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Development and Validation of Stability-Indicating UV-Spectrophotometric Methods for the Determination of Flunarazine Dihydrochloride In Dosage Form

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ABSTRACT

Two sensitive, precise and cost-effective UV-spectrophotometric methods are described for the determination of flunarazine dihydrchloride (FNH) in bulk drug and tablets. The proposed methods are based on the measurement of the absorbance of FNH either in 0.1 M HCl (method A) or in acetonitrile (method B) at 253 nm. As per the International Conference on Harmonization (ICH) guidelines, the methods were validated for linearity, accuracy, precision, limits of detection (LOD) and quantification (LOQ) and robustness and ruggedness. Beer's law is obeyed over the concentration ranges of 2.5-30.0 µg mL^{-1} in method A and 1.0-20.0 µg mL^{-1} in method B. The calculated molar absorptivity values are 2.12×10^4 and 2.47×10^4 L mol^{-1} cm^{-1} for method A and method B, respectively. The proposed methods were applied successfully to the determination of FNH in tablets with good accuracy and precision without any detectable interference from common excipients. The accuracy of the proposed methods was further assessed by the recovery studies *via* a standard addition method. In addition, forced degradation of FNH was conducted in accordance with the ICH guidelines. Acidic and basic hydrolysis, thermal stress, peroxide and photolytic degradation were used to assess the degradation behavior of the drug. Substantial degradation was observed during oxidative degradation and no degradation was observed under other stress conditions.

Keywords: Flunarazine dihydrchloride, degradation studies, spectrophotometry, pharmaceuticals.

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INTRODUCTION

Stability testing and stress testing (forced degradation studies) are critical components of drug development strategy. The studies help us to understand the mechanism of a drug's decomposition, which further helps in obtaining information on physical and chemical factors that result in instability[1] These factors are then controlled to stabilize the drug or drug formulation, resulting in increased shelf-life or improved efficacy. Stress testing is defined as the stability testing of drug substances and drug products under conditions exceeding those used for accelerated testing. These studies are undertaken to elucidate the intrinsic stability of the drug substance. According to International Conference on Harmonization (ICH) guideline Q1A (R2), the stability testing of drug substances should be carried out under different stress conditions such as hydrolysis, oxidation, photolysis and thermal degradation to validate the stability-indicating supremacy of analytical methods used for the analysis of stability of drugs[2] The standard conditions for photo stability testing are described in ICH guideline Q1B[3]. These tests allow accurate and precise quantification of drugs and their degradation behavior.

Flunarizine dihydrochloride (FNH) (figure 1) is the difluorinated derivative of cinnarizine dihydrochloride. It is chemically known as [trans-1-cinnamyl-4-(4,4-difluorobenzenhydryl) piperazine dihydrochloride] and has antihistaminic and CNS depressant effect, but it is mainly used for migraine prophylaxis [4].

FNH has official monographs in European Pharmacopeia [4] and British Pharmacopeia [5] which describe potentiometric titration for its assay using sodium hydroxide. A literature survey regarding quantitative analysis of the drug in pharmaceuticals revealed that attempts have been made to develop analytical methods using chromatographic techniques like high performance liquid chromatography (HPLC) [6,7] and high performance thin layer chromatography (HPTLC) [8]. One spectrofluorimetry [9] and several visible spectrophotometric methods [9,10-19] have also been reported.

UV-spectrophotometry has the advantages of speed, sensitivity and reliability. In spite of these advantages, only three methods have been reported for the determination of FNH by UV-spectrophotometry. A method for simultaneous determination of propranolol hydrochloride and FNH in their combined dosage formulation was reported by Doshi et al [20], in which the absorbance of methanolic solutions of drug was measured between linearity range 24-64 μg mL^{-1} at 289 nm for propranolol hydrochloride and between 6-16 μg mL^{-1} at 253 nm for FNH. Patil et al [21] reported two methods for simultaneous determination of propranolol hydrochloride (PPH)

and FNH in capsule dosage form in methanol medium. First method involves formation of Q-absorbance equation at two wavelengths 272.8 nm (isoabsorptive point) and 253 nm (λ_{max} of FNH). While, second method involves the measurement of absorbance at wavelength 289 nm for PPH in the concentration range of 08 - 48 µg mL^{-1} and at 253 nm for FNH in the concentration range 03 – 18 µg mL^{-1}. In a method reported by Busaranon et al [22], UV absorbance was measured in the concentration range of 6-24 µg mL^{-1} methanolic FNH both directly and by the first derivative measurements at 254 and 268 nm, respectively.

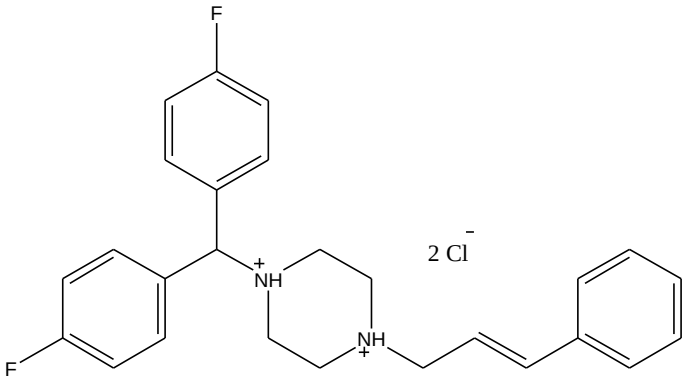


Figure 1: Structure of Sumatriptan succinate.

So far, to our knowledge, no stability indicating UV-spectrophotometric assay has been reported for the determination of FNH using the ICH approach of stress testing. The focus of the study was to develop a simple, rapid, accurate and precise stability indicating UV-spectrophotometric methods for the determination of FNH in bulk drug and tablet dosage form.

MATERIALS AND METHOD

Apparatus

All absorbance measurements were made on Shimadzu Pharmaspec 1700 UV/Visible spectrophotometer provided with 1-cm matched quartz cells.

Reagents and Standards

All chemicals and reagents used were of analytical or pharmaceutical grade and distilled water was used throughout the experiment.

Sodium Hydroxide (1.0 M):

Prepared by dissolving 10 g of sodium hydroxide pellets (Merck, Mumbai, India) in 250 ml of water.

Hydrochloric Acid (1.0 M and 0.1 M):

Concentrated acid (Merck, Mumbai, India, sp. gr. 1.18) was appropriately diluted with water to get the required concentration.

Acetonitrile:

Spectroscopic grade acetonitrile (Merck Pvt. Ltd., Mumbai, India) used as such.

5% hydrogen peroxide:

30% w/v hydrogen peroxide (Loba Chemie Pvt. Ltd., Mumbai, India) diluted appropriately in water to get the required concentration.

Preparation of Standard FNH Solution

A standard stock solution of FNH (200 µg mL^{-1}) was prepared by dissolving calculated quantity of pure drug in 0.1 M HCl (method A), acetonitrile (method B) and diluted to 50 µg mL^{-1} (method A) and 40 µg mL^{-1} (methods B) using the respective solvents.

Pharmaceutical grade FNH (99.78%) was received from Inga Pharmaceuticals, Mumbai, India, as a gift sample and used as received. Two brands of tablets containing FNH, Flunatrac-10 (10 mg) (Minova Life Sciences Ltd., Bangalore, India) and Flunarin-5 (5 mg) (FDC Ltd., Goa, India) used in the investigation were purchased from local commercial sources.

Assay Procedures

Method A

Varying aliquots (0.25, 0.5, 1.0, 2.0,……5.0 mL) of 50 µg mL^{-1} standard FNH solution in 0.1 M HCl were taken into a series of 10 ml calibrated flask, the content was diluted to the mark with 0.1 M HCl and mixed well. The absorbance of each solution was measured at 253 nm *versus* 0.1 M HCl.

Methods B

Different aliquots (0.25, 0.5, 1.0, 2.0,……5.0 mL) of a standard FNH (40 µg mL^{-1}) solution were accurately transferred into a series of 10 mL volumetric flasks and the volume was made up to the mark with acetonitrile. The absorbance of each solution was measured at 253 nm against acetonitrile.

Calibration graphs were prepared by plotting the increasing absorbance values *versus* concentrations of FNH. The concentration of the unknown was read from the respective calibration graph or deduced from the regression equation derived using the Beer's law data.

Procedure for tablets

Twenty tablets were weighed accurately and ground into a fine powder. An accurately weighed amount of the powdered tablet equivalent to 20 mg of FNH was transferred to a 100 mL calibrated flask and shaken with 60 ml of 0.1 M HCl (method A) and with acetonitrile (method B) for about 20 min, then made up to the mark with the respective solvents, mixed and filtered using a Whatman No. 42 filter paper. The filtrate containing 200 µg mL^{-1} FNH was appropriately

diluted with the respective solvents to obtain the working concentrations of 50 µg mL^{-1} for method A and 40 µg mL^{-1} for method B and the assay was completed by following the recommended procedures.

Procedure for placebo blank and synthetic mixture analysis

A placebo blank of the composition: talc (10 mg), starch (5 mg), acacia (5 mg), methyl cellulose (10 mg), sodium citrate (5 mg), magnesium stearate (10 mg) and sodium alginate (5 mg) was made and its solution was prepared as described under "Procedure for tablets" and then subjected to analysis using the procedures described above.

To the 10 mg of the placebo blank of the composition described above, 20 mg of FNH was added and homogenized, transferred to a 100 ml calibrated flask and solution prepared as described under "Procedure for tablets". The synthetic mixture solutions were subjected to analyses according to the recommended procedures. This analysis was performed to study the interference by common excipients such as talc, starch, acacia, methyl cellulose, sodium citrate, magnesium stearate and sodium alginate.

Preparation of acid and base induced-degradation products

A 10.0 mL of 50 µg mL^{-1} standard FNH solution in 0.1 M HCl (method A) or 10.0 mL of 40 µg mL^{-1} standard solution of FNH in acetonitrile (method B) was taken separately in triplicate in 25 ml calibrated flasks, 5.0 ml each of 1.0 M HCl (acid hydrolysis) and 1.0 M NaOH (alkaline hydrolysis) were added separately to each flask. The flasks were kept on a water bath for 2.0 h at 80 °C, and then cooled to room temperature. The flasks were neutralized with 5.0 ml of 1.0 M NaOH (for acid hydrolysis) and 5.0 ml of 1.0 M HCl (for alkaline hydrolysis) followed by making the flasks to the mark with the respective solvent (0.1 M HCl in method A or acetonitrile in method B). The absorption spectrum for each flask was performed from 200-400 nm versus the corresponding blank.

Procedure for oxidative degradation

To 10.0 mL of 50 µg mL^{-1} FNH solution in 0.1 M HCl (method A) or to 10.0 mL of 40 µg mL^{-1} FNH solution in acetonitrile (method B) taken in a 25 ml calibrated flask, 5 ml of 5% hydrogen peroxide was added. The flasks were kept on a water bath at 80 °C for 2.0 h. The flasks were cooled to room temperature, made up to the mark with the respective solvent and the absorption spectrum was run from 200-400 nm against the corresponding blank.

Procedure for dry heat and photo-degradation

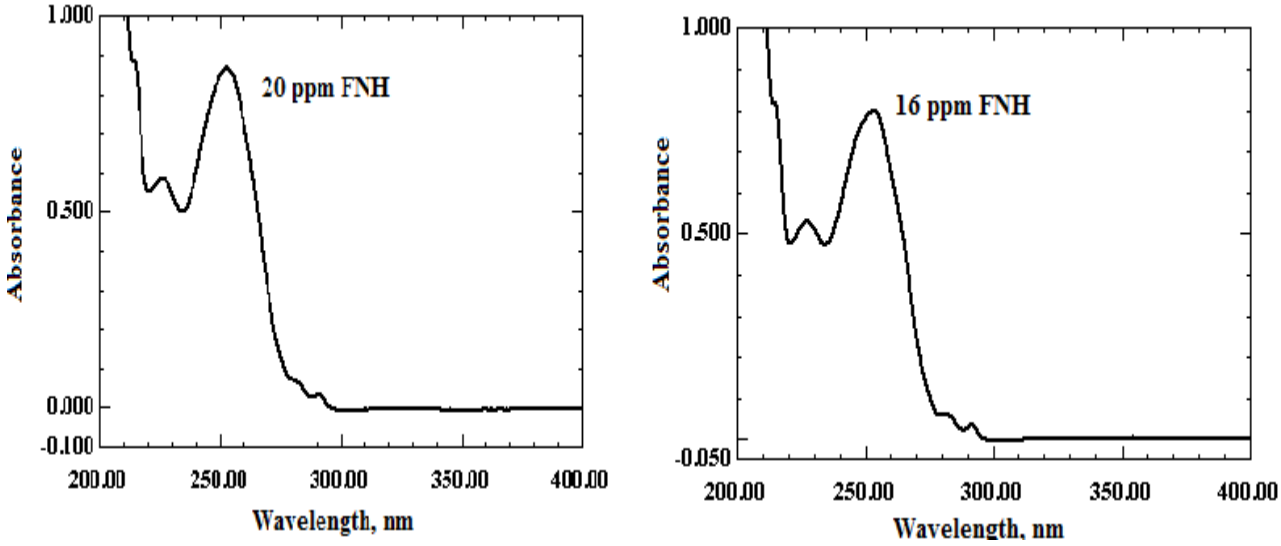
The powdered sample (0.5 g) of FNH was taken on a Petri dish and kept in the oven at 105 °C for 24 h, the sample cooled to room temperature and used to prepare 20 µg mL^{-1} FNH in 0.1 M

HCl (method A) and 16 µg mL^{-1} FNH in acetonitrile (method B). Also, another powdered sample (0.5 g) of FNH was taken on a Petri dish, exposed to UV radiation in a UV chamber of 1200 lux-hr for 48 h and used to prepare 20 µg mL^{-1} FNH in 0.1 M HCl (method A) and 16 µg mL^{-1} FNH in acetonitrile (method B). The absorption spectrum of each solution was run from 200-400 nm against the corresponding solvent.

RESULTS AND DISCUSSION

Spectral characteristics

The absorption spectra of 20 µg mL^{-1} FNH solution in 0.1 M HCl (method A) and 16 µg mL^{-1} FNH solution in acetonitrile (method B) were recorded between 200 and 400 nm and showed a maximum absorption at 253 nm, for both method A and method B. At this wavelength, 0.1 M HCl and acetonitrile had insignificant absorbance. Therefore, the analysis of FNH was carried out at 253 nm, for both method A and method B (Figure. 2).



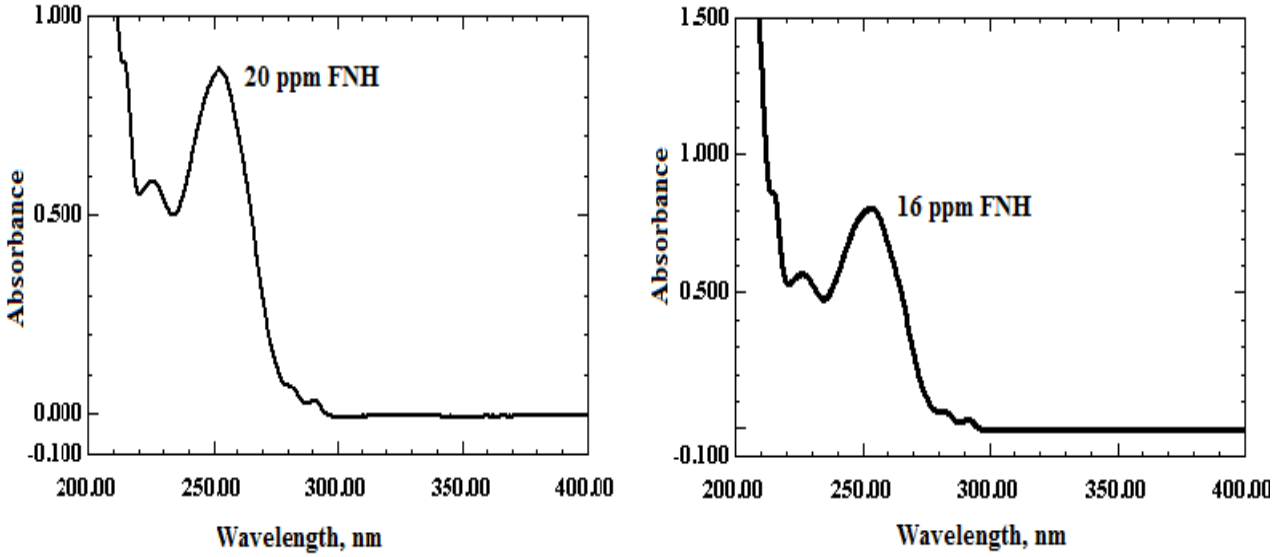
(a) **(b)**

Figure 2: Absorption spectra of FNH in 0.1 M HCl(method A) & in acetonitrile(method B).

Forced degradation study

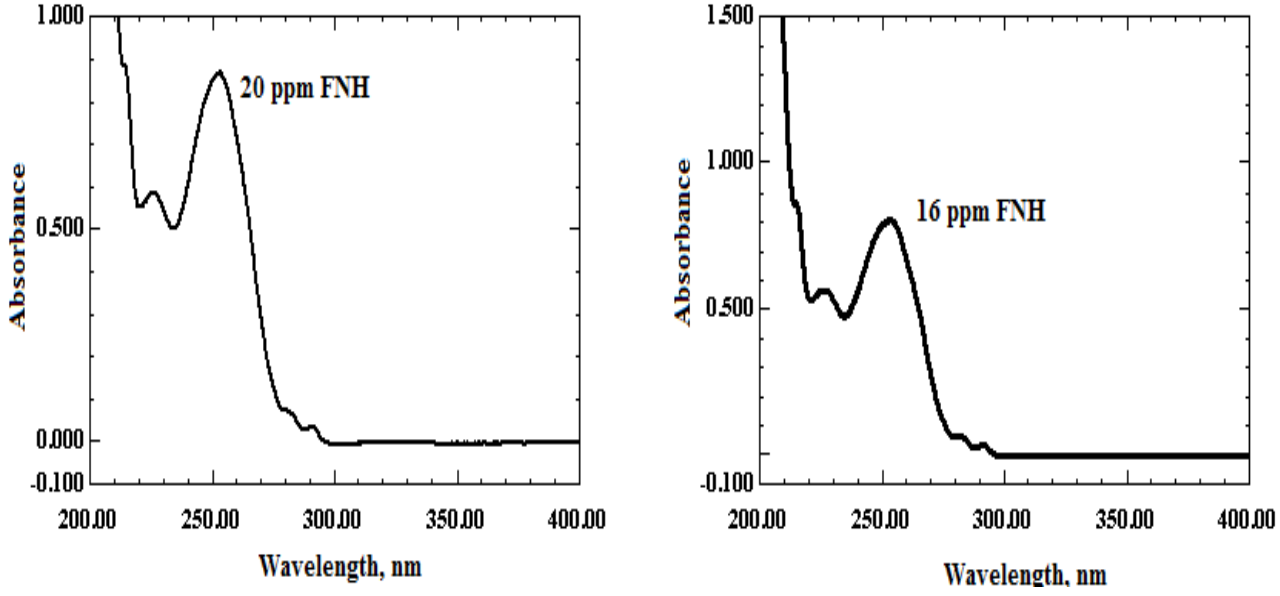
The absorption spectra of the FNH solutions in 0.1 M HCl and acetonitrile treated with acid, base and water hydrolysis, hydrogen peroxide, dry heat and UV radiation were run in the range of (200-400 nm). The degradation was evaluated based on the comparison of the UV spectra of "stressed FNH samples" with that of the "standard FNH solution" [23]. The resulting UV spectra of stress FNH solutions (20 µg mL^{-1} in 0.1 M HCl) and (16 µg mL^{-1} in acetonitrile) subjected to acid hydrolysis showed the same spectra (Figure. 3) of the standard solution which indicated that FNH does not undergo degradation under this condition. Under alkaline condition FNH solutions

in 0.1 M HCl and in acetonitrile do not undergo degradation at the respective wavelength (Figure. 4). The UV spectra of stress FNH samples subjected to dry heat treatment and UV-degradation were similar to that of the standard FNH sample in both methods and it showed that FNH did not undergo degradation under these conditions (Figure. 5). The absorption spectra of FNH solutions in 0.1 M HCl and in acetonitrile treated with hydrogen peroxide showed that FNH undergoes significant degradation in both methods since undetectable absorbance was observed in both the methods (Figure. 6). The overall degradation summary was compiled in table 1.



(a) **(b)**

Figure 3: Acidic degradation. a) (method A), b) (method B)



(a) **(b)**

Figure 4: Basic degradation. a) (method A), b) (method B)

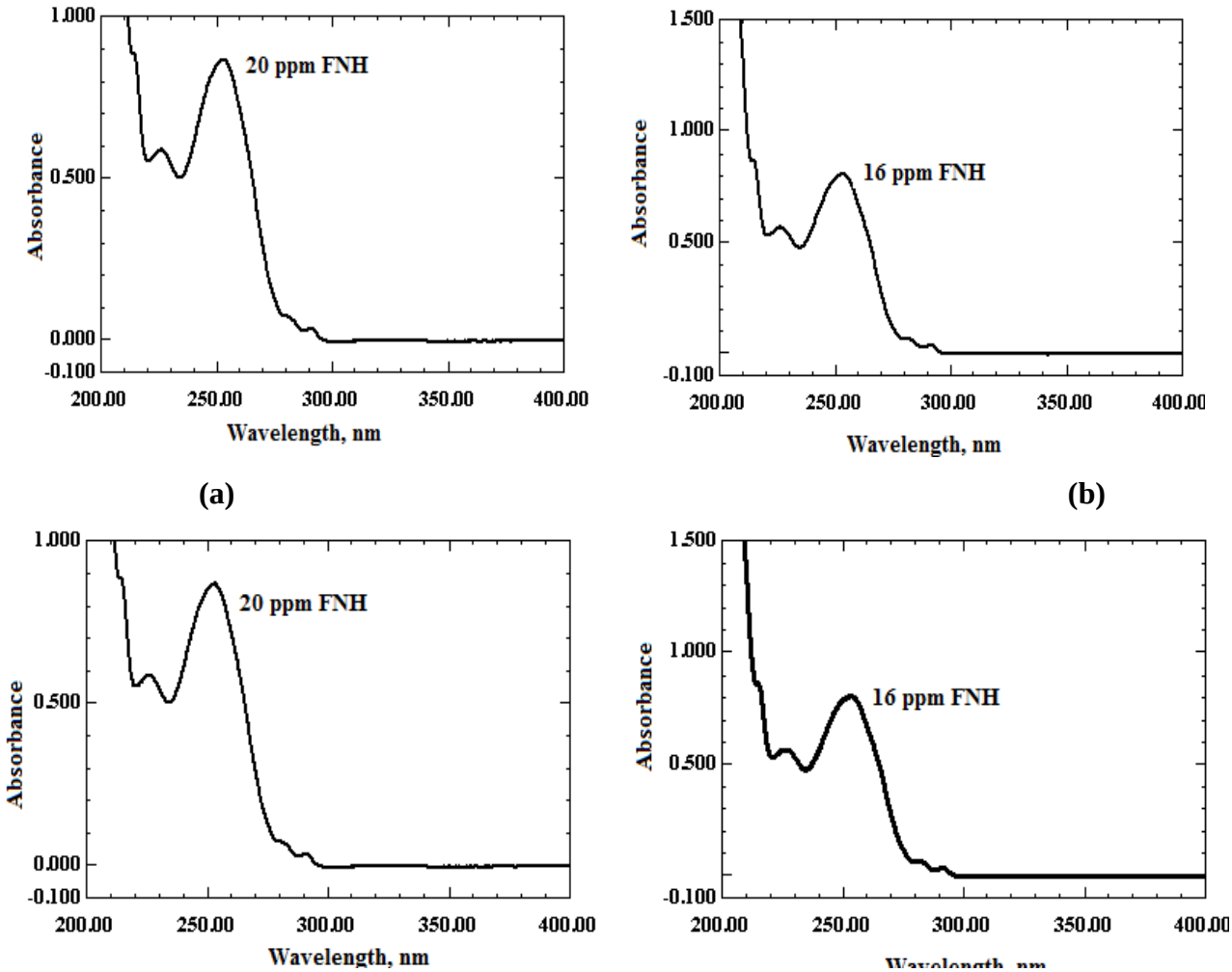


Figure 5. Thermal degradation a) (method A), b) (method B) Photo degradation c) (method A), d) (method B)

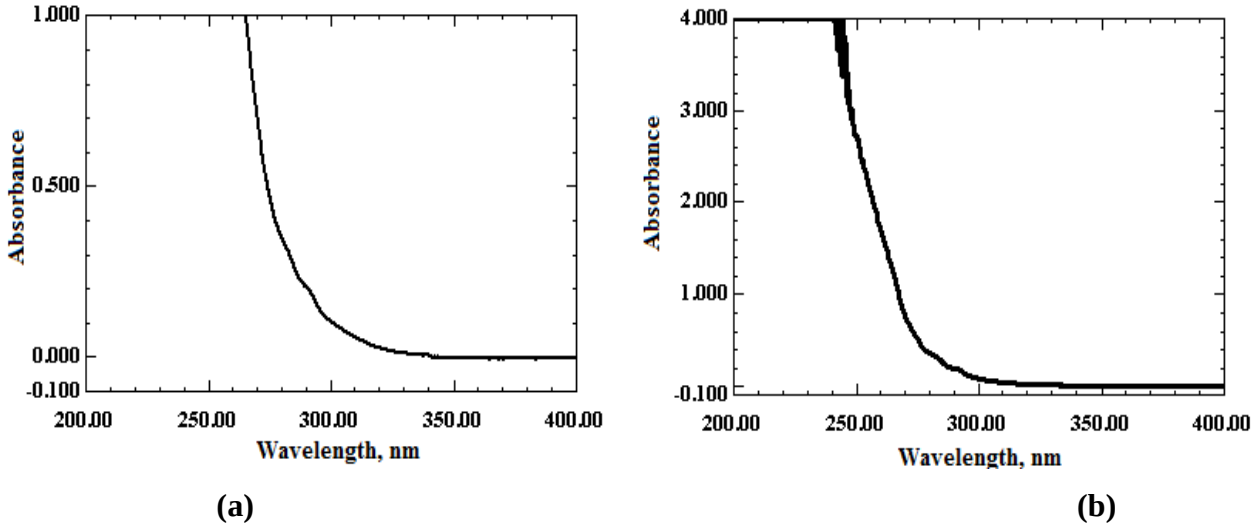


Figure 6: Peroxide degradation a) (method A), b) (method B)

Table 1. Forced degradation summary

Degradation condition	% Assay* (method A)	% Assay* (method B)	Observation
Control sample	99.8	99.8	Not applicable
Acid hydrolysis (1M HCl , 80°C, 2 hours)	99.8	98.7	No degradation observed
Base hydrolysis (1M NaOH , 80°C, 2 hours)	-	-	No degradation observed
Oxidation (5% H_2O_2 , 80°C, 2 hours)	-	-	Extensively degraded
Thermal (105°C, 3 hours)	98.9	99.1	No degradation observed
Photolytic (1.2 million lux hours)	99.5	98.8	No degradation observed

*** Percentage against standard FNH.**

METHOD VALIDATION

Linearity and sensitivity

The regression parameters calculated from the calibration graphs data, along with the standard deviations of the slope (S_b) and the intercept (S_a) were presented in Table 2. Beer's law was obeyed over the concentration ranges 1.25-25.0 µg mL^{-1} in method A and 1.0-20.0 µg mL^{-1} in method B. The linearity of calibration graphs was demonstrated by the high values of the correlation coefficient (r) and the small values of the y-intercepts of the regression equations. The molar absorptivity, Sandell sensitivity values of both methods were also amassed in Table 2. The sensitivity of the methods can be determined, through the limit of detection (LOD) and limit of quantification (LOQ). The limits of detection and quantification were calculated as per the current ICH guidelines [2] and reported in Table 2.

Table 2. Regression and analytical parameters.

Parameter	Method A	Method B
λ_{max}, nm	253	253
Beer's law limits (µg mL^{-1})	1.25-25.0	1.0-20.0
Molar absorptivity (L mol^{-1} cm^{-1})	2.15×10^4	2.47×10^4
Sandell sensitivity* (µg cm^{-2})	0.0222	0.0201
Limit of detection (µg mL^{-1})	0.24	0.23
Limit of quantification (µg mL^{-1})	0.72	0.69
Regression equation, Y**		
Intercept,(a)	0.0058	0.0047
Slope,(b)	0.0433	0.0493
Correlation coefficient (r)	0.9997	0.9999
Standard deviation of intercept (S_a)	0.00517	0.00243
Standard deviation of slope (S_b)	0.00037	0.00129

[a]Limit of determination as the weight in μg per mL of solution, which corresponds to an absorbance of A = 0.001 measured in a cuvette of cross-sectional area 1 cm^2 and l = 1 cm.

[b] $Y = a + bX$, where Y is the absorbance, X is concentration in μg mL^{-1}, a is intercept, and b is slope.

Precision and accuracy

The precision of the methods was calculated in terms of intermediate precision (intra-day and inter-day). Three different concentrations of FNH were analyzed in seven replicates during the same day (intra-day precision) and five consecutive days (inter-day precision). The %RSD values of intra-day and inter-day studies showed that the precision was satisfied (RSD ≤ 1.57) (Table 3). The accuracy was evaluated as percentage relative error between the measured concentrations and taken concentrations for FNH (Bias %). The results obtained are cumulated in Table 3 and showed that the accuracy was good (RE ≤ 2.01).

Table 3. Evaluation of intra-day and inter-day precision and accuracy.

Method	FNH taken (µg mL^{-1})	Intra-day (n = 7)			Inter-day (n = 5)		
		FNH found[a] (µg mL^{-1})	%RSD[b]	%RE[c]	FNH found[a] (µg mL^{-1})	%RSD[b]	%RE[c]
Method A	5.00	5.09	1.13	1.89	5.10	1.33	2.01
	10.0	10.09	0.73	0.85	10.10	1.01	1.02
	15.0	14.92	1.36	0.54	14.90	1.57	0.66
Method B	4.00	3.97	1.27	0.63	3.97	1.52	0.76
	8.00	7.92	0.50	0.95	7.91	0.83	1.11
	12.0	12.12	1.02	1.04	12.14	1.27	1.16

Mean value of five determinations; [b.] Relative standard deviation (%); [c.] Relative error (%).

Selectivity

The proposed methods were tested for selectivity by placebo blank and the resulting absorbance readings in both methods were same as reagent blank, inferring no interference from the placebo. Non interference from placebo was further confirmed by carrying out recovery study from synthetic mixture with percent recoveries of 99.63 ± 2.14 and 101.3 ± 1.96 for method A and method B, respectively. These results confirm the selectivity of the proposed methods in the presence of the inactive ingredients added to the tablet formulation.

Ruggedness and robustness

Table 4. Robustness and ruggedness.

Method	FNH taken, µg mL^{-1}	Method robustness	Method ruggedness	
		Wavelengths, nm[a] RSD, %(n = 3)	Inter-analysts RSD, %(n = 3)	Inter- cuvettes RSD, %(n = 3)
	7.50	0.97	1.68	1.34
Method A	10.0	1.21	1.35	1.56
	12.5	1.40	1.39	1.83
Method B	6.00	0.79	1.01	1.41
	8.00	1.03	0.98	1.77
	10.0	1.19	1.52	1.65

[a]Wavelengths used were 251, 253 and 255 in both method A and B.

Ruggedness of the proposed methods was expressed as % RSD of the same procedure done by three analysts and also by a single analyst performing the analysis with three different cuvettes. The results presented in Table 4 showed that no statistical differences between different analysts and cuvettes suggesting that the proposed methods were rugged (%RSD ≤ 1.83).

Method robustness was tested by measuring the absorbance at different wavelengths (251,253 and 255 nm) and the intermediate precision, expressed as % RSD was found within the acceptable limits as shown in the Table 4.

Applications to analysis of tablets

The proposed methods were successfully applied to the determination of FNH in two brands of tablets (Flunaract-10 and Flunarin-5) and the results are summarized in Table 5. The results obtained were compared with those of the published literature method [7] which describes HPLC determination of FNH in tablets using LiChrospher 100 RP-18 column with methanol–ion pair solution as mobile phase at a flow rate of 0.7 mL min^{-1} and UV detection at 254 nm. When the results were statistically compared with those of the reference method by applying the Student's t- test for accuracy and F- test for precision, the calculated t- value and F-value at 95% confidence level did not exceed the tabulated values of 2.78 and 6.39 respectively, for four degrees of freedom. The tests indicate that there is no significant difference between the proposed methods and the reference method with respect to accuracy and precision.

Table 5. Results of analysis of tablets by the proposed methods

Tablet Brand name	Label claim mg/tablet	Found (Percent of label claim ±SD)[a]		
		Reference method	Proposed methods	
			Method A	Method B
Flunaract-10	10.0	100.1±0.46	99.16 ± 0.79 **t** =2.30 **F**=2.95	101.1 ± 0.90 **t** =2.21 **F** =3.83
Flunarin-5	5.00	100.9±1.05	101.7 ± 1.57 **t** =0.95 **F** =2.24	102.0 ± 1.39 **t** =1.41 **F**=1.75

[a]Mean value of five determinations.

Tabulated t-value at the 95% confidence level is 2.78.

Tabulated F-value at the 95% confidence level is 6.39.

Recovery study

To further assure the accuracy and reliability of the methods, recovery experiments were performed by applying the standard-addition technique. Pre-analyzed tablet powder was spiked with pure FNH at three different levels and the total was found by the proposed methods. Each

determination was repeated three times and the percent recovery of pure FNH added (Table 6) was within the permissible limits indicating the absence of the interference from inactive ingredients in the assay.

Table 6. Results of recovery study by standard addition method.

Tablets Studied	Method A				Method B			
	FNH in tablets, µg mL^{-1}	Pure FNH added, µg mL^{-1}	Total found, µg mL^{-1}	Pure FNH recovered*, Percent±SD	FNH in tablets, µg mL^{-1}	Pure FNH added, µg mL^{-1}	Total found, µg mL^{-1}	Pure FNH recovered*, Percent±SD
	4.96	2.5	7.53	102.8±0.86	4.04	2.0	6.00	98.00±2.21
Flunaract	4.96	5.0	10.01	101.0±1.39	4.04	4.0	7.99	98.75±1.68
-10	4.96	7.5	12.35	98.53±2.02	4.04	6.0	10.14	101.7±1.77
	5.09	2.5	7.62	101.2±1.32	4.08	2.0	6.11	101.5±0.88
Flunarin-	5.09	5.0	10.05	99.20±1.67	4.08	4.0	8.04	99.00±1.77
5	5.09	7.5	12.50	98.80±1.50	4.08	6.0	10.22	102.3±2.05

Mean value of three determinations.

CONCLUSION

Stress testing is an important aspect of the drug development process. The present study reports two simple stability-indicating methods for quantification of FNH in bulk drug as well as in tablets. The previously reported HPLC method[6] for degradation studies are complex and cumbersome and require expensive and sophisticated instrument. In contrast, the proposed methods rely on the use of simple technique but provide sensitivity comparable to that achieved by sophisticated and expensive technique like HPLC. From this study it can be conclude that FNH quite stable to several stress conditions such as acid and base hydrolysis, photo and dry heat treatment conditions whereas it undergoes degradation under oxidative condition. Moreover, the proposed methods have the advantages of simplicity and high sensitivity and are free from experimental variables such as heating or extraction step. The methods have been demonstrated to be free from interference by common tablet excipients and additives, so they can be used as alternative for rapid and routine determination of bulk sample and formulation.

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