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Development and validation of an innovative HPLC-UV method for determination of Metalaxyl-M in flowable suspension fungicide formulas

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A B S T R A C T

Metalaxyl-M is a modern and more efficient version of metalaxyl which is designated to provide effective control of damaging fungal diseases. This work aimed to develop and validate a low-cost, accurate, fast, and simple analytical method for determination of active ingredient metalaxyl-M using high-performance liquid chromatography with ultraviolet detection (HPLC-UV). The development of the novel method was performed on reversed-phase brownlee™ C-18 column at constant temperature 30 ºC. The mobile phase consists of acetonitrile and distilled water in volumetric ratio 62.5:37.5, a flow rate of 1.2 mL/min, and the detective wavelength at 270 nm. The validation protocol underscore in our study was SANCO/3030/99 rev. 5. Specificity, linearity, LOD, LOQ, precision, recovery, and accuracy parameters were calculated and discussed. The values for multiple correlation coefficient ($R^2 \geq 0.990$), relative standard deviation (RSD < 1.5 %), recoveries ranged from (99.21 - 101.16 %) were found within acceptance criteria.

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1. Introduction

Pesticides are agents formulated to eliminate, control, destroy, or deter pests that hinder agricultural operations. The accumulation of pesticides in the plant's parts is not only the major problem but the transfer of it through air, soil, and water, destroying ecosystems.[1,2] So that, there is a strong need for the design of new analysis methods for determination of active ingredients in pesticide formulas to avoid its circulation at unauthorized high concentrations that cause harmful effect on plants and the ecosystem. There are different types of pesticides formulas as reported.[3] The main formula that discussed in the articles was Flowable Suspensions (FS). Flowable suspensions (FS) is the modified version of suspension concentrates (SC) which both have identical manufacturing technologies, the only difference was that more thickeners and antisettling agents are typically added to avoid separation of the dispersed phase because of its applied straight to the seeds without dilution. The flowable suspensions (FS) is applied directly to the seed surface, facilitating the direct penetration of pesticides into the target, hence greatly enhancing pesticide utilisation and augmenting agricultural production. Nowadays, it is widely used in the safest and most effective manner in essential important crops, such as maize.[4,5]

Metalaxyl-M is an (R)-enantiomer of a chiral compound that called metalaxyl which is thousand time more active than its (S)-enantiomer.[6] Metalaxyl-M active ingredient (**Fig. 1**), generally used in different pesticides formulations such as suspension concentrate (SC) or Flowable Suspensions (FS) for protecting seed germination and the initial growing stages of crop plants. Mode of action for metalaxyl-M can be concluded that metalaxyl-M disrupts the growth of the mycelium and spores.[7] It was found in literature surveys,[8,9] that metalaxyl-M can be estimated separately or simultaneously with chlorothalonil. Our innovation addresses a chromatographic analytical method that is accurate and precise required to generate data for authorization and post-registration control and monitoring purposes for a product Flowdo-Z 5% FS® which

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consists of two active ingredients 2.5% metalaxyl-M and 2.5% fludioxonil. The mentioned product that is under study in the article produced by KZ for pesticides and chemicals. It is well-developed and designed to provide effective control of damaging fungal diseases in crops.

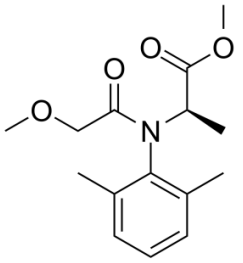

Fig. 1. Chemical structure of Metalaxyl-M.

2. Materials and Methods

2.1 Reagents and chemicals

The analytical standard of metalaxyl-M with a purity of 92% w/w manufactured by Zhejiang Heben Pesticide & Chemicals Co., Ltd, China was granted from Kafr El-Zayat for Pesticides and Chemicals Company, Egypt. Pure HPLC-grade acetonitrile purchased from Scharlab in Spain. MS® 0.45 µm nylon membrane filters procured from Membrane Solutions, LLC, located in Seattle, USA.

2.2 Equipment

The chromatographic analysis was performed on a PerkinElmer HPLC system consisted of a Series 200 liquid chromatograph, a Series 200 UV/vis spectrophotometric detector has a range of 190 to 700 nm, and the pump can generate pressure up to 6100 psi. Utilising a computer system running Microsoft's approved operating system (Windows XP-pack 2) for data handling output. Brownlee™ C-18 reversed phase analytical column (stainless steel, 250 × 4.6 mm i.d., 5 µm). Elmasonic S40H ultrasonic bath used for degassing the mobile phase.

2.3 Preparation of Standard Solutions

Stock solutions of metalaxyl-M were prepared by dissolving 51.8 mg of the pure analytical standards in acetonitrile in a 100 ml volumetric flask. Then, the prepared stock solution was ultrasonicated for 5 min and stored in a refrigerator at 4 ºC. In order to prepare five different standard concentrations 51.8, 103.6, 155.4, 207.2 and 259 µg/mL. These standard concentrations were prepared by taking (1, 2, 3, 4 and 5) mL of each stock solution and completed by acetonitrile into a 10 mL volumetric flask.

3. Results and Discussion

Development of a new analytical method for measurement of active component metalaxyl-M in wastewater was done using HPLC-UV. Separation of this component was carried out using a Brownlee™ C-18 reversed phase analytical column (stainless steel, 250 × 4.6 mm i.d., 5 µm). Generally, C-18 columns are made to have great peak shape and high efficiency with all kinds of samples. They are especially beneficial when using reverse-phase liquid chromatography (RP-LC), to separate acidic, basic, and other highly polar substances.[10] It was concluded that the mobile phase, which included acetonitrile/water (62.5/37.5, V/V%), isocratic elution with a flow rate of 1.2 mL/min, constant column temperature at 30 ºC, and UV detection at 270 nm, produced the best separation and symmetrical peak shape of the metalaxyl-M analytical standard. The chromatographic peak of metalaxyl-M was high, thin, and symmetrical under these chromatographic conditions, and a smooth baseline was produced (**Fig. 2**). The retention time of metalaxyl-M was 3.52 min, while the run time of analysis was roughly 5 min. Because of the short run time, only a little amount of organic solvent is needed for this analysis, which makes this procedure both economically and ecologically friendly. The optimized chromatographic conditions for this new method were summarized in **Table 1**.

Table 1. Optimized chromatographic conditions for metalaxyl-M developed method.

No.	Parameter	Condition
1	Equipment	PerkinElmer HPLC Series 200
2	Column	Brownlee™ C-18 reversed phase column (250 × 4.6 mm, 5 µm)
3	Mobile phase	Distilled water: Acetonitrile (37.5:62.5) (v/v)
4	Flow rate	1.2 ml/min
5	Injection Volume	20 µL
6	Wavelength	270 nm
7	Column temperature	30°C
8	Mode of separation	Isocratic
9	Run time	5 min.
10	Retention time	Metalaxyl-M: 3.518 min.

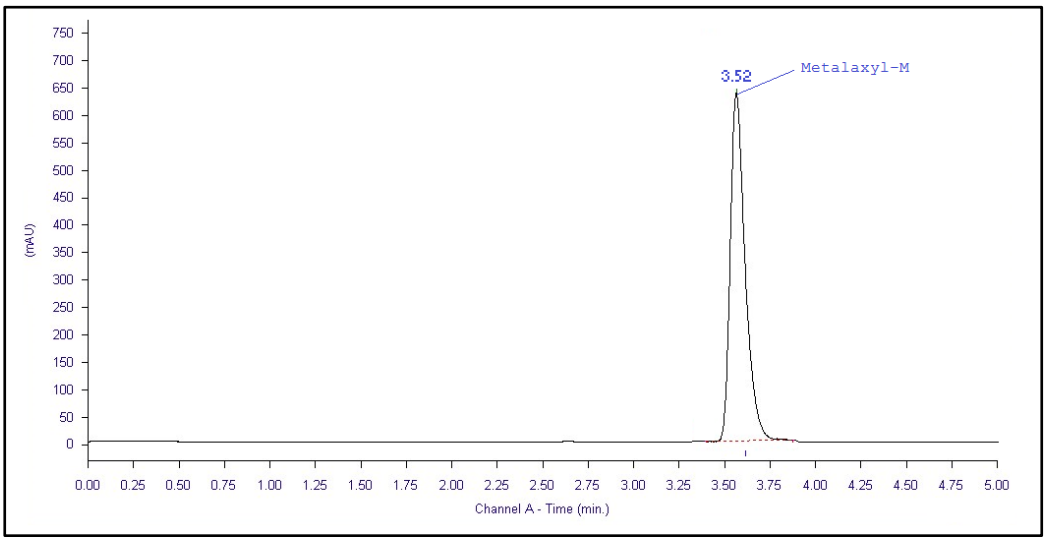

Fig. 2. illustrates the chromatogram of metalaxyl-M analytical standard analyzed in this study.

The established method was validated by testing specificity, selectivity, linearity, range, recovery, LOD, LOQ, precision, and accuracy in compliance with SANCO guidelines,[11] to see whether it was suitable for its recommended application. The specificity and selectivity of the new applicable method were assessed by detecting the peak of interest and determining the index of peak purity. The identification of the analyte was conducted by comparing its retention time in the standard solution with that in the sample. The chromatograms of the blank sample that contain all formulation component except the active ingredients (**Fig. 3**) indicate that, there are no coeluted peaks that cause interference at the retention time mentioned for metalaxyl-M as shown in (**Fig. 4**) that proven the selectivity of the new developed method.

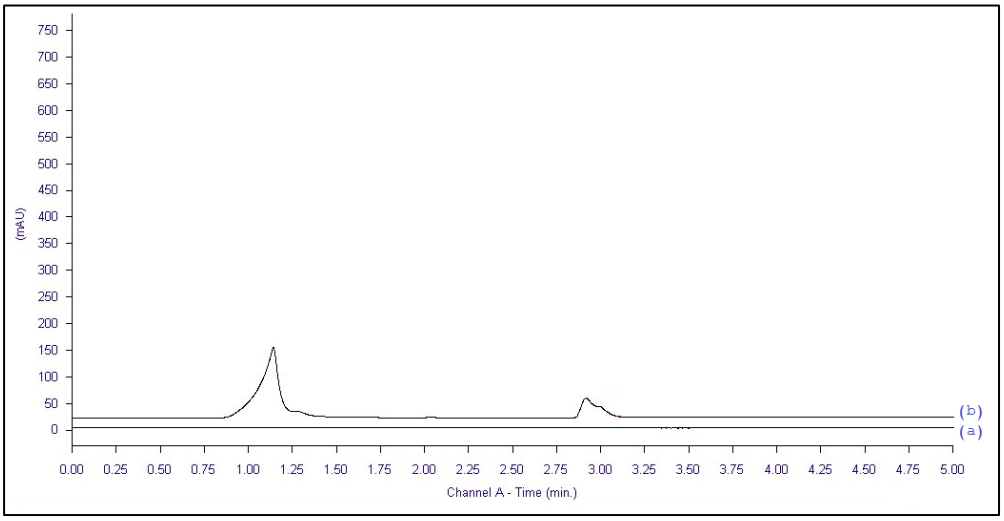

Fig. 3. (a) Represents the HPLC-UV base line of mobile phase, (b) illustrates the chromatogram of blank sample.

The linearity and range of the method were evaluated by creating calibration curves that illustrate the relationship between peak area and the injected quantity of analyte (**Fig. 5**). The correlation coefficient (R^2) criteria should be more than 0.990.[12] A set of five working standard solutions with varying concentrations between 51.8 and 259 µg/mL have been prepared for this purpose. The developed method exhibits high linearity with non-linear trends or outliers according to the findings obtained in the **Table 2**. The limit of detection (LOD) and the limit of quantitation (LOQ) can be calculated from the linearity of the calibration curve using the following equations,[12,13] $LOD = 3.3\sigma/S$ and $LOQ = 10\sigma/S$,respectively. According to the results data, the LOD was 0.422 µg/mL and the LOQ was 1.28 µg/mL. These results have proven the sensitivity of the method at lower concentrations.

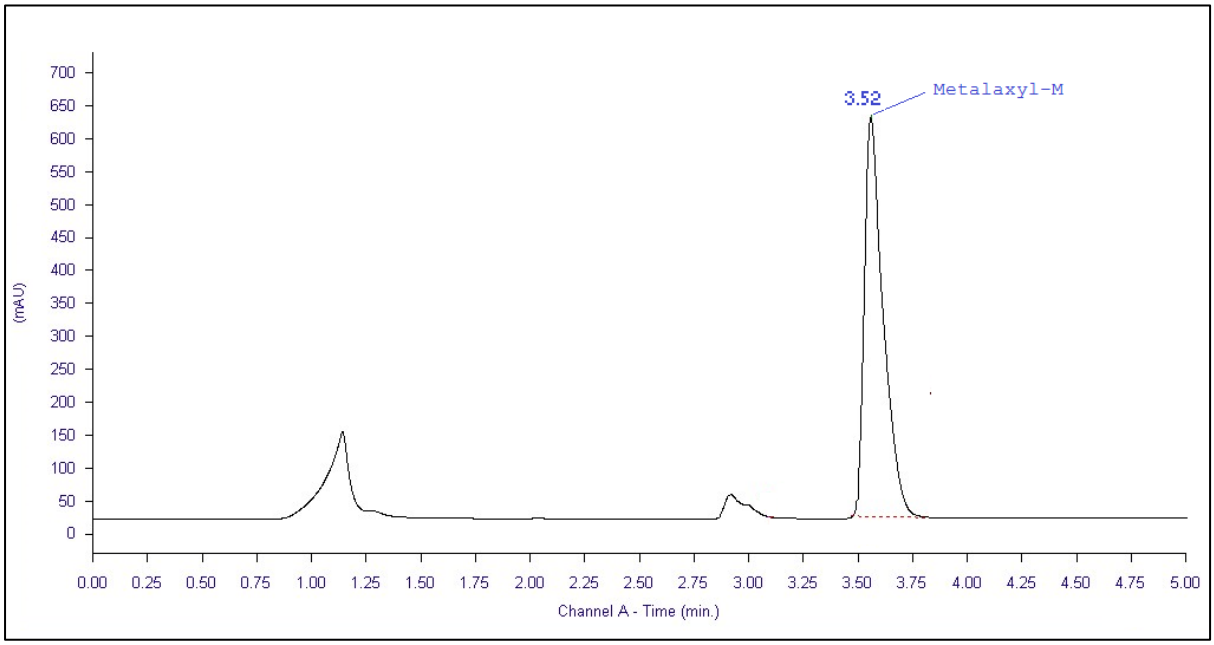

Fig. 4. The chromatogram of the FS formula containing metalaxyl-M active ingredients.

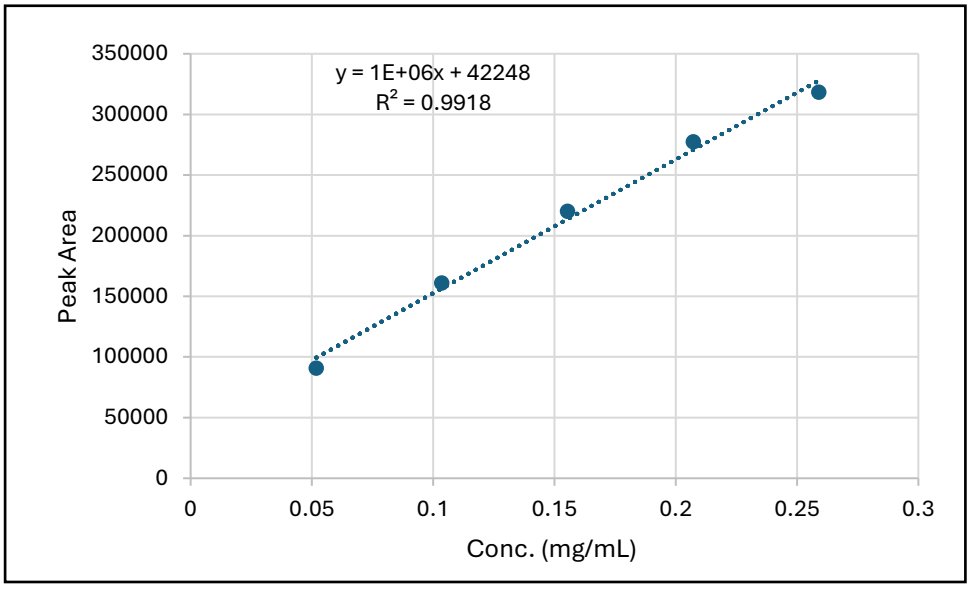

Fig. 5. The linear response of peak area against metalaxyl-M concentration.

Table 2. Range and linearity of the newly developed method.

Conc. (mg/mL)	Replicates	Peak Area (P. A.)	Average P. A.	Statistical data	
0.0518	R_1	91040.09	90986.14	R^2	0.992
	R_2	90076.05			
	R_3	91842.29			
0.1036	R_1	161823.71	161083.72	Regression equation	$y = 10^6 x + 42248$
	R_2	161505.70			
	R_3	159921.75			
0.1554	R_1	219073.85	220311.42	Linearity range (µg/mL)	51.8- 259
	R_2	222664.11			
	R_3	219196.31			
0.2072	R_1	277912.72	277452.52	LOD (µg/mL)	0.422
	R_2	276950.94			
	R_3	277493.91			
0.259	R_1	318874.38	318500.13	LOQ (µg/mL)	1.28
	R_2	318840.65			
	R_3	317785.38			

Precision of the method was expressed by measuring repeatability of the obtained results. The relative standard deviation (RSD) of the peak areas of three replicate injections of standard solutions with five concentrations of calibration curve 51.8, 103,6, 155.4, 207.2, and 259 ppm was calculated to assess the repeatability of this new method. The results were in between 0.17% and 0.97% that was less than the criteria 1.5%.[12] These findings demonstrate a very good precision of the new method.

Accuracy is the parameter responsible for evaluating the degree of agreement between the value that is identified as true or as a reference and the result obtained by the method being evaluated.[14] It is determined by assessing the analyte recovery percentage (R%) according to equation The accuracy results of the tested range (33.33% -166.67%) of the target concentration of 100% (100 µg/mL) were found to be within the range of acceptable criteria levels (98–102%),[14] as demonstrated in the **Table 3**.

Table 3. Shows accuracy and recovery results of the newly developed method.

Theoritical Conc. (%)	Replicaes	Peak Area	Actual Conc. (%)	Mean Actual Conc. (%)	R(%)
33.33	R_1	91413.63	33.35	33.66	101.00
	R_2	93866.52	34.24		
	R_3	91554.68	33.40		
100	R_1	223064.71	99.65	99.21	99.21
	R_2	222035.09	98.41		
	R_3	224672.13	99.58		
166.67	R_1	381522.03	169.10	168.60	101.16
	R_2	379659.11	168.27		
	R_3	380015.76	168.43		

The designed HPLC-UV method was successfully applied for separation and determination of the content of metalaxyl-M in analyzed fungicide formulation Flowdo-Z 5% FS®. The mean obtained result was 2.53% which is in acceptable limits with the value reported by the manufacturer. This study confirms the high importance of applied sciences in different fields as reported before in several scientific papers.[15-18]

4. Conclusion

The present study produces a novel accurate, simple, fast, and selective HPLC-UV method for estimation of the active ingredient metalaxyl-M. This method shows excellent validation results that makes it highly recommended for use by any analytical laboratory to estimate the active ingredient content of metalaxyl-M in different pesticide formulas. The linearity of the developed method was greater than 0.99 and RSDs ranged from 0.17% to 0.97%, indicating the method's stability and reliability. All validation parameters discussed in the study were in acceptable limits in accordance with SANCO/3030/99 rev.5 guidelines. The retention time of metalaxyl-M was about 3.52 min with run time of analysis was about 5 min. This method requires a little amount of mobile phase that makes it a cost-effective method.

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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