Determination of Carbendazim in Suspension Concentrates by High-Performance Liquid Chromatography (HPLC)

Parra, J.; Matamoros, O.; Barrantes, D. Formulaciones Químicas S.A.: Puntarenas, Costa Rica.

■ *ABSTRACT*

An analytical method using high-performance liquid chromatography (HPLC) is presented for the quantification of carbendazim (methyl 2-benzimidazole carbamate) in commercial formulations. The procedure employs a C18 octadecyl reversed-phase column and ultraviolet (UV) detection at 254 nm. Analyte extraction is performed using methanol, with carbaryl as an internal standard to correct for variations in the analytical process. Statistical calculations of repeatability, ANOVA, and trueness justify the sensitivity and applicability of the method as a simple way to evaluate the quality of concentrated suspensions.

■ INTRODUCTION

Carbendazim, $C_9H_9N_3O_2$ or methyl *1H-benzimidazol-2-yl carbamate* (MBC), CIPAC 263, is a broad-spectrum systemic fungicide widely used to control various pathogens in agriculture. Its most frequent application is as a post-harvest treatment for the control of pathogenic fungi in fruits, vegetables, ornamental plants, and grasses. This molecule is commonly formulated as suspension concentrates (SC), given its chemical nature that allows it to disperse easily in water with the aid of high-HLB surfactants. The determination and regulation of this substance are essential, as oral exposure can lead to its decomposition into secondary metabolites such as 5-hydroxy-2-benzimidazole carbamate (5-HBC), which exhibits mutagenic and endocrine-disrupting effects in mammals.

Quantitative determination of this carbamate by gas chromatography (GC) presents significant difficulties due to its thermal degradation and column adsorption phenomena. On the other hand, direct spectrophotometric techniques frequently lack the necessary specificity in the presence of the degradation subproducts. Consequently, reversed-phase HPLC is selected as the optimal alternative due to its ability to operate at room temperature and its high resolution.

■ EXPERIMENTAL

Instrumentation: A high-performance liquid chromatograph (HPLC) equipped with a UV detector (Waters Model 48 or equivalent) and an octadecyl C18 reversed-phase column (250 × 4.6 mm, 5 µm) was utilized.

Reagents: Technical-grade carbendazim, technical-grade carbaryl, and HPLC-grade methanol were used.

Solution Preparation:

1. **Technical Active Ingredient (IAGT) Solution:** Technical - grade carbendazim (25 mg) was weighed on an analytical balance and dissolved in 100 mL of methanol.
2. **Internal Standard (STD) Solution:** Technical - grade carbaryl (250 mg) was dissolved in 500 mL of methanol.

3. **Working Standard Solution:** Technical - grade carbendazim (25 mg) was dissolved in 100 mL of the internal standard solution (Solution B).
4. **Sample Preparation:** An amount of commercial formulation (SC) equivalent to 25 mg of carbendazim was weighed, dissolved in 100 mL of the internal standard solution, and mechanically stirred for a few minutes. The mixture was then filtered through a 25-mm diameter, 0.45-µm Econofilter nylon membrane (Agilent®).

Operating Conditions: A flow rate of 1 mL/min, a run time of 7 min, a detection wavelength of 254 nm, and a mobile phase consisting of acetonitrile and water (60:40, v/v) were employed.

Environmental Conditions: To ensure precise and reliable analytical results, the laboratory temperature must be maintained between 20 °C and 28 °C, while the relative humidity must be kept between 20% and 80%.

■ RESULT & DISCUSSION

Below, the discussion of the results obtained from the chromatographic analysis of the working standard and a commercial sample of a carbendazim suspension concentrate (SC) of approximately 50% w/v is developed, integrating a comprehensive descriptive statistical analysis.

Repeatability measures the variability of results over a short period under the same operating conditions (same analyst, instrument, and reagents). To evaluate the precision of the calibration system and the analytical method, the chromatographic peak areas for carbendazim and carbaryl (IS) were compiled, and their peak area ratios were calculated. The mean, variance, standard deviation, and RSD were obtained for the peak areas of both standards and the carbendazim sample:

Mean: $\bar{x} = \frac{\sum_{i=1}^{n} x_i}{n}$ [1]

Sample Variance: $s^2 = \frac{\sum_{i=1}^{n} (x_i - \bar{x})^2}{n-1}$ [2]

Standard Deviation: $s = \sqrt{s^2}$ [3]

%RSD: $(\text{RSD }\%) = \left(\frac{s}{\bar{x}}\right) \times 100$ [4]

Table I. Results of peak area integration obtained from HPLC determinations of standards and samples.

.

Compound	*Mean ($\bar{x}$)*	*Variance (s^2)*	*Standard dev. (s)*	*RSD (%)*
Carbendazim (Analyte)				
Standard	3589245	111810176,3	10574,03	0,29%
Sample (50 SC)	4592231	2633565313	51318,27	1,12%
Carbaryl (Internal Standard)				
Standard	10077359	222559550,3	14918,43	0,15%
Sample (50 SC)	10443791	239432844,5	15473,62	0,15%

Based on the obtained results, the mass percentage of carbendazim in the sample is calculated using Equation 5.

Equation [5]:

$$\%\text{Carbendazim} = \frac{A_{\text{Carben-M}}}{A_{\text{Carba-M}}} \times \frac{A_{\text{Carba-STD}}}{A_{\text{Carben-STD}}} \times \frac{mg_{\text{Carben-STD}}}{mg_{\text{Carben-M}}}$$

Where:

- $A_{\text{Carben-M}}$: Carbendazim sample area.
- $A_{\text{Carba-M}}$: Carbaryl sample area.
- $A_{\text{Carba-STD}}$: Carbaryl STD area
- $A_{\text{Carben-STD}}$: Carbendazim STD area.
- $mg_{\text{Carben-STD}}$: mg of carbendazim on STD.

- $mg_{Carben-M}$: mg of carbendazim on sample
- P: Purity of the carbendazim analytical standard.

Equation 5 was used to determine the concentration of carbendazim present in the commercial sample, which corresponds to a suspension concentrate (SC). The obtained results are tabulated in the following table.

Table II. Mass percentage of carbendazim present in the suspension concentrate.

Statistical Parameter	*Value*
Replica 1	50,155
Replica 2	50,848
Mean	50,5015
Variance	0,2401
Standard Deviation	0,49
RSD (%)	0,9703

According to Miller & Miller, an RSD below 1% demonstrates excellent precision and repeatability of the extraction and analytical method for quantification in commercial formulations, such as the one evaluated in this study. The relative standard deviation of the standard (0.29% according to Table I) and the sample (0.97%) are well below the commonly established acceptance limit for HPLC methods in formulation quality control, which justifies that the chromatographic system possesses excellent instrumental repeatability. Furthermore, the use of peak area ratios relative to the internal standard guarantees very tight control over the dispersion of the chromatographic data.

To verify whether a systematic bias exists in the quantification of carbendazim using this analytical method, the average concentration obtained from the commercial sample of the suspension concentrate was compared against its declared nominal value of 50% active ingredient, employing a one-sample Student's t-test for small sample sizes using the following equation:

$$t = \frac{(\bar{x}-\mu)\cdot\sqrt{n}}{s} \qquad [6]$$

t = 1,44

For a sample size of n = 2, the associated degrees of freedom are (v = n - 1); thus, (v = 1). The critical value of the two-tailed distribution at a 5% significance level is $t_{critical}$ = 12,7062. This value is substantially larger than the calculated t-value of 1.44; consequently, the null hypothesis (H_0), which asserts that there is no significant difference between the nominal or declared concentration of the product (reported as 50 SC) and the mean concentration obtained by the HPLC determination method, is accepted as true (confirming the trueness of the method).

To further examine the structure of the chromatographic system's variability and to assess whether there is a statistically significant difference in the peak area ratios obtained for the working standards and the commercial carbendazim 50% suspension concentrate (SC) sample, a one-way analysis of variance (ANOVA) was performed. Although chemical differences in the ratios are expected because they correspond to different concentration levels, this analysis serves to demonstrate the power of the model and to evaluate the variability both within the groups (residual variability or instrumental error) and between the groups.

Total sum of squares:

$$SS_{total} = \sum_{i=1}^{p} \sum_{j=1}^{n_i} \left(x_{ij} - \bar{x}_{grand}\right)^2 \quad [7]$$

Between-groups sum of squares:

$$SS_{between} = \sum_{i=1}^{p} n_i \left(\bar{x}_i - \bar{x}_{grand}\right)^2 \quad [8]$$

Within-groups sum of squares (residual):

$$SS_{within} = \sum_{i=1}^{p} (n_i - 1)\, s_i^2 \quad [9]$$

F-statistic:

$$F = \frac{MS_{between}}{MS_{within}} = \frac{SS_{between}/(p-1)}{SS_{within}/(N-p)} \quad [10]$$

Using Equations 7 to 10, the parameters required to discriminate the variance of the obtained results are established. The results are tabulated in the following table:

Table III. ANOVA parameters.

Statistical Parameter	*Between Groups*	*Within Groups*	*Total*
Sum of Squares (SS)	8,37x10^{-3}	2,02x10^{-5}	8,39x10^{-3}
Degrees of Freedom (df)	1	3	4
Mean Square (MS)	8,37x10^{-3}	6,73x10^{-6}	—
F-statistic	**1243.13**	—	—
p-value	5,02x10^{-5}	—	—
Critical F (95% conf.)	**10.128**	—	—

The calculated test statistic, defined as the ratio of mean squares $MS_{between}/MS_{within}$, was 1243.13 units. Comparing this result with the boundary value of the distribution at a 5% significance level (0,05), it significantly exceeds the critical value from Miller & Miller's tables ($F_{critical}$ = 10,128), yielding a remarkably small p-value. Therefore, the strong rejection of the null hypothesis (which states that there is no difference between the averages) at a 95% confidence level is fully consistent with the experimental design of this methodology. The pure standard and the commercial carbendazim 50% suspension concentrate (SC) formulation were subjected to independent dilution and extraction processes; thus, the significant difference between their area ratios demonstrates that the method discriminates with outstanding sensitivity the variations in analyte concentration within the working range.

According to the convention of the Eurachem Guide (Section 6.2) and IUPAC criteria, the estimation of the Limit of Detection (LOD) and the Limit of Quantification (LOQ) of an instrumental method is based on the standard deviation of signals at low concentration levels. By tabulating the peak area data from Table I, the following function is obtained (see Appendix 1)

$$f(x) = 3.07823 \cdot x \quad [11]$$

The standard deviation of the working area ratio is approximately s_{ratio} = 0,0010, which allows for the estimation of the limit of detection and quantification of the method. The standard deviation of the ratios expressed in concentration, s_c is obtained as follows:

$$s_c = \frac{s_{ratio}}{b} \quad [12]$$

From Equation 12, a value of is obtained. By multiplying this value by specific absolute factors, the respective detection and quantification limits of the HPLC method for carbendazim determination can be established:

$$LOD = 3s_c = 0{,}00098 \quad [13]$$

$$LOQ = 10s_c = 0{,}00328 \quad [14]$$

To express these limits in concentration units of carbendazim injected into the HPLC system, the obtained ratio is multiplied by the fixed concentration of carbaryl (internal standard) used in the

working solutions (500 mg/L, prepared by dissolving 250 mg of technical - grade carbaryl in 500 mL of methanol):

Absolute LOD = (0,00098) (500 mg/L)
= 0,49 mg/L
Absolute LOQ = (0,00328) (500 mg/L)
= 1,64 mg/L

■ CONCLUSIONS

The alternative analytical method by reversed-phase HPLC using a C18 column and UV detection proved to be a chemometrically and analytically robust tool for the determination and quality control of carbendazim in suspension concentrate (SC) formulations. The use of carbaryl as an internal standard was proven to be highly selective and effective for correcting and normalizing any instrumental or injection volume variations in the chromatographic system.

The method exhibits excellent precision under repeatability conditions, with a mass repeatability standard deviation of only 0.49% and a relative standard deviation (RSD) of 0.9703% for the commercial sample. Being significantly below the suggested limit of 2.0% for this type of concentrated formulation, the rapid methanol-assisted extraction process is validated as a highly reproducible and optimal preparation protocol for routine laboratory analyses.

The trueness of the analytical method was thoroughly confirmed by a one-sample Student's t-test. The average carbendazim concentration obtained (50.50%) compared to the manufacturer's nominal value (50.0%) showed no statistically significant differences at a 95% confidence level, guaranteeing an accurate method free of systematic bias or errors.

The method demonstrated high metrological sensitivity, with a Limit of Detection (LOD) of 0,49 mg/L and a Limit of Quantification (LOQ) of 1,164 mg/L of injected carbendazim. This defines a safe working and linear range extending from the LOQ to concentrations above 55 mg/L. Within this range, the method operates with negligible analytical uncertainty, ensuring the delivery of reliable and technically valid results.

The described method is simple, rapid, and highly sensitive, allowing the precise identification and quantification of carbendazim at low concentrations. The implementation of carbaryl as an internal standard ensures the stability of the results against fluctuations in flow rate or injection volume. This protocol is suitable for integration into the quality control and stability programs of Formuquisa, meeting the technical requirements for analytical validation.

■ REFERENCES

Miller, J. C.; Miller, J. N. *Statistics and Chemometrics for Analytical Chemistry*, 6th ed.; Pearson Education: Harlow, Essex, Reino Unido, 2010.

Morillas Bravo, P. P., Colabs. *Guía Eurachem: La adecuación al uso de los métodos analíticos – Una Guía de laboratorio para la validación de métodos y temas relacionados*, 1ª ed.; Eurolab España: Madrid, España, 2016.

Mathur, R. P.; Sharma, S.; Bhushan, R. HPLC Determination of Carbendazim in Formulations. *J. Liq. Chromatogr.* **1988**, *11* (12), 2621–2628.

Hashim, M.; Al-Attar, A.; Abu Zeid, I. Toxicological Effects of Carbendazim: A

Review. *Acta Sci. Med. Sci.* **2023**, *7* (7), 218–225. DOI: 10.31080/ASMS.2023.07.1528.

■ APPENDIX

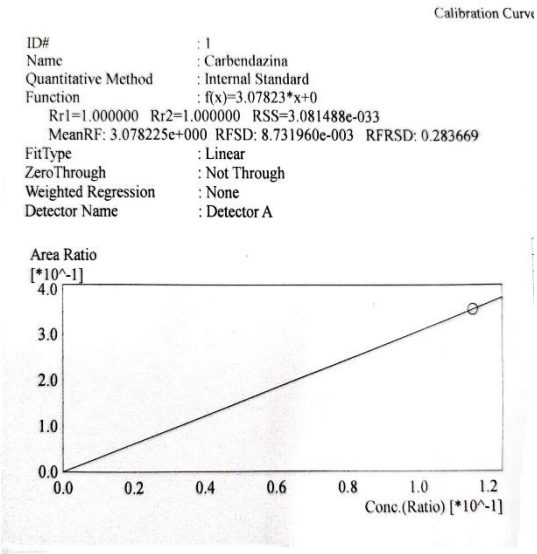

Figure 1. Instrumental calibration curve obtained from peak area ratios using HPLC-UV.

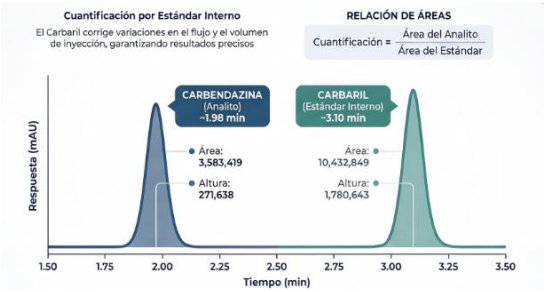

Figure 2. Chromatographic profiles obtained by HPLC-UV at 254 nm.