In Vitro Comparative Degradation Study Between Two Brands Of Amytriptyline Hydrochloride Tablet Using UV Spectrophotometer

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Abstract

The objective of this study was to develop the degradation studies of different brands of amytriptyline hydrochloride in market. Forced degradation is a process that involves degradation of 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. Amitriptyline (AMT) belongs to tricyclic dibenzocycloheptadiene derivatives. It acts primarily as a serotonin-norepinephrine reuptake inhibitor, used for the treatment of several psychiatric disorders. This drug was subjected to different stress conditions as per International Conference on Harmonization guidelines (ICH). An ultraviolet UV spectroscopic method was developed for analysis of the drug in the presence of the degradation products. Distilled water was used as a solvents. The amount of degraded drugs was calculated by taking the absorbance at 222 nm. According to the assay limit of USP specified that the content should not be less than 95% and not more than 105% of labelled amount. All brands were degraded on basic pH and on acidic pH. In addition to heat exposure all brands were also degraded. It was concluded that all brands degraded from ranges for all the stresses applied for degradation studies.

Keywords - Amytriptyline hydrochloride, dibenzocycloheptadine, degradation studies, assay.

Introduction

Chemical stability of pharmaceutical molecules is a matter of great concern as it affects the safety and efficacy of the drug product. The FDA and ICH guidance's state the requirement of stability testing data to understand how the quality of a drug substance and drug product changes with time under the influence of various environmental factors Knowledge of the stability of molecule helps in selecting proper formulation and package as well as providing proper storage conditions and shelf life, which is essential for regulatory documentation. Forced degradation is a process that involves degradation of 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. The ICH guideline states that stress testing is intended to identify the likely degradation products which further helps in determination of the intrinsic stability of the molecule and establishing degradation pathways, and to validate the stability indicating procedures used [1].

Tricyclic antidepressants (TCAs) are heterocyclic chemical compounds used primarily as antidepressants. The TCAs were first discovered in the early 1950s and were subsequently introduced later in the decade [2] they are named after their chemical structure, which contains three rings of atoms. Amitriptyline (AMT) belongs to tricyclic dibenzocycloheptadiene derivatives and chemically AMT is 3-(10,11-dihydro-5H-dibenzo[a,d]cycloheptene-5-ylidene)-N,N-dimethyl-1-propanamine hydrochloride (Figure 1). AMT is official in Indian Pharmacopoeia (IP), British Pharmacopeia (BP) and United States Pharmacopeia (USP) [3–4]. Its molecular formula is $C_{20}H_{23}N{\cdot}HCl$ having molecular weight of 313.86 g/mol [5]. It is a white, colourless, crystalline compound which is freely soluble in water. It has anticholinergic activity. Amitriptyline acts primarily as a serotonin-norepinephrine reuptake inhibitor, with strong actions on the serotonin transporter and moderate effects on the nor-epinephrine transporter. It is used for the treatment of several psychiatric disorders [4,6–8]. The usual recommended dose varies between 50 and 200 mg daily. Despite the beneficial effects of amitriptyline hydrochloride the overdoses of the drug had many undesirable side effects and may lead to some disorders like unconsciousness, convulsions, hyper-reflexia and cardiac depression [9].

Several researchers have been reported the determination of the AMT in biological fluids and/or pharmaceutical formulations. These include chromatographic techniques like HPTLC [10], HPLC with liquid-liquid micro

extraction technique [11], GC [12], GC-MS [13], LC-MS [14], UPLC-MS [15], electro generated chemiluminescence [16], chemometric methods [17] and spectrophotometry [18–25]. The aim of present work was to develop and validate a simple UV spectrophotometric method to be applied for analysis of amytriptyline hydrochloride degradation in tablets as per ICH guidelines, which serves as a tool for the quality control of pharmaceutical dosage forms. These types of degradation studies of drugs and these are very helpful for health care professionals [26–28].

Materials and Methods

Reagents

Analytical grade reagents were used 0.1N sodium hydroxide, 0.1N hydrochloric acid, de-mineralized water and distilled water.

Glasswares

Volumetric flask, funnel, beakers, Measuring cylinder, pipette, and stirrer used were of Pyrex type and were washed followed by thorough washing with water and finally rinsed with distilled or de-mineralized water which was freshly prepared in the laboratory.

Instruments

Theses include

- Spectrophometer: UV-vis spectrophotometer, UV mini-1240, Shimadju.
- Corvettes
- Weighing Balance: Precision balance, LF224DR, Shinko Denshi Co., ltd.
- Water Bath: Stainless-steel, thermo station, HH-S

Wavelength Selection

About 100 ppm of amytriptyline hydrochloride was accurately prepared in distill water. The wavelength maxima (λmax) was observed at 222 nm and this wavelength was adopted for absorbance measurement.

Preparation of 0.1 N Sodium Hydroxide

0.4 grams of sodium hydroxide was taken and transferred it in 100ml volumetric flask and dissolve it in small quantity of water and finally make up the volume up to the mark of the flask with de-mineralized water.

Preparation of 0.1 N Hydrochloric Acid

8.36 ml analytical grade hydrochloric acid (37%, 12N) was taken in a volumetric flask and de-mineralized water was added to making up to the volume.

Standard Stock Solution

The two brands were purchased from a local medicine shop located in Bayezid Bostami, Chittagong. All tablets of brand were labelled to contain Amytriptyline hydrochloride 10 mg per tablet. Showing manufacturing and expire date of different brands (Table 1). Weigh and finally crushed tablets accurately for making primary solutions of Amytriptyline 10 mg, Tryptin 10 (0.0826 gm) Square Pharmaceuticals Ltd., Amilin 10 (0.0920 gm) Opsonin Pharma Ltd., were weighed accurately and introduced in 100 ml volumetric flasks. Distill water was added and shaken vigorously and was making up the volume up to 100 ml to make the strength of the solution 100ppm in 100 ml.

Procedure

For Acid

To study the effect of acid, take 5 ml of 100 ppm solution of each brand in two separated test tubes then 5ml of 0.1N HCl was added in each test tube. They were then left for a period of 30 min. Upon completion of time period, solutions were transferred to a cuvette separately and then absorbance of the solutions was recorded at the wavelength of 222 nm.

For Base

To study the effect of acid, 5 ml of 100 ppm solution of each brand in two separated test tubes then 5 ml of 0.1N NaOH was added in each test tube. The samples were then left for a period of 30 min. Upon completion of time period, solutions were transferred to a cuvette separately and then absorbance of the solutions was recorded at the wavelength of 222 nm.

For Heat

To study the effect of heat, 5 ml of 100 ppm solution of each brand was taken in two separated test tubes each containing 5 ml of water, than place these solutions in water bath for 30 min and absorbance of the solutions was recorded at the wavelength of 222 nm.

Result and Discussion

This research was performed with the purpose to compare the degree of degradation in two different brands of amytriptyline hydrochloride 10 mg tablet. Table 2 shows the variation in absorbance after the effect of different degradation parameters. After acidic pH and basic pH effect, percent of the assay was found 39.38 %-78.80 % (Table 3, 4). In addition to heat exposure percent of the assay was found 59.67 %-62.49 % (Table 5).

The limit of the assay by USP specified that the content should not be less than 95% and not more than 105% of labeled amount. According to this USP specified limit, all brands were degraded in acidic and basic pH. After the heat exposure all brands were also degraded. Effect of acidic pH, basic pH or heat exposure no brands of amytriptyline hydrochloride does comply with this USP specified limit.

Conclusion

It was used to study the stress degradation studies as per ICH guidelines. Amytriptyline was found to be degraded in almost all types of stress conditions and was found to be less stable. The method was used is accurate and precise as well as reproducible and economical and can be successfully used degradation studies of different dosage form. It was concluded that all brands degraded from ranges for all the stresses applied for degradation studies.

Acknowledgement

The authors greatly acknowledge to Department of Pharmacy, International Islamic University Chittagong (IIUC), for supports of valuable instruments and equipments during period of laboratory works.

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Table 1: Showing manufacturing and expire date of different brands

Sl. No.	Brand name	Mfg. Date	Exp. Date
1.	Tryptin	July, 2014	July, 2017
2.	Amilin	September, 2014	September, 2016

Table 2: Showing absorbance of drug in different parameters

Sl. No.	Brand name	Absorbance of standard	Absorbance after acidic pH effect	Absorbance after basic pH efeect	Absorbance after heat effect
1.	Tryptin	2.986	1.915	1.176	1.782
2.	Amilin	2.605	2.053	1.126	1.628

Table 3: Showing effect of acid

Sl. No	Brands	% Assay
1.	Tryptin	64.13 %
2.	Amilin	78.80%

Table 4: Showing effect of base

Sl. No	Brands	% Assay
1.	Tryptin	39.38 %
2.	Amilin	43.22%

Table 5: Showing effect of heat

Sl. No	Brands	% Assay
1.	Tryptin	59.67 %
2.	Amilin	62.49 %

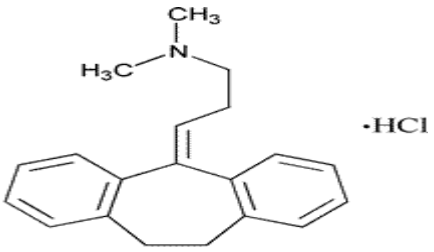

Figure 1: Amytriptyline hydrochloride structure