RESEARCH PAPER

Development and Validation of a Robust Spectrophotometric Method for the Determination & Stability Profiling of Flunarizine Dihydrochloride by UV-Visible Spectroscopy

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ABSTRACT:

OBJECTIVES: A Stability indicating UV spectroscopic method for the accurate, precise and robust estimation of Flunarizine Dihydro chloride in bulk and its dosage form and the stability studies was performed for the drug as per ICH Guidelines (Q2)(R1) Guidelines.

Materials & Methods: FLUNARIZINE DIHYDROCHLORIDE shown its maximum absorbance at 254 nm by using 25% v/v methanol. The calibration curve was drawn over a concentration range of 10-60 µg/ml with a correlation coefficient (r^2) of 0.9992.

Results: The developed method was validated in accordance with ICH guidelines and shown acceptance for all the parameters. Accuracy was assessed through recovery studies and was found within 98-102%. stability studies were performed for the drug and its drug product was found to be stable at all stress conditions.

Conclusion: The stability studies were monitored and characterized to understand the stability profile of drug. This validated method can be readily applied for routine quality control analysis of Flunarizine di hydrochloride in bulk and pharmaceutical dosage forms.

KEYWORDS: Method development, Calcium channel blocker, Flunarizine di hydrochloride, stability studies.

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1. INTRODUCTION:

Flunarizine (FLN) is a chemically 1-[Bis (4-fluorophenyl) methyl]-4-[(2E)-3-phenyl-2-propenyl] Piperazine. So, Flunarizine Dihydro HCl is a piperazine derivative that exhibits pharmacological activity as a mixed T-type and L-type calciumchannel blockerand also blocks sodium channels. Flunarizine is mainly employed in the prophylaxis of migraine and in the management of vestibular disorders such as vertigo and also useful in neurological conditions including

epilepsy. It may also known as Flunarizine 2HCL and was marketed under the brand name of sibelium, flunarin. Molecular formula of flunarizine is $C_{26}H_{26}F_2N_2 \cdot 2HCl$. Chemical structure of Flunarizine dihydrochloride was shown in Figure 1

Flunarizine is a selective calcium entry blocker that prevents cellular calcium overload. It stabilizes neurons against cortical spreading depression and inhibits H1 and D2 receptors to prevent migraines and vertigo.

The aim of the present study was to develop and validate a sensitive and economical UV spectrophotometric analytical method for the estimation of Flunarizine dihydrochloride in pharmaceutical dosage form.

The Development of a stability- indicating method is essential for evaluating the stability of a drug over time in both its pure form and pharmaceutical formulation. It plays an important role in meeting regulatory requirements and more importantly in ensuring patient safety.

II. MATERIALS AND METHODS

In this study, the drug Flunarizine dihydrochloride was sourced from *Yarrowchem (Mumbai)*. The dosage form used in our investigation was a marketed formulation, containing a labelled dose of 5mg of Flunarizine dihydrochloride

For the experimental work, methanol and distilled water were used as solvents. To analyze the drug, UV-Visible double beam spectrophotometer (SHIMADZU.) equipped with UV Probe software to record the absorption spectrum was used.

To ensure proper sample preparation for UV-Vis analysis, ultrasonication was utilized to aid the dissolution and dispersion of the drug into the solvent. This technique helps produce a more uniform and reliable solution for accurate spectrophotometric measurements.

III. METHODOLOGY:

Selection of solvent:

To select a suitable solvent for the determination of Flunarizine dihydrochloride Various solvents were used such as Methanol, Methanol (25%v/v), Ethanol, water are tested for solubility studies and the drug was freely soluble in Methanol (25%v/v) and was selected as a solvent.

Preparation of standard solution:

10mg of Flunarizine dihydrochloride was accurately weighed and transferred into 10ml volumetric flask, make up the volume up to the mark with solvent (25% v/v Methanol), The concentration of the prepared solution is 1000 µg/ml (Stock solution). From the stock solution, respective dilutions were prepared using 25% methanol &75% distilled water.

Preparation of sample solution:

Accurately weighed 10 tablets and determined the average weight of each tablet, drug equivalent 10mg was weighed and transfered into 10ml volumetric flask. 10 ml of solvent Methanol (25%v/v) was added and the solution filtered using whattman's filter paper, if necessary, Sonicated for 10 min. The concentration of this solution was 1000µg/ml. Further dilutions were prepared by using solvent.The 0.5ml of stock solution was diluted to 10 ml with solvent to give 50µg/ml. The absorbance of the resulting solution was measured against the respective blank solution (25%v/v Methanol) in the UV region of 200-400nm, which shows maximum absorbance at 254 nm.

IV. VALIDATION OF THE METHOD:

i. Linearity and Range:

To prepare the calibration curve for flunarizine dihydrchloride, six different concentrations ranging from 10 to 60 µg/ml were prepared. For each concentration, the appropriate volume was pipetted into a 10 ml standard volumetric flask, and the volume was made up to the mark using solvent(25% v/v methanol). The absorbance of each solution was then measured at 254 nm using a UV spectrophotometer, with 25% methanol (v/v) serving as the blank. Finally, the recorded absorbance values were plotted against the corresponding concentrations to construct the calibration graph, which helps in determining the linear relationship between concentration and absorbance.

ii. Precision:

Precision of the method was evaluated by analyzing multiple homogeneous samples and expressing the results as % Relative Standard Deviation (% RSD). Both intra-day and inter-day precision studies were performed to assess the consistency of the method over time.

Intra-day:

To evaluate short-term repeatability, 0.5 ml (50 µg/ml) of the stock solution was measured and analyzed six times at different intervals throughout the same day.

Inter-day:

For long-term consistency, the same 0.5 ml (50 µg/ml) sample was analyzed six times across different days. The % RSD was then calculated for both sets of measurements to determine the method's reliability and reproducibility over time.

iii. Assay:

Weigh 4 tablets accurately and calculate the average weight per tablet. Using a mortar and pestle, carefully grind the tablets into a fine powder. From this powder, weigh exactly 220mg, which corresponds to the appropriate amount of active ingredient, and transfer it into a 10 ml volumetric flask.

After, add 10 ml of 25% methanol (v/v) to the flask as the solvent. To ensure the drug is fully dissolved and evenly dispersed, sonicate the mixture for 6 minutes.

Once the stock solution is prepared, pipette out 5ml of this solution—corresponding to a concentration of 50 µg/ml—into another 10 ml volumetric flask. Top it up to the mark with solvent. Finally, measure the absorbance at 254 nm using a UV – visible spectrophotometer, and repeat the measurement three times to ensure accuracy and consistency.

iv. LOD AND LOQ:

The Limit of Detection (LOD) and Limit of Quantification (LOQ) were determined using the slope of the calibration curve and the standard deviation obtained from the precision study. These values help define the smallest amount of flunarizine dihydrochloride that can be reliably detected or quantified using the developed method.

v. Robustness:

Robustness is a way to check how reliable a method is when small, intentional changes are made to the testing conditions. It gives an idea about method overall consistency. In this study, the method was tested by slightly varying the wavelength (±3 nm) and the solvent ratio (±5% v/v). After making these changes, the results were evaluated, and the % RSD was calculated to see if the method remained stable and dependable.

vi. Accuracy:

"The accuracy of the proposed method was evaluated through recovery studies performed at three concentration levels (80%, 100%, and 120%) with nine determinations, covering the specified range in replicates and the results are shown in the table 5. The % recovery studies at each level fall within the range of 98.0–102.0%.

vii. Force degradation studies:

Forced degradation studies were carried out to assess the stability of flunarizine dihydrochloride under different stress conditions, including acidic, alkaline, thermal, oxidative, and photolytic environments.

For these studies, an optimized concentration of 50µg/ml of flunarizine dihydrochloride was prepared from a stock solution. This solution was then treated with 0.1 M HCl for acid hydrolysis, 0.1 N NaOH for alkaline hydrolysis, 3% v/v hydrogen peroxide (H_2O_2) for oxidative degradation. Photolytic degradation, was conducted by exposing the sample solution to UV light in a UV chamber, and the thermal degradation, a separate sample of the same concentration was placed in a hot air oven.

V. RESULTS AND DISCUSSION:

Method Optimization:

The sensitivity of the UV method depends upon the proper selection of wavelength. A sample solution of flunarizine dihydrochloride about 50 µg/ml were prepared and checked for the UV absorbance against the blank solution and the λ_{max} for the drug (standard and sample) was observed at 254nm.

VI. VALIDATION PARAMETER

i. Linearity:

The calibration curve, which plots absorbance against concentration, showed a linear relationship across the range of 10 to 60 µg/ml. The correlation coefficient (r^2) was found to be 0.9992, indicating a very strong linear relationship. This means that the test results were directly proportional to the concentration of the analyte in the sample, confirming the method's reliability for quantitative analysis. Observe the overlay spectrum and calibration curve of flunarizine dihydrochloride in figure 3 & 4. R^2 is 0.9992 which is within the limit (0.9992) and hence the linearity has been passed. Results of linearity shown in Table 3.

ii. Precision:

For a method to be considered precise, the % Relative Standard Deviation (% RSD) of assay results from six replicates should be no more than 2%. In this study, the intra-day precision showed an RSD of 1.151%, while the inter-day precision had an RSD of 1.22%. These values are **well within the acceptable limit as mentioned in table 4, confirming that the method is reliable and consistent in its performance over time.**

iii. Accuracy:

To assess the accuracy of the method, a standard addition study was carried out using three different concentrations of flunarizine dihydrochloride at 40, 50, and 60 µg/ml. The results showed % recovery values ranging between 98% and 102.0%, indicating that the method is both accurate and capable of reliably measuring the drug at various concentration levels as presented in table 5.

iv. LOD AND LOQ:

The Limit of Detection (LOD) and Limit of Quantification (LOQ) were studied, helped to establish the smallest amounts of the drug that could be reliably

detected and accurately measured by the method and the results were shown in table 6.

v. Robustness:

Robustness studies were conducted by altering the optimised conditions, wavelength and solvent composition were slightly adjusted. The method still produced consistent and reproducible results. This confirms that the method remains reliable under small variations, supporting its robustness and validation and the results are presented in Table 7.

Assay:

The assay was performed by following the same sample preparation procedure outlined in the method validation section. The results showed that the % purity of flunarizine dihydrochloride was within the acceptable range confirming the quality of the formulation indicating that the sample meets quality standards. The % purity of sample was shown in table 8.

Stability Studies:

The drug was tested under various stress conditions, including acidic, basic, oxidative, photolytic, and thermal environments. The drug was found to be stable across all these conditions, indicating the stability and the results were shown in table 9.

VII. CONCLUSION:

The developed UV spectroscopic method proved to be simple, accurate, precise, and robust for analysing flunarizine dihydrochloride in both its bulk and tablet formulation. The method demonstrated good specificity, with no interference observed from excipients or other additives present in the commercial product. In the stability studies, noticeable degradation was observed under stress conditions, confirming the method's ability to detect changes in the drug's stability. The statistical analysis of the results further supports that this method is suitable for accurately determining flunarizine dihydrochloride, even in the presence of degradation products. Therefore, the method can be confidently considered a stability-indicating assay and is well-suited for routine use in quality control laboratories within the pharmaceutical industry.

VIII. ACKNOWLEDGEMENT

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X. ABBREVATIONS:

FLN :Flunarizine ; UV: Ultraviolet; ICH: International Council for Harmonisation; LOD: Limit of Detection; LOQ: Limit of Quantification

X. CONFLICT OF INTEREST

There is no conflict of interest.

XI. REFERENCES:

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FIGURES:

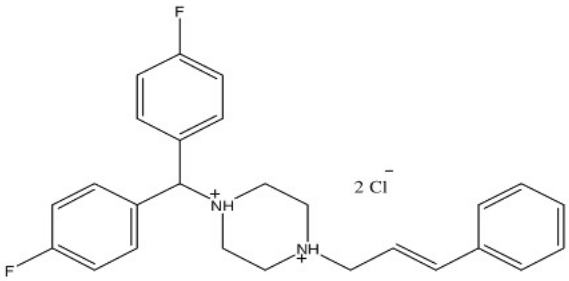

Fig 1 : Chemical structure of Flunarizine dihydrochloride

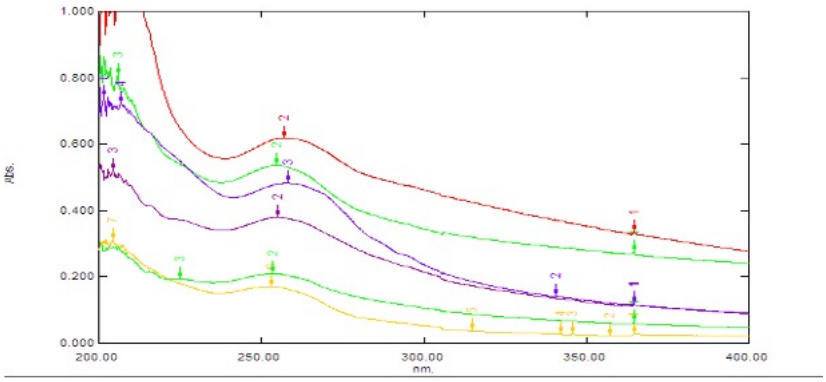

Fig 2 : Overlay spectrum of Flunarizine dihydrochloride at 254nm

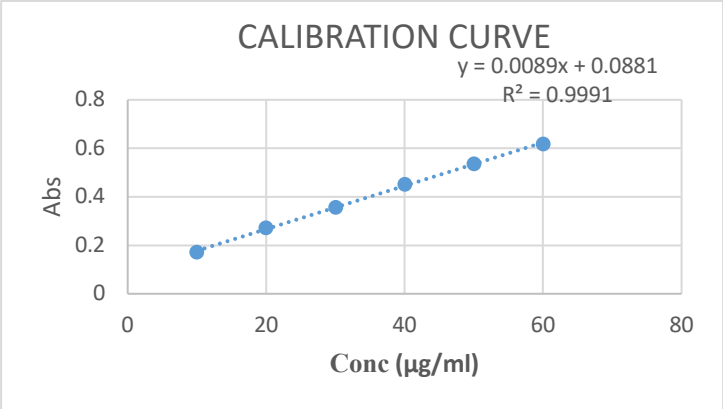

Fig 3 : Calibration curve of Flunarizine dihydrochloride

Table:1 Results of Linearity

S. No	Concentration (µg/ml)	Absorbance
1.	10	0.171
2.	20	0.271
3.	30	0.357
4.	40	0.452
5.	50	0.537
6.	60	0.618

Table:2. Results of Precision

S.NO	INTRADAY	INTERDAY
1.	0.523	0.526
2.	0.533	0.520
3.	0.531	0.520
4.	0.526	0.528
5.	0.523	0.525
6.	0.522	0.526
Mean	0.526	0.524
Std.Dev	0.004633	0.003371
%RSD	0.8807	0.6433

Table No: 3. Results of Accuracy

S.NO	Spike level	Amount taken (µg/ml)	Amount added (µg/ml)	Absorbance	Amount recovered	% Recovery	Mean (%) Recovery
1.	80%	40	90.057	0.874	90.569	100.56	100.76
2.				0.871	90.259	100.22	
3.				0.882	91.398	110.49	
4.	100%	50	100.063	0.968	100.310	100.24	100.10
5.				0.961	99.79	99.72	
6.				0.969	100.414	100.35	
7.	120%	60	110.069	1.047	108.497	98.57	100.26
8.				1.083	112.288	101.96	
9.				1.065	110.362	100.26	

Table No: 4 Results of LOD and LOQ

S.NO	Parameter	Slope	Std dev	Observation (µg/ml)	Formula
1.	LOD	0.0089	0.004848	1.79 µg/ml	LOD=3.3x σ/S
2.	LOQ			5.44 µg/ml	LOQ=10x σ/S

Table No: 5 Results of robustness

Parameter	Condition	Condition	Absorbance
Wave length (nm) ±4	Low WL	251 nm	0.527
	Optimized	254nm	0.532
	High WL	257 nm	0.533
Mobile phase (ratio) min±10 ratio	Low	70:30	0.536
	Optimized	75:25	0.537
	High	80:20	0.53

Table No: 6 %Assay

SAMPLE	STANDARD (Absorbance)	SAMPLE (Absorbance)
Sample 1	0.537	0.539
Formulation Average weight (Tablet)	440mg	
Standard weight	5mg	
Sample weight (Tablet Formulation)	110 mg	
Label Claim	5 mg	
Assay (% Purity)	99.62%	