

Forced Degradation Tests of Ampicillin in Medication Forms

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Abstract

Ampicillin is a β lactam antibiotic having broad spectrum having activity against Gram +ve, Gram -ve bacteria as well as enterococci. It is indicated for respiratory tract, urinary tract and soft tissue susceptible infections. Forced degradation commonly includes exposure of drug to heat, range of pH values, UV light. forced degradation study helps to determine the loss of drug substance and the degradation product under diverse conditions. According to ICH guidelines Ampicillin was exposed to different stress conditions. For the analysis of drug in the presence of degradation products, UV spectroscopic method was developed which calculate the amount of the degraded product. According to the BP, the official assay limit of the content should NLT 95% and NMT 105% of labelled amount. AMP, AMP.L, AMP.M on exposure to acidic (1 N HCl) and basic (1 N NaOH) medium shows heavy degradation. However, when AMP, AMP.L, AMP.M are exposed to heat and UV light negligible difference in availability was determined. After 8 days, the effect of time shows that AMP shows no degradation whereas AMP.L and AMP.M shows less degradation as compared to other stress degradation factors. The method was found to be simple, cost effective and less time consuming. Hence this method can be successfully used.

Keywords: Ampicillin; Degradation Studies; API; Formulations

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1. Introduction

An acid-stable semisynthetic β lactam antibiotic *i.e.* ampicillin (α -amino-benzyl penicillin) or (6[D(-)-aaminophenylacetamido] penicillanic acid) (figure 1) was synthesized in 1961. It has a broad spectrum of antimicrobial activity than penicillin G and it is extensively effective against Gram+ve (Group A Streptococcus pyogenes, Staphylococcus pyogenes Smith (penicillin sensitive) and Diplococcus pneumonia) and Gram-ve organisms (Klebsiella pneumoniae, Proteus mirabilis, Salmonella typhimurium and Escherichia coli), as well as for enterococcal infections resistant to penicillin G, but is ineffective against penicillinase-producing staphylococci [1-5]. It is indicated for respiratory tract, urinary tract and soft tissues susceptible

infections. It is also useful in the prophylaxis and treatment of exacerbations of chronic bronchitis [6]. Mechanism of action of penicillin (e.g. ampicillin) with trans peptidase and β -lactamase enzymes involves the opening of β -lactam ring by a serine hydroxyl group which kill these bacterial enzymes by their lethal action than the bacteria resist these bactericides by using their method of defence. As a result of these reactions, an ester of penicilloic acid (penicilloyl enzyme intermediate) is produced by the addition of hydroxylic group across the β -lactamic bond. The β -lactamic bond is broken down and the hydroxylic hydrogen atom transfers to the nitrogen atom of β -lactam amide [7].

Spectrophotometry method is commonly preferred chiefly by small-scale industries as the equipment cost is less and the problems of maintenance are

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economical. The analytical technique is based on the determining the absorption of a monochromatic light by colorless complex in the near (UV) ultraviolet region (200-380 nm). For stress-degradation studies of ampicillin UV spectrophotometry can also be use. As per ICH guidelines the active pharmaceutical substance is focused to several forced degradation conditions which include, basic, acidic and photo conditions [8].

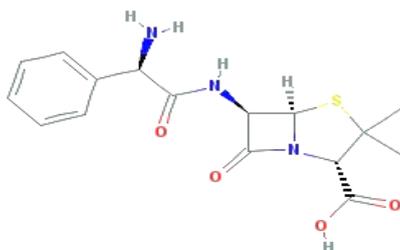


Figure 1. Ampicillin

In the early development process forced degradation activities must be performed to ensure that the method is selective before effort, lot of time, and money have been spent. It is essential to find out the conditions responsible for degradation of drug [9].

Forced degradation is capable of demonstrating that the proposed method is stability indicating i.e. the method uses to identify the increase in the degradation product and the succeeding loss of in active components [10].

2. Materials and Methods

2.1 Parameters in forced degradation

The characteristic forced degradation studies on drug substance involves photo degradation, Acid/base Stress testing, pH variation (high and low), Temperature and or with humidity, and Time.

2.2 Acid/base stress testing

Forced degradation of a drug substance is evaluated by acid/base stress testing. It involves degradation of a drug substance by exposure to acidic or basic environment over time to its primary (monomeric) degradation products. Acid/base hydrolysis is carried out by labile carbonyl functional groups which include alcohols, carbamates, amides (lactams), aryl amines, esters (lactones), imides and imines.

2.3 Thermal/Thermal/humidity stress testing

Thermal or thermal/humidity stress testing is carried out by exposing the drug substance to thermal/humidity conditions over time which causes the substance to degrade forcefully to its primary components.

2.4 Degradation by UV light

UV degradation is a major problem in several UV-instable products which are made up of synthetic and natural polymers as they crack or disintegrate when exposed to continuous sunlight. As the attack is dependent on the extent and degree of exposure, continuous exposure is a more serious problem than intermittent exposure [9].

2.5 Experimental Ampicillin

The Ampicillin brands used were Penbritin 250 mg capsule of Glaxo Smith Kline Pakistan ltd and ampicillin 250 mg capsule of Pliva Pakistan (pvt) ltd.

2.6 Reagents

Analytical grade reagents were used which includes 1N sodium hydroxide, 1N hydrochloric acid and deionized water used was double distilled, deionized and filtered.

2.7 Glasswares

volumetric flask, funnel, beakers, measuring cylinder, pipette, and stirrer used were of Pyrex type and were washed with chromic acid followed by thorough washing with water and finally rinsed with double distilled or de-ionized water which was freshly prepared in the laboratory.

2.8 Instruments

- Spectrophometer: PG Instrument (T80 uv/vis spectrometer) along with a pair of 5 cm quartz cuvettes
- Weighing Balance: Pioneer OHAIUS (Item PA214C)
- Water Bath: DT; Digital constant temperature tank HH-4
- UV Lamp: Power: 8N, LF-204.LS, Serial N 045571, 4W-254 nm,4W-365 nm.

2.9 Preparation of 1 N Sodium hydroxide

Weigh 40 gm of NaOH, dissolve in small quantity of water taken in 100ml volumetric flask and make up the volume up to mark with de ionized water.

2.10 Preparation of 1 N Hydrochloric acid

Take 8.36ml analytical grade hydrochloric acid (37%, 12N) in a volumetric flask and add de-ionized water to make up the volume.

2.11 Preparation of ampicillin solution

Separately weigh the intact capsule of each of the brands than empty the capsule n weigh the empty shell.

Finally weigh granules of each brand capsule individually. Triturate the granules separately in mortar pestle. powder equivalent to 25 mg of ampicillin trihydrate accurately weigh i.e. Penbritin (0.0230gm), ampicillin (0.0258gm) for making primary solutions of ampicillin while active (0.0250 gm) is directly weigh. Once weighed samples were introduced into three separate 100ml volumetric flask. Hot water of 70 ml was use to dissolve the powdered material and shake and finally make up the volume to 100 ml respectively for each sample. Solutions obtained of desired concentration (200 ppm) were transferred individually to cuvette to determine the absorbance at max 226nm by using spectrophotometer.

2.12 Procedure for Degradation Studies

2.12.1 For Acid

To study the effect of acid, take 5 ml of 100 ppm solution of ampicillin(local), Penbritin (multinational) and ampicillin(active) in three separated test tubes then 5ml of 1 M HCl is added in each test tube. The samples were then left for a period of 30 minutes. Upon completion of time period, solutions were transferred to a cuvette separately and then absorbance of the solutions was recorded at the wavelength of 226 nm.

2.12.2 For Base

To study the effect of acid, 5 ml of 100 ppm solution of ampicillin(local), Penbritin (multinational) and ampicillin(active) in three separated test tubes then 5ml of 1 N NaOH is added in each test tube. The samples were then left for a period of 30 minutes. Upon completion of time period, solutions were transferred to a cuvette separately and then absorbance of the solutions was recorded at the wavelength of 226 nm.

2.12.3 For UV light

To study the effect of UV light, take 5 ml of 100 ppm solution of ampicillin(local), Penbritin (multinational) and ampicillin(active) in three separated test tubes then 5 ml water is added in each test tube and place these solutions in UV light

and absorbance of the solutions was recorded at the wavelength of 226 nm.

2.12.4 For Heat

To study the effect of UV light, take 5 ml of 100 ppm solution of ampicillin(local), Penbritin (multinational) and ampicillin(active) in three separated test tubes each containing 5 ml of water, then place these solutions in UV light and absorbance of the solutions was recorded at the wavelength of 226 nm.

2.12.5 For time

To study the effect of time on degradation, we take 100 ppm solution of ampicillin(local), Penbritin (multinational) and ampicillin(active) in cuvette individually and initial absorbance (at 226nm) of each solution was observed then sample were left for a period of 7 days, after completion of specified time interval absorbance of the solutions was recorded at the wavelength of 226 nm.

3. Results and Discussions

When have conducted study on Active of ampicillin(AMP) and 22 brands of it i.e. ampicillin (AMP.L) and penbritin (AMP.M). when AMP, AMP.L and AMP.M are subjected to 1 N HCl, the active and two of its brands showed increased availability (187.68%, 164.57%, 173.23%) respectively absorbance and percentages are given in table 1 and 2 and figures 2-4. When AMP, AMP.L and AMP.M are subjected to 1 N NaOH, the drug showed increased availability (188.45%, 174.56%, 176.70%) respectively. Similarly, when AMP, AMP.L and AMP.M are subjected to heat for 30 minutes, the AMP do not show any changes whereas AMP.L and AMP.M showed minor change in availability (105.63%, 103.74%) respectively. Negligible changes also observed when AMP, AMP.L and AMP.M are exposed to U.V light (101.49%, 104.75%, 98.84%) respectively. When AMP, AMP.L and AMP.M keep for 8 days insignificant changes also observed (99.18%, 89.42%, 94.72%) respectively.

According to our results we can conclude that AMP when introduced in acidic medium i.e. 1N HCl shows degradation to a larger extend i.e. 87.68% whereas AMP.M shows 61.8% degradation while the AMP.L shows the least degradation 45.31% in the acidic medium.

Similarly, when active (AMP) and the 2 brands of ampicillin (AMP.L, AMP.M) are added in basic medium i.e. 1 N NaOH. All of them show degradation i.e. AMP degraded 88%. AMP.L degraded 55.3% and AMP.M degraded 65.27% which shows that AMP degraded the most.

AMP, AMP.L and AMP.M when heated for 30 min and evaluated for degradation, all of them shows negligible changes in concentration i.e.0.36%, 13.63% and 7.69% respectively from the concentration before treatment which concluded that no degradation occurs after heating the samples. They are in the official limits i.e. 95-105% of the drug should be available in % assay.

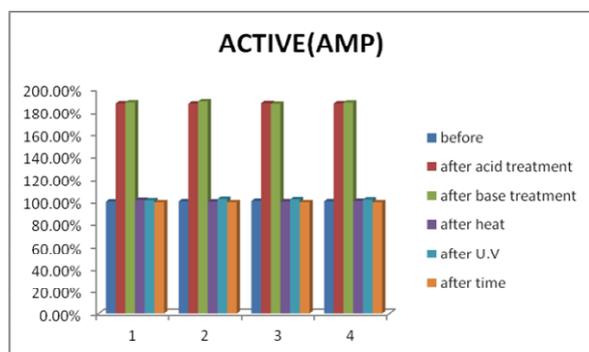


Figure 2. Degradation in API

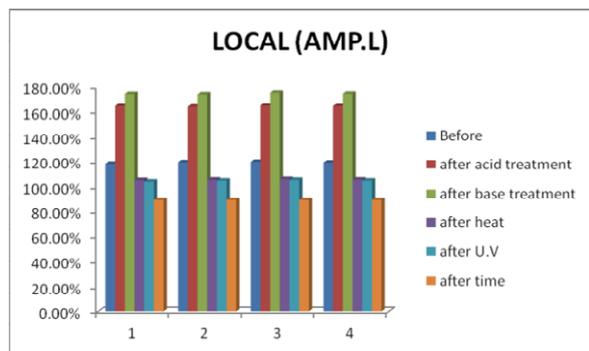


Figure 3. Degradation in Local Formulation

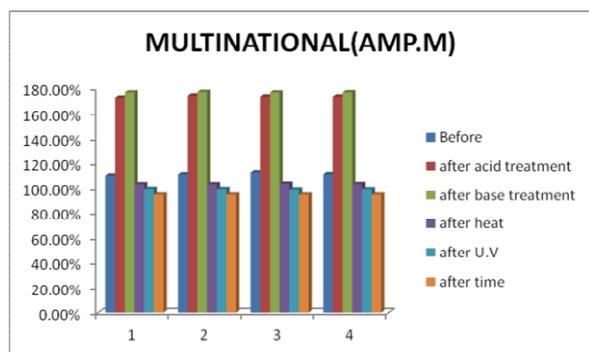


Figure 4. Degradation in Multinational Formulation

When AMP, AMP.L and AMP.M are placed in U.V light for 30 min minor changes in concentrations were observed i.e.1.49%, 14.51 and 12.59% respectively from the concentration initially calculated. They are in the official limits i.e. 95-105% of the drug should be available in % assay.

In the same way when the sample of AMP, AMP.L and AMP.M are left for 8 days of interval and availability was evaluated, AMP shows negligible difference in concentration i.e. 0.82%, it is in the official limits (95 to 105%) whereas AMP.L and AMP.M shows changes in concentration from the initial values i.e. 29.84% and

16.71% which resulted in degradation and out of the official limits

4. Conclusion

According to the specification in BP, the official assay limit of the content should NLT 95% and NMT 105% of labelled amount. From our experiment we can conclude that ampicillin degrades most in acidic and basic medium whereas little degradation also occurs with time, in addition UV light and heat has negligible degradation effect on ampicillin.

Table 1. Absorbance of drugs

Absorbance of blank(drug +water)			
S. No	A	B	C
1	0.646	0.766	0.714
2	0.647	0.774	0.72
3	0.649	0.776	0.73
average	0.647333	0.772	0.721333
Absorbance of drug +acid			
1	1.214	1.271	1.243
2	1.213	1.267	1.255
3	1.216	1.274	1.249
average	1.214333	1.270667	1.249
Absorbance of drug+base			
1	1.22	1.259	1.273
2	1.226	1.256	1.276
3	1.212	1.266	1.273
average	1.219333	1.260333	1.274
Absorbance of drug after heating			
1	0.655	0.76	0.747
2	0.646	0.762	0.747
3	0.647	0.766	0.75
average	0.649333	0.762667	0.748
Absorbance of drug after u.v			
1	0.652	0.751	0.714
2	0.66	0.757	0.713
3	0.658	0.761	0.711
average	0.656667	0.756333	0.712667
Absorbance of drug after time			
1	0.642	0.695	0.683

Table 2. Degradation Studies

Active(AMP)						
S.No	before	after acid treatment	after base treatment	after heat	after U.V	after time
1	99.79%	187.63%	188.56%	101.23%	100.77%	99.18%
2	99.95%	187.48%	189.48%	99.84%	102.00%	99.18%
3	100.26%	187.94%	187.32%	100%	101.70%	99.18%
average	100.00%	187.68%	188.45%	100.36%	101.49%	99.18%
Local (AMP.L)						
	Before	after acid treatment	after base treatment	after heat	after U.V	after time
1	118.33%	164.60%	174.37%	105.26%	104.01%	89.42%
2	119.57%	164.10%	173.96%	105.54%	104.84%	89.42%
3	119.88%	165.02%	175.34%	106.09%	105.40%	89.42%
average	119.26%	164.57%	174.56%	105.63%	104.75%	89.42%
Multinational(AMP .M)						
	Before	after acid treatment	after base treatment	after heat	after U.V	after time
1	110.30%	172.39%	176.56%	103.60%	99.02%	94.72%
2	111.23%	174.06%	176.97%	103.60%	98.89%	94.72%
3	112.77%	173.23%	176.56%	104.02%	98.61%	94.72%
average	111.43%	173.23%	176.70%	103.74%	98.84%	94.72%

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