Bayer CropScience

Triadimenol
398

GLC method
Method Extension

Report to CIPAC
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1. INTRODUCTION

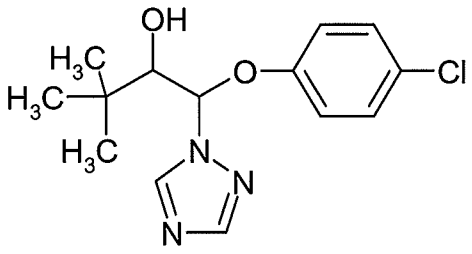
1.1 Scope

So far the CIPAC method 398 for the determination of triadimenol covers only Technical (TC), Wettable Powder (WP) and Emulsifiable Concentrates (EC).

The existing FAO specification covers beside TC, WP and EC also the formulation types DP, OL, DC, GR and WG.

The validity of CIPAC method 398 for the formulation types Suspension Concentrates (SC), Flowable Concentrate for Seed Treatment (FS) and Emulsion, oil in water (EW) was investigated in frame of this method extension study.
Therefore, method details and validation data are provided in this report in order to demonstrate that the method is also applicable to these formulation types.
For comparison reasons in addition to SC, FS and EW one representative sample of the formulation types WP, EC and WG was selected respectively and precision determined.

1.2 General information on the active substance



ISO common name:	triadimenol
CAS index name:	1H-1,2,4-Triazole-1-ethanol, .beta.-(4-chlorophenoxy)-.alpha.-(1,1-dimethylethyl)- (9Cl)
IUPAC Name:	1-(4-Chlorophenoxy)-3,3-dimethyl-1-[1,2,4]triazol-1-yl-1butan-2-ol (unstated stereochemistry)
CAS-No.:	55219-65-3
Empirical formula:	$C_{14}H_{18}ClN_3O_2$
RMM:	295.8 g/mol
Note:	Triadimenol is a diastereomeric mixture (A(threo) : B(erythro) = 7 : 3); A = (1R,2S)- + (1S,2R)-enantiomer (CAS-No. 89482-17-7); B = (1R,2R)- + (1S,2S)-enantiomer (CAS-No. 82200-72-4)
m.p.:	diastereomeric form A: 138.2 °C, B: 133.5 °C
Solubility at 20 °C:	dichlormethane 100 - 200 g/L; acetone 190 g/L; toluene 10 – 30 g/L
Description:	Form: white powder
Formulations:	WP, EC, DC, GR, WG, SC, FS and EW.

2. METHOD DESCRIPTION

Triadimenol
398/TC/(M)/-

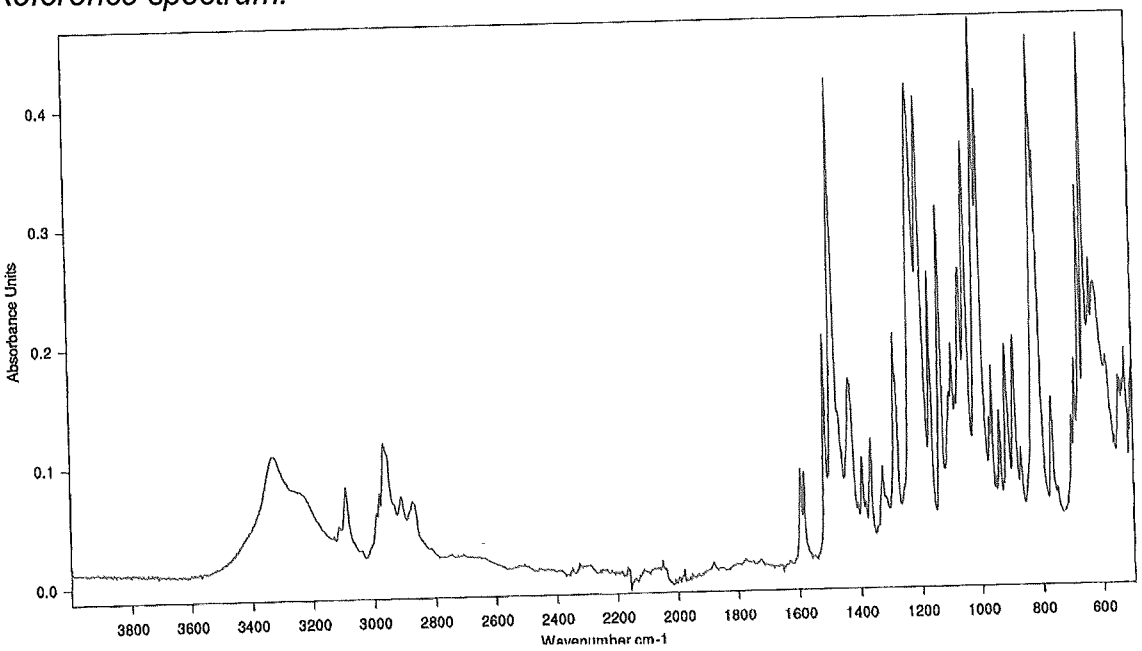
1 Sampling. Take at least 100 g.

2 Identity tests.

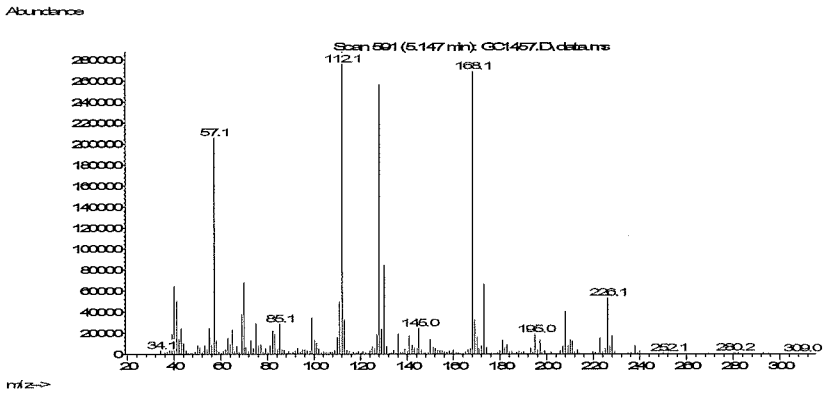
2.1 GLC.
Use the GLC method below. The difference between the retention times of triadimenol and the internal standard for the sample solution should not deviate by more than 10 s from that for calibration solution.

2.2 Infrared spectroscopy.
FT-IR Infrared Spectrometer with ATR accessories (e.g. Diamond ATR)
Disperse the test substance homogeneously on the crystal and record the IR spectrum 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:



2.3 Mass spectrometry (GC-MS).
Use the sample preparation below. Record the MS spektrum using GC-MS and compare it qualitatively with the reference spectrum. The test is considered to be positive when the spectrum is identical with the reference spectrum.
Reference spectrum:



3 Triadimenol
OUTLINE OF METHOD
Triadimenol is dissolved in, or extracted with acetone, di-(2-ethylhexyl) phthalate is added as internal standard, and the content of triadimenol is determined by capillary gas chromatography with on-column cold injection. Alternatively to on-column cold injection split injection is applicable when the temperature program is adjusted appropriately.

REAGENTS
Acetone
Triadimenol standard of known purity, at least 950 g/kg
Di-(2-ethylhexyl) phthalate internal standard
Calibration solution. Prepare in duplicate calibration solutions. Weigh (to the nearest 0.1 mg) about 200 mg of the of triadimenol standard (s mg) and 200 mg of di-(2-ethylhexyl) phthalate (*r* mg) into a 20 ml volumetric flask, fill to the mark with acetone and dissolve. Transfer with a pipette exactly 1.0 ml of the solution to a 100 ml volumetric flask, fill to the mark with acetone and homogenize.

APPARATUS
Gaschromatograph with flame ionization detector and on-column capillary column accessory (alternatively split injector)
Electronic integrator or lab data system
Column Quartz capillary, 30 m x 0.53 (i.d.) mm, coated with silicone ODER 1.1 µm.
Ultrasonic bath

PROCEDURE
(a) Praparation of sample solution. Weigh (to the nearest 0.1 mg) about 200 mg of sample (*w* mg) into a 20 ml volurimetric flask, add 200 mg of internal standard di-(2-ethylhexyl) phthalate (*q* mg), fill to the mark with acetone and dissolve for 10 min using an ultrasonic bath. Transfer with a pipette exactly 1.0 ml of the solution to a 100 ml volumetric flask, fill to the mark with acetone and homogenize. Prepare two solutions for each sample.

(b) Operating conditions (typical)
Column temperature 2 min at 80°C, then 10°C/min up to 280 °C

Injection port temperature	*cold on column injection*
Detector temperature	280 °C
Flow rate carrier gas	*helium: 5 ml/ min*
Flow rate make up gas	*nitrogen: 30 ml/ min*
Flow rate air and hydrogen	*as recommended by the manufacturer*
Retention time	*triadimenol: about 18 min*
	di-(2-ethylhexyl) phthalate: about 23 min

(c) Determination. Inject at least two times 1 µl aliquots of each calibration solution and calculate the single response factors f_i using the formula below. The individual values should agree within 2 %, otherwise repeat the calibration. If the response is satisfactory, inject 1 µl aliquots of each sample solution bracketing them by injections of the calibration solutions as follows: calibration solution 1, sample solution1, sample solution 2, calibration solution 2. Measure the relevant peak areas. Calculate the mean value *f* of each pair of response factors bracketing two sample injections and use this value for evaluating the two bracketed sample runs.

(d) Calculation

$$\text{Response factor } f_i = \frac{I_r \times P \times s}{H_s \times r}$$

Where:

f_i	= single response factors of triadimenol
I_r	= peak area of the internal standard in the calibration solution i
P	= purity of triadimenol standard, in g/kg
s	= mass of triadimenol in the calibration solution i (mg)
H_s	= peak area of triadimenol in the calibration solution i
r	= mass of the internal standard in the calibration solution i (mg)

$$\text{Triadimenol content} = \frac{f \times H_w \times q}{I_q \times w} \text{ g/kg}$$

Where:

f	= average response factor
H_w	= *peak area of triadimenol in the sample solution*
q	= *mass of internal standard in the sample solution (mg)*
I_q	= *peak area of internal standard in the sample solution*
w	= *mass of sample taken (mg)*

The content of triadimenol (sum of both diastereometric forms) is the mean value of the results of the solutions.

Repeatability r_{95} = 12.4 g/kg at 967 g/kg active ingredient content
Reproducibility R_{95} = 26.5 g/kg active ingredient content

Triadimenol Wettable Powder
398/WP/(M)/-

1 Sampling. Take at least 500 g.

2 Identity tests.
2.1 GLC. As for 398/TC/(M)/2.1.
2.2 Infrared

REAGENTS and APPARATUS
Tetrachlormethan
Ultrasonic bath
Centrifuge 3000 rpm

PROCEDURE
Weigh an amount of the formulation containing about 50 mg of active ingredient into a 10 ml flask and add tetrachlormethane. Sonificate the suspension for 15 min then centrifuge the suspension and apply a portion of the clear supernatant uniformly to the crystal. Verify the identity by comparing the spectrum with those of triadimenol standard.

2.3 Mass spectrometry (GC-MS). As for 398/TC/(M)/2.3

3 Triadimenol. As for 398/TC/(M)/3 except:
(a) Preparation of sample solutions. Homogenize the sample and weigh (to the nearest 0.1 mg) into a 20 ml volumetric flask enough sample (*w* mg) to contain about 200 mg of pure triadimenol, add 200 mg of internal standard (*q* mg). Fill to the mark with acetone and extract for 30 min in an ultrasonic bath. Transfer with a pipette exactly 1.0 ml of the solution to 100 ml volumetric flask, fill to the mark with acetone and homogenize. Centrifuge the suspension and inject 1 µl aliquots of the supernatant liquid. The concentration of triadimenol should be similar to its concentration in the calibration solution. Prepare two solutions of each sample.

Repeatability r_{95}	= 3.29 g/kg at 148 g/kg active
Reproducibility R_{95}	= 5.09 g/kg at 148 g/kg active

4 Suspensibility.
(a) Preparation of suspension according MT 15.1 / MT 184 (i)
(b) Determination of sedimentation according MT 15.1/ MT 184 (ii)
(c) Determination of triadimenol in the bottom 25 ml suspension. After removal of the top 225 ml of suspension add to the remaining 25 ml in the cylinder 75 ml of N,N-dimethylacetamid containing an amount of internal standard that corresponds to the amount of active ingredient. Analyze according to the method 398/TC/M/3 using an injection port temperature of 160 °C.

Triadimenol Emulsifiable Concentrate
398/EC/(M)/-

1 Sampling. Take at least 500 ml.

2 Identity tests.
2.1 GLC. As for 398/TC/(M)/2.1.
2.2 Mass spectrometry (GC-MS). As for 398/TC/(M)/2.1.

3 Triadimenol. As for 398/TC/(M)/3 except:
(a) Preparation of sample solutions. Thoroughly shake the sample container to ensure that the emulsion is homogeneous. Immediately weigh (to the nearest 0.1 mg) enough sample (*w* mg) to contain about 200 mg of pure triadimenol into a 20 ml volumetric

flask, add 200 mg of internal standard (*q* mg). Fill to the mark with acetone and dissolve for 10 min in an ultrasonic bath.. Transfer with a pipette exactly 1.0 ml of the solution to 100 ml volumetric flask, fill to the mark with acetone and homogenize. Inject 1 µl aliquots of the solution. The concentration of triadimenol should be similar to its concentration in the calibration solution. Prepare two solutions of each sample.

Repeatability r_{95} = 4.6 g/kg at 217 g/kg active ingredient content
Reproducibility R_{95} = 10.0 g/kg at 217 g/kg active ingredient content

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

1 Sampling. Take at least 500 g.

2 Identity tests.
2.1 GLC. As for 398/TC/(M)/2.1.
2.2 Infrared As for 398/WP/(M)/2.2
2.3 Mass spectrometry (GC-MS). As for 398/TC/(M)/2.1.

3 Triadimenol. As for 398/TC/(M)/3 except:
(a) Preparation of sample solutions. Homogenize the sample and weigh (to the nearest 0.1 mg) into a 20 ml volumetric flask enough sample (*w* mg) to contain about 200 mg of pure triadimenol, add 200 mg of internal standard (*q* mg). Fill to the mark with acetone and extract for 30 min in an ultrasonic bath. Transfer with a pipette exactly 1.0 ml of the solution to 100 ml volumetric flask, fill to the mark with acetone and homogenize. Centrifuge the suspension and inject 1 µl aliquots of the supernatant liqid. The concentration of triadimenol should be similar to its concentration in the calibration solution. Prepare two solutions of each sample.

4 Suspensibility.
(a) Preparation of suspension according MT 15.1 / MT 184 (i)
(b) Determination of sedimentation according MT 15.1/ MT 184 (ii)
(c) Determination of triadimenol in the bottom 25 ml suspension. After removal of the top 225 ml of suspension add to the remaining 25 ml in the cylinder 75 ml of N,N-dimethylacetamid containing an amount of internal standard that corresponds to the amount of active ingredient. Analyze according to the method 398/TC/M/3 using an injection port temperature of 160 °C.

Triadimenol Suspension Concentrates
398/SC/(M)/-

1 Sampling. Take at least 500 g.

2 Identity tests.
2.1 GLC. As for 398/TC/(M)/2.1.
2.2 Infrared. As for 398/WP/2.2
2.3 Mass spectrometry (GC-MS). As for 398/TC/(M)/2.1.

3 Triadimenol. As for 398/TC/(M)/3 except:
(a) Preparation of sample solutions. Thoroughly shake the sample container to ensure that the suspension is homogeneous. Immediately weigh (to the nearest 0.1 mg) into a 20 ml volumetric flask enough sample (*w* mg) to contain about 200 mg of pure triadimenol, add 200 mg of internal standard (*q* mg). Fill to the mark with acetone and extract for 30 min in an ultrasonic bath. Transfer with a pipette exactly 1.0 ml of the solution to 100 ml

volumetric flask, fill to the mark with acetone and homogenize. Centrifuge the suspension and inject 1 µl aliquots of the supernatant liquid. The concentration of triadimenol should be similar to its concentration in the calibration solution. Prepare two solutions of each sample.

4 Suspensibility.
(a) Preparation of suspension according MT 161/MT 184 (i)
(b) Determination of sedimentation according MT 161 / MT 184 (ii)
(c) Determination of triadimenol in the bottom 25 ml suspension. After removal of the top 225 ml of suspension add to the remaining 25 ml in the cylinder 75 ml of N,N-dimethylacetamid containing an amount of internal standard that corresponds to the amount of active ingredient. Analyze according to the method 398/TC/M/3 using an injection port temperature of 160 °C.

Triadimenol Flowable Concentrate for Seed Treatment

398/FS/(M)/-

1 Sampling. Take at least 500 g.

2 Identity tests.
2.1 GLC. As for 398/TC/(M)/2.1.
2.2 Infrared As for 398/WP/2.2
2.3 Mass spectrometry (GC-MS). As for 398/TC/(M)/2.1.

3 Triadimenol. As for 398/TC/(M)/3 except:
(a) Preparation of sample solutions. Thoroughly shake the sample container to ensure that the suspension is homogeneous. Immediately weigh (to the nearest 0.1 mg) into a 20 ml volumetric flask enough sample (*w* mg) to contain about 200 mg of pure triadimenol, add 200 mg of internal standard (*q* mg). Fill to the mark with acetone and extract for 30 min in an ultrasonic bath. Transfer with a pipette exactly 1.0 ml of the solution to 100 ml volumetric flask, fill to the mark with acetone and homogenize. Centrifuge the suspension and inject 1 µl aliquots of the supernatant liqid. The concentration of triadimenol should be similar to its concentration in the calibration solution. Prepare two solutions of each sample.

4 Suspensibility.
(a) Preparation of suspension according MT 161/MT 184 (i)
(b) Determination of sedimentation according MT 161 / MT 184 (ii)
(c) Determination of triadimenol in the bottom 25 ml suspension. After removal of the top 225 ml of suspension add to the remaining 25 ml in the cylinder 75 ml of N,N-dimethylacetamid containing an amount of internal standard that corresponds to the amount of active ingredient. Analyze according to the method 398/TC/M/3 using an injection port temperature of 160 °C.

Triadimenol Emulsion, Oil in Water

398/EW/(M)/-

1 Sampling. Take at least 500 ml.

2 Identity tests.

2.1 GLC. As for 398/TC/(M)/2.1.

2.2 Mass spectrometry (GC-MS). As for 398/TC/(M)/2.1.

3 Triadimenol. As for 398/TC/(M)/3 except:

(a) Preparation of sample solutions. Thoroughly shake the sample container to ensure that the emulsion is homogeneous. Immediately weigh (to the nearest 0.1 mg) enough sample (*w* mg) to contain about 200 mg of pure triadimenol into a 20 ml volumetric flask, add 200 mg of internal standard (*q* mg). Fill to the mark with acetone and dissolve for 10 min in an ultrasonic bath. Transfer with a pipette exactly 1.0 ml of the solution to 100 ml volumetric flask, fill to the mark with acetone and homogenize. Inject 1 µl aliquots of the solution. The concentration of triadimenol should be similar to its concentration in the calibration solution. Prepare two solutions of each sample.

3. METHOD ASSESSMENT

Toluene is not suitable to dissolve or extract the triadimenol sufficiently out of each formulation type. Therefore we propose to use acetone for the sample preparation. Acetone is suitable for all relevant formulation types. When acetone is used the column temperature needs to be slightly changed at the beginning of the temperature program (originally: 2 min at 100 °C, then 10 °/min to 280 °C; changed: 2 min at 80 °C, then 10°C/ min to 280°C). No further method modifications were needed.
The analytical procedure for the SC, FS, WG and EW is very similar to those of the WP or EC.
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

25 g/kg (a.i.) to 970 g/kg

New formulation types:

Triadimenol SC 312	Active ingredient content: 312 g/L	(279 g/kg)
Triadimenol FS 60	Active ingredient content: 56 g/kg	
Triadimefon EW 50	Active ingredient content: 50 g/kg	

The new formulation types are within the content range of the existing CIPAC method 398 triadimenol.

3.2 Selectivity test

For each of the considered formulation types representative formulations were selected and blank formulations checked. In all cases no peak at expected retention was observed. In case of some recipes (formulation types) peaks were observed close to the analyte or to the internal standard. In those cases standard addition of the reference item to the respective formulation and / or blank formulation was performed.

Method: Standard addition of reference item to blank formulation / to formulation

Additionally the MS spectrum of the analyte in the respective sample was found to be identical with the reference item.

The method was found to be specific. No relevant interferences were observed.

3.3 Precision (Repeatability)

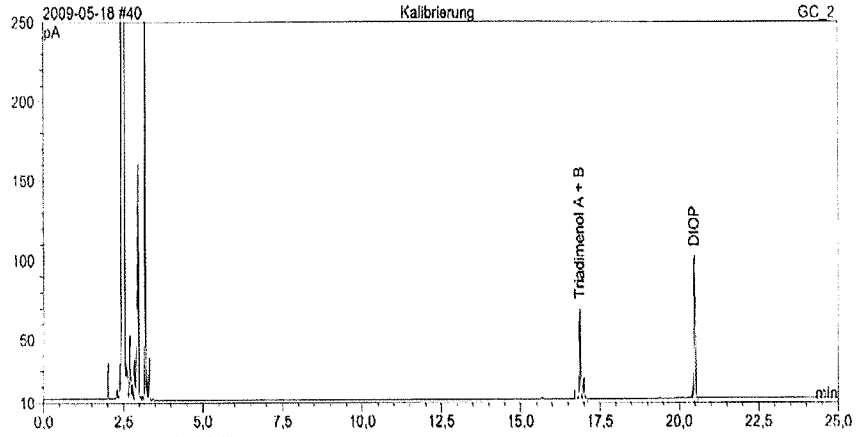
	Statistical evaluation					
Formulation type	WP 25*	EC 250*	WG 5**	SC 312	FS 60	EW 50
Content a.i., mean values in %	24.5	23.4	4.84	28.1	5.58	4.71
Repeatability r in %	0.40	0.15	1.01	1.19	1.71	1.49
Modified Horwitz Criterion	1.65	1.67	2.10	1.62	2.06	2.11

* Formulation type already described in the existing method.
** Formulation Type already described in the existing FAO specification.
The determination was done using certified triadimenol standard:
Batch 940627ELB04, 98.3 %

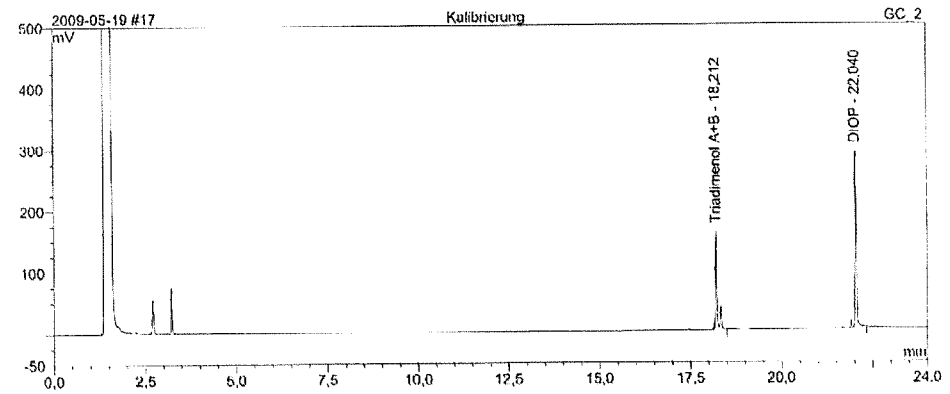
All repeatability figures were below the modified Horwitz criterion.

4. REPRESENTATIVE CHROMATOGRAMS

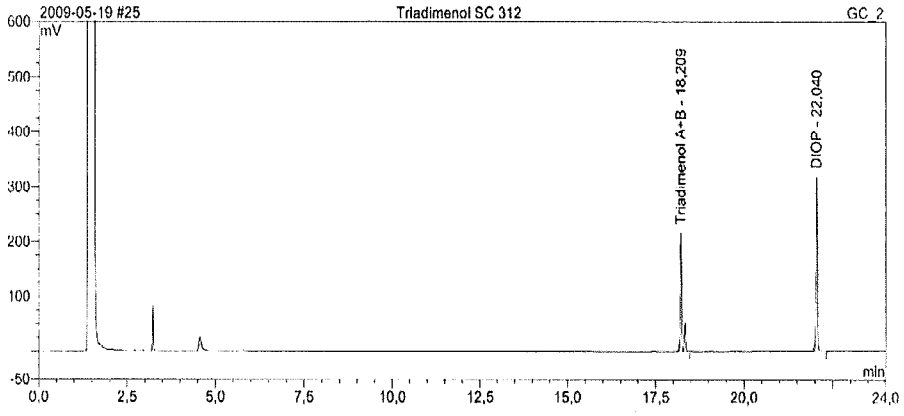
(1) Triadimenol standard (calibration) in toluene



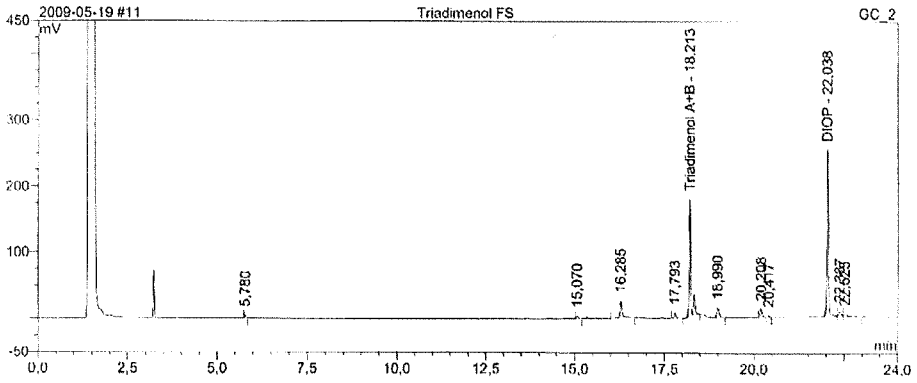
(2) Triadimenol standard (calibration) in acetone



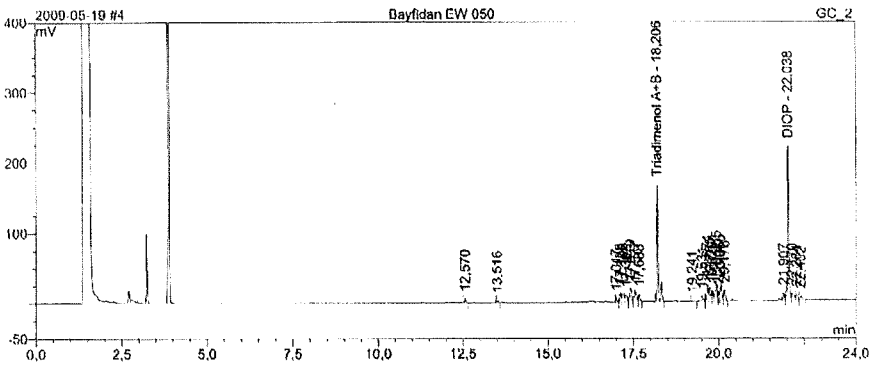
(3) Triadimenol SC 312, batch 2005-003151



(4) Triadimenol FS 60, batch PF90219429 (containing a second a.i.; overall a.i. content = 145.2 g/l)



(5) Triadimenol EW 50, batch PF90070705



Sample	Content Triadimenol in the original sample	Content Triadimenol in the spiked sample	Recovery in %
S 1	188,64 mg (4.7 %)	228,39 mg (5.7 %)	100.2 %
S 2	188,64 mg (4.7 %)	228,30 mg (5.7 %)	101.9 %
S 3	188,64 mg (4.7 %)	229.27 mg (5.7 %)	101.1 %

5. CONCLUSION

The shown validation data demonstrate the validity of the CIPAC method 398 for the determination of triadimenol in WP, EC, WG, SC, FS and EW formulations when the method is slightly changed.
The method was found to be specific. Application to the new formulation types will not induce any systematic errors.
The repeatability figures of the new examined formulation types showed them to be comparable to those of the original study.

Therefore, we propose to extend the existing CIPAC method 398 to SC, FS and EW formulation type.