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The accurate and reliable HPLC-UV based method for detecting the active ingredient hexaflumuron in some plant protection products

Ismael M. Elkamhawy[1,2]*, Mostafa G. Emara[2], Alaa E. Ali[1], Mohamed A. Khashaba[3]

[1] Damanhour University, Faculty of Science, Chemistry Department, Damanhour, 22511, Egypt
[2] Chromatographic Analysis Laboratory, KZ for Pesticides and Chemicals Company, Nubaria City, Egypt
[3] Alexandria University, Faculty of Science, Chemistry Department, Alexandria, Egypt

Abstract

The main objective of this study was to present a new selective, sensitive, and validated RP-HPLC analytical method for measuring the concentrations of hexaflumuron (HFM), which is a well-known active ingredient that is widely used as an insecticide to protect crops, especially fruits and vegetables. Quantification was carried out using a reversed phase HPLC system that was equipped with a UV detector. The development of the novel method was performed on a reversed-phase C-18 (stainless steel, 5 µm, 250 × 4.6 mm) column at a constant temperature of 30°C. The mobile phase consists of acetonitrile and distilled water in a volumetric ratio of 85:15, a flow rate of 1 mL/min, and the detection wavelength at 220 nm. Retention time of separation at 3.84 min. The method was validated by testing specificity, linearity, precision, recovery, LOD, LOQ, and accuracy according to the CIPAC (Collaborative International Pesticides Analytical Council) and complies with the guidelines of SANCO/3030/99 rev.5. guidelines. The method revealed an acceptable linearity regression R^2 (0.9974). The method was found to be accurate from concentration levels 50–250 µg/mL with high accuracy (99.3-100.7%). The method's validation results make it suitable for use as a standardization tool in the evaluation of Emulsion Concentrate formulations containing this active ingredient.

Keywords: Hexaflumuron, HPLC analysis, method validation, precision, accuracy

1. Introduction

Hexaflumuron (HFM) is one of the widely used insecticides since its registration in the Environmental Protection Agency (US EPA) in 1994 [1]. HFM chemical name is [1-(3,5-dichloro-4-(1,1,2,2-tetrafluoroethoxy) phenyl)-3-(2,6-difluorobenzoyl)urea] and its chemical structure is indicated in Fig. 1. HFM is a white crystal or powder with the melting point range between "202 °C – 205 °C" and its molar mass of 461.1 g/mol. It is insoluble in water (27 µg/l), soluble in methanol and xylene 11.3 and 5.2 (gm/l), respectively [2,3]. HFM chemically belongs to the class of benzoylphenylureas, a significant group of potent insect growth regulators (IGRs). HFM action's mode through the inhibition of chitin synthesis in the cuticle of insects that cause disrupt hormonal balance with exchanging in molting process and makes it also effective in controlling immature stages of insects. Benzoylphenylureas including HFM have been used to control a wide range of agricultural pests worldwide due to their high selectivity, insecticidal activity, and low acute toxicity to mammals [4–6].

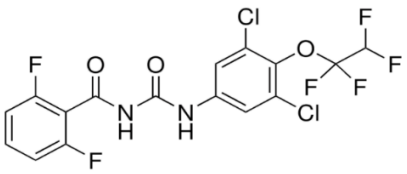

Figure 1. Hexaflumuron structural diagram, $C_{16}H_8Cl_2F_6N_2O_3$

Chromatographic separation techniques are one of the most important, straightforward, and effective methods for both qualitative and quantitative analysis. At the moment, high performance liquid chromatography (HPLC) is used for carrying out structural and functional analysis, and purification of a wide range of molecules within a short time, that makes it the most effective technique for achieving a superior and ideal separation in all applied fields. HPLC is

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***Author of correspondence:** Ismael.elkamhawy@sci.dmu.edu.eg
Ismaelelkamhawy@yahoo.com
Tel: +20 106 2515962 **Fax:** N/A
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accurate, simple to use, and produces fast results [7,8]. The literature survey has only reported a limited number of methods [9–11] using different techniques or detector types other than used in this article. HPLC can be used successfully in pesticide analysis, which is the main topic in our article. We here aimed to report a new validated method for determining the concentration of hexaflumuron active ingredients in some EC products such as SCORCH 10% EC®. Our new applicable method addresses researchers and chemists who need these types of methods for essential purposes such as generating data for authorization, stability studies, post-registration control, and quality control monitoring.

2. 2. Materials and methods

2.1. Chemicals and Reagents

Hexaflumuron analytical standard was supplied by Shandong Luba Chemical Co., Ltd., China, as a gift sample from Kafr El-Zayat for Pesticides and Chemicals Company, Egypt, with a known purity ≥ 95 % w/w. Acetonitrile with a pure HPLC grade was purchased from Scharlab, Spain. MS® 0.45 µm nylon membrane filters were purchased from Membrane Solutions, LLC., Seattle, USA.

2.2. Instrumentation and chromatographic conditions

The HPLC system consisted of a Series 200 liquid chromatograph, a Series 200 UV/vis spectrophotometric detector with a range of 190 to 700 nm, and the pump can generate pressure up to 6100 psi. Brownlee™ C-18 reversed phase column with definite specifications (stainless steel, 250 × 4.6 mm i.d., 5 µm particle size) was procured from PerkinElmer Instruments, LLC, USA. The Merit Water still instrument model W4000 was used to obtain the distilled water. The mobile phase was prepared by mixing 850 mL acetonitrile and 150 mL distilled water to prepare a 1 L solution. The mobile phase was filtered through a 0.45 µm membrane filter and then degassed before use by using an Elmasonic S40H ultrasonic bath (220–240 V~). The pump flow rate reached 1 mL/min with UV/vis detection at 220 nm. The column oven was adjusted to 30° C with an injection volume of 20 µL. The retention time of HFM was about 3.84 min (Fig. 2).

2.3. Preparation of standard solution

The stock solution (1000 ppm) was prepared by weighing accurately about 26.3 mg of hexaflumuron analytical standard and then diluting it to 25 mL by using acetonitrile. After that, 100 µg/mL is prepared for injection by taking 1 mL from the standard stock solution, and the volume is completed to 10 mL using acetonitrile.

2.4. Method validation parameters

CIPAC document 3807 [12] and the document SANCO/3030/99 rev.5 [13] outlines the validation requirements for analytical methods used for the quality analysis of technical aspects and commercial formulations of plant protection products.

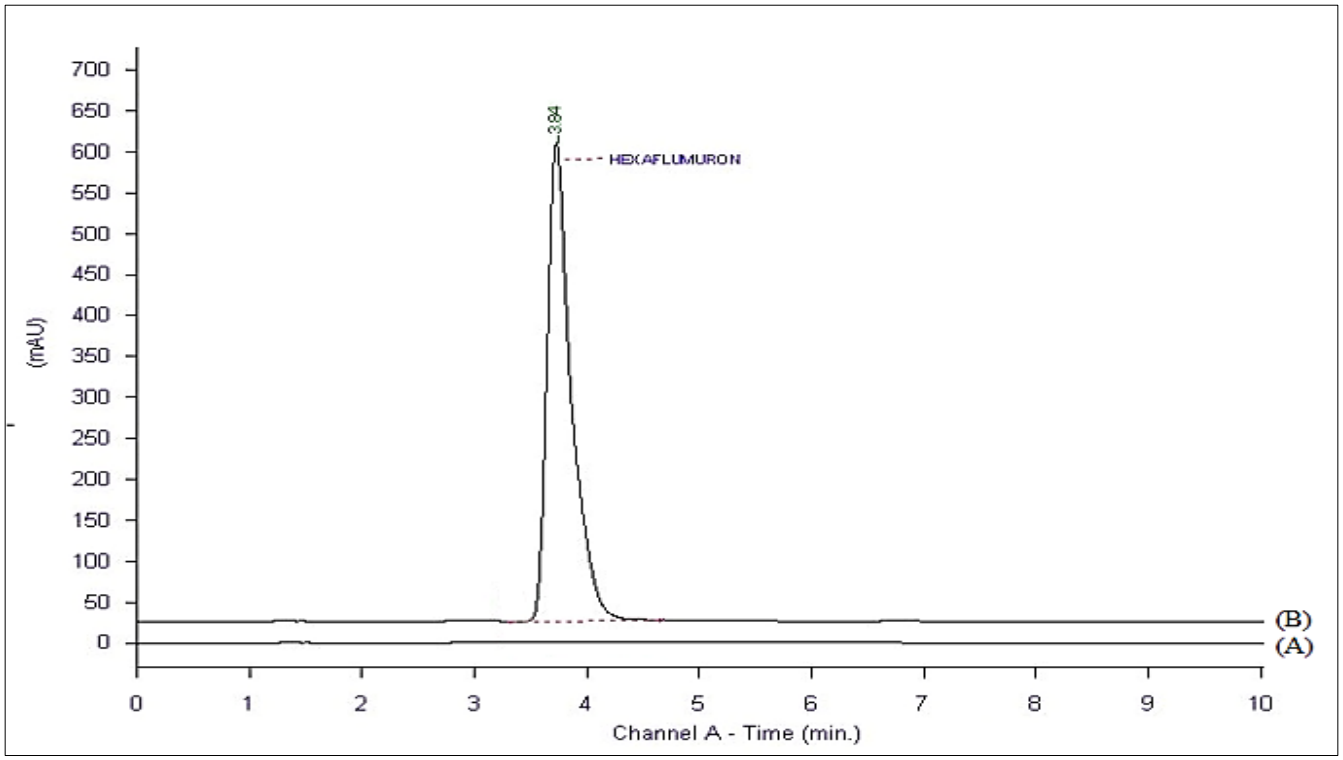

Figure 2. (A) Represents the HPLC-UV baseline of the blank sample, and (B) illustrates the chromatogram of the HFM analytical standard analyzed in this study.

Validation is required for specific and non-standardized analytical methods used in plant protection product formulations. This includes calculating and discussing the specificity, linearity, recovery, accuracy, LOD, LOQ, and precision of the method.

2.4.1. System suitability

As usual, the instrument's performance and the system's adaptability have been confirmed. After that, chromatography was performed first to ensure that there were no interfering peaks in any of the sample solutions that were established.

2.4.2. Specificity

When a method provides a result for just one analyte, it is referred to as specific [14]. The specificity has been investigated by injecting the excipients to ensure that there are no interferences from other peaks or distortions with the target peak.

2.4.3. Linearity and range

Linearity is evaluated by calculating the correlation coefficient, R^2, which must be ≥ 0.99 [14]. The regression linearity equation (1):

$$Y = aX \pm b \quad (1)$$

Where (Y) represents the response of the average peak area, (X) represents the claimed working concentration in ppm, (a) represents the slope, and (b) is the intercept of the calibration curve. The recommended procedure for determining linearity is by preparing the stock solution of higher concentration and then diluting it to at least five different concentrations. The working range is the range where the matrix gives results with acceptable uncertainty that is determined by observing the concentrations between the minimum and the maximum concentration in the linearity test [15,16].

Linearity was performed by preparing 5 different concentrations (50, 100, 150, 200, and 250 ppm) of HFM analytical standard. The stock solution (10000 ppm) was prepared by weighing accurately 250 mg of HFM analytical standard and dissolving it in 25 mL of acetonitrile in a calibrated volumetric flask. Then, serial dilutions were prepared by taking (0.25 mL, 0.5 mL, 0.75 mL, 1 mL, and 1.25 mL) from the stock solution, respectively, and completing to 50 mL with acetonitrile, and then each concentration was injected in triplicate.

2.4.4. Limit of detection (LOD) and limit of quantification (LOQ)

LOD is the lowest concentration in a sample that can be detected but not necessarily quantified under the stated experimental conditions. LOQ is the lowest concentration of analyte that can be determined with acceptable precision and accuracy [17]. From the linearity of the calibration, LOD and LOQ could be estimated according to the following equations;

$$LOD = 3.3\sigma/S \quad (2)$$

$$LOQ = 10\sigma/S \quad (3)$$

where (σ) is the standard deviation of response (peak area) and (S) represents the slope of the linearity calibration curve.

2.4.5. Repeatability and Precision

The precision of an analytical method represents the closeness among repeatable measurements acquired through a method under normal conditions. The analytical variation and a measure of the test method's precision are provided by the replicates' relative standard deviation (RSD), which ≤ 1.5% is accepted [15,18]. The following formula (4) can be used to estimate the RSD% of this method:

$$RSD\ (\%) = \frac{\sigma}{\bar{x}} \times 100 \quad (4)$$

Precision was determined in the current new method by triplicate analyses at a concentrations of 50 µg/mL, 100 µg/mL, 150 µg/mL, 200 µg/mL, and 250 µg/mL of standard HFM solution using the developed method.

2.4.6. Accuracy and recovery

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 [18]. It is determined by assessing the analyte recovery percentage (R%) according to equation (5). The new test method's accuracy was evaluated in triplicate at three different concentration levels 50% (0.05 mg/mL), 100% (0.1 mg/mL), and 150% (0.15 mg/mL). Three sets were prepared and injected in triplicate at each concentration. The calculated R% for each level should be between 98.0% and 102.0% [19,20].

$$R\% = \frac{Actual\ Conc.\ (\%)}{Theoritical\ Conc.\ (\%)} \times 100 \quad (5)$$

2.5. Preparation of sample solution

Weigh about 250 mg from the product under study (SCORCH 10% EC®), then dilute to 25 ml with acetonitrile in a 25 mL volumetric flask. After that, prepare the injected solution by taking 1 mL from the previous flask, and the volume is completed to 10 mL using acetonitrile.

3. Results and discussion

3.1. Method development

HPLC-UV was used to develop a new analytical technique for determining the active ingredient hexaflumuron in the pesticide formulation SCORCH 10% EC®, using the Brownlee™ C-18 reversed phase column (250 × 4.6 mm, 5 µm), which is made for successfully separating the analyte with high efficiency and excellent peak shape with all pesticide sample types. It was found that the mobile phase, which included acetonitrile/water (85/15, v/v), isocratic elution with a flow rate of 1.0 mL/min, constant column temperature at 30 ºC, and UV detection at 220 nm, produced the best separation and symmetrical peak shape of the pesticide under investigation. In these chromatographic conditions, a zero-response baseline was obtained, and the chromatographic peak of HFM was clear, narrow, and symmetrical, as shown in Fig. 2. The optimized chromatographic parameters for the developed HPLC-UV method are illustrated in Table 1.

Table 1. Optimized chromatographic conditions for the hexaflumuron reversed phase HPLC-UV developed method

No.	Parameter	Condition
1	Column	Brownlee™ C-18 reversed phase column (250 × 4.6 mm, 5 µm)
2	Mobile phase	Acetonitrile: distilled water (85%:15%) (v/v)
3	Flow rate	1 mL/min
4	Injection Volume	20 µL
5	Wavelength	220 nm
6	Column temperature	30°C
7	Mode of separation	Isocratic
8	Run time	10 min.
9	Retention time	Hexaflumuron: 3.845 min.

3.2. Method validation

3.2.1. *Method suitability*

A reverse phase high-performance liquid chromatography (RP-HPLC) method was designed with consideration of system suitability factors, including tailing factor (T), number of theoretical plates (N), runtime, and cost-effectiveness. System suitability tests are essential in method development and are employed to verify the correct operation of the chromatographic system. The advanced method produced the elution of HFM at 3.845 min, where the total run time is 10 minutes, as shown in Fig. 2. Retention time, number of theoretical plates, and peak tailing factor (T) were assessed for six replicate injections of the standard at working concentration. The final results are given in Table 2.

Table 2. System suitability results of Hexaflumuron

Parameters	Hexaflumuron (HFM)
Retention time (min)	3.845
Number of theoretical plates (N)	4823
Tailing Factor (T)	1.15

3.2.2. *Specificity*

The analyte was identified by comparing its retention time with that of the sample and the standard solution that was completely identical. Moreover, the positioned peak of the pure analytical standard of HFM was used to corroborate the identification. As can be seen from the chromatograms in Figure 2, there were no other coeluted peaks that interfered with the HFM peak when a blank sample was injected.

3.2.3. *Linearity and range*

As can be easily seen by reviewing the data in Figure 3, the calibration data don't show any non-linear trends or outliers. According to Table 3, the calibration range was 0.05 mg/mL to 0.25 mg/mL, and linearity was evaluated using the correlation coefficient (R^2). The slope value was $y = 4\times10^7x + 174405$ and appeared high linearity with $R^2 = 0.9974$.

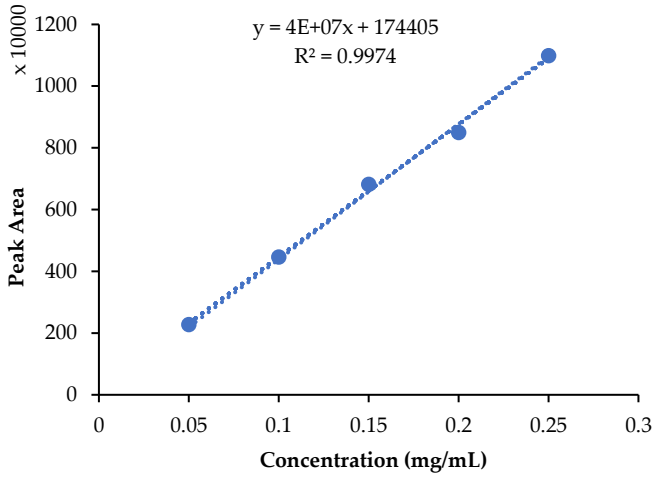

Figure 3. The linear response of peak area against hexaflumuron concentration.

3.2.4. *LOD & LOQ*

The linearity calibration data of HFM could be used for easily determining the LOD and LOQ limitations. The results showed that the LOQ was 41.6 µg/mL and the LOD was 13.7 µg/mL.

3.2.5. *Repeatability and precision*

As shown in Table 3, trireplicate injections of HFM standard solutions with five concentrations of 50 µg/mL, 100 µg/mL, 150 µg/mL, 200 µg/mL, and 250 µg/mL were used to assess the analyte's repeatability using the RSD% of peak areas. The results were found to be below the 1.5% acceptability threshold. These findings demonstrate the repeatability of the existing HFM determination method.

Table 3. Results of the linearity study of the HPLC-UV method

Conc. (mg/mL)	Rep.	Peak Area	Average	STDEV	RSD%
0.05	R1	2253316.25	2282300.03	29813.53	1.31
	R2	2280704.60			
	R3	2312879.25			
0.1	R1	4425770.96	4462113.24	31742.17	0.71
	R2	4484406.82			
	R3	4476161.95			
0.15	R1	6892058.69	6822192.34	69631.53	1.02
	R2	6821720.31			
	R3	6752798.04			
0.2	R1	8448178.54	8498632.79	45345.77	0.53
	R2	8511734.95			
	R3	8535984.89			
0.25	R1	10996251.52	10992666.12	56672.10	0.52
	R2	11047460.39			
	R3	10934286.44			

Conc: Concentration, **Rep.:** Replicates

3.2.6. Accuracy and recovery

The accuracy results of the tested range (50% -150%) of the target concentration of 100% (100 µg/mL) were found to be within the range of acceptable criteria levels (98–102%), as demonstrated in Table 4.

Table 4. Accuracy and recovery results

Theoretical Conc. (%)	Rep.	Peak Area	Actual Conc. (%)	Mean Actual Conc. (%)	R(%)
50	R1	2319572.20	50.39	50.35	100.70
	R2	2365003.87	51.38		
	R3	2268531.62	49.28		
100	R1	4502659.95	97.82	98.48	98.48
	R2	4575316.55	99.40		
	R3	4521545.61	98.23		
150	R1	6811329.70	147.97	148.99	99.32
	R2	6960648.22	151.22		
	R3	6802656.43	147.79		

Conc: Concentration, **Rep.:** Replicates

3.3. Method application

In order to verify the applicability of the new validated method to a commercial formulation, SCORCH 10% EC® was analyzed at working concentration, and it is shown in Fig. 4. The sample peak was first identified by comparing the retention time with the analytical standard of HFM. System suitability parameters fell within the acceptance ranges. Integration of separated peak area was done, and product concentration was determined by triplicate injection and then using the average peak area concentration relationship obtained in the standardization step. These good results that are shown in Table 5 revealed that the validated method was found to be resolute, selective, and specific for the HFM peak. Also, these results are compatible with the acceptable limit provided by the manufacturer.

Table 5. The values of hexaflumuron in commercial sample that determined by the new method

Product Name	Replicates	Peak Area	Actual Conc. (%)	Mean Actual Conc. (%)
SCORCH® 10% EC	R1	4407754.93	9.72	9.79
	R2	4459302.19	9.83	
	R3	4468061.92	9.85	

4. Conclusion

This stated method can be used effectively to determine the amount of hexaflumuron in some pesticide products while simultaneously obtaining precise chromatographic profiles for chemometric analysis.

The new method showed good linear regression, $R^2 > 0.99$. The method was reliable and stable, with RSDs

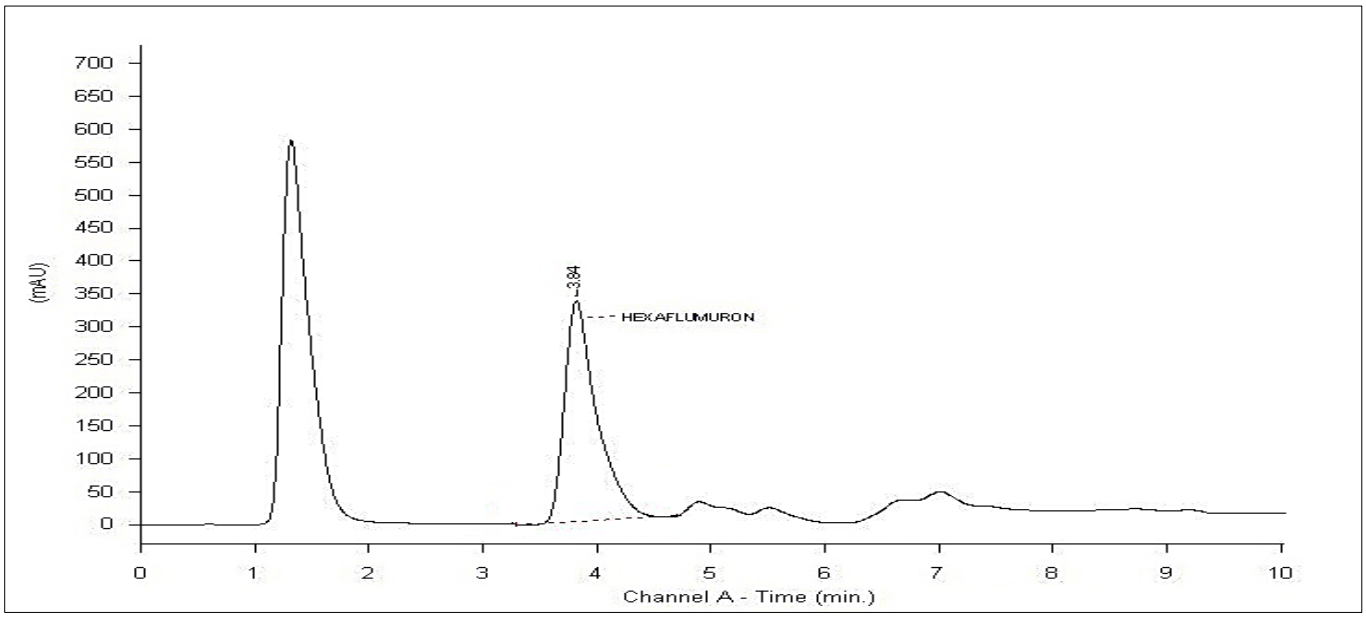

Figure 4. The chromatogram of the commercial formulation containing HFM pesticide

between 0.52 and 1.31 %. These validation results were excellent compared to other methods reported in literature, making our new method widely applicable. The retention time of HFM was about 3.84 min with run time of the analysis was about 10 min. This analysis requires a small amount of organic solvent, as indicated by the justified run time, making this method both economical and environmentally benign. The appropriate quantification limit and reliable results proved the method's ability to be used routinely in the chemical analysis laboratories.

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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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