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Title page

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Stability indicating RP-HPLC method for determination of Flubendazole in pharmaceutical dosage forms.

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ABSTRACT

In this manuscript stability study of Flubendazole (FLUB); a benzimidazole carbamate derivative with anthelmintic activity; has been carried out. In order to investigate the stability of drug, FLUB was subjected to stress degradation under different conditions recommended by International Conference on Harmonization (ICH). A validated HPLC-DAD method has been established to resolve FLUB from all its degradation products obtained under acidic, basic, neutral, oxidation, photo-degradation and thermal conditions and also from its suspension additives. Chromatographic separation was attained on ZORBAX Eclipse Plus C18 column in an isocratic mode of elution using a mixture of water: acetonitrile (50:50, v/v) as a mobile phase at a flow rate of 1 mL/min where FLUB was well resolved from all degradation products at a t_R of 5.35 min. The column thermostated compartment was adjusted at 25^0C and the effluent was monitored by a photo diode array detector at 254 nm where FLUB was linear in the range of 0.5-10 µg mL^{-1}. The method was validated in terms of accuracy, precision, specificity, robustness and ruggedness as per USP guidelines. It was successfully applied to quantify FLUB in bulk powder and pharmaceutical formulations with complete resolution between FLUB and suspension additives. Statistical analysis between the suggested method and the official HPLC method using student's-t and F-ratio tests reveals that the suggested method is as accurate and precise as the reported one.

Keywords:

Flubendazole; Stability indicating assay method; HPLC-DAD; Stress degradation study; Pharmaceutical formulations.

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1. Introduction

Flubendazole (FLUB); chemically is identified as, [5-(4-Fluorobenzoyl)-1 H-benzimidazol-2-yl] carbamic acid methyl ester [1]. It is a benzimidazole carbamate derivative, with an anthelmintic effect and activity against most nematodes and some other worms. Activity against some larval stages and ova has also been demonstrated. It inhibits or destroys cytoplasmic microtubules in the worm's intestinal or absorptive cells leading to inhibition of glucose uptake and depletion of glycogen stores, hence death of the worm within several days occurs [2].

A thorough literature survey revealed the presence of few methods for determination of FLUB. European Pharmacopoeia [3] has reported a RP-HPLC assay of FLUB. Also, different HPLC-MS for determination of FLUB; in pig [4] and in eggs and poultry muscle [5] with its metabolites or in environmental [6] and wastewater [7] samples; has been described. The drug was also analyzed along with its metabolites in plasma by UV photodiode-array and fluorescence detection [8]. Additionally, FLUB and other benzimidazole compounds were resolved by HPLC methods in bovine liver [9] and milk samples [10]. Moreover, it was determined by different polarographic methods in either pharmaceutical formulations [11] or biological fluids [12]. Furthermore, an HPLC-UV method for simultaneous determination of FLUB and febantel in swine and poultry feeds has been published [13]. Till now, there is no stability indicating method for determination of FLUB in presence of its degradation products or suspension additives has been previously established.

Current state-of-the-art argues that stability is the most important quality requirement for a pharmaceutical product. Stable preparations directly emphasize the quality of the product, assuring its precise delivery. Moreover, the drug product shelf life depends on the analytical studies at normal and stressed conditions [14]. The present drug stability test guidelines Q1A (R2) [15,16] issued by the International Conference on Harmonization (ICH) recommend carrying out of stress testing on drug substance to establish its inherent stability characteristics. Analysis of stability samples should be performed through the use of validated stability-indicating analytical methods (SIAM) [17]. It is essential for SIAM procedures to be able to accurately measure the active ingredients, without interference, from the degradation products, excipients, impurities or other potential process contaminations [18, 19].

As previously mentioned, a comprehensive HPLC study of the degradation behavior of FLUB under various ICH prescribed stress conditions has been lacking. Accordingly, the present manuscript argues the degradation behavior of FLUB under acidic, basic, neutral,

oxidation, photo-degradation and thermal conditions and develops a RP-HPLC-DAD method to separate the drug and its degradation products at a reasonable retention time and it was successfully applied for determination of FLUB in pharmaceutical dosage forms.

2. Experimental

2.1. Instrument

HPLC (Agilent 1260 Infinity, Germany) instrument was equipped with Agilent 1260 Infinity preparative pump (G1361A), Agilent 1260 Infinity Diode array detector VL (G131SD), Agilent 1260 Infinity Thermostated column compartment (G1316A) and Agilent 1260 Infinity preparative Autosampler (G2260A). Separation and quantitation was performed on ZORBAX Eclipse Plus C18 column (250 × 4.6 mm i.d, 5µm particle size (USA).

2.2. Materials

- Pure standard

Flubendazole was given as a gift from Alexandria CO. for Pharmaceuticals and Chemical Industries, Alexandria, Egypt. Its purity was found to be 100.22 ± 1.271 %, according to the official method [3].

- Pharmaceutical formulations

a) Fluvermal® tablets (Batch No. DCE1053) and suspension (Batch No. FE1824) manufactured by Minapharma Egypt, under license of Janssen Pharmaceutica Belgium. It is claimed to contain 100 mg of FLUB per tablet or 5mL suspension.

b) Fluver® tablets (Batch No.3139001) and suspension (Batch No.3209033) manufactured by Alexandria CO. for Pharmaceuticals and Chemical Industries, Alexandria, Egypt. It is claimed to contain 100 mg of FLUB per tablet or 5mL suspension.

- Chemicals and reagents

a) Methanol and acetonitrile of HPLC grade obtained from Chromasolv® (Sigma-Aldrich Chemie GmbH, Germany), Fisher Scientific (UK) and Poch SA (Poland).

b) Deionized water (SEDICO pharmaceuticals Co., Cairo, Egypt).

c) Hydrochloric acid, sodium hydroxide and hydrogen peroxide (analytical grade) were purchased from El- Nasr Pharmaceutical Chemicals Co., Abu- Zabaal, Cairo, Egypt.

2.3. Standard solutions

a) Stock standard solution of FLUB (1 mg/mL): it was prepared by accurately weighing 0.1 g FLUB in 100-mL volumetric flask and dissolving in 0.1M methanolic HCl.

b) Working standard solution of FLUB (0.1 mg/mL): it was prepared by suitable dilution of FLUB stock solution with water: acetonitrile (50:50, v/v).

3. Methods

3.1. Chromatographic Condition

Chromatographic separation was achieved on ZORBAX Eclipse Plus C18 column (250 × 4.6 mm i.d, 5µm particle size using a mobile phase consisting of a mixture of water and acetonitrile (50: 50, v/v) in isocratic mode. Separation was performed at 25 ^{0}C using a 1 mL/min flow rate and the run time of 8 min. The injection volume was 50 µL and the photodiode array detector was adjusted at 230 and 254 nm.

3.2. Linearity and Construction of Calibration Curve

To establish the linearity of analytical method, a series of dilutions ranging from 0.5-10 µg/mL of pure FLUB were prepared from its working standard solution (0.1 mg/mL) using a mixture of water: acetonitrile (50:50, v/v) as a solvent. Triplicate 50 µL injections were made for each concentration and chromatographed as under the previously mentioned chromatographic conditions. The relative peak areas at 254 nm (using 5 µg/mL of FLUB as an external standard) were plotted against the corresponding concentrations to obtain the calibration graph.

3.3. Stability Study of Flubendazole

Flubendazole forced degradation study was carried out under conditions recommended in ICH guidelines such as acidic, basic, neutral, oxidation, photo-degradation and thermal conditions, degradation study was fulfilled in the dark for all conditions except for photo-degradation, in order to exclude the possible degradation effect of light on FLUB. Stock standard solution of FLUB (1 mg/mL) was used throughout stress decomposition study to provide an indication of the stability indicating property and specificity of proposed method. Using the previously computed regression equation, the remaining FLUB concentration in each sample was determined and % FLUB degradation was calculated.

3.3.1. Acidic degradation

Acid decomposition study was performed by refluxing 5 mL of FLUB stock solution with 5 mL 0.1M and 1M HCl solution at 80 ^{0}C for 5 hours, respectively. The resultant solutions were cooled to room temperature, neutralized to pH 7 with 1N NaOH and diluted to 25 mL

with the mobile phase to prepare solutions of 200 µg/mL of each. A concentration of 10 µg/mL of each produced sample was prepared and 50 µL was injected into the LC system.

3.3.2. Alkaline degradation

The study under alkaline condition was carried out by separately mixing 5 mL of FLUB stock solution with 5 mL 0.1M and 1M NaOH and the solutions were refluxed for 5 hours at 80 ^{0}C. The resultant solutions were neutralized with 1N HCl and the volume was adjusted to 25 mL with the mobile phase. Then the solution was diluted to a concentration of 10 µg/mL and 50 µL was injected into the LC system.

3.3.3. Neutral degradation

Neutral decomposition study was accomplished by refluxing 5 mL of FLUB stock solution with 5 mL deionized water at 80^{0}C for 5 hours. The resultant solutions were diluted to a concentration of 10 µg/mL and 50 µL was injected into the LC system.

3.3.4. Oxidation degradation

To study hydrogen peroxide induced degradation, studies were performed in 3% and 30% hydrogen peroxide. By separately mixing 5 mL of FLUB stock standard solution with 5 mL of either 3% or 30% hydrogen peroxide in two 25 mL volumetric flasks at 80^{0}C for 5 hours. Then the solutions were heated in a boiling water bath for 10 min to expel the excess hydrogen peroxide. The resultant solutions were diluted with the mobile phase to obtain a concentration of 200 µg/mL each which was then diluted to obtain a concentration of 10 µg/mL, then 50 µL was injected into the LC system.

3.3.5. Photo-degradation

The photochemical stability of the drug was studied by exposing 5 mL of FLUB stock solution to direct sunlight for 4 days (about 20 hours average). The solution was then diluted with the previously prepared mobile phase to a concentration of 10 µg/mL from which 50 µL was injected into the LC system.

3.3.6. Thermal degradation

Flubendazole 5 mg was kept at 80°C for 5 hours in an oven. The sample was then transferred to 25-mL volumetric flask, dissolving in 5 mL 0.1 M methanolic HCl and then the volume was adjusted with acetonitrile: water (50:50, v/v) to obtain sample solution of 200 µg/mL. A concentration of 10 µg/mL was then prepared from which 50 µL was injected into the LC system.

3.4. Application to Pharmaceutical formulations

- *Fluvermal® and fluver ® Tablets:* Ten tablets each of Fluvermal® and fluver® Tablets were separately weighed, powdered and mixed well. Amount of powdered tablets from each dosage form equivalent to 100 mg of FLUB was taken and dissolved in 75 mL 0.1 M methanolic HCl for 15 min, the volume was adjusted to 100 mL with 0.1 M methanolic HCl and then filtered. Suitable dilution of the prepared solutions with water: acetonitrile (50:50, v/v) was performed to obtain working solution (0.1 mg/mL) of each pharmaceutical formulation.
- *Fluvermal® and fluver® Suspensions:* 5 mL from each suspension was accurately transferred into two separate 100-mL volumetric flasks. 75 mL 0.1 M methanolic HCl was added and ultra-sonicated for 10 min, the volume was completed to the mark with 0.1 M methanolic HCl and then filtered to obtain stock solution of 1mg/mL FLUB. Working solutions of 0.1mg/mL were then prepared in water: acetonitrile (50:50, v/v) mixture.
- Different concentrations of each formulation were prepared and then the procedure under chromatographic conditions has been followed. In order to assess the accuracy of the method, recovery studies have been performed by spiking the pre-analyzed FLUB sample (4 µg/mL) with extra 80, 100 and 120% of pure FLUB then the mean % recovery of the pure added drug was calculated.

4. Results and Discussion

According to the International Conference on Harmonization (ICH) guidelines, requirements for establishing SIAMs have become more clearly mandatory [15, 16]. The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors, such as temperature, light, oxygen, pH and moisture. In this work a stability study of FLUB was carried out for the first time using HPLC-DAD method. The developed method was able to resolve the drug from all degradation products in a single run; moreover, the drug was completely resolved from suspension excipients. Additionally, the stress degradations conditions were followed on FLUB pharmaceutical tablets and the same results were obtained as the pure standard drug.

4.1. Method Development and optimization

Many parameters must be evaluated and optimized during the method development and optimization so as to separate the parent drug along with suspension additives and degradation products within a single run without any interference.

In order to select a suitable mobile phase for the analysis of FLUB, the pure drug along with its degradation products and pharmaceutical formulations were injected and run in different solvent systems on the basis of trial and error, taking in consideration, the system suitability parameters, retention time, tailing factor, number of theoretical plates and HETP. Initially methanol and water in different ratios were tried. However, the pure drug started to be eluted very late (after more than 20 min), therefore, methanol was replaced by acetonitrile. Water: acetonitrile mixture was used in different ratios and it was found that using a mixture of water and acetonitrile (50:50, v/v) as a mobile phase at a flow rate of 1 mL/min gave acceptable retention time (t_R), theoretical plates and good resolution between the drug, suspension excepients and degradation products.

Different stationary phases were tested such as ZORBAX Eclipse Plus C18 and C8 columns. The two stationary phases nearly gave the same the system suitability parameters.

In addition, the mobile phase was delivered at different flow rates (0.8, 1 and 1.5 mL/min) in order to obtain maximum resolution within short analysis time. Pumping the mobile phase at 1 mL/min resulted in good resolution within 8 min analysis time.

The photodiode array detector (DAD) was adjusted at different detection wavelengths (230 and 254 nm) in order to detect any degradation products produced in any forced degradation conditions and increasing method sensitivity. It was found that the number of detected degradation products was the same at both wavelengths but scanning at 254 nm gave higher sensitivity for FLUB than scanning at 230 nm.

The thermostated column compartment temperature was optimized by testing different temperatures (20, 25 and 30^0C). It was observed that column temperature neither affected the chromatographic separation nor the peak shape.

Finally, the chromatographic separation of FLUB, its degradation products and suspension excepients was carried out on ZORBAX Eclipse Plus C18 column with a mixture of water: acetonitrile (50:50, v/v) delivered at 1mL/min, maintaining the column temperature at 25^0C and detection at 254 nm.

4.2. Results of Stress Degradation Studies

Summary of results of FLUB stability studies is given in **Table 1.**

4.2.1. Acidic degradation

The drug was found to be stable to acidic degradation. Initially 0.1 M HCl was used at 80 ^{0}C and degradation was followed during 5 hours but no degradation was observed. So the strength of acid was increased to 1 M HCl for 5 hours, where the drug was slightly degraded with the appearance of two small peaks at 2.21 and 2.47 min, **Fig. 1**.

4.2.2. Alkaline degradation

Conversely to acidic condition, FLUB was found to be very sensitive to alkaline degradation. On using 0.1 M NaOH at 80 ^{0}C for 5 hours, an additional peak at 2.52 min was generated while using 1 M NaOH lead to nearly complete degradation of the parent drug giving three degradation products at 2.22, 2.7 and 2.8 min. **Fig. 1**.

4.2.3. Neutral degradation

The drug was found to be highly stable to neutral hydrolysis. There was very little change in peak area upon refluxing the drug with deionized water, **Fig. 1**.

4.2.4. Oxidation degradation

Flubendazole was highly affected by oxidative degradation. On oxidation with 3% H_2O_2, a significant degradation was observed with the production of two new peaks at 2.47 and 3.22 min, also on using 30% H_2O_2, two additional peaks at 2.49 and 3.2 min were observed, **Fig. 2**.

4.2.5. Photo-degradation

Exposing FLUB to direct sunlight up to 4 days resulted in minor degradation with appearance of small peaks at 3.15, 3.52 and 3.78 min, **Fig. 2**.

4.2.6. Thermal degradation

The studied drug was found to be thermally stable as no additional peaks were observed when the drug was subjected to dry heat as shown in **Fig. 2**.

4.3. Application of the Method

The second step after method optimization is its application to determine FLUB in its pure form and different pharmaceutical formulations. The method showed good linearity when the relative peak area (using 5 µg/mL of FLUB as an external standard) was plotted against FLUB concentration in the range of 0.5-10 µg/mL and the computed regression equation was found to be:

$$A=0.1944C + 0.0197 \qquad r = 0.9998$$

Where A is the integrated relative peak area, C is the concentration in µg/mL and r is the correlation coefficient. Calibration curve parameters are given in **Table 2**.

In order to test the ability of the method to quantify FLUB in its marketed formulations, it was applied to determine FLUB in Fluvermal® and Fluver® tablets and suspensions. Good percentage recoveries were obtained as given in **Tables 3& 4,** also the chromatograms in **Fig. 3** showed complete resolution between peak of the parent drug from all peaks of suspension additives. Also results of recovery studies was found to be acceptable at all tested levels, proving the high accuracy of the proposed method, **Table 3& 4.**

Results obtained by applying the proposed HPLC-DAD method for the determination of FLUB in bulk powder was statistically compared with those obtained by the official HPLC method [3] using student's t-test and variance ratio F-test, revealing no significant difference between the performance of the two methods regarding accuracy and precision, **Table 5.**

4.4. System suitability testing

The system suitability parameters were calculated to ascertain the suitability and effectiveness of the operating system. It was evaluated by comparing the obtained parameter values given in **Table 6**, with the acceptance criteria of the FDA guidance document [20]. The capacity factors was found to be within the range of $0.8 < k' > 10$, the resolution (Rs) between two adjacent peaks was greater than 2, the column efficiency (number of theoretical plates, N) was >2000, and the tailing factor is 1.02, **Table 6.**

4.5. Method Validation

Method validation has been performed as recommended in USP [21].

Linearity

Data from the linearity curve (n=11) showed that the method demonstrated good linearity over the concentration range of 0.5-10 µg/mL as revealed from the high value of the correlation coefficient (0.9998), **Table 2**.

Accuracy

It was calculated as the percentage recoveries of pure FLUB, and it was evaluated by determining nine concentrations covering the working range of the drug, by using the relative peak area and the previously computed regression equation, the mean percentage recovery was calculated and found to be 100.57%. It was further assessed by application of standard

addition technique at three levels where results obtained at each level showed that the developed method is accurate and reliable, **Tables 3& 4.**

Precision

Precision was evaluated by testing intraday (repeatability) and interday (intermediate) variations and was determined using standard solutions of 4, 8 and 10 µg/mL. For intraday assay each solution was injected three times within day, precision was calculated as the percent relative standard deviation (RSD%, n=9) of the total peak areas of FLUB. Similarly, interday variation was evaluated by determining the same standard solutions (n=9) on three successive days and calculating RSD%. The obtained values of RSD% were less than 2% proving that the developed method possesses good precision**, Table 2**.

Limits of Detection and Quantitation (LOD and LOQ)

The sensitivity of the method was determined with respect to LOD and LOQ. Concentrations of FLUB in the lower part of the linear range of the calibration curve and the equations LOD = 3.3 × N/B and LOQ = 10 × N/B were used, where N is the standard deviation of the response and B is the slope of the corresponding calibration. The values of LOD and LOQ were found to be 0.11 and 0.34 µg/mL, respectively, **Table 2**.

Specificity

Specificity is the ability of a method to discriminate between the intended analyte and other components in the sample. The specificity of the LC method is illustrated in **Figs. 1-3,** where complete separation of FLUB, suspension excipients and various degradation products formed under different stress conditions was observed. The peaks obtained were sharp and had clear baseline separation. Moreover, the peak purity was checked by using DAD detector and the purity factor was found to be 999.73 and purity threshold was 996.36. The purity factor was more than the purity threshold, indicating that no additional peaks were co-eluted with the parent drug and thus confirming the ability of the method to determine the analyte of interest in the presence of potential interferences.

Robustness

It is the measure of the performance of a method when small, deliberate changes are made to the specified method parameters [22]. It is evaluated during method development when conditions are optimized in order to identify critical parameters for the successful application of the method.). Insignificant difference in peak area was observed when small deliberate changes have been made as confirmed from the calculated %RSD. For changing % acetonitrile (± 2%) it was found to be 0.95, mobile phase flow rate (±0.05 min) was 0.95 and for column temperature (±2^0C) was 1.09.

Ruggedness

It is expressed as %RSD and it estimates the degree of reproducibility of the results obtained under variety of conditions, such as performing the analysis by two different analysts where % RSD was 0.39 and using acetonitrile of different manufactures [(Sigma-Aldrich, Chromasolv®, Germany), (Fisher Scientific, UK)] and (Poch SA, Poland) and % RSD was found to be 0.55. The peak area of the drug was not significantly affected by these changes as evident from the low value of %RSD indicating that the method is rugged.

Conclusion

In this paper drug stability guidelines issued by the international conference of harmonization (ICH) have been followed to establish the inherent stability of FLUB under different stress conditions such as, acidic, alkaline, neutral, oxidative, photo-degradation and thermal degradation conditions. This stability study has been performed by sensitive, specific, accurate and validated stability indicating HPLC-DAD method. The drug was found to be liable to alkaline and oxidative conditions and the degradation products were well separated from the drug substance demonstrating the stability indicating power of the method. Moreover, the method was capable to resolve suspension formulation additives from the parent drug when applied to real marketed samples. The information presented herein could be very useful for quality monitoring of FLUB bulk samples and also be employed to check the quality of drug during stability studies. The developed LC method fulfilled all the requirements to be identified as a reliable and feasible method, including accuracy, linearity, recovery, and precision data.

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Figures

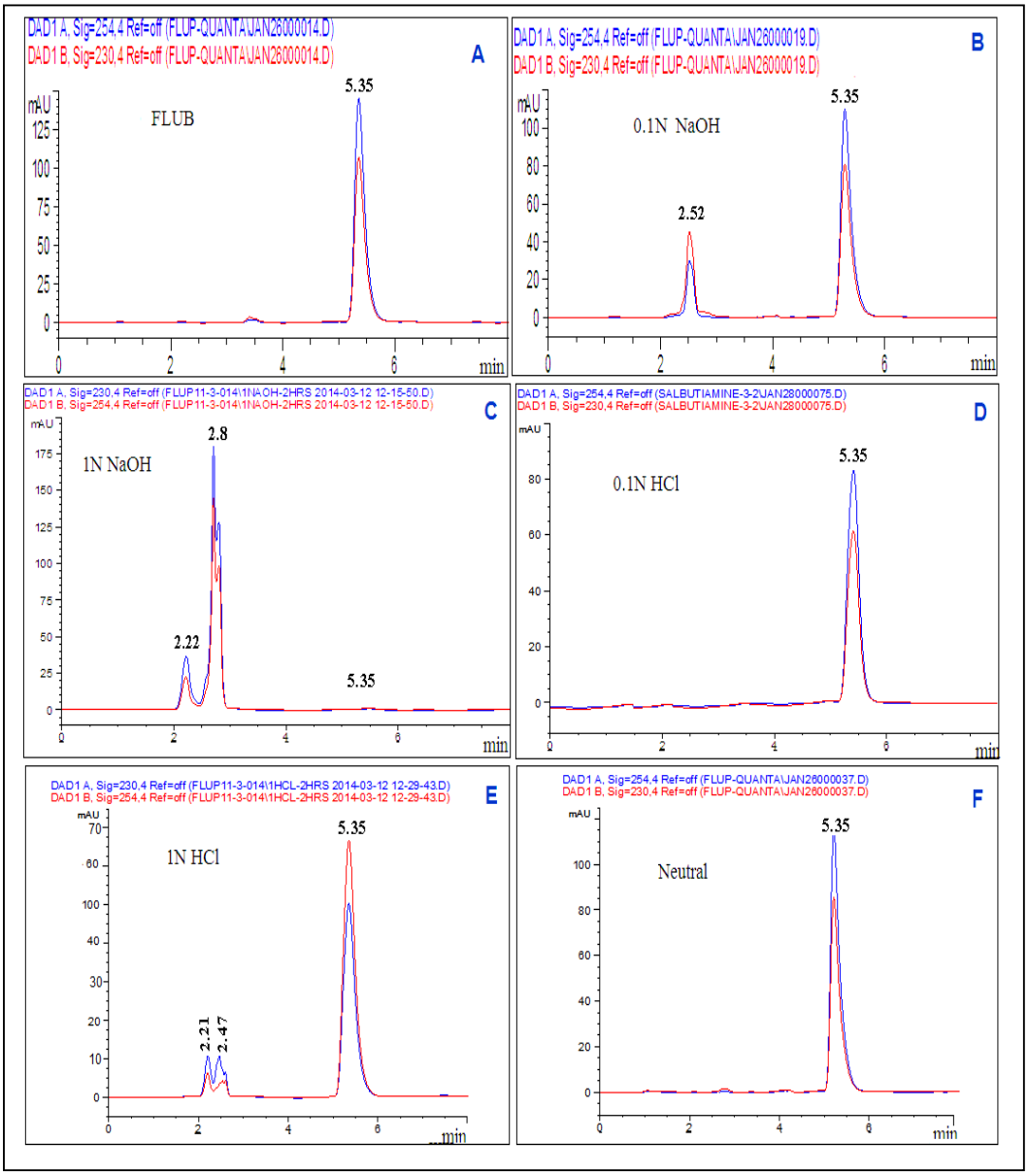

Figure 1: HPLC chromatogram of (A) 10 µg/mL of pure FLUB, (B) alkaline degradation using 0.1N NaOH, (C) alkaline degradation using 1N NaOH, (D) acidic degradation using 0.1 N HCl, (E) acidic degradation using 1N HCl and (F) neutral degradation under neutral condition.

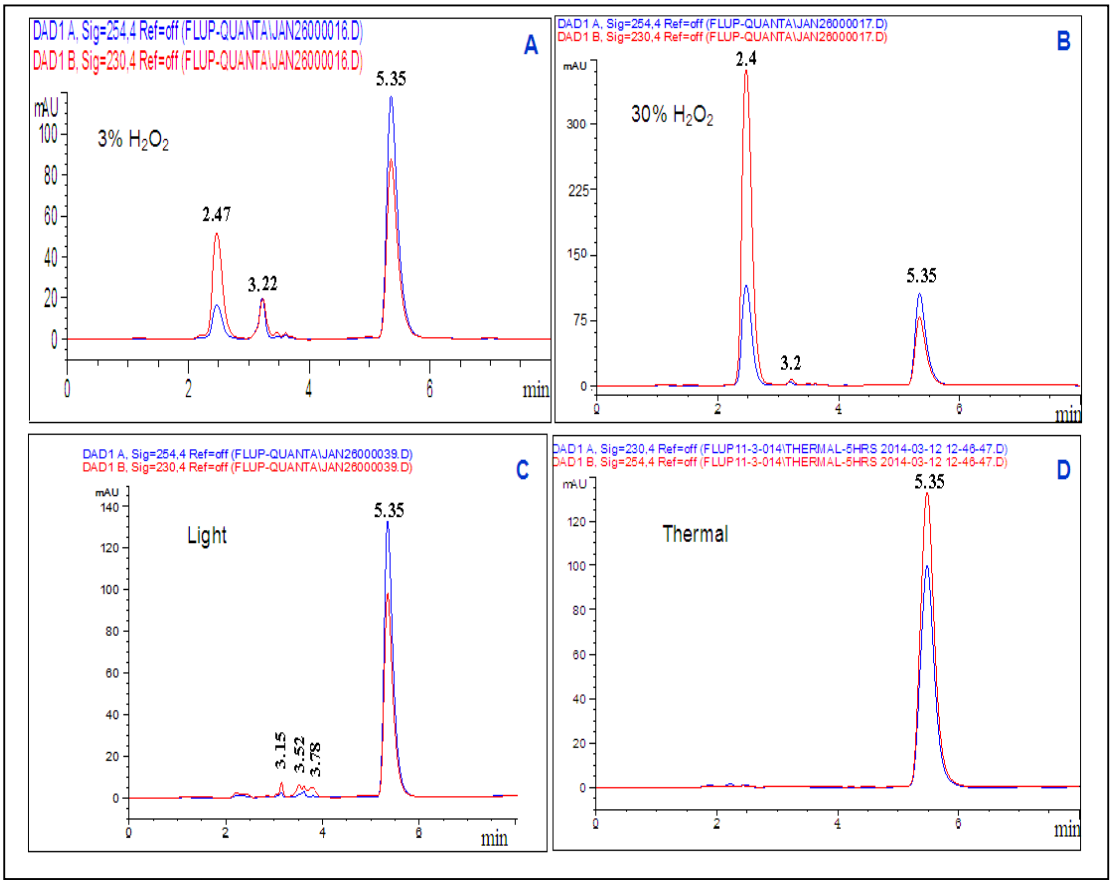

Figure 2: HPLC chromatogram of (A) degradation using 3% H_2O_2, (B) degradation using 30% H_2O_2, (C) photo-degradation under light condition and (D) degradation under thermal condition.

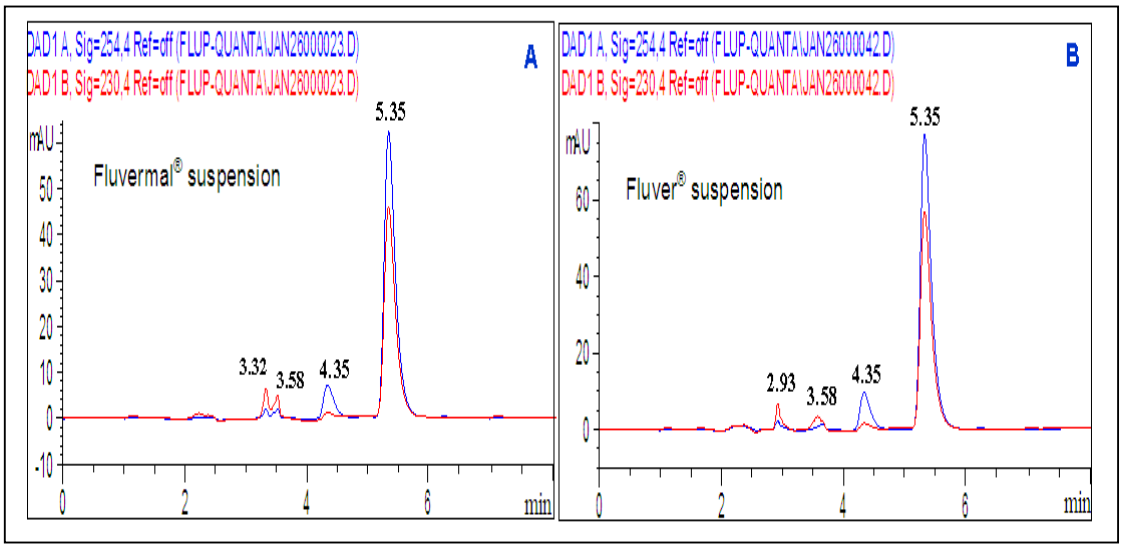

Figure 3: HPLC chromatogram of (A) Fluvermal® suspension and (B) Fluver® suspension using the proposed LC method.

Tables

Table 1: Summary of stress degradation studies of Flubendazole under different conditions.

Stress conditions	Time of degradation (hrs)	t_R of the obtained degradation products (min)	% Degradation
0.1 N NaOH at 80^0C	5	2.52	22.5 %
1 N NaOH at 80^0C	5	2.22, 2.7 and 2.8	99.25 %
0.1HCl at 80^0C	5	No degradation	Zero %
1HCl at 80^0C	5	2.21 and 2.47	33.10 %
H_2O at 80^0C	5	No degradation	Zero %
3% H_2O_2 at 80^0C	5	2.47 and 3.23	17.9 %
30%H_2O_2 at room temperature	5	2.49 and 3.20	43.66 %
Photo-degradation	20	3.15, 3.52 and 3.78	8.50 %
Dry heat at 80^0C	5	No degradation	Zero %

Table 2: Regression and analytical parameters of the proposed HPLC method for determination of Flubendazole (FLUB).

Parameters	FLUB
Linearity	
Range (µg/mL)	0.5 - 10
Slope	0.1944
SE of the slope	0.0079
Intercept	0.0197
SE of the intercept	0.0013
Correlation coefficient	0.9998
Accuracy (mean ± %RSD)	100.57± 1.47
Precision (%RSD)	
Repeatability [a]	1.01
Intermediate precision [b]	1.02
LOD (µg/mL)	0.11
LOQ (µg/mL)	0.34

[a] The intraday (n = 9), average of three different concentrations (4, 8 and 10 µg/mL) repeated three times within day.

[b] The interday (n = 9), average of three different concentrations (4, 8 and 10 µg/mL) repeated three times in three successive days.

Table 3: Determination of Flubendazole in Fluvermal® formulations by the proposed HPLC method and results of standard addition technique.

Pharmaceutical formulation	Taken	Found	% Found [a] ±%RSD	Standard addition technique	
				Pure added (µg/mL)	% Found [b]
Fluvermal® tablets (B. N. DCE1053) claimed to contain 100 mg FLUB/tablet	**4.00**	**4.03**	**100.75 ± 1.763**	4.00	101.00
				5.00	99.20
				6 .00	97.33
	Mean ± % RSD				**99.18 ± 1.85**
Fluvermal® suspension (B. N. DFE1824) claimed to contain 100 mg FLUB/t5 mL	**4.00**	**4.26**	**106.50 ± 0.133**	4.00	100.25
				5.00	100.60
				6.00	97.50
	Mean ± % RSD				**99.45 ± 1.71**

[a] Average of 6 determinations.

[b] Average of 3 determinations.

Table 4: Determination of Flubendazole in Fluver® formulations by the proposed HPLC method and results of standard addition technique.

Pharmaceutical formulation	Taken	Found	% Found [a] ±%RSD	Standard addition technique	
				Pure added (µg/mL)	% Found [b]
Fluver® tablets (B. N. 3139001) claimed to contain 100 mg FLUB/tablet	**4.00**	**4.01**	**100.25 ± 0.439**	4.00	100.00
				5.00	103.40
				6 .00	101.83
		Mean ± % RSD			**101.74 ± 1.67**
Fluver® suspension (B. N. 3209033) claimed to contain 100 mg FLUB/5 mL	**4.00**	**4.17**	**104.25± 1.322**	4.00	99.25
				5.00	97.40
				6.00	98.33
		Mean ± % RSD			**98.33 ± 1.33**

[a] Average of 6 determinations.

[b] Average of 3 determinations.

Table 5: Statistical comparison of the results obtained by applying the proposed HPLC method and the official HPLC one for determination of Flubendazole and in pure form.

Items	HPLC method	Official method [a] [3]
Mean	100.56	100.22
%RSD	1.47	1.27
Variance	2.19	1.62
n	9	8
Student's t-test	0.52 (2.13) [b]	
F- test	1.36 (3.73) [b]	

[a] RP-HPLC using a gradient mixture of ammonium acetate: acetonitrile at a flow rate of 1.2 mL/min and UV detection at 250 nm.

[b] Figures between parenthesis represent the corresponding tabulated values of t and F at P = 0.05.

Table 6: Parameters of system suitability of the developed RP-HPLC method for the determination of Flubendazole.

Parameters		Obtained value	Reference value [20]
		FLUB	
Tailing factor (T)		1.02	≈ 1
Capacity factor (K')		3.86	1- 10 acceptable
Number of theoretical plates (N)		2488.36	Increase with efficiency of the separation
HETP		0.01005	The smaller the value the higher the column efficiency
Resolution (R_s)	0.1 N NaOH	9.91	R > 2
	1 N NaOH	7.52	
	1N HCl	8.08	
	3 % H_2O_2	7.03	
	30 % H_2O_2	8.64	
	Neutral	9.65	
	Light	5.53	
	Fluvermal® suspension	2.95	
	Fluver® suspension	2.95	
Selectivity (α)	0.1 N NaOH	2.99	> 1
	1 N NaOH	2.50	
	1N HCl	3.10	
	3 % H_2O_2	2.01	
	30 % H_2O_2	2.02	
	Neutral	3.63	
	Light	1.16	
	Fluvermal® suspension	1.31	
	Fluver® suspension	1.31	

HETP = Height equivalent to theoretical plate, cm/plate.