

Stability Indicating HPLC Method for the Determination of Diflubenzuron Insecticide

Vinayak Ambike, Anant Argekar*

Department of Chemistry, The Institute of Science, Mumbai, India

Email address:

apargekar@gmail.com (A. Argekar)

*Corresponding author

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Abstract: A new, fast, accurate, reliable and stability indicating HPLC method for the determination of Diflurobenzuron (DFB), in technical and formulation samples in presence of related and degraded impurities has been developed using intersil – 3 stainless steel C. 18 column (5 μ m, 250 mm length x 4.6 mm id), acetonitrile: water: 1.4 dioxane (58:39:0.03 v/v) as mobile phase and diphenyl as internal standard. The flow rate of the mobile phase was 2 cm³ min⁻¹. Detection was carried out at 260 nm using UV detector. The retention times were 1.55 min, 1.75 min, 6.3 min, 7.9 min and 10.7 min for 2, 6 difluorobenzoic acid, 2, 6 difluorobenzamide, difluorobenzuron, difur (an impurity) and diphenyl (internal standard) respectively. The linearity range of DFB was 0.5 to 15 mg per 100 cm³. The LOD and LOQ values for DFB were 0.142 to 0.432 respectively. When various technical and formulated samples were analysed by this proposed method the percentage recoveries were found to be 99.20-101.80% with RSD between 0.01% to 0.45%.

Keywords: Diflubenzuron, Stability Indicating HPLC, Diphenyl Internal Standard

1. Introduction

Diflubenzuron (DFB), 1-(4-chlorophenyl)-3-(2, 6 difluorobenzoyl).

Urea (CAS RN-35367-38-5), is a well-known insecticide used for the control of a wide range of insects, pests and larvae of flies, mosquitoes and locusts. It is also ecto parasiticide. DBF is a chitin synthesis inhibitor that act as an anti-moulting agent, leading to death of larvae and pupae [1].

An up to date literature survey indicates that DFB has been analysed by various analytical instrumental techniques like GLC [2, 3, 4] HPLC [5, 6, 7, 8, 9, 10] SFC [10, 12, 13] HPTLC [14] etc. The analytical method for DFB, recommended by CIPAC [15], is HPLC.

In this method C-8 (octyl) column, or alternate, mobile phase containing acetonitrile, water and 1:4 Dioxan, in proportion of 45:40:10 and "Linuron" as internal standard are used. However, due to high concentration of 1:4 Dioxan, the column efficiency is reduced and resolution of DFB and related impurity peaks are affected. Also Linuron, the internal standard, used in this method, is a specific agro

product, and may not be available readily in expected pure form.

However, in the present method, C-18 (octadecyl) column and mobile phase consisting of Acetonitrile: Water: 1:4 Dioxan in the proportion of 58:39:03 (V/V/V) and Biphenyl is used as an internal standard are used. C-18 columns are readily available and are more economic than C-18 (Octyl) columns; content of 1:4 Dioxan is very less in the mobile phase and Biphenyl is cheaply available with simple structure and high purity. Moreover, DFB and its related as well as degraded impurities are very well resolved in this method.

2. Experimental

2.1. Instrumentation

A high-pressure liquid chromatograph, SHIMADZU, equipped with LC-10 AD pump, SPD-10 AV UV/VIS detector with variable wavelength and Controller Bus Module CBM-10 A was used. A photodiode array detector (Shimadzu SPD M 10 A) was also used for confirming the

peak purities.

2.2. Chemicals and Solvents

DFB (technical) procured from M/s. Shimac, China with purity of 95% was used as a reference standard. Formulation samples of DFB were procured from market.

Acetonitrile and Dioxan used were of HPLC grade supplied by S D Fine Chemicals and SRL respectively, whereas water used was double distilled prepared in the laboratory.

2.3. Stationary Phase

Inertsil-3 stainless steel C-18 column (5 μ m; 250 mm length X 4.6. mm i. d.) from GE Science Inc., Japan was used as a stationary phase.

2.4. Mobile Phase

Acetonitrile: water: 1:4 Dioxan in the volume ratio of 58:39:03.

2.5. Preparation of Stock Solutions

Stock solution (A) of DFB was prepared by dissolving 100 mg of DFB in 50 cm³ of 1:4 Dioxan followed by sonication for about 15 minute and diluted to 100 cm³ with 1:4 Dioxan.

The stock solution (B) of internal standard was prepared by dissolving about 120 mgs of Biphenyl in 50cm³ of 1:4 Dioxan followed by sonication for about 15 min. and diluted to 100cm³ with 1:4 Dioxan.

2.6. Preparation of Working Standard Solution

10cm³ of solution (A) was taken in a 100cm³ volumetric flask and 5cm³ of solution (B) was added. The solution was diluted to 100cm³ with the mobile phase.

2.7. Preparation of Sample Solutions

Various formulation samples namely technical, wettable powder (WP), wettable dispersible granules (WDG) and suspoconcentrate (SC) were accurately weighed equivalent to 10mg of DFB, in duplicate, and transferred to 100cm³ flask containing 50cm³ of 1:4 Dioxan and sonicated for 15 minutes and then diluted to 100cm³ with 1:4 Dioxan. 10 cm³ of this solution was mixed with 5cm³ of internal standard (solution B) and diluted to 100cm³ with mobile phase. This solution was filtered through Whatman no.1 filter paper to obtain clear solution before injection.

In the case of WDG samples, the material was crushed to fine powder in a mortar before weighing and in case of suspo-concentrate formulation (SC) 20cm³ of water was added to ensure complete dispersion before dissolving in 50cm³ of 1:4 Dioxan.

2.8. Chromatographic Conditions

Column: Inertsil-3 stainless steel C-18 column, 5 μ m; 250 mm length X 4.6 mm i. d.
Flow rate: 2cm³ min⁻¹

Detector: UV-260 nm

Range: 1.0 AUFS

Attenuation: 9 AUFS

Injection volume: 20 μ l

2.9. Procedure for Calibration

Into a series of 100cm³ flasks, varying amount of stock solution A (1 to 16cm³) were taken and 5 cm³ of internal standard solution (B) was added. The contents were diluted up to the mark with the mobile phase. 20 μ l of each solution was injected into the column and peak area ratios were recorded for all the Chromatograms. Calibration curve constructed by plotting peak area ratio (Y-axis) against the amount of DFB in mg/cm³ (X-axis) and the linear relationship was evaluated by calculation of regression line by the method of least squares.

2.10. Assay Procedure

Each of the sample solution, prepared as above in duplicate, was injected in the column and the peak area was recorded as described in calibration procedure. The amount of DFB was computed by internal standard quantification using following equation:

$$\text{DFB content, percent by mass} = \frac{M_1 \times A_2 \times A_3 \times P}{M_2 \times A_1 \times A_4}$$

Where

M_1 = mass, in mgs. of DFB ref. standard taken

M_2 = mass, in mgs. of DFB sample taken

A_1 = Peak area of DFB ref. standard obtained

A_2 = Peak area of DFB sample obtained

A_3 = Peak area of internal standard obtained in sample

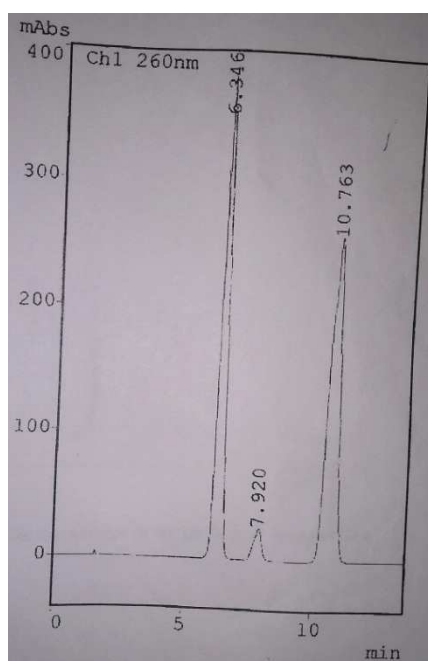
A_4 = Peak area of internal standard obtained in ref. Std.

P = Percentage purity of ref. standard

3. Results and Discussions

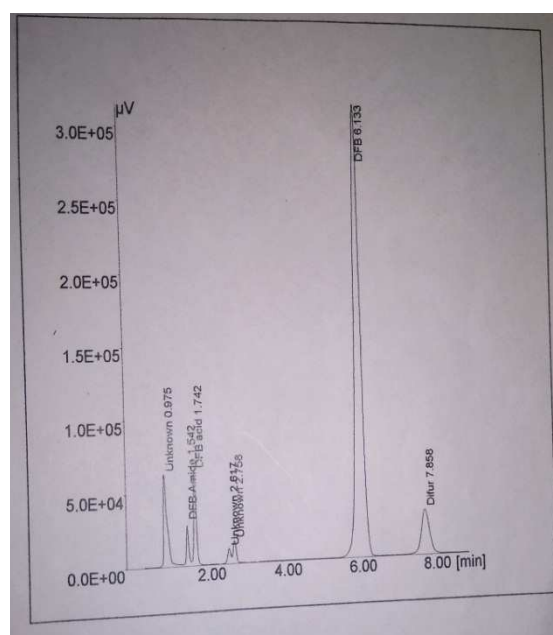
3.1. Chromatography

The mobile phase comprising of Acetonitrile: water: 1:4 Dioxan in the proportion of 58:39:03 was selected because it was ideal to resolve DFB, Difur (an impurity) and Biphenyl (internal standard) with retention times of 6.3 min., 7.90 min. and 10.7 min. respectively (Figure 1). Also the impurities, formed due to degradation, namely 2, 6 difluorobenzamide and 2, 6 Difluoro Benzoic acid are also well resolved from DFB (Figure 2). Solutions of DFB, Difur and Biphenyl in 1:4 Dioxan gave maximum absorption at 259 nm, 262 nm and 248 nm respectively. 260nm was selected for detection, because at this wavelength all the three components gave measurable absorbance (Figure 3). Since 1:4 Dioxan content in the mobile phase was minimum, the column performance was not affected and the results obtained were highly reproducible. The peak purities of DFB Difur and Biphenyl were confirmed using PDA detector. The chromatographic parameters of the system are given in Table-3.



1 DFB 2 Difur 3 Biphenyl

Figure 1. Chromatogram of DFB, Difur and Biphenyl.



1 Unknown 2 DFB-amide 3 DFB acid 4 Unknown 5 Unknown 6 DFB 7 Difur

Figure 2. Chromatogram of DFB and impurities.

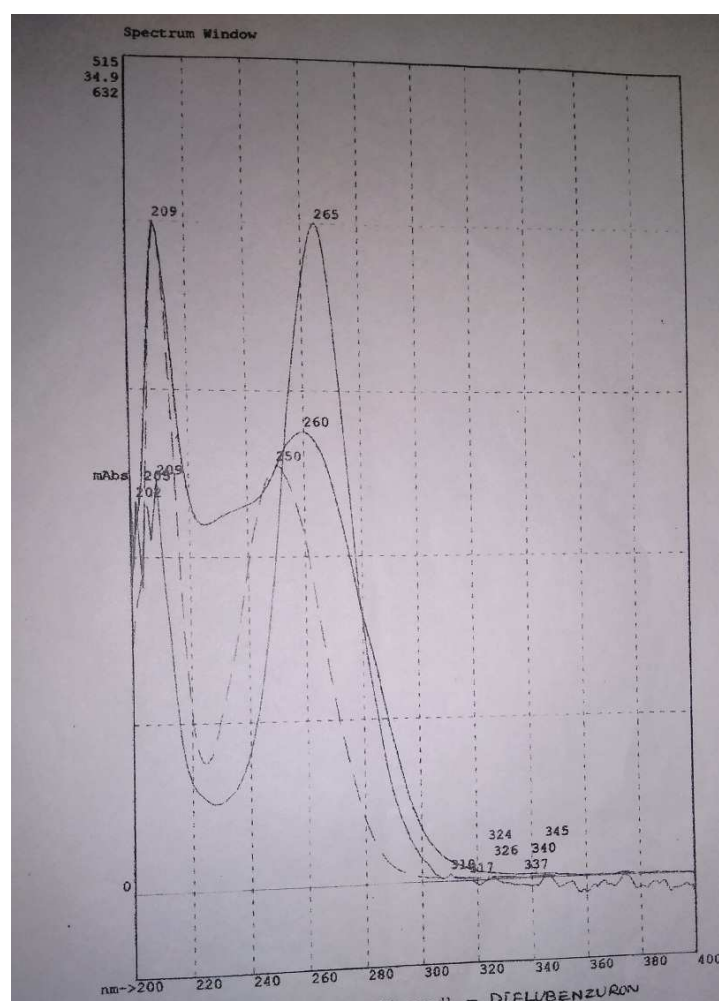


Figure 3. PDA scan of DFB, Difur and Biphenyl.

3.2. Linearity, Limit of Detection and Limit of Quantification

The plot of peak area ratios versus the respective concentration of DFB was found to be linear in the range from 0.5 mg to 15 mg/100cm³ (Table-2). The limit of detection (LOD) and limit of quantification (LOQ) were calculated by using the equations,

$$\text{LOD} = 3.3 \times \sigma/S \text{ and } \text{LOQ} = 10 \times \sigma/S$$

Where, 'σ' the noise elimination, is the standard deviation

of the blank responses (six injections) and 'S' is the slope of corresponding calibration curve of DFB. LOD and LCQ values were found to be 0.142 and 0.432 respectively

3.3. Assay

Contents of DFB found in the commercial brand of samples by the present method are as shown in the Table-3. The low values of RSD indicate that the method is precise and accurate.

Table 1. Chromatographic Parameters.

Parameter	Values (mean)		% RSD (N=5)	
	DFB	BIPHENYL	DFB	BIPHENYL
Theoretical plates	7408	9963	1.7	2.19
Capacity	659.2	1157	0.07	0.11
Asymmetry	1.4	1.44	1.88	1.38
Retention time	6.34	10.76	0.11	1.13
Resolution	--	12.04	--	1.14

Table 2. Linearity Study.

Amount of DFB std Taken in mgs	Area ratio (n = 3)	% RSD
0.5	0.047	0.45
1.0	0.10	0.31
2.0	0.184	0.52
5.0	0.496	0.12
10.0	0.930	0.18
15.0	1.396	0.07

Regression Output:

Slope: 0.09288

R Squared: 0.99995

Std. Err. Of Y Est (: 0.00401

No. of Observations: 6

Degree of Freedom: 4

'X' Coefficient: 0.00141

Table 3. Sample Analysis (assay).

Sr. No	Sample	Qty in mgs	Amt of DFB found in mg	Purity%	%RSD
1	Techn. tel#1	11.74	11.23	97.5	0.24
		9.66	9.22	95.4	
2	Tech # 2	16.84	10.35	95.5	0.27
		9.95	9.48	95.3	
3	25 WB # 1	40.86	10.50	25.7	1.09
		42.08	10.61	25.1	
4	25 WB # 5	41.76	10.73	25.7	0.83
		42.08	10.61	25.4	
5	25 WDG#1	40.32	9.59	23.8	1.47
		409	9.93	24.3	
6	25 WDG#2	42.20	10.55	24.9	0.42
		43.72	1097	25.1	
7	5 SC#6	152.23	7.61	5.0	2.29
		201.52	9.87	4.8	
8	25 SC#5	43.46	10.95	25.2	0.28
		40.26	10.10	25.1	

3.4. Accuracy and Precision

To study the accuracy and precision of the present method, the recovery experiments were performed by standard addition technique. Four different levels of standards were added to pre-analyzed samples and each level was repeated thrice. The

percentage recoveries in the samples were in the range of 99.4 to 100.25 for technical, 99.7 to 101.7 for 25 WP, 99.2 to 101.5 for 25 WDG and 99.4 to 101.8 respectively (Table-4). The results indicate that there is no interference due to excipients present in the formulations (% RSD \leq 0.4 max.).

Table 4. Sample Recovery Study.

Blank value in mgs	Amount of std added in mgs	Avg. area ratio(n = 3)	Amount of DFB recovered in mgs	Recovery in %	RSD %
DFB Tech. # (purity = 95.5%)	Sample weights = 104.8 mgs				
10.35	NIL	1.061	10.29	99.40	0.01
10.35	0.5	1.123	10.88	100.25	0.10
10.35	1.0	1.182	11.34	99.91	0.36
10.35	2.0	1.293	12.24	99.08	0.40
10.35	3.0	1.430	13.66	100.12	0.20
DFB. (25 WB) #1 (A% = 25.7%)	Sample weights = 408.6 mgs				
10.5	NIL	1.156	10.47	99.7	0.14
10.5	0.5	1.241	11.19	101.70	0.22
10.5	1.0	1.304	11.56	100.5	0.35
10.5	2.0	1.46	12.62	100.96	0.12
10.5	3.0	1.596	13.47	99.76	0.14
DFB. (25 WDG) #2 (A% = 25%)	Sample weights = 437.2 mgs				
10.97	NIL	1.076	10.88	99.20	0.18
10.97	0.5	1.152	11.61	101.20	0.24
10.97	1.0	1.227	11.97	100.0	0.42
10.97	2.0	1.382	13.17	101.54	0.16
10.97	3.0	1.537	14.07	100.87	0.17
DFB. (5 SC) # 6 (A% = 4.8%)	Sample weights = 2015.2 mgs				
9.87	NIL	1.056	9.91	100.4	0.14
9.87	0.5	1.112	10.40	99.40	0.24
9.87	1.0	1.168	10.85	99.85	0.45
9.87	2.0	1.289	11.96	100.78	0.28
9.87	3.0	1.424	13.10	101.80	0.25

3.5. Stability Indicating Ability of the Method

In a series of five volumetric flasks of 100 cm³ capacity, 10 mgs of DFB were taken and 5 ml of 1 N NaOH were added to each flask and kept aside for undergoing alkali hydrolysis. After every 24 hrs. one of the flasks were treated with 1 N HCl to neutralize to pH 7 and the solution was diluted up to the mark with mobile phase and 20 μ l of each were then injected in the HPLC column and the present procedure was followed to analyze DFB. It was observed that the decomposed neutralized solution gave a number of low retention products,

the percentage of which significantly increased from 1 to 5 days as shown in Table-5. The impurities decomposition products at retention times 1.55 min. and 1.75 min. were confirmed to be 2, 6 Difluorobenzamide and 2, 6 Difluoro Benzoic acid by spiking the standards of these compounds however, other impurities/products could not be identified due to non-availability of standards.

These results indicate that a present method is a stability indicating method since the method can be not only for assay of DFB but also the impurities like Difur, 2, 6 Difluoro Benzoic acid, 2, 6 Difluorobenzamide without interference.

Table 5. Decomposition Study.

	Imp.-1 (1 min.) %	Imp-2 (1.5 min.) %	Imp-3 (1.8 min.) %	Imp-4	Imp-5	DFB	Difur
Day 1	14.3	0.27	3.5	1.07	1.26	71.78	7.58
Day 2	35.4	0.23	3.28	0.93	1.05	52.50	6.61
Day 3	40.2	0.21	2.93	0.88	1.00	48.91	5.54
Day 4	44.5	0.16	2.17	0.87	1.28	45.75	4.97
Day 5	49.0	0.17	2.50	0.77	0.84	41.97	4.67

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