Int J Biomed Investig (2025) Vol 8 (2)

International Journal of Biomedical Investigation

journal homepage: https://ijbi.edwiserinternational.com

Original Article

A novel RP-UPLC method development and validation for quantification of flubendazole in bulk and pharmaceutical dosage form

Mageshwari Anand[1,*], P.G. Sunitha[1], N. Deattu[1], T. Hariharan[1], Hemanath R[1], Jawaharsamuvel R[2]

[1]Department of Pharmaceutical Chemistry, College of Pharmacy, Madras Medical College, Affiliated to The Tamil Nadu Dr. M.G.R Medical University, Chennai, Tamil Nadu, India
[2]Department of Pharmaceutical Chemistry, College of Pharmacy, Sri Venkateswaraa University, Redhills, Chennai, Tamil Nadu, India

ARTICLE INFO

Received 10 June 2025
Revised 09 July 2025
Available Online 15 July 2025

Keywords:
RP-UPLC
Flubendazole
Validation
ICH guidelines
Method development

ABSTRACT

RP-UPLC approach was used to produce a straightforward, accurate, and exact method for estimating flubendazole. For chromatography, stationary phase C18 (4.6 x50 mm,5 µm) was utilized. Acetonitrile and HPLC-grade water in 700:300 ratio was used as the mobile phase. The flow rate was kept at 0.45 ml/min, the detection wavelength was set at 230nm.six standard injections was used to study the system suitability characteristics, and the finding fell well within the acceptable range. The r2 value was 1.0000 after a linearity analysis was conducted at 80% to 120%level. The results showed that the precision was 0.66 for intermediate precision and 0.73 for repeatability. Respectively the LOD and LOQ values was found to be0.002 µg/ml and 0.006 µg/ml.

Introduction

Flubendazole (FLUB) chemically is identified as [5(4Fluorobenzoyl)1 H benzimidazol2yl] carbamic acid methyl ester1 gives 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].

*Corresponding author: Mageshwari Anand. Department of Pharmaceutical Chemistry, College of Pharmacy, Madras Medical College, Affiliated to The Tamil Nadu Dr. M.G.R Medical University, Chennai-600 003, Tamil Nadu, India.

https://doi.org/10.31531/2581-4745.1000164

UPLC

Ultra-Performance Liquid Chromatography (UPLC) is specially designed to withstand higher system pressures during chromatographic analysis so that it enables significant decreases in separation time and solvent consumption. The UPLC columns packed with 1.7 µm sized particles provides not only increased efficiency but also the ability to work at increased linear velocity without loss of efficiency but also the ability to work at increased linear velocity without loss of efficiency, providing both resolution and speed. Using advantages of UPLC, a number of applications in different fields including pharmacy, clinical analysis, pesticide analysis and tetracycline in human urine have been reported. The UPLC is based on the principle of use of stationary phase consisting of particles less than 2µm, while UPLC columns are typically filled with particles of 3 to 5 µm. The underlying principles of this evolution are governed by the Van Deemter equation, which is an empirical

formula that describes the relationship between linear velocity (flow rate and plate height (HETP or column efficiency). The Van Deemter curve, governed by an equation with three components shows that the usable flow range for a good efficiency with a small diameter particle in much greater than for larger diameter.

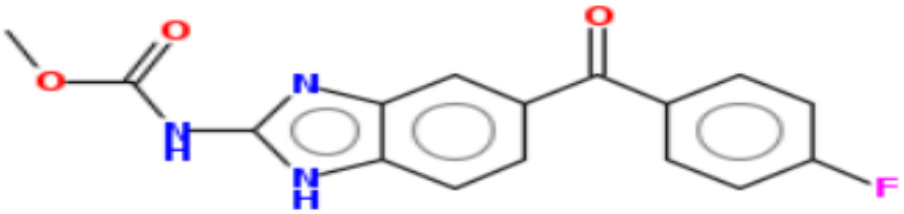

Figure 1: Structure of Flubendazole.

Method Development

The following criteria have been utilized to build the Flubendazole estimation method based on drug solubility and Pka Value.

Optimized Chromatographic Conditions:

Column: C18 (4.6 mm x 50 mm, 5 microns)

Mobile phase: Acetonitrile and water (700:300)

Flow rate: 0.45 ml/min

Detection wavelength: 230nm

Temperature: 30ºC

Injection Volume: 5 µL.

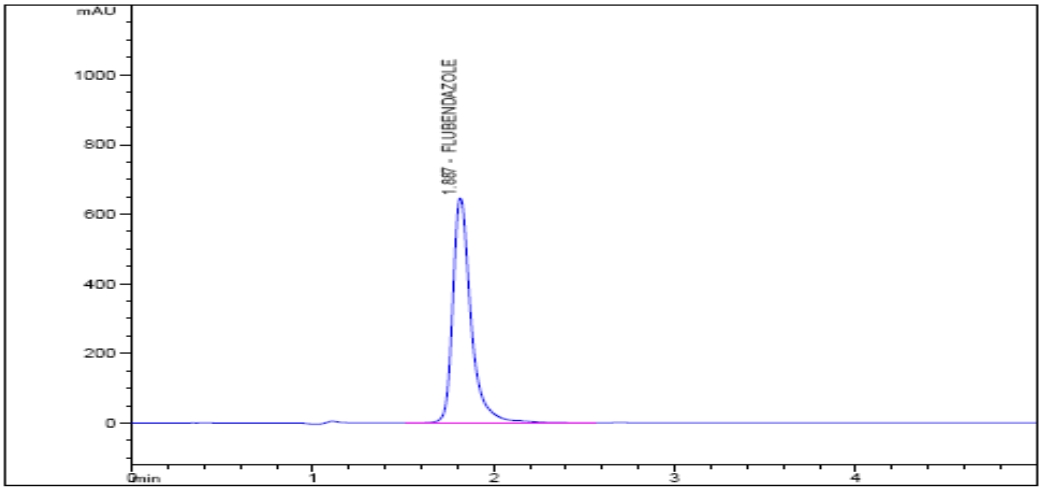

Figure 2: Optimized chromatogram.

Materials and Methods

Diluent: According to the drug's solubility, 50% ACN and 0.1M methanolic HCl was used.

Preparation of Standard stock solution

Around 20 mg of the flubendazole was transfer into standard 25 ml volumetric and dissolved in 0.1M methanolic HCl and made up the volume.

Preparation of Standard working solution (100% solution)

1ml of standard stock solution was transfer into 10ml volumetric flask made up to the volume with 50% ACN.

Preparation of Sample stock solution

Around 20mg of Flubendazole sample into 25 ml volumetric flask. It was dissolved and made up to the volume with 0.1M Methanolic HCl and filter through 0.45 µm membrane filter. 1ml of the solution was transfer into 10ml volumetric Flask and makeup with 50% ACN.

Preparation of Standard stock solution

Weigh accurately and transfer around 20 mg of Flubendazole standard into 25 ml volumetric standard flask.it was dissolved and made up to the volume with 0.1M Methanolic HCl.

50% Standard solution

1.0ml standard stock was pipetted out and made up to 20 ml.

75% Standard solution

0.7ml standard stock was pipetted out and made up to 10 ml.

100% Standard solution

1.0ml standard stock was pipetted out and made up to 10 ml.

125% Standard solution

1.3ml standard stock was pipetted out and made up to 10 ml.

150% Standard solution

1.5ml standard stock was pipetted out and made up to 10 ml.

Accuracy

Preparation of 80% solution

Around 16mg of Flubendazole was transfer into 25 ml volumetric flask. It was dissolved and made up to the volume with 0.1M Methanolic HCl and filter through 0.45 µm membrane filter. 1ml of the solution was transfer 10ml volumetric Flask and made up with 50% ACN.

Preparation of 100% solution

Around 20mg of Flubendazole was transfer into 25 ml volumetric flask. It was dissolved and made up to the volume with 0.1M Methanolic HCl and filter through 0.45 µm membrane filter. 1ml of the solution was transfer 10ml volumetric Flask and made up with 50% ACN.

Preparation of 120% solution

Around 24mg of Flubendazole was transfer into 200 ml volumetric flask. It was dissolved and made up to the volume with 0.1M Methanolic HCl and filter through 0.45 µm membrane filter. 1ml of the solution was transfer 10ml volumetric Flask and made up with 50% ACN.

Acceptance criteria

The % Recovery for each level should be between 98.0%- 102.0%.

Robustness

Small deliberate changes in method like Flow rate and Wavelength were made. Robustness conditions like Flow minus, Flow plus, Wavelength decreasing and Wavelength increasing was maintained and samples were injected in duplicate manner.

LOD and LOQ

The calibration curve method was used to calculate LOD and LOQ independently. Using the devised UPLC method, the compound's LOD and LOQ were determined by injecting progressively lower quantities of the standard solution.

Results and Discussions

System suitability

Following protocol, a standard solution of flubendazole was made and injected into the UPLC apparatus five times. By determining the percentage RSD of retention time, tailing factor, theoretical plates, and peak areas from five replicate injections that fall within range, standard chromatograms were used to assess the system appropriateness criteria.

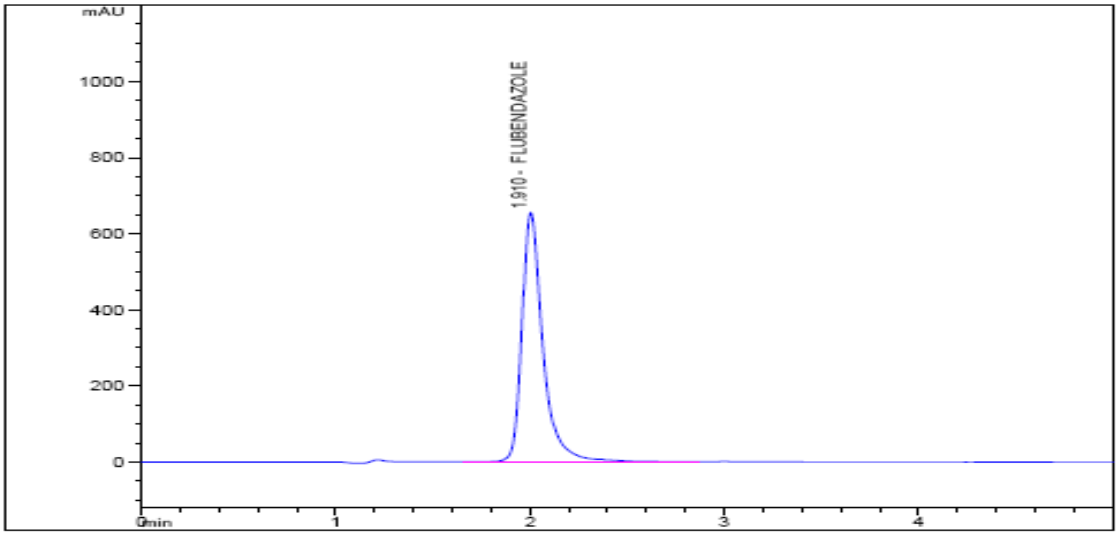

Figure. 3: System suitability Chromatogram.

Table 1: Peak Name – Flubendazole.

S.No.	Peak name	RT	Area	Theoretical plate count	Tailing factors
1.	Flubendazole	1.900	4616.130	2010	1.419
2.	Flubendazole	1.892	4659.480	2014	1.415
3.	Flubendazole	1.897	4593.252	2021	1.42
4.	Flubendazole	1.898	4540.714	2013	1.454
5.	Flubendazole	1.895	4503.282	2045	1.409
Mean		**1.90**	**4582.572**	**2021**	**1.423**
SD		0.003	61.632	14.22	0.018
%RSD		**0.16**	**1.34**	**0.70**	**1.24**

Precision

Repeatability

Six working sample solutions of 80ppm are injected and the percentage amount was calculated and %RSD was found to be 0.6 and chromatogram was shown.

Five working sample solutions of 80ppm are injected on the next day of the preparation of samples and the % amount found was calculated and %RSD was found to be 0.6.

Repeatability Chromatogram

Intermediate precision

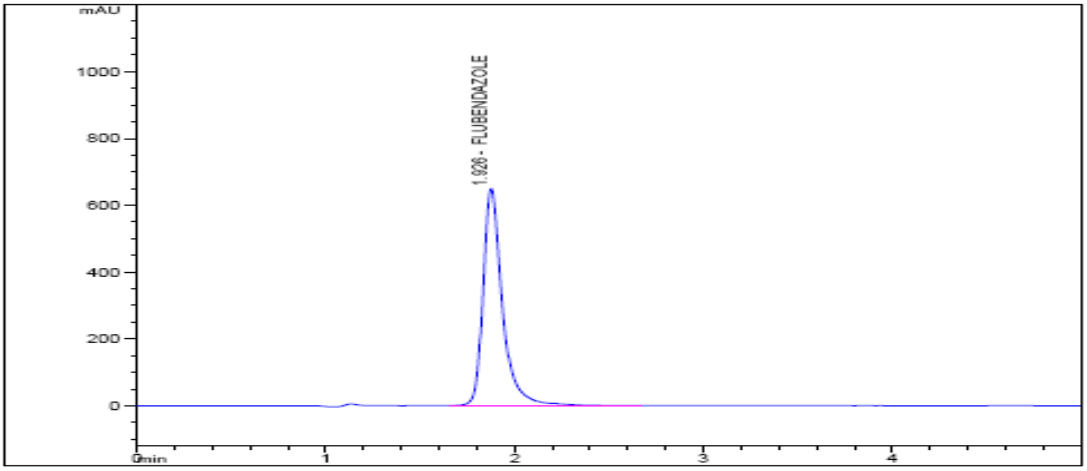

Figure 4: Intermediate precision Chromatogram.

Linearity

To demonstrate the linearity of assay method, inject 6 standard solutions with concentrations of about 40 ppm to 120 ppm of Flubendazole. Average areas were mentioned above and linearity equations obtained for Flubendazole y = 57.9909 x + 0.1666 Correlation coefficient obtained was R^2 = 1.0000 for the Flubendazole.

Table 2: Linearity Concentration and Responses.

Linearity Level (%)	Concentration (ppm)	Area
50	40.25	2334.26
70	56.35	3267.715
100	80.50	4667.397
125	100.62	5836.584
150	120.74	7001.531

Accuracy

Three injections of 80%, 100%, and 120% concentrations were made in triplicate, and the recovery percentage was determined to be 99.30.

LOD

Detection limit of the Flubendazole in this method was found to be 0.002 µg/ml.

Table 3: Accuracy data.

% Level	Sample wt. (mg)	Area	Content (mg)	Content (%)
80%	48.65	3680.158	98.870	98.87
	49.05	3679.481	98.046	98.05
	48.23	3685.468	99.875	99.88
100%	60.36	4599.361	99.593	99.59
	61.03	4600.789	98.530	98.53
	60.78	4611.145	99.158	99.16
120%	72.09	5520.360	100.086	100.09
	72.78	5528.390	99.281	99.30
	72.21	5536.158	100.206	100.21

LOQ

Detection limit of the Flubendazole in this method was found to be 0.006 µg/ml.

Robustness

Small, intentional adjustments are made to this procedure, such as temperature minus, temperature plus, mobile phase minus, mobile phase plus, flow minus, and flow plus. The above conditions %RSD is computed.

Table 4: Robustness Data.

Parameter	%RSD
Flow Minus(0.35ml/min)	0.83%
Flow Plus(0.55ml/min)	0.55%
Wavelength Minus (228nm)	0.43%
Wavelength Plus(232nm)	0.95%

Assay of Marketed Formulation

Standard solution and sample solution were injected separately into the system and chromatograms were recorded and drug present in sample was calculated using before mentioned formula.

Table 5: Assay of Formulation.

Standard	Sample	%Assay
4645.451	4621.451	99.62

Conclusion

To estimate the medication flubendazole in pharmaceutical dosage form, the UPLC method was created. The UPLC method was refined through the investigation of various circumstances and media. The suggested approach was validated in accordance with ICH requirements in all appropriate parameters, and the sample recoveries in each formulation were in good agreement with their corresponding labelled claims. This technique can be applied to the quality control testing of pharmaceutical dosage forms medication flubendazole.

Author Contributions

All authors contributed equally to this research. All authors read and approved the final manuscript.

Acknowledgements

The authors are thankful to the Department of Pharmaceutical Chemistry, College of Pharmacy,

Madras Medical College, Chennai, Tamil Nadu for helping in carrying out this work.

Conflict of Interest

The authors declare no conflict of interest.

Funding

The authors did not receive any fundings from any private or government sources.

Ethical Approvals

This study does not involve experiments on animals or human subjects.

References

1. Muheem A, Shakeel F, Zafar S, Jahangir MA, Warsi MH, Jain GK, Ahmad FJ. Development and validation of stability indicating liquid chromatographic (RP-HPLC) method for estimation of ubidecarenone in bulk drug and formulations using quality by design (QBD) approach. Brazilian Journal of Pharmaceutical Sciences. 2017;53(4):e17293.
2. Martindale, The Extra Pharmacopoeia the Complete Drug Reference, 35th Ed.K. Profitt (Ed.). Royal Pharmaceutical Society. London UK ;2006.
3. Viktor Cvilink, Lenka Skálová. LC–MS–MS identification of albendazole and flubendazole metabolites formed ex vivo by Haemonchus contortus. Analytical and bioanalytical chemistry.2008; 391:337–343.
4. Hendrik De Ruyck, Els Daeseleire. Determination of Flubendazole and Its Metabolites in Eggs and Poultry Muscle with Liquid Chromatography-Tandem Mass Spectrometry. Journal of agricultural and food chemistry.2001; 49, 610−617.
5. Guglielmo Dusi. Rapid determination of the antiparasitic drugs flubendazole and febantel in feeds by HPLC with ultraviolet detection. Journal of Pharmaceutical and Biomedical Analysis.2005; 38 (2005) 375–379.