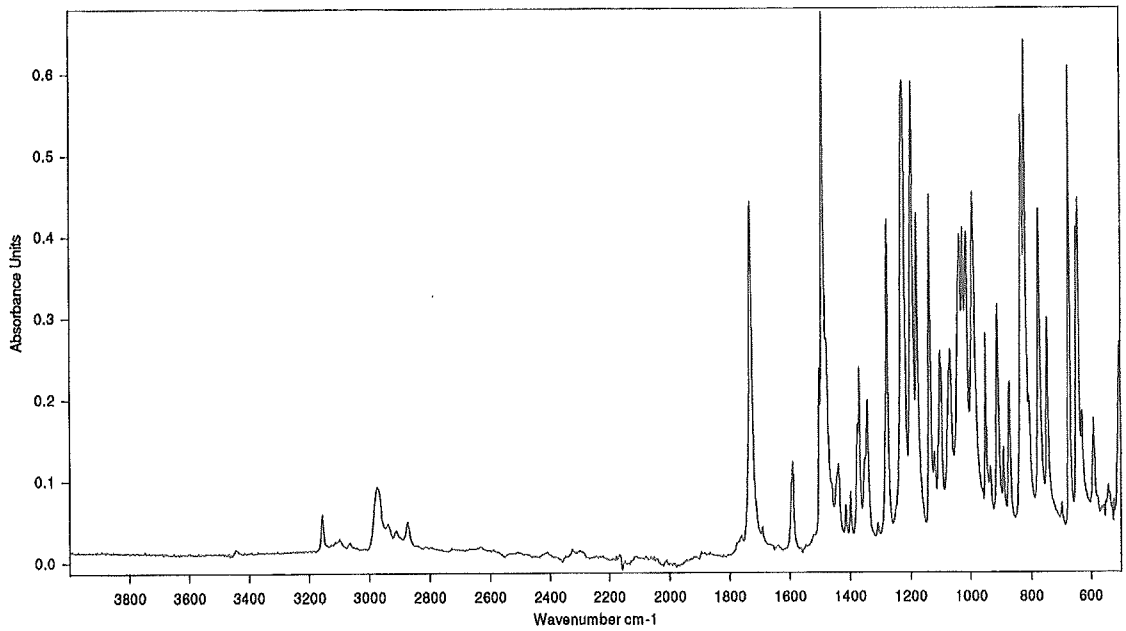


Fig. 138 IR spectrum of triadimefon technical (KBr disc).

(b) FT-IR Infrared Spectrometer with ATR accessories (e.g. Diamond ATR)
Disperse the test substance homogeneously on the crystal and record the IR spectrum of the test substance in the range 4000 - 600 cm^{-1} . Compare it qualitatively with the reference spectrum. The test is considered to be positive when the spectrum is qualitatively identical with the reference spectrum.

Reference spectrum:



2.2 HPLC. The identity is checked simultaneously with the determination of triadimefon and is confirmed if the retention time of triadimefon for the sample solution does not deviate by more than 1.5 % from that for the calibration solution.

3 Triadimefon

OUTLINE OF METHOD

Triadimefon is dissolved in acetonitrile-water and separated by high performance chromatography on a 5 µm Spherisorb-ODS-column with acetonitrile-water (49+51) (v/v) and UV-detection (276 nm). The active ingredient content is determined from peak areas using an external standard.

REAGENTS

Acetonitrile HPLC grade

Water HPLC grade

Eluant 49 % (v/v) acetonitrile / 51 % (v/v) water (degas before use)

Triadimefon standard of known purity

Calibration solution. Weigh (to the nearest 0.1 mg) about 60 mg of the of triadimenol standard into a 50 mL volumetric flask, dissolve and made up to volume with the eluant.

APPARATUS

High performance liquid chromatograph equipped with an ultraviolet spectrophotometric detector equipped with a detector suitable for operation at 276 nm and an injection system capable to inject 20 µl.

Electronic integrator or data system

Column. Stainless steel, length 125 mm, internal diameter 4 mm, packed with Spherisorb ODS 5 µm or any commercially available column with at least 5000 theoretical plates (Hypersil ODS was also tested) .

Ultrasonic bath

PROCEDURE

(a) Chromatographic conditions (typical)

Eluant flow rate:	*2 ml/min.*
Column temperature	40 °C
Injection volume	20 µL
Detector wavelength	276 nm
Run time	approximately 8 min
Retention time	approximately 2.7 min (adjust the operating parameters to a retention time of triadimefon between 2 and 3 min).

(b) Equilibration of the system. Pump sufficient eluent through the column to equilibrate the system. Inject 20 µl portions of the calibration solution and repeat the injections until retention times and peak areas vary by less than ± 1.5 % of the mean for 5 successive injections.

(c) Linearity check. Check the linearity of the response by injecting solutions with half and two times the concentration of the calibration solution. T*he linear range must not be exceeded otherwise the sample weights have to be reduced accordingly.*

(d) Sample preparation. Weigh (to the nearest 0.1 mg) about 60 mg (*w* mg) of the technical sample into a 50 ml volumetric flask, dissolve and make up to volume with the eluant.

(e) Determination. Inject 20 µl portions of two calibration solutions (C_1, C_2) of which the triadimefon content differs approximately by 10 %. Inject each calibration solution at least twice and calculate the avarage quotient of the peak area and the corresponding mass. The individual values should not deviate from the mean by more than 0.4 % otherwise repeat the calibration. Then inject in duplicate 20 µl portions of each sample solution (S_n). A series of more than 4 sample runs requires a repetition of the calibration test at the end of the series. The following injection sequence is proposed:

$$C_1, C_1, C_2, C_2, S_1, S_1, S_2, S_2, C_1 S_3, S_3, S_4, S_4 C_1, ...,S_n, C_2$$

Measure the relevant peak areas.

(f) Calculation

$$\text{The content of triadimefon} = \frac{F \times s \times P}{F_1 \times w} \text{ g/kg}$$

Where:

F1	= peak area of triadimefon standard
s	= mass of triadimefon standard, in mg
P	= purity of triadimefon standard, in g/kg
F	= peak area of triadimefon sample
W	= mass of sample, in mg

Repeatability r_{95} = 0.97 % at 90 – 100 % a.i. (ISO 5725)
Reproducibility R_{95} = 1.59 % at 90 – 100 % a.i. (ISO 5725)

Triadimefon Wettable Powder

352/WP/(M)/-

1 Sampling. Take at least 500 g.

2 Identity test. As for 352/TC/(M)/2.

3 Triadimefon

Reagents and Apparatus. As for 352/TC/(M)/3 together with
Ultrasonic bath
Centrifuge 3000 rpm

Procedure. As for 352/TC/(M)/3, except:
(b) Sample preparation. Homogenize the sample and weigh (to the nearest 0.1 mg) sufficient sample (*w* mg) to contain about 60 mg of triadimefon into a 50 ml volumetric flask. Add about 40 ml of eluant and extract the active ingredient by treating the sample in an ultrasonic bath for about 15 min. Allow to equilibrate to room temperature, make up to volume with eluant and homogenize. Before injecting centrifuge in order to eliminate inert ingredients.

Repeatability r_{95} = 0.55 % at 25 % a.i. (ISO 5725)
Reproducibility R_{95} = 0.80 % at 25 % a.i. (ISO 5725)

4 Suspensibility

(a) Preparation of suspension according MT 15.1, MT 184.
(b) Determination of sedimentation according MT 15.1/ MT 184
(c) Determination of triadimefon in the bottom 25 ml of suspension. After removal of the top 225 ml of suspension transfer the remaining 25 ml of the suspension to a 50 ml volumetric flask. Fill up to the mark with eluant. Determine the mass of triadimefon as described in the method procedure

Triadimefon Emulsifiable Concentrate

352/EC/(M)/-

1 Sampling. Take at least 500 g.

2 Identity test. As for 352/TC/(M)/2.

3 Triadimefon

Reagents and Apparatus. As for 352/TC/(M)/3 together with
Ultrasonic bath

Procedure. As for 352/TC/(M)/3, except:
(b) Sample preparation. Thoroughly shake the sample container to ensure that the emulsion is homogeneous. Immediately weigh (to the nearest 0.1 mg) sufficient sample (*w* mg) to contain about 60 mg of triadimefon into a 50 ml volumetric flask. Add about 40 ml of eluant and dissolve the active ingredient by treating the sample in an ultrasonic bath for about 15 min. Allow to equilibrate to room temperature, make up to volume with eluant.

Triadimefon Water Dispersable Granules
352/WG/(M)/-

1 Sampling. Take at least 500 g.

2 Identity test. As for 352/TC/(M)/2.

3 Triadimefon
Reagents and Apparatus. As for 352/TC/(M)/3 together with
Ultrasonic bath
Centrifuge 3000 rpm

Procedure. As for 352/TC/(M)/3, except:
(b) Sample preparation. Homogenize the sample and weigh (to the nearest 0.1 mg) sufficient sample (*w* mg) to contain about 60 mg of triadimefon into a 50 ml volumetric flask. Add about 40 ml of eluant and extract the active ingredient by treating the sample in an ultrasonic bath for about 15 min. Allow to equilibrate to room temperature, make up to volume with eluant and homogenize. Before injecting centrifuge in order to eliminate inert ingredients.

4 Suspensibility
(a) Preparation of suspension according MT 161, MT 184.
(b) Determination of triadimefon in the bottom 25 ml of suspension. After removal of the top 225 ml of suspension transfer the remaining 25 ml of the suspension to a 50 ml volumetric flask. Fill up to the mark with eluant. Determine the mass of triadimefon as described in the method procedure.

Triadimefon Granules
352/GR/(M)/-

1 Sampling. Take at least 500 g.

2 Identity test. As for 352/TC/(M)/2.

3 Triadimefon
Reagents and Apparatus. As for 352/TC/(M)/3 together with
Ultrasonic bath
Centrifuge 3000 rpm

Procedure. As for 352/TC/(M)/3, except:
(b) Sample preparation. Homogenize the sample and weigh (to the nearest 0.1 mg) sufficient sample (*w* mg) to contain about 60 mg of triadimefon into a 50 ml volumetric flask. Add about 40 ml of eluant and extract the active ingredient by treating the sample in an ultrasonic bath for about 15 min. Allow to equilibrate to room temperature, make up to volume with eluant and homogenize. Before injecting centrifuge in order to eliminate inert ingredients.

3. METHOD ASSESSMENT

In comparison to the original method description the only change in the analytical procedure for the EC, WG, GR formulations is the reduction of the injection volume from 30 to 20 µl. No further method modifications were needed.
In accordance with the CIPAC method extension guideline a selectivity test was performed and the precision under repeatability conditions was determined for each of the formulation types.

3.1 Check of the acceptability range

Scope of the existing CIPAC method

1000 g/kg (a.i.) – 10 g/kg

New formulation types:

Triadimefon EC 100 Active ingredient content: 100 g/L (108 g/kg)

Triadimefon WG 50 Active ingredient content: 500 g/kg

Triadimefon GR 1 Active ingredient content: 10 g/kg

The new formulation types are within the content range of the existing CIPAC method 352 triadimefon.

3.2 Selectivity test

For each of the considered formulation types representative formulations were selected and blank formulations checked. In all cvases no peak at expected retention time or close nearby was observed. Additionally the UV spectrum of the analyte in the sample was compared with the reference item.

Method: Standard addition of reference item to blank formulation

No interferences were observed for the active ingredient with the formulants.

3.3 Precision (Repeatability)

	Statistical evaluation					
Formulation type	*WP 5	*WP 25	TK 70	EC100	WG 50	GR 1
Content a.i., mean values in %	5.3	25.5	72.4	11.1	52.6	0.962
Repeatability r in %	1.23	0.42	0.45	0.68	0.37	2.30
Modified Horwitz Criterion	2.09	1.65	1.41	1.87	1.48	2.70

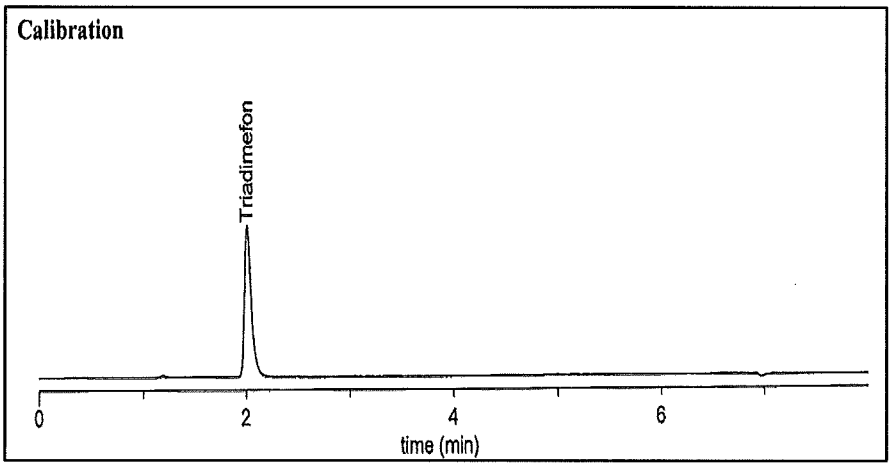
* Formulation type already described in the existing method.

The determination was done using certified triadimefon standard: Batch 920427ELB02, 99.8 %

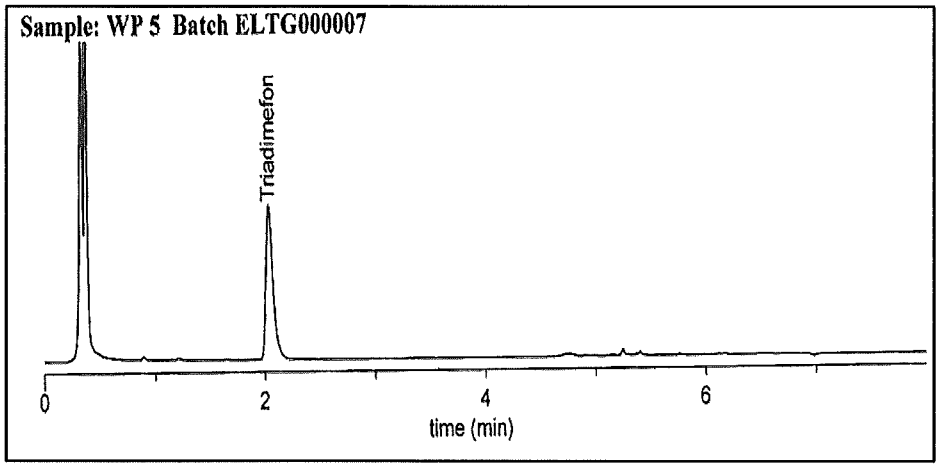
All repeatability figures were below the modified Horwitz criterion.

4. REPRESENTATIVE CHROMATOGRAMS

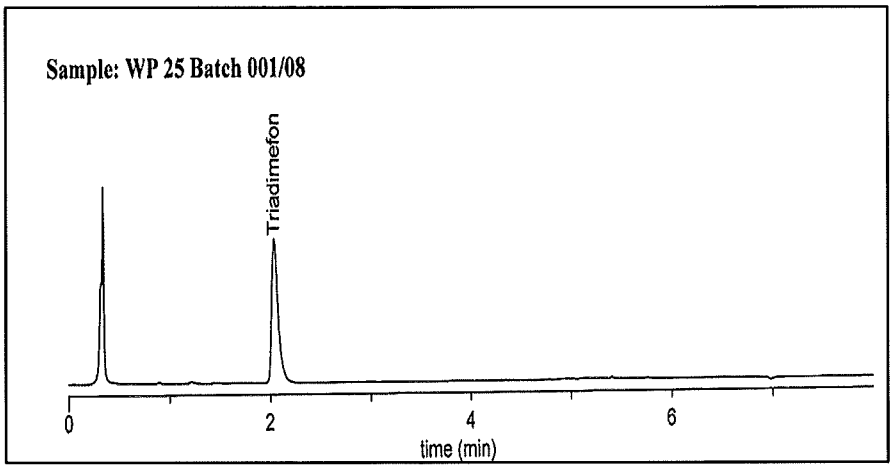
(1) Triadimefon standard (calibration)



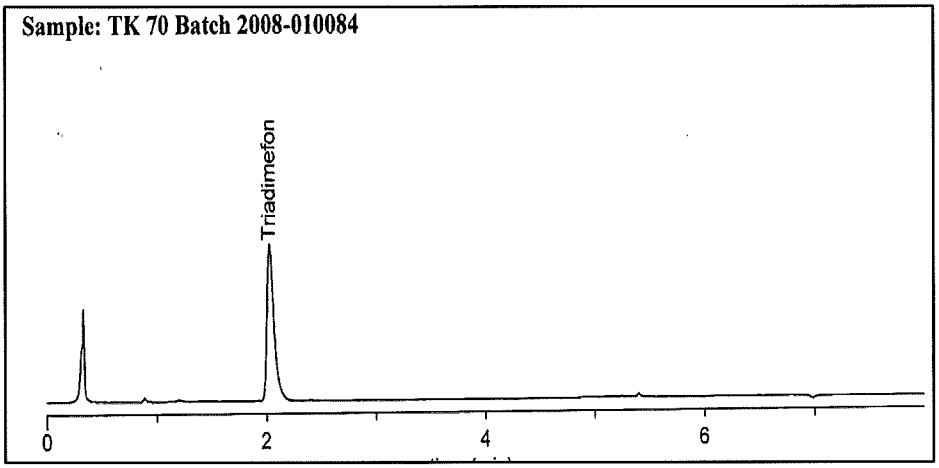
(2) Triadimefon WP 5 , batch ELTG000007



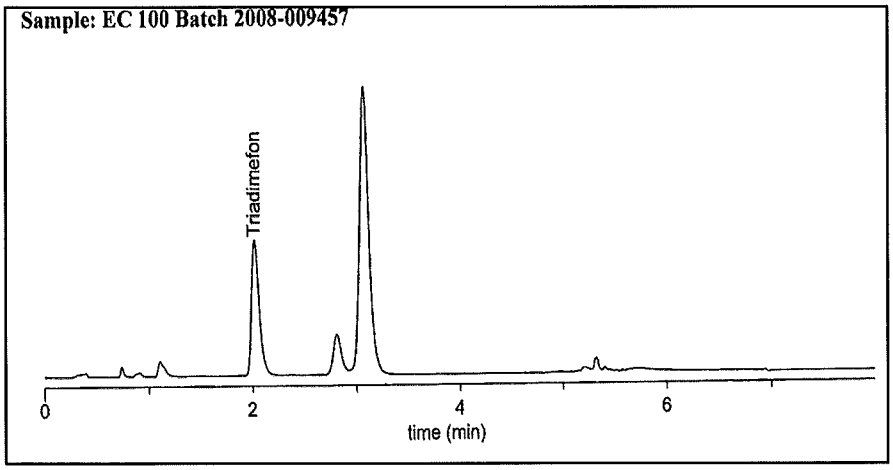
(3) Triadimefon WP 25, batch 001/08



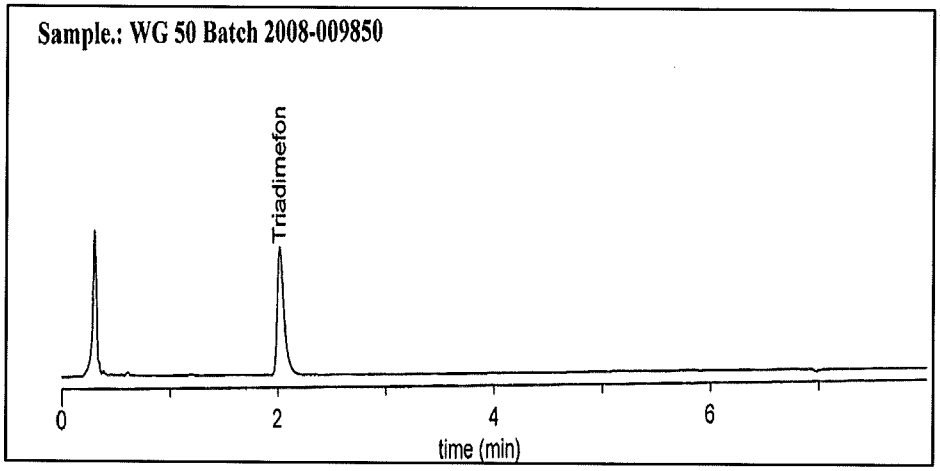
(4) Triadimefon TK 70, batch 2008-010084



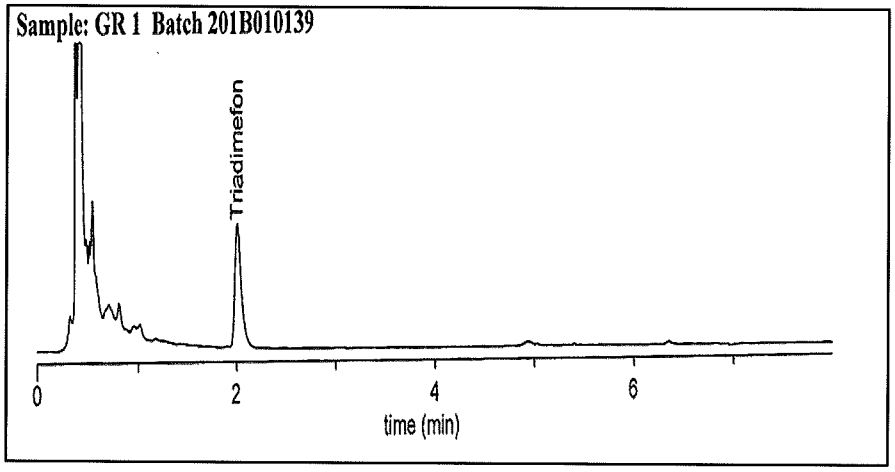
(5) Triadimefon EC 100, batch 2008-009457



(6) Triadimefon WG, batch 2008-009850



(7) Triadimefon GR, batch 201B010139



5. CONCLUSION

The shown validation data demonstrate the validity of the CIPAC method 352 for the determination of triadimefon in TC and in WP, EC, WG and GR formulations

Therefore, we propose to extend the existing CIPAC method 352 to EC, WG and GR formulation type.