SIMULTANEOUS DETERMINATION OF THREE ACTIVE INGREDIENTS OF A PESTICIDE, USING GAS CHROMATOGRAPHY-FLAME IONIZATION DETECTOR

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Abstract: *A gas chromatographic method developed and validated for simultaneous determination of spiroxamine, tebuconazole and triadimenol of a pesticide formulation (Falcone) for quality evaluation, using a glass capillary HP5 column (30 m x 0.32 mm; 0.25 μm), temperature programming with flame ionization detector and dicyclohexylphthalate as an internal standard. The calibration graphs were found linear in the concentration range of 1 μg/mL to 1000 μg/mL for three active ingredients with correlation coefficient of 0.999, 0.999 and 0.998 respectively and co-efficient of variation for intra-day and inter-day repeatability studies at different concentration levels was found to be less than 2%. The accuracy of method ranges between 98.5% to 100.8%. Specificity were also within the acceptable range. The method is highly sensitive with LOD and LOQ as 0.5 and 2 μg/mL for spiroxamine, tebuconazole and triadimenol respectively. The developed method was successfully applied to the simultaneous quantitative analysis of spiroxamine, tebuconazole and triadimenol and therefore can be used for the routine quality control, ensuring efficiency and efficacy of the formulation for the desired purpose.*

Keywords*: Gas chromatography, spiroxamine, simultaneous determination tebuconazole, triadimenol,*

INTRODUCTION

Ever since the combination formulations consisting of more than one technical grade pesticide were allowed around a decade ago in Albania, there has been a large number of combination formulations being introduced in the market [1]. The experience with the combination formulations has been encouraging, as their efficacy has been found to be better than the formulations containing single ingredient [3,5]. The analysis of three components individually using three different methods is high costs and time consuming, especially in routine control.

The aim of the present work was to develop and validate an analytical method using a gas chromatographic technique for simultaneous determination of spiroxamine, tebuconazole & triadimenol which is comparatively faster with significant precision, accuracy and resolution.

The Plant Protection Product - Falcon EC 460 is a fungicide based on three different active substances to provide broad spectrum disease control in cereals and grapes. It is formulated as an emulsifiable concentrate containing 250 g/l spiroxamine & 167 g/l tebuconazole & 43 g/l triadimenol [9,10].

EFFICACY DATA

Spiroxamine is a member of the spiroketalamine class of chemicals. In contrast to triazoles which inhibit the C-14-demethylase, it mainly blocks the Δ14-reductase and thus belongs to a different class of sterol-biosynthesis-inhibitors (SBI's). It is a systemic fungicide

with protective and curative action and shows a very fast uptake into plant tissue. On cereals, spiroxamine shows a good efficacy against powdery mildew (*Blumeria graminis*), a good to moderate control of rusts (*Puccinia species*), net blotch (*Drechslera teres*), leaf scald (*Rhynchosporium secalis*) and has a side effect against Septoria leaf spot (*Septoria tritici*). On grape, it shows a particularly high efficacy against *Uncinula necator*. Spiroxamine is registered in formulations alone and in mixture with tebuconazole or prothioconazole [6, 9].

Tebuconazole is a systemic broad-spectrum fungicide with protective and curative action. It is a triazole compound that acts as a demethylation inhibitor (DMI) of fungal sterol biosynthesis. It is absorbed into the vegetative parts of the plant and is translocated acropetally in the xylem. Thanks to its protective and curative action, tebuconazole provides reliable efficacy before and after infection by the pathogen. Tebuconazole is a well-established fungicide, used commercially for many years to control a wide range of crop diseases throughout the world. It is used straight and in mixture in a range of foliar formulations and seed treatments [8,9].

Triadimenol is also a triazole fungicide that acts as a demethylation inhibitor (DMI) of fungal sterol biosynthesis. It is a systemic fungicide with protective and curative action which provides reliable efficacy before and after pathogen infection. It is rapidly taken up by the plant tissue and acropetally transported via the xylem into young growing tissue. Triadimenol has been marketed for many years to control to control a wide range of diseases and can be used as a seed dressing or a foliar spray [8, 9].

Spiroxamine & tebuconazole & triadimenol EC 460 contains these three different sterol biosynthesis inhibitors (SBI). Tebuconazole & triadimenol are de-methylation inhibitors (DMI) with partial but incomplete cross resistance profiles. Spiroxamine also inhibits sterol biosynthesis, but at a different position in the biochemical pathway in common with morpholine and piperidine fungicides. The combination of these three actives ingredients with different and complementary spectra allows a high level of performance at controlling cereal diseases [8, 10].

The GC method is applicable for the simultaneous determination of the content of Spiroxamine, tebuconazole and triadimenol in formulations *(e.g.* Spiroxamine + Tebuconazole + Triadimenol EC 460).

Principle of the method: After the addition of a known weight of dicyclohexylphtalate as internal standard and dilution with dichloromethane the contents are determined by capillary gas chromatography using a FID detector.

MATERIAL AND METHODS

The reference standards Spiroxamine (purity: 99%), Tebuconazole (purity: 99.6%) and Triadimenol (purity: 99.4%) were obtained from Fluka chemicals. Dibutyl phthalate having purity 98.0% was procured from Aldrich chemistry. Formulations were procured from the local market.

Gas chromatography analysis was carried out using SHIMADZU GC2010 Gas Chromatograph equipped with split/splitless injector, a flame ionization detector was used for the measurements and glass capillary HP-5, ID: 0.32 mm, length: 30 m, film thickness: 0.25 µm; packed with non-polar polymer [(5%-phenyl)-methylpolysiloxane].

Preparation of internal standard stock solution (A): Dicyclohexylphthalate) (DCHP)

Approximately 0.0700 g of dibutyl phthalate was accurately weighed and transferred into 50 mL volumetric flask, dissolved and diluted to volume using HPLC grade dichloromethane.

Preparation of reference standard solutions (B)

Approximately 80 mg each of reference standard of spiroxamine, tebuconazole and triadimenol were weighed accurately and transferred into 25 mL volumetric flask. To this, 1 mL of internal standard stock solution (A) was added using a pipette and total volume was made up to the mark by HPLC grade dichloromethane.

Preparation of sample solutions (C)

Approximately 90 mg each of three formulations were accurately weighed individually and transferred into three different 25 mL volumetric flask. To this, 1 mL of internal standard stock solution (A) was added and the total volume was made up to the mark using HPLC grade dichloromethane.

Gas chromatographic conditions

Column*:* Glass capillary HP-5, ID: 0.32 mm, length: 30 m; film thickness: 0.25 µm; Carrier gas: Helium at a flow rate of 1.6 mL/min.; Injector mode*:* Split of 1:100; Injection volume*:* 1 µL; Injector temperature*:* 260 C; Oven temperature*:* Initial 200 C (12 min) @ 5 C/ min, to 280 C (5 min); Detector*:* Flame ionization detector at 280 C [7]

Method development and validation.

The method was validated for the determination of spiroxamine, tebuconazole and triadimenol for the following parameters; specificity, linearity, precision, accuracy, limit of detection, limit of quantitation [2].

Linearity

Linearity of the method was determined by plotting a calibration curve for spiroxamine, tebuconazole and triadimenol for concentration *vs.* detector response (area counts in mV). For this purpose, a combined standard stock solution containing spiroxamine, tebuconazole and triadimenol was prepared by dissolving about 65.5 mg (accurately weighed) each of the reference standards in 25 mL of volumetric flask and made up to volume with HPLC grade dichloromethane. From the combined standard stock solution of lindane and carbaryl, aliquots of 10, 5, 2., 1.0, 0.5, mL were taken into five individual 25 mL volumetric flask. To all the flasks, 1 mL of the internal standard solution (solution B) was added and the total volume was made up to the mark with dichloromethane. From each of these calibration standards 1 µL was injected into the GC. The calibration curve obtained was subjected to regression analysis by the least square method to calculate the calibration equation and the correlation coefficient (r).

Specificity

The specificity of the method was determined by analyzing the sample solution (solution C) containing all the components *i.e.* spiroxamine, tebuconazole , triadimenol and the internal standard that is DBP. For this purpose 1 µL of one of the sample solutions was injected into the gas chromatograph and the specificity of the method was measured in terms of the resolution between the two peaks.

Precision

The precision of the method was determined in terms of repeatability or reproducibility. Repeatability was determined by evaluating five replicates of the three different concentrations *i.e.* 10, 50 and 100 µg/mL of the combined calibration standard solution of spiroxamine, tebuconazole and triadimenol on the same day (intra-day) under the mentioned chromatographic conditions. Co-efficient of variation or the percent RSD was calculated in each case.

Accuracy

The accuracy of the method was evaluated by spiking different known concentrations of spiroxamine, tebuconazole and triadimenol into the pre-analyzed sample. One of the five sample solutions containing approx. 76 ppm each of spiroxamine, tebuconazole and

triadimenol was spiked` with varying standard concentrations of spiroxamine, tebuconazole and triadimenol *i.e.* 50 ppm of each, 100 ppm of each and 150 ppm of each so as to give a total concentration of 126 ppm, 176 ppm and 226 ppm**.** 1µL of each of these solutions was injected onto gas chromatograph and the closeness of the results to the true value was determined [2,4].

Limit of detection and limit of quantification

For the measurement of limit of detection, standard deviation of the background was determined. Thereafter, standard solutions of very low concentration levels *i.e.*0.1, 0.5, 1.0, 2.0 and 5.0 µg/mL were injected till the signal obtained was thrice the standard deviation of the background. For the measurement of limit of quantification again, known concentration of standard spiroxamine, tebuconazole and triadimenol solutions were injected till the signal obtained was reproducible for five replicate injections [4].

RESULTS AND DISCUSSIONS

The chromatogram (Figure 1) for the combined standard solution containing approximately 65 µg/mL of each spiroxamine, tebuconazole and triadimenol shows well resolved peaks for the spiroxamine (RT.3.1&3.3 min), tebucxonazole (RT. 5.9 min.). and triadimenol (RT. 4.0 min.). DCHP used as internal standard elutes at retention time, 6.2 minutes**.**

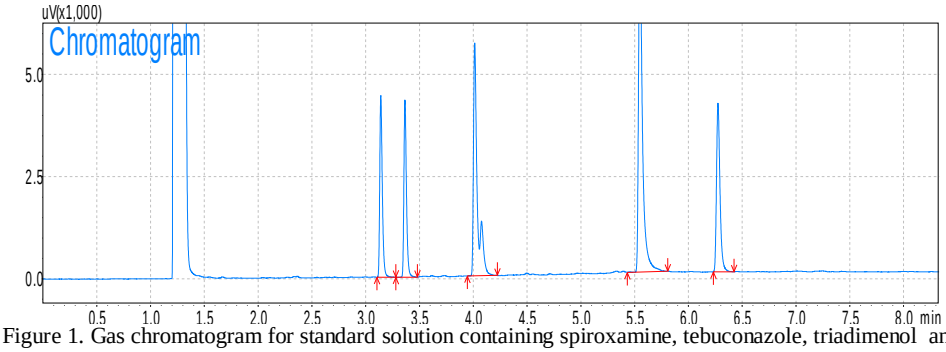


Figure 1. Gas chromatogram for standard solution containing spiroxamine, tebuconazole, triadimenol and DCHP (Internal standard).

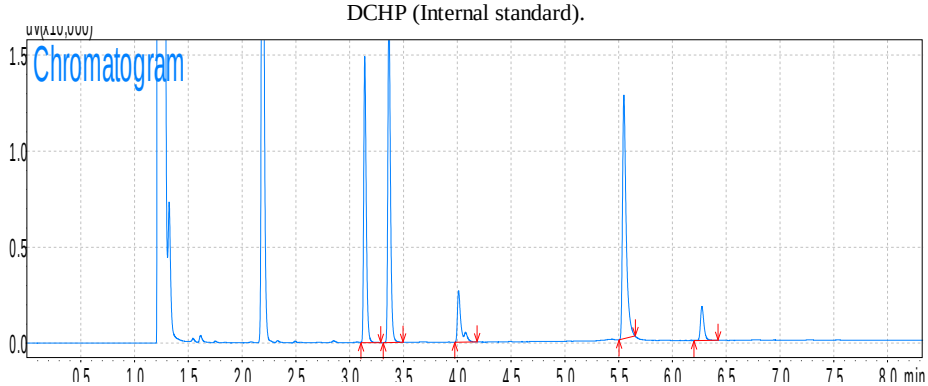


Figure 2. Gas chromatograms of six solutions of formulation. Peak at R.T.2.4 min is due to impurity from formulation.

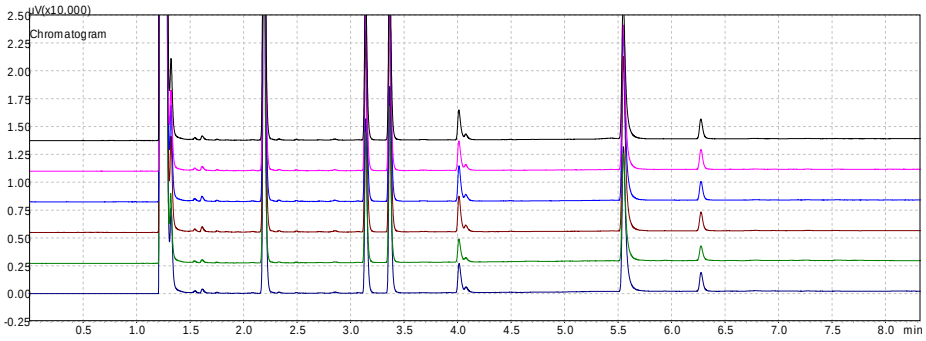


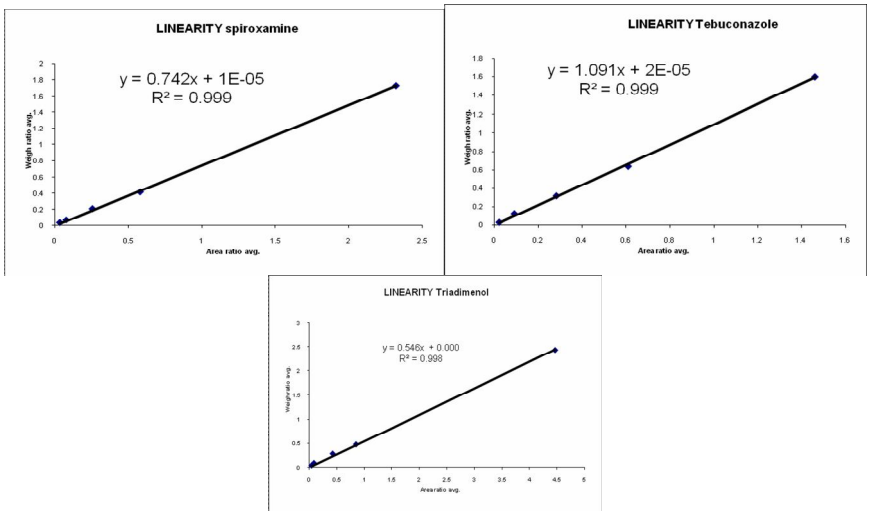
Figure 3. Chromatograms showing specificity of the method (a) dichlormethane solvent (R.T.1.2 min); (b) spiroxamine isomers reference standard (R.T.3.1, 3.3 min); (c) triadimenol reference standard (R.T.4.0 min); (d) tebuconazole reference standard (R.T.5.9 min); (e) DchP Reference Standard (R.T. 6.9 min)

VALIDATION OF THE DEVELOPED METHOD

Linearity

5 samples with double measurements, correlation coefficient spiroxamine: R2 = 0.9996, tebuconazole: R2 = 0.999, triadimenol: R2 = 0.9984, regression equation spiroxamine: y = 0.7421x + 0.0001, tebuconazole: y = 1.0916x + 0.0001 triadimenol: y = 0.5466x + 0.0001, chromatograms are given; The calculation of linearity has been performed by using absolute areas, whereas the determination is based on internal standard.

Calibration graphs were plotted for the calibration range 1 to 1000 µg/mL for spiroxamine, tebuconazole and triadimenol which gave linear response over the studied range of concentration and the least squares linear regression analysis of the data provided excellent correlation coefficient (r) of more than 0.999 for three compounds.



Precision

3 samples from one batch, duble injection; no outliers, acceptable according to the modified Horwitz equation, relative standard deviation of the active substances (RSD): spiroxamine: 1.07%, tebuconazole: 0.69%, triadimenol: 0.86%.

Accuracy

5 samples of laboratory-prepared synthetic formulation: spiroxamine: mean recovery: 99.97 %, RSD: 0.641 %; tebuconazole: mean recovery: 99.87 %, RSD: 0.364 %; triadimenol: mean recovery: 1 0 1 .99 %, RSD: 0.517%.

Specificity

The specificity of the method is indicated in Figure 3, which shows chromatogram for each of the individual components, *i.e.* spiroxamine, tebuconazole and triadimenol .The peak corresponding to each component elutes at different retention times, thereby causing no overlapping of the peaks.

Limit of detection and limit of quantitation

The limit of detection (LOD) was calculated on the basis of S/N (signal to noise) ratio of 1:3 and was found to be 0.5 µg/mL for three active ingredients. The limit of quantitation (LOQ) was obtained as 2.0 µg/mL evaluated on the basis of reproducible signal obtained on injecting 5 replicates of the same concentration.

CONCLUSIONS

A validated gas chromatographic method has been developed for the simultaneous determination of spiroxamine, tebuconazole and triadimenol in a formulation containing spiroxamine, tebuconazole and triadimenol using dicyclohexylphthalate (DCHP) as an internal standard. The work described in this paper has shown that the analytical method developed is precise, accurate and sensitive for the determination of three analytes. The method is specific to the analysis of spiroxamine, tebuconazole and triadimenol in the formulations without any interference from the other ingredients including the impurities. The developed method was successfully applied to the simultaneous quantitative analysis of spiroxamine, tebuconazole and triadimenol and therefore can be used for the routine quality control, ensuring efficiency and efficacy of the formulation for the desired purpose.

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