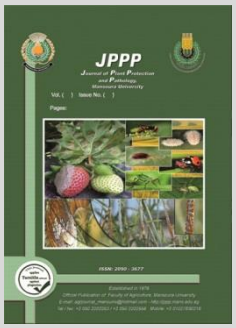
**Journal of Plant Protection and Pathology**

**Journal homepage: www.jppp.mans.edu.eg**
**Available online at: www.jppp.journals.ekb.eg**

# Analytical Method Determination of Penconazole in some Emulsifiable Concentrate (EC) Formulations Using Gas Chromatography

**Khalil, N. S.***

Central Agric. Pesticides Lab., Agric. Res. Center, Dokki, Giza 12618




## ABSTRACT

A method for simultaneous determination of penconazole content in some Emulsifiable Concentrate (EC) formulations has been described. Determination and quantification of penconazole were performed by gas chromatography equipped with a flame ionization detector (GC/FID) using an external standard of high and known purity. Validation parameters based on the Australian Pesticides and Veterinary Medicines Authority (APVMA) guidelines and ISO/IEC 17025 definition including method specificity, linearity, precision, accuracy, limit of detection (LOD) and limit of quantitation (LOQ) were evaluated. Under the optimum conditions, the linearity was found to be high with correlation coefficient values ($R^2$> 0.999) for the target penconazole formulations. The results showed precise and accurate method. The RSD% was in the range of 1.40% for formulation of 200 g (a.i.)/L and 1.43 % for formulation 100 g (a.i.)/L respectively. Selectivity showed no interference from any other possible adjuvants or components. It can be concluded that the GC-FID described method is reliable, suitable and successfully applied to the estimation of the target penconazole determination. GC- MS and IR spectroscopy were used as a type of quality control to ascertain the presence of penconazole.

***Keywords:*** GC - FID, penconazole, EC formulations, validation, GC - MS, APVMA

## INTRODUCTION

In recent years, the use of pesticides has been increased for quantity and quality of agricultural products (Ergonen *et al.*, 2005 and Melgar *et al.*, 2010). A large group of fungicides have been introduced in agriculture for the protection, prevention of plant diseases and improve crop yield (Hof *et al.*, 2001). Azoles especially triazoles fungicides are used on many different types of plants, including field crops, fruit trees and vegetables (Ribas *et al.*, 2016). These fungicides are highly effective against different fungal diseases, especially powdery mildews, rusts, and fungi/fungal including leaf-spotting. Penconazole is a systemic triazole fungicide with preventive and curative properties widely used for control powdery mildew (JMPR. 848, 1992; 2016 and Sun *et al.*, 2004). In Egypt it is currently recommended on different fruits and vegetables according to the Agriculture Pesticide Committee (APC), the competent authority responsible of the registration of agricultural pesticides (APC, 2020). Regardless their importance of pesticides uses, it is necessary for the analytical laboratories to assess simultaneous determination of active ingredient in order to provide accurate and reliable data of its concentration in different formulations. The accurate and reliable data are the basis for the decision making for complying or not complying of the pesticide formulations with the estimated specifications and regulations. For that reason, most and probably all countries have their regulatory authorities that responsible to monitor the quality of pesticide products. Also, there is a need for reliable and sensitive analytical methods that are able to quantify a large number of compounds even at the low limits set by legislation (Fintschenko *et al.*, 2010).

In this study, the diazole fungicide analyzed is Penconazole. It is primarily available in emulsifiable concentrate (EC) and emulation oil in water (EW) formulations. Penconazole was analyzed and quantified in two EC formulation products with concentrations 100 and 200 g a.i./L formulation. The aim was to develop and validate a simultaneous rapid method for the determination of the active ingredient penconazole in the commercial formulation products. The method performance for the determination meets the required criteria (European Guide, 1998; Taverniers *et al.*, 2004 and APVMA, 2004).

The results of validation of the GC-FID method for the measurement were based on the ISO/IEC 17025 definition (ISO/IEC 17025, 2005 and APVMA, 2004) with emphasized on the following validation parameters: method Specificity, linearity, precision (repeatability), accuracy, limit of detection (LOD) and limit of quantitation (LOQ).

## MATERRIALS AND METHODS

**Materials: EC penconazole samples**.

The EC formulation samples with concentrations 100 and 200 g (a.i.)/L formulation currently applied in Egypt and under registration process respectively (Topas 100 g/L and super-penco 200 g/L) were provided from the Research Department of Pesticide Analysis at the Central agricultural pesticides Laboratory with the analytical standard.

**Reagents and Standards**

Methanol HPLC grade

* **Corresponding author.**
**E-mail address: nasr_khalil@yahoo.com**
**DOI: 10.21608/jppp.2021.206738**

Analytical standard of known purity 97.5 – 99.5% as certified by manufacturer(s). The penconazole identity is shown in (Table 1).

**Equipment**

Analytical balance, capable of measuring to 0.1 mg

Ultrasonic bath

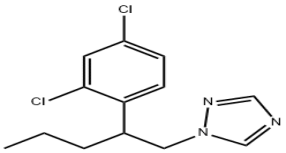
**Preparation of standard solution**

Weigh accurately 0.01 g of 100 % penconazole reference standard (0.0101 g of 99 % penconazole reference standard) into 25 ml volumetric flask and add 25 ml of methanol. Shake well to homogenize**.**

**Preparation samples solutions:**

For penconazole determination, weigh accurately a quantity of the sample (0.1 g penconazole 100 g/L EC and 0.05 g of penconazole 200 g/L EC) equivalent to 0.01 g of penconazole 100 %reference standard purity, into a 25 ml volumetric flask and add 25 ml of methanol. Shake well to homogenize.

**Table 1. Identity of Penconazole**

| **ISO Common name:** | **Penconazole** |
|---|---|
| Chemical name (s) | |
| IUPAC: | (RS)-1-[2-(2,4-dicholorophenyl)pentyl]-1H-1,2,4-triazole |
| CA: | 1-[2-(2,4-dichlorophenyl)pentyl]-1H-1,2,4-triazole |
| CAS Registry number | 66246-88-6 |
| CIPAC number: | 446 |
| Structural formula: |  |
| Molecular formula | C13H15Cl2N3 |
| Molecular mass | 284.2 g |

**Apparatus**

**GC – FID**

Apparatus GC was Agilent 7890B gas chromatograph equipped with a flame ionization detector (FID), a 7693B automatic sampler and a GC computerized data system.

**GC/FID Conditions:**

The conditions of GC were performed according to optimized analytical conditions as shown in (Table 2).

**Table 2. Optimized analytical conditions of GC – FID**

| **Apparatus** | **Agilent 7890B** |
|---|---|
| Column | capillary column HP-50+ (30 m x 0.53 mm I.D., 1 µm film thickness) |
| Oven column temperature | isothermal at 180ºC for 1 min, then ramp 20ºC /min to 260ºC (isothermal for 5 min), Detector FID at 250ºC, Injector 250ºC with splitless mode. |
| Carrier Gas | Nitrogen with Flow rate 8 ml/min |
| injection volume | 1 µl was employed |
| Total run time | 10 min. |
| H2 | 40 ml/ min |
| Air Flow | 400 ml / min |
| Retention time of penconazole | 5.31 min. |

**GC-MS analysis**

GC – MS was used as a quality control and qualitative analysis, and to prove no present of any other not specified active ingredient or banned components. The procedures were performed according to optimized analytical conditions as shown in (Table 3) and the GC-MS electron ionization mass spectrum of penconazole is shown in Fig. (3). The mass spectra were identified using Nist and Wiley mass spectral data base Library.

**Table 3. Optimized analytical conditions of GC / MS**

| **Apparatus** | **Agilent 7890B model, equipped with 5977 A MSD.** |
|---|---|
| Column | fused silica capillary column HP-5MS (30 m x 0.25 mm x 0.25 µm film thickness). |
| Temperature program | isothermal at 50ºC for 0.5 min, then ramp 10ºC /min to 190ºC (isothermal for 1 min), followed by ramp 10ºC /min to 300ºC and held for 2 min. The injector temperature was set at 280ºC. The mass spectra were identified using Wiley mass spectral data base Library. |
| Carrier Gas | Helium with 1.0 ml/min pulsed split mode. |
| injection volume | 1 µl was employed |
| Total run time | 28.5 min |
| Retention time of penconazole | 20.55 min. |

**Calculations**

**Active ingredient content, percent m/v = ((W1 X A2 X P) / (W2 X A1)) X D**

**Where**

**W1: mass in g of standard penconazole in standard solution. W2 = mass in g of sample taken for test. A1 = peak area of penconazole in the chromatogram of standard solution. A2: peak area of penconazole in the chromatogram of sample solution. P: percent purity of penconazole standard. D: density of formulation**

## RESULTS AND DISCUSSION

**Gas chromatography (GC – FID) analysis**

GC is used for analysis and determination of triazole fungicides e.g. propiconazole (FAO propiconazole specification, 2019). A certain volume of standard was injected into a GC system under optimized analytical conditions (Table 2). The out signal was monitored using GC ChemStation version installed by Agilent. Chromatographic conditions for confirmation of peak identity identification under the conditions selected was based on a retention time and concentration based on Area. The peak area for each injected was recorded and compared with reference standard.

**Method Validation Parameters**

Validation of the method was performed according to APVAMA (Australian Pesticides and Veterinary Medicines Authority) guidelines.

**Specificity**

Specificity was evaluated to ensure that nothing interfered with the target analyte which is penconazole in the current experiment. Injection of sample solvent (blank), penconazole sample and penconazole analytical standard, each injection alone under the optimized analytical conditions. Examinations of chromatograms showed no impurities interfered and no significant matrix peaks observed in the retention times.

**Linearity:**

The linearity of the calibration is the ability of analytical to induce a signal (response or test results) that is directly proportional to the concentration of the given analytical parameter. within a given concentration range. Linearity can be investigated for the method as a whole and thus becomes a trueness and as function of the concentration of the analyte.

In this study, the calibration curves established with five different concentrations levels 100, 200, 400, 800 and 1600 mg (a.i.)/L with three replicates for each level. Linearity of the method is usually expressed in terms of the results (area and absorbance). In this current study the linearly with the penconazole concentration is considered with the given working range. The linearity of the tested method is expressed by the regression coefficient value (Fig.1 and 2).

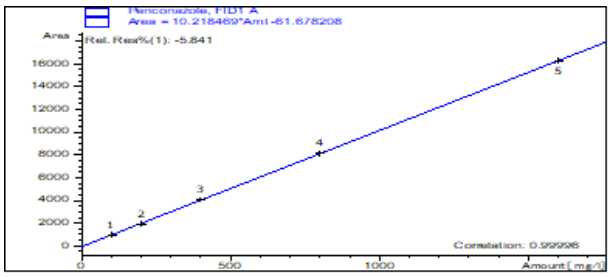



**Fig. 1. Linearity of the calibration curve of penconazole of active ingredient concentration 100 g/L EC**

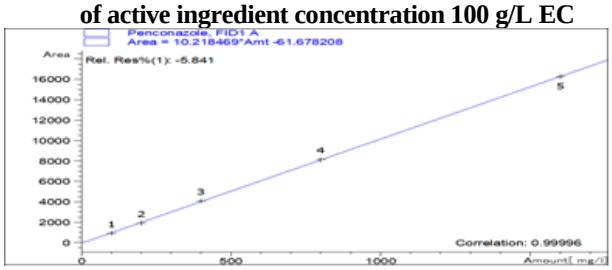



**Fig. 2. Linearity of the calibration curve of penconazole of active ingredient concentration 200 g /L EC**

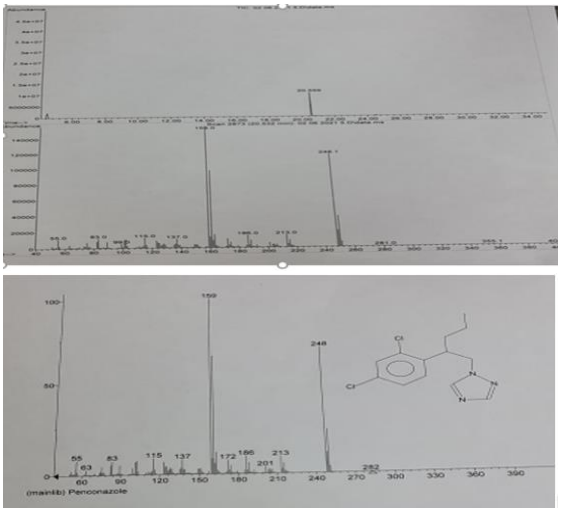


**Fig. 3. GC-MS electron ionization mass spectrum of penconazole from Nist and Wiley mass spectral data base Library.**

**Assay Accuracy and Precision**

**Precision:**

The precision of an analytical procedure expresses the closeness of a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be a measure of the degree of repeatability/reproducibility of the tested analytical method under worked condition. Repeatability was determined using two different concentrations of pesticide prepared and analyzed (Tables 5, 6 ,7 & 8). The precision of a tested analytical method is usually expressed as the standard deviation (SD) of a series of measurements. According to Australian Pesticides & Veterinary Medicines Authority (APVMA) guidelines for the validation of analytical methods, the accepted precession levels are shown in (Table 4).

**Table 4. Recommended levels of precision according to APVMA.**

| Component measured in sample | Precision |
|---|---|
| ≥10.0% | ≤ 2% |
| 1.0 up to 10.0% | ≤ 5% |
| 0.1 up to 1.0% | ≤ 10% |
| < 0.1% | ≤ 20% |

**Table 5. Repeatability, STDEV and RSD% for Penconazole 100 g/L EC with concentration 400 mg/L of EC formulation.**

| Prepared concentration 400 mg (a.i.) /L | Retention time RT: min | Area (Average of two injections) | Estimated concentration for the form. g/L |
|---|---|---|---|
| Rep. 1 | 5.314 | 4032.68213 | 99.73202615 |
| Rep. 2 | 5.312 | 4005.72754 | 99.06541376 |
| Rep. 3 | 5.313 | 4089.51282 | 101.1375026 |
| Rep. 4 | 5.314 | 4056.42163 | 100.3191263 |
| Rep. 5 | 5.313 | 3988.183715 | 9.863153845 |
| Rep. 6 | 5.310 | 4110.75781 | 101.6629112 |
| Rep. 7 | 5.312 | 4048.3833 | 100.1203309 |
| Rep. 8 | 5.312 | 4166.62476 | 103.0445535 |
| Mean | | 4062.286713 | 10.04641754 |
| SDEV. | | 58.27455459 | 0.14411846 |
| RSD% | | 1.434525889 | 1.434525889 |

**Table 6. Repeatability, STDEV and RSD% for Penconazole 100 g/LEC**

| Prepared concentration 100 mg (a.i.) /L | Retention time RT: min | Area |
|---|---|---|
| Rep. 1 | 5.309 | 902.79187 |
| Rep. 2 | 5.309 | 902.56238 |
| Rep. 3 | 5.307 | 907.9447 |
| Rep. 4 | 5.309 | 904.08673 |
| Rep. 5 | 5.309 | 893.44382 |
| Mean | | 902.1659 |
| SDEV. | | 5.33169372 |
| RSD% | | 0.590988167 |

**Table 7. Repeatability, STDEV and RSD% for Penconazole 200 g/L EC**

| Prepared concentration 400 mg a.i. /L | Retention time RT: min | Area Average of two injections | Estimated concentration g/L |
|---|---|---|---|
| Rep. 1 | 5.314 | 4765.957275 | 199.5974894 |
| Rep. 2 | 5.312 | 4808.40576 | 201.3752248 |
| Rep. 3 | 5.313 | 4688.79956 | 196.3661372 |
| Rep. 4 | 5.314 | 4700.85498 | 196.8710162 |
| Rep. 5 | 5.313 | 4832.491215 | 202.3839196 |
| Rep. 6 | 5.310 | 4890.358155 | 204.8073773 |
| Rep. 7 | 5.312 | 4763.809085 | 199.5075235 |
| Rep. 8 | 5.312 | 4751.500975 | 198.992062 |
| Mean | | 4775.272126 | 199.9875938 |
| SDEV. | | 66.99179863 | 2.805605263 |
| RSD% | | 1.402889655 | 1.402889655 |

**Table 8. Repeatability, STDEV and RSD% for Penconazole 100 g/L EC**

| Prepared concentration 100 mg (a.i.) /L | Retention time RT: min | Area |
|---|---|---|
| Rep. 1 | 5.309 | 1236.2428 |
| Rep. 2 | 5.309 | 1230.90649 |
| Rep. 3 | 5.307 | 1218.80933 |
| Rep. 4 | 5.309 | 1233.4364 |
| Rep. 5 | | 1237.88171 |
| Mean | | 1231.455346 |
| SDEV. | | 7.555881957 |
| RSD% | | 0.613573361 |

**Accuracy:**

The data generated in Tables (5 and 7), used to calculate the accuracy of the method where the accuracy was expressed as the recovery determined as the percentage of ratio of the concentration of penconazole detected relative to the concentration of penconazole. The results showed that recoveries lie between 98 and 106 % recovery.

**Limit of detection and Quantification (LOD &LOQ)**

The detection limit of an analytical procedure is the lowest amount that can be detected, but not quantitated as an exact value where the limit of quantification (LOQ) is the lowest concentration that could be determined with accepted accuracy and precision. To determine the LOD value, the S / N ratio was used as 3: 1 and for LOQ value, the S / N ratio was used as 10: 1.

The results showed that the estimated LOD and LOQ for the method are 0.18 mg and 0.63 mg respectively.

## CONCLUSION

The method described facilitates the quantitative and qualitative determination of penconazole in EC formulations. The procedure described is relatively fast, simple, precise, and applicable for routine pesticides analysis laboratories.

## REFERENCE


APC, 2020. Agricultural Pesticide Committee, Ministry of Agriculture and Land Reclamation – Egypt.

APVAMA, 2004. Australian Pesticides and Veterinary Medicines Authority. Guidelines for the validation of analytical methods for active constituent, agricultural and veterinary chemical products.

Ergonen, A.T, Salacin S, Ozdemir M.H, 2005. Pesticide use among greenhouse workers in Turkey. The Journal of Clinical Forensic Medicine. 12, 205–208 .

European Guide, 1998. The Fitness for Purpose of Analytical Methods A Laboratory Guide to Method Validation and Related Topics.. Available at https://www.eurachem.org/.

FAO, 2019. Specifications and evaluations for agricultural pesticides propiconazole.

Fintschenko Y, Krynitsky J.A, and Wong W.J, (2010). Emerging pesticide residue issues and analytical approaches, J. Agric. Food Chem. 58 5859–5861.

Hof, H. 2001. Critical Annotations to the Use of Azole Antifungals for Plant Protection. Antimicrob. Agents Chemother. 45, 2987–2990. [CrossRef] [PubMed]

ISO/IEC 17025: 2005**.** General requirements for the competence of testing and calibration laboratories. 2nd Edition.

JMPR. 848. Penconazole. 1992 19/11/15. Available online: http://www.inchem.org/documents/ jmpr/jmpmono/v92pr14.htm (accessed on 6 May 2016).

Melgar, M.J, Santaeufemia, M, Garcìa, M.A, 2010. Organophosphorus pesticide residues in raw milk and infant formulas from Spanish northwest, Journal of Environmental Science and Health, Part B, 45, 595–600.

Ribas, A.D.R.E.; Spolti, P.; Del Ponte, E.M.; Donato, K.Z.; Schrekker, H.; Fuentefria, A.M. 2016. Is the emergence of fungal resistance to medical triazoles related to their use in the agroecosystems? A mini review. Braz. J. Microbiol. 47, 793–799. [CrossRef]

Sun, X.H, Song, J.R., Tan, Z.C, Di, Y.Y, Ma, H.X, Wang, M.H, Sun, L.X, (2004). Heat capacity and enthalpy of fusion of penconazole, Thermochimica Acta, 413(1-2), 261-265.

Taverniers, I.; Loose, M.D.; Van Bockstaele, E. Trends in quality in the analytical laboratory, II: 2004. Analytical method validation and quality assurance. Trac-Trend Anal. Chem. 23, 535-552. DOI: http://dx.doi.org/ 10.1016/j.trac.2004.04.001.

Taverniers, I.; Van Bockstaele, E.; Loose, M.D. 2004. Trends in quality in the analytical laboratory I Traceability and measurement uncertainty of analytical results Trends in Anal. Chem. 23, 480-490 DOI: http://dx.doi.org/ 10.1016/S0165-9936 (04)00733-2.


## طريقة تحليل لتقدير مبيد بنكونازول في بعض مستحضرات المبيدات في صورة مركزات قابلة للإستحلاب EC بإستخدام جهاز كروماتوجرافي الغاز


**نصر صبحي خليل**

**المعمل المركزي للمبيدات ـ مركز البحوث الزراعية ـ الدقى ـ جيزة ١٢٦١٨ـ مصر**



في هذا الدراسة تم وصف طريقة لتحليل وتقدير محتوي المادة الفعالة بنكونازول (مبيد فطري) في بعض مستحضرات المبيدات والتي في صورة مركزات قابلة للإستحلاب(EC) Emulsifiable Concentrate. شملت الطريقة تحديد وتقديرالمادة الفعالة Penconazole (مبيد بنكونازول) بإستخدام جهازتحليل كروماتوجرافي الغاز المزود بكاشف تأين اللهب (FID) GC - flame ionization detector) وبإستخدام المادة القياسية للمبيد ذات النقاوة العالية والمعروفة (.Reference St). تم تقييم وإثبات صحة الطريقة بإستخدام معايير التحقق المستندة إلى إرشادات هيئة مبيدات الآفات والأدوية البيطرية الإسترالية (APVMA) وتعريف معايير الإعتماد (أيزو) ISO / IEC 17025 . وتشمل هذه المعايير التي شملتها الدراسة، الاختصاص النوعي أو خصوصية الطريقة (Specificity)، الخطية وتشمل المدي (Linearity)، الإحكام – تكرار القياس (Precision)، الدقة (accuracy) و حدود القياس الكمي (Limit of detection & quantitation). أظهرت النتائج في ظل الظروف المثلى للطريقة المستخدمة أن معامل الارتباط الخاص بالخطية (linearity) (R2> 0.999) لمستحضرات مبيد البينكونازول المستهدفة. كما أظهرت النتائج أن الإنحراف النسبي RSD ٪ في حدود ١,٤٠ ٪ للمستحضرات ذات التركيز ٢٠٠ جم (مادة فعالة) / لتر و ١,٤٣ ٪ للمستحضرات ذات التركيز ١٠٠ جم (مادة فعالة) / لتر على التوالي. لم تظهر النتائج أي تداخل من أي مواد مساعدة أو مكونات أخرى محتملة في مستحضرات المبيدات مع المادة الفعالة وتم استخدام جهازي GC-MS و IR لهذا الغرض أيضاً و كنوع من مراقبة الجودة للتأكد من وجودالمادة الفعالة بنكونازول في المستحضرات.والمستخاص أن الطريقة موضع الدراسة بإستخدام جهاز GC-FID مناسبة، دقيقة ويمكن تطبيقها بنجاح لتحديد وتقدير المادة الفعالة Penconazole للمستحضرات موضع الدراسة في المختبرات الروتينية لتحليل المبيدات .