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A STABILITY-INDICATING UV SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF AMOROLFINE HYDROCHLORIDE

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Keywords:

Amorolfine hydrochloride, Stability indicating method UV Bulk, Forced degradation

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ABSTRACT: A stability-indicating method by UV spectrophotometry has been developed for the specific determination of Amorolfine HCl in bulk in the presence of its degraded products. The method is simple, accurate, precise, and robust. Experiments were designed for determining linearity, the limit of detection and quantitation, accuracy, precision, and specificity of this analytical method as per the International Organization for Standardization guidelines. The drug substance was exposed to thermal, photolytic, hydrolytic, and oxidative stress conditions, and the stressed samples were analyzed using the proposed method to demonstrate the specificity of the method. The proposed method was found to be linear in the concentration ranges from 2-20µg/ml with the linear correlation coefficient of R2= 0.990, and the mean recovery was 95.59 % at a detection wavelength of 215 nm. Although the degradation products of stressed conditions had not been identified, the method had been able to detect the changes due to stress conditions. The stated method can be used as a stability-indicating method with a high degree of linearity, accuracy, and precision for assay of Amorolfine hydrochloride in the routine pharmaceutical analysis of Amorolfine hydrochloride in bulk. The Limit of Detection (LOD) and Limit of Quantification (LOQ) values were found to be 0.052µg/ml and 0.157µg/ml, respectively.

INTRODUCTION: Amorolfine hydrochloride (2R,6S)-2, 6-dimethyl-4- [2-methyl-3- [4-2-methylbutan-2-yl) phenyl] propyl] morpholine, hydrpchlorde is a antifungal drug. It inhibits the fungal enzymes D14 reductase and D7-D8 isomerase [1-3]. This inhibition, depleting ergosterol and causing ignosterol to accumulate in the fungal cytoplasmic cell membranes [4, 5].

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Knowledge of the stability of the molecule helps in selecting. proper formulation, packaging, storage conditions, and shelf life, which is essential for regulatory documentation. Forced degradation is a process that involves degradation of effects fungal sterol synthesis pathways drug products and drug substances at conditions more severe than accelerated conditions and thus generates degradation products that can be studied to determine the stability of the molecule [6, 7, 8, 9, 10].

Stability studies of Amorolfine HCl by spectrophotometric and HPLC-UV have already been reported [11, 12]. In context, the aim was to develop and validate simple and precise stability-

indicating UV Spectrophotometric method for Amorolfine Hydrochloride in bulk [13, 14, 15, 16].

MATERIALS AND METHODS: A gift sample of Amorolfine HCl was provided Lexine Techno Chem Pvt Ltd. Vadodara. The identity and purity of drug were confirmed by recording the FTIR spectra; methanol was used as a solvent. The reagents used were Hydrochloric acid, Sodium hydroxide & Hydrogen Peroxide 3% solution [17, 18, 19, 20].

APPARATUS: Double Beam UV-visible spectrophotometer (Shimadzu, model 1800) having two matched quartz cells with 1cm light path.

- UV- probe 2.35 software
- Electronic analytical balance (AUW-220d) , Shimadzu
- Volumetric flask – 10 , 25 ,50 ,100 ml (Durasil)
- Pipettes – 1, 2, 5, 10 ml

METHODS: [21, 22, 23, 24, 25]

Spectrophotometric Conditions: Spectro photo metric analysis was carried out at an ambient temperature, methanol was used as a solvent, and a standard solution of 10µg/ml was analyzed at 215 nm.

Preparation of Stock and Standard Solution: Accurately weighed 100 mg of Amorolfine HCl was transferred to 100 ml volumetric flask to make a stock solution of 1000µg/ml. Suitable aliquots were taken into 100 ml volumetric flask to make standard solutions in the range of 2-20 µg/ml.

Preparation of Calibration Curve: The calibration curve was prepared in the concentration range 2-20 µg/ml by analyzing each solution in triplicate and plotting the concentration (µg/ml) against absorbance.

The correlation coefficient and equation of the line were determined. The spectrum of fresh drug solution (10µg/ml) is shown in **Fig. 1** and the calibration curve in **Fig. 2**. The data is shown in **Table 2**.

Forced Degradation Studies: The forced degradation of Amorolfine HCl was done in each of the mentioned below stress conditions at a concentration of 20 µg/ml.

The degradation was confirmed in each case by recording the changes in the ultraviolet spectra of each stressed sample, comparing it with that of fresh drug solution.

Acid Degradation: 20ml stock solution of Amorolfine HCl was heated on a water bath at 60 ºC with 20 ml of 0.1N HCl. The sample was taken at 0 min, 30 min, 60 min, 90 min, and absorbances were measured, then % Drug recovery and % Drug degraded were calculated.

Alkaline Degradation: 20ml of stock solution and 20 ml of 0.1 N NaOH were mixed and kept at 60 °C. The sample was taken at 0 min, 30 min, 60min, 90 min, and absorbances were measured, then % Drug recovery and % Drug degraded were calculated.

Oxidation Induced Degradation Method: 20ml of stock solution and 20 ml of 3% H_2O_2 were mixed and kept at 60 °C. The sample was taken at 0 min, 30 min, 60 min, 90 min, and absorbances were measured, then % Drug recovery and % Drug degraded were calculated.

Thermal Degradation: 0.05 mg of Amorolfine HCl in solid-state was at 80 ºC for 4 h in an oven equipped with temperature control.

The sample was taken after 4 h, and absorbance was measured, then % drug recovery and % drug degradation were calculated.

Photo Degradation: 0.05mg of Amorolfine HCl was kept in the solid state for 4 h in sunlight directly, and absorbance was measured at 215 nm and the % drug recovery, % drug degradation was calculated.

RESULTS AND DISCUSSION:

Recording of Spectrum of Standard Solution: The spectrum of the standard drug was recorded with 10µg/ml solution and scanned in the range of 400-200 nm. 215 nm was found to be the suitable wavelength for analysis.

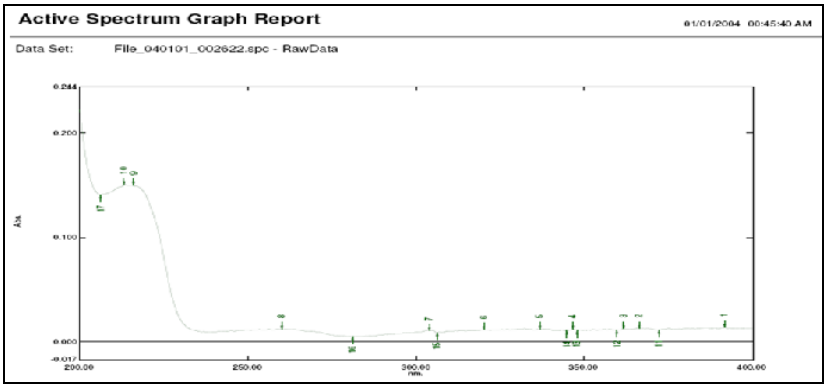

FIG. 1: SPECTRUM OF FRESH STANDARD SOLUTION OF AMOROLFINE HCL (20MG/ML)

Degradation Behavior of Amorolfine HCl: The amount (percent) of Amolorfine HCl remaining determined from the absorbance of the fresh drug sample relative to that of the stressed drug sample. The results are shown in **Table 8**.

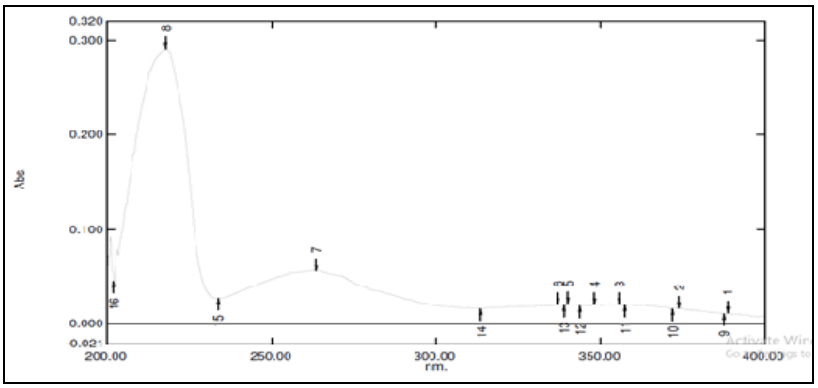

FIG. 2: SPECTRUM OF ACID DEGRADED AMOROLFINE HCL

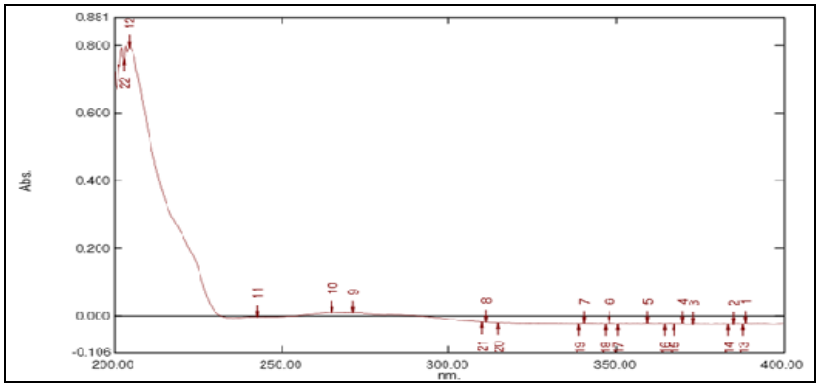

FIG. 3: SPECTRUM OF BASE DEGRADED AMOROLFINE HCL

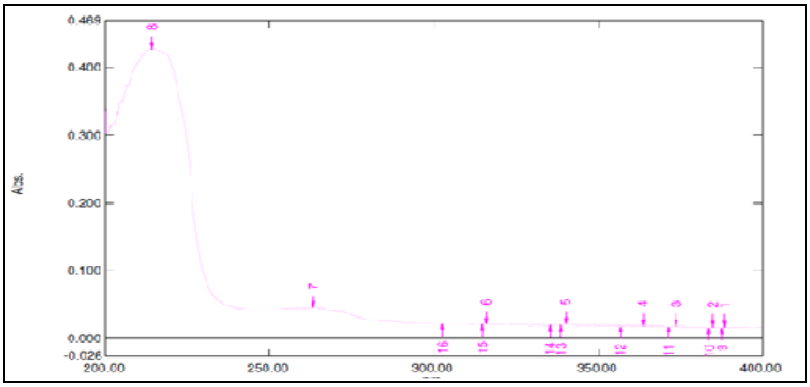

FIG. 4: SPECTRUM OF OXIDATIVELY DEGRADED AMOROLFINE HCL

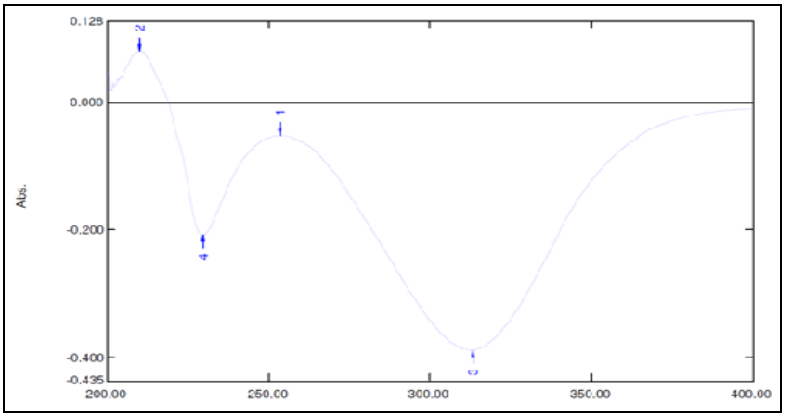

FIG. 5: SPECTRUM OF THERMALLY DEGRADED AMOROLFINE HCL

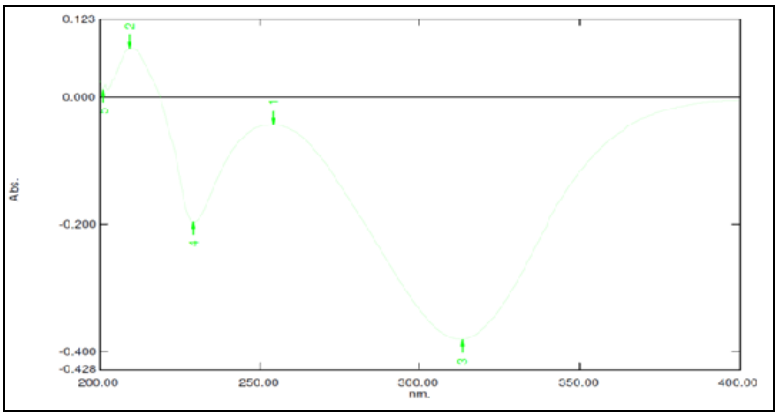

FIG. 6: SPECTRUM OF PHOTO DEGRADED AMOROLFINE HCL

TABLE 1: % DRUG DEGRADED UNDER EACH STRESS CONDITION

Sr. no	Stress Condition	Absorbance	% Drug Degraded	% Drug Remain
1.	Fresh solution	0.529	0	100 %
2.	Acidic	0.203	61.63 %	38.37
3.	Alkaline	0.346	34.61%	65.40%
4.	Oxidative	0.322	39.14 %	60.86%
5.	Thermal	0.044	91.69 %	8.31%
6.	Photo	0.043	91.88%	8.12%

Method Validation:

A. Accuracy: The accuracy of the method was evaluated by spiking the drug at three concentration levels (10,20 & 30 µg/ml) to the original 20 µg/ml solution. The percent recovery of the added drug was calculated from the linearity plots. The results are shown in **Table 2**.

TABLE 2: RESULTS OF RECOVERY STUDIES

Preanalyzed Sample Solution (µg/ml)	Level of Addition(%)	Drug recovered (µg/ml)	% Recovery	%RSD
Amolorfine HCl	50	28.25	93.50%	0.61834
	100	38.45	95.16%	1.0648
	150	49.47	98.13%	0.82548
Average of %RSD				0.836206

B. Linearity and Range: The linearity of the method was established by preparing a calibration curve in methanol in the range of 2-20 µg/ml.

Triplicates of each of the solutions were analyzed, and a calibration curve was recorded. The mean (n=3) absorbance was plotted against concentration (µg/ml).

The correlation coefficient and equation of line were determined. The results are shown in **Fig. 7** and **Table 3**.

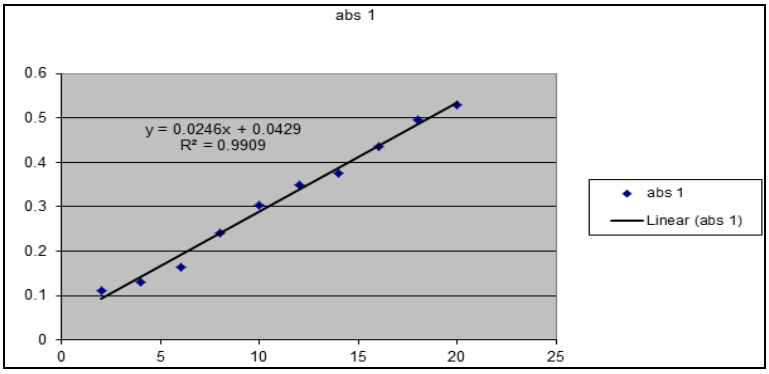

FIG. 7: CALIBRATION CURVE OF STD AMOLORFINE HCL

TABLE 3: LINEARITY STUDY OF AMOLORFINE HCL AT 215 nm

Sr.no	Con. (µg/ml)	Absorbance Mean ± S.D	% RSD
1	2	0.112 ±0.0006	0.515491
2	4	0.112±0.001	0.892857
3	6	0.164±0.0006	0.352043
4	8	0.242±0.0006	0.238574
5	10	0.303±0.0006	0.190545
6	12	0.349±0.0006	0.16543
7	14	0.376±0.001	0.266667
8	16	0.436±0.0006	0.13242
9	18	0.497±0.0006	0.116401
10	20	0.529±0.0006	0.10914

C. Precision: The inter day and intraday precision was determined by calculation of the % RSD values on triplicates of each concentration. The mean (n=3) absorbance of each concentration was compared with that of the second run on the same day (intraday) and with that on the next day (inter day) and the percent relative deviation calculated. The results are shown in **Table 4**.

TABLE 4: RESULTS OF INTRA-DAY AND INTER-DAY PRECISION STUDIES

Drug		Intra day Precision		Interday Precision	
Amolorfine HCl	Con(µg/ml)	Mean ± S.D	% RSD	Mean±S.D	% RSD
	6	5.96±0.000577	0.35348	5.96±0.001	0.613497
	12	11.96±0.0006	0.16	11.89±0.00058	0.16654
	20	19.96±0.000577	0.109278	19.92±0.001	0.189753
Average % RSD			0.1748026		0.323263

D. Ruggedness: Mean absorbance (n=3) was measured for the 20µg/ml solution analyzed by two different analysts on different days, and the percent relative deviation between two trials was calculated. The results are shown in **Table 5**.

TABLE 5: RESULTS OF RUGGEDNESS STUDIES

Drug	Amount Taken	Analyst-I		Analyst-II	
Amorolfine HCl		Amount found±S.D	%RSD	Amount found±S.D	%RSD
	20 µg/ml	20±0.001549	0.292853	20±0.001643	0.310324

E. Robustness: The Specificity of the method was established through the determination of the drug in the presence of its degradation products with high degree of precision. The spectrum homogeneity was confirmed by analyzing the ratio chromatograms at the wavelengths 210 nm and 215 nm. are shown in **Table 6**.

TABLE 6: RESULTS OF ROBUSTNESS STUDIES

Drug	Amount taken	WL-1		WL-II	
Amorolfine HCl	20 µg/ml	Amount found ± S.D	%RSD	Amount found ± S.D	%RSD
		19.98±0.00114	0.2157	19.96±0.00071	0.133922

F. Repeatability: Mean absorbance (n=6) was measured for the 20µg/ml solution analyzed at the same wavelength on the same day, and the % standard deviation was calculated. The results are shown in **Table 7**.

TABLE 7: RESULTS OF REPEATABILITY STUDIES

Drug	Amount taken	Amount found±S.D	%RSD
Amolorfine HCl	20 µg/ml	19.98±0.000816	0.154444

G. Limit of Detection and Limit of Quantitation: The limit of detection and limit of Quantitation was calculated based on standard deviation(σ) and the slope(S) of the calibration plot, using the formulae LOD= 3.3σ/s and LOQ=10σ/S as defined by ICH. The LOD was found to be 0.052µg/ml and LOQ 0.157µg/ml.

H. System Suitability: The system suitability parameters Linearity and range, accuracy, precision, and specificity were determined and are shown in **Table 8**.

TABLE 8: SYSTEM SUITABILITY PARAMETERS

Validation parameters	values
Range (Linearity)	2-20 (µg/ml)
Regression equation (y=mx+C)	y = 0.024x + 0.042
Correlation coefficient R^2	0.990
LOD	0.052005
LOQ	0.15759
Recovery(% RSD)	0.297957
Intraday Precision	0.1748026
Interday Precision	0.323263
Ruggedeness	0.301
Robustness	0.174
Repeatability	0.154

CONCLUSION: A validated stability-indicating assay method has been developed for the determination of Amolorfine hydrochloride in bulk. The analytical performance of the UV method was established, and the method was validated in terms of precision, accuracy, robustness, ruggedness, repeatability detection, and quantitation limits. The results show that the developed method was accurate, precise, simple, economical, fast, and specific. Amorolfine HCl is most prone to degradation under stress, followed in order by the stress Photo (91.88%) thermal (91.69%), acidic (61.63%), oxidative (39.14%) basic (34.61%). Degradation was found to a lesser extent in oxidative and basic conditions, while found greater in thermal and photo methods. The method can be widely used as a standard technique for stability-indicating, rapid and accurate quantitative determination of Amolorfine HCl in bulk.

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CONFLICTS OF INTEREST: The authors declare that there are no conflicts of interest regarding the publication of this paper.

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