International Journal of Pharmaceutical Chemistry

ISSN: 2249-734X (Online)
Journal DOI:10.7439/ijpc
CODEN:IJPCI4 (American Chemical Society)

Research Article

Development of force degradation profile of atenolol and chlorthalidone in combine tablet dosage form by (RP-HPLC)

M. S. Charde[*1], A. S. Welankiwar[1] and R. D. Chakole[2]

[1]*Government College of Pharmacy, Kathora Naka, Amravati, India.*
[2]*Department of Pharmacy, Govt. Polytechnic, Gadge Nagar, Amravati, India.*

Abstract

A force degradation profile of Atenolol (ATN) & Chlorthalidone (CTN) in combine tablet dosage form on RP-HPLC was developed using Comosil RP-C18 (4.6 x 250mm, 5µm) in an gradient mode with mobile phase comprising of Methanol: Water (pH 3 using OPA) The flow rate was 1 mL/ min and effluent was monitored at 226 nm. The stress conditions selected on the basis of literature review and drug profile. The analysis of the marketed formulation shows the % RSD of 0.37 and 0.99 for ATN & CTN which fully agrees with system suitability. All the system suitability parameters were fully obeyed during generation of force degradation profile.

Keywords: ATN, CTN, RP-HPLC, Force, Stress, Degradation

1. Introduction

Forced degradation studies are also known as stress testing, stress studies, stress decomposition studies, forced decomposition studies, etc. 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. But these guidelines are very general in conduct of forced degradation and do not provide details about the practical approach towards stress testing. Although forced degradation studies area regulatory requirement and scientific necessity during drug development, it is not considered as a requirement for formal stability program[17-18]. The review of literature[7-16] suggested that no stability indicating assay for the above combination is reported. The present work was undertaken with an objective to develop the force degradation profile for the above combination on RP-HPLC so as to support the development of stability testing program. Atenolol [Figure 1] Chemically is RS)-2-{4-[2-Hydroxy-3-(propan-2 ylamino)propoxy]phenyl}acetamide. It is white to almost white powder used as anti- hypertensive having solubility in methanol and water, sparingly soluble in ethanol the pKa is 9.6. While chlorthalidone [Figure 2] chemically is (RS)-2-chloro-5-(3-Hydroxy-1-oxoisoindolin-3-yl) benzenesulphonamide. It is white to yellowish white crystalline and practically odorless used as anti-hypertensive having solubility in methanol and insoluble in water, slightly soluble in ethanol the pKa is 9.4.[5, 6.-19, 20]

Figure 1 Chemical Structure of Atenolol

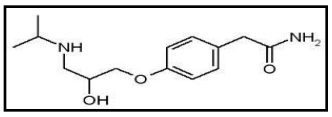
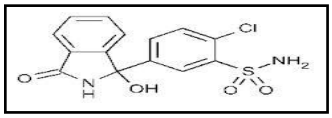


Figure 2 Chemical Structure of Chlorthalidone



2. Experimentals

2.1 Reagents & Chemicals: Standard samples of ATN & CTN were received as gift samples from the leben laboratories akola (Maharashtra) and IPCA Laboratories mumbai (Maharashtra). The marketed formulation Tenoric (IPCA Laboratories) was purchased from the local market containing ATN 50 mg and CTN 12.5 mg and all the chemicals used were are of analytical grade.

2.2 Instruments: HPLC System of Younglin Quaternary pump with UV- VIS detector (190-990 nm) Software – Autochro. Analytical balance of citizen model CY 104 (microanalytical balance) was used for weighing purpose also the ultrasonicator servewell instruments model RC-SYSTEM MU-1700 used for sonication purpose.

2.3 Preparation of Standard Solutions

2.3.1 Standard Stock Solution (A) Accurately weighed quantity of ATN (40.0 mg) was transferred to 10.0 mL volumetric flask and dissolved in methanol. The volume was made up to mark with methanol to get final concentration of 4000 µg/mL. The resultant solution was then sonicated for 10.0 min in ultrasonicator.

2.3.2 Standard Stock Solution (B) Accurately weighed quantity of CTN (10.0 mg) was transferred to 10.0 mL volumetric flask and dissolved in methanol. The volume was made up to mark with methanol to get final concentration of 1000 µg/mL. The resultant solution was then sonicated for 10.0 min in ultrasonicator.

2.3.3 Working Standard Solution (C) 0.1 mL of solution (A) and 0.1 mL of solution (B) was transferred to 10.0 mL volumetric flask and then the volume was made up to the mark with mobile phase to get final concentration of (40.0 µg/mL of ATN & 10.0 µg/mL of CTN) respectively. The resultant solution was then sonicated for 10.0 min in ultrasonicator.

2.4 Optimization of Mobile Phase and Chromatographic Conditions

Procedure The chromatographic conditions were set as per the optimized parameters. The mobile phase was allowed to equilibrate with stationary phase as was indicated by a steady baseline. Solution (C) was injected in the Rheodyne injector (20.0 µl) and the respective chromatograms were recorded. Various mobile phases were tried by permutations and combinations and also by varying column, flow rate, column temperature and type of buffers with varying pH and solvents. The various mobile phases tried are as follows.

- **Trial 1** Methanol: Water (80: 20) pH 7

* Correspondence Info

Dr. M. S. Charde
Government College of Pharmacy,
Kathora Naka, Amravati, India
E-mail: manojudps@rediffmail.com

- **Trial 2** Methanol: Water (60: 40) pH 7
- **Trial 3** Methanol: Water (50:50) pH 7
- **Trial 4** Methanol: Water (35: 65) pH 7
- **Trial 5** Acetonitrile: Methanol: Water (15: 30: 55) pH 7
- **Trial 6** Methanol: Water (60: 40) pH 3

Above mentioned various mobile phases were tried. The mobile phase containing Methanol: water (60: 40) at pH 3, injection volume-20.0 µL flow rate of 1mL/min was selected, due to its high resolving power, sensitivity and suitability, for the determination of ATN and CTN. The chromatogram is shown in **Figure 3**. Hence the following optimized chromatographic parameters were selected to carry out further experimentation.

- **Column** : Comosil RP-C18 (4.6 x 250mm, 5µm)
- **Flow Rate** : 1 mL/min
- **Wavelength** : 226.0 nm
- **Injection Volume** : 20.0 µL
- **Column Temperature** : Ambient
- **Run Time** : 20.0 min
- **Mobile Phase** : Methanol: Water (60:40)
- **pH** : 3 (Using OPA)

2.5 System Suitability Studies

System suitability is a pharmacopoeial requirement and is used to verify, whether the resolution and reproducibility of the chromatographic system are adequate for analysis to be carried out. It is performed to ensure that the system is operating properly and read to deliver results with acceptable accuracy and precision. The tests were performed by collecting data from five replicate injections of standard solutions.

Procedure: The chromatographic conditions were set as per the optimized parameters and mobile phase was allowed to equilibrate with stationary phase as was indicated by the steady baseline. Five replicate injections of mixed working standard solution (C) were injected in to the system, the chromatograms were recorded for both the drugs and the results are shown in **Table 1 & 2.**

2.6 Analysis of Marketed Formulation

Preparation of Standard Solutions: Prepared as per the methodology adopted for system suitability studies.

Preparation of Sample Solutions: Ten Tablets were weighed accurately and ground to fine powder. An accurately weighed quantity of Tablet powder equivalent to (50 mg of ATN & 12.5 mg of CTN) were transferred to 50.0 mL of volumetric flask and dissolved in sufficient amount of methanol. Then the volume was made up to the mark with methanol. The resultant solution was then filtered through whatman filter paper (no. 41). The filtered solution was then sonicated in ultrasonicator for 10.0 min. aliquot portions of 0.8 mL was then transferred to the three separate 10.0 mL volumetric flask and then the volume was mad up to the mark with mobile phase to get final concentration of (80.0 µg/mL of ATN and 20.0 µg/mL of CTN) respectively.

Procedure: Equal volume (20.0 µL) of standard and sample solution was injected separately after equilibrium of stationary phase. The chromatograms were recorded and the response i.e. peak area of major peaks were measured. The amount of drug in a Tablet was calculated using following formula

$$\text{mg/Tablet} = \frac{AT1 \times WS1 \times Ds \times P1}{AS1 \times WT \times Dt} \times \text{Avg. wt}$$

Where,

AT1 = Average area of ATN/CTN peaks in Test chromatogram
AS1 = Average area of ATN/CTN peaks in Standard chromatogram
Ds = Dilution factor for standard
Dt = Dilution factor for test
P1 = Potency of working standards of ATN/CTN of % w/w basis
Avg. wt = Average weight of 10 Tablets

Further calculate the amount of ATN/CTN present in % of Label claim using following formula

$$\% \text{ Label Claim} = \frac{\text{Assay (mg/Tablet)} \times 100}{\text{Label claim of ATN/CTN}}$$

The results are shown in **Table 3**, while chromatogram is shown in **Figure 4.**

2.7 Force Degradation Studies

In order to establish the force degradation profile and to determine whether the analytical method for assay was stability indicating, the Tablet formulation of ATN and CTN were subjected to various stress conditions to conduct forced degradation studies. Stress studies were carried out under the condition of acid/alkali hydrolysis, oxidation, neutral and thermal degradation in accordance with ICH Q1A (R2) guideline. Selection of stress conditions was primarily depends on the literature review and drug profile

The % degradation was evaluated by the following formula:

$$\% \text{ Degradation} = \frac{\text{Area of unstressed} - \text{Area of Stressed}}{\text{Area of unstressed}} \times 100$$

Approaches for Force Degradation

2.7.1 Acid Degradation: An accurately weighed quantity of tablet powder equivalent to (50 mg of ATN & 12.5 mg of CTN) was transferred to a round bottom flask to which 50.0 mL of 0.1N HCL has been added as degradation medium. It is then subjected to reflux on water bath at 70^0C for 1 hr. the resultant solution was then filtered through whatman filter paper (no. 41). Then 0.8 mL of filtered solution was then transferred to 10.0 mL volumetric flask and volume was made up to the mark with mobile phase to get final concentration of (80.0 µg/mL of ATN and 20.0 µg/mL of CTN) respectively.

2.7.2 Alkali Degradation: An accurately weighed quantity of Tablet powder equivalent to (50 mg of ATN & 12.5 mg of CTN) was transferred to a round bottom flask to which 50.0 mL of 0.1N NaOH has been added as degradation medium. It is then subjected to reflux on water bath at 70^0C for 1 hr. the resultant solution was then filtered through whatman filter paper (no. 41). Then 0.8 mL of filtered solution was then transferred to 10.0 mL volumetric flask and volume was made up to the mark with mobile phase to get final concentration of (80.0 µg/mL of ATN and 20.0 µg/mL of CTN) respectively.

2.7.3 Oxidative Degradation: An accurately weighed quantity of Tablet powder equivalent to (50 mg of ATN & 12.5 mg of CTN) was transferred to a round bottom flask to which 50.0 mL of 3% H_2O_2 has been added as degradation medium. It is then subjected to reflux on water bath at 70^0C for 1 hr. the resultant solution was then filtered through whatman filter paper (no. 41). Then 0.8 mL of filtered solution was then transferred to 10.0 mL volumetric flask and volume was made up to the mark with mobile phase to get final concentration of (80.0 µg/mL of ATN and 20.0 µg/mL of CTN) respectively.

2.7.4 Neutral Degradation: An accurately weighed quantity of Tablet powder equivalent to (50 mg of ATN & 12.5 mg of CTN) was transferred to a round bottom flask to which 50.0 mL of water has been added as degradation medium. It is then subjected to reflux on water bath at 70^0C for 1 hr. the resultant solution was then filtered through whatman filter paper (no. 41). Then 0.8 mL of filtered solution was then transferred to 10.0 mL volumetric flask and volume was made up to the mark with mobile phase to get final concentration of (80.0 µg/mL of ATN and 20.0 µg/mL of CTN) respectively

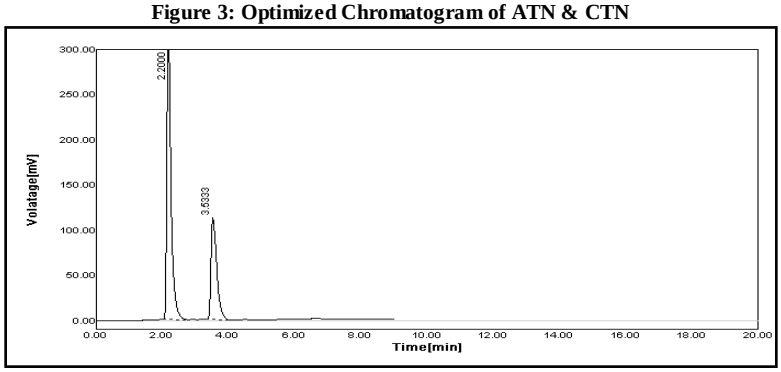
2.7.5 Thermal Degradation: An accurately weighed quantity of Tablet powder equivalent to (50 mg of ATN & 12.5 mg of CTN) was transferred to a heating mantle. It is then subjected to heat 70^0C for 1 hr. It is then transferred to 50.0 mL volumetric flask and then volume was made up to the mark with methanol. The resultant solution was then filtered through whatman filter paper (no. 41). Then 0.8 mL of filtered solution was then transferred to 10.0 mL volumetric flask and volume was made up to the mark with mobile phase to get final concentration of (80.0 µg/mL of ATN and 20.0 µg/mL of CTN) respectively.

Procedure: Equal volume (20.0 µL) of each stress sample was injected separately after equilibrium of stationary phase. The chromatograms were recorded and the response i.e. peak area, retention time of the major peaks were measured. The respective chromatograms are shown in **Figure 5 to 9.**

3. Results and Discussion

3.1 Optimization of Mobile Phase and Chromatographic Conditions

Figure 3: Optimized Chromatogram of ATN & CTN



Observation: Good resolution with minimized tailing also proper peak shape and system suitability was observed within the limits. Hence the above chromatographic parameters are finalized.

3.2 System Suitability Studies

Table 1 Result of System Suitability Studies for (ATN)

System Suitability Test (ATN)					
Sr. No	**Area Reproducibility**	**Retention Time**	**Tailing Factor**	**Resolution**	**Theoretical Plates**
1	2840	2.3	1.71	0	3303
2	2853	2.3	1.71	0	3303
3	2856	2.31	1.71	0	3322
4	2854	2.31	1.71	0	3322
5	2860	2.31	1.72	0	3322
Mean	2852.6	2.306	1.712	0	3314.4
SD	7.536	0.0054	0.0044	0	10.40
%RSD	0.264	0.237	0.261	0	0.313
Limit	**NMT 2%**	**NMT 1%**	**< 2**	**> 2**	**> 2000**

Observation: All the parameters of system suitability are observed within the limits for ATN.

Table 2 Results of System Suitability Studies for (CTN)

System Suitability Test (CTN)					
Sr. No	**Area Reproducibility**	**Retention Time**	**Tailing Factor**	**Resolution**	**Theoretical plates**
1	1366	3.51	1.75	3.31	2460
2	1376	3.51	1.75	3.31	2460
3	1379	3.51	1.81	3.27	2460
4	1363	3.51	1.75	3.31	2460
5	1375	3.5	1.75	3.27	2698
Mean	1371.8	3.508	1.762	3.294	2507.6
SD	6.90	0.0044	0.0268	0.021	106.43
%RSD	0.503	0.127	1.52	0.665	4.24
Limit	**NMT 2%**	**NMT 1%**	**< 2**	**> 2**	**> 2000**

Observation: All the parameters of system suitability are observed within the limits for CTN.

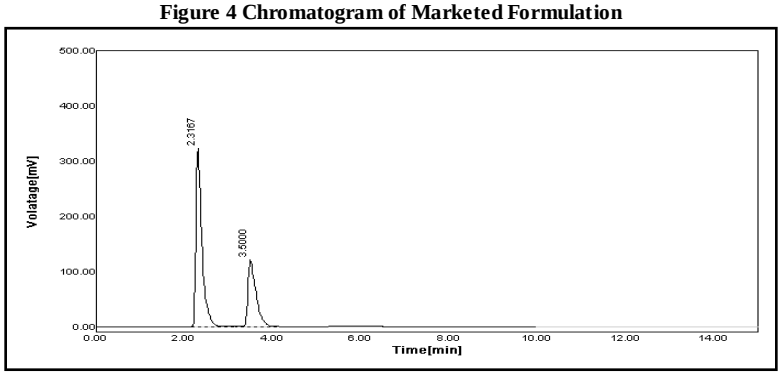
3.3 Analysis of Marketed Formulation

Table 3: Results of Marketed Formulation Analysis

Analysis of Marketed Formulation												
Sr. No	**Standard Amount Taken [µg/mL]**		**Sample Amount Taken [µg/mL]**		**Area of Standard**		**Area of Sample**		**Amount Found [µg/mL]**		**% Amount Found**	
	ATN	**CTN**	**ATN**	**CTN**	**ATN**	**CTN**	**ATN**	**CTN**	**ATN**	**CTN**	**ATN**	**CTN**
1			80	20			3068	1510	79.9	19.9	99.875	99.5
2	80	20	80	20	3102	1540	3080	1521	80.5	20.3	100.62	101
3			80	20			3075	1517	80.2	20.1	100.25	100.5
						Mean	3074.3	1516	80.2	20.1	100.25	100.5
						SD	6.0	5.56	0.3	0.2	0.375	1
						%RSD	0.19	0.367	0.374	0.99	0.374	0.995

The proposed method was applied to the determination of ATN & CTN in marketed formulation the **mean % amount** found was **100.25 (ATN) & 100.5 (CTN)** with **% RSD** values is **NMT 2.0%** indicates the developed method was successfully applied for analysis of marketed formulation. All the results found are in good agreement with the label content of marketed formulation.

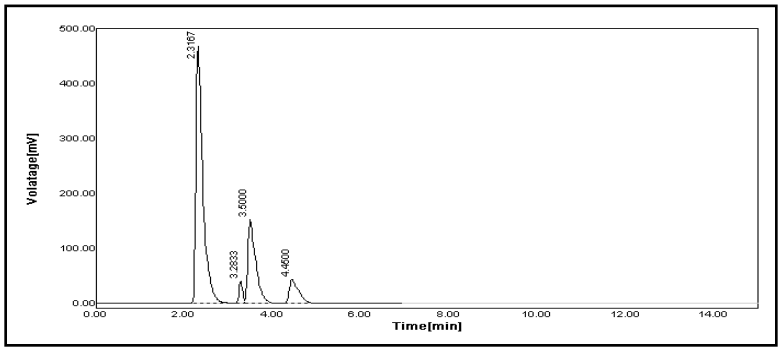
Figure 4 Chromatogram of Marketed Formulation



3.4 Force Degradation Studies

3.4.1 Acid Degradation

Figure 5 Chromatogram of Acid Degradation



Acid degradation studies revels that both the drugs are capable of undergoing a strong degradation in acidic medium (0.1 N HCL) since both the drugs are basic in nature. A strong degradation was observed because of stronger ionization of both drugs under acidic medium owing to higher pKa of both the drugs.

Table 4 Overview of Acid Degradation

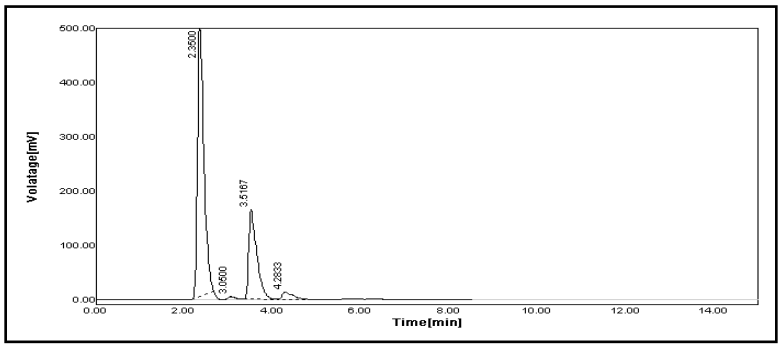
Acid Degradation (0.1 N HCL)			
Name	**Retention Time**	**Area**	**% Area**
Atenolol	2.31	5115	65.13
Chlorthalidone	3.5	1871	23.82
Deg-I	3.28	226	2.89
Deg-II	4.45	641	8.16

Table 5 Summary of Acid Degradation

Acid Degradation			
Stress Condition	**Drugs**	**% Degradation**	**% Assay After Degradation**
0.1 N HCl Reflux in water bath At 70^0C for 1 hr	ATN	6.8	96.8
	CTN	8	92

3.4.2 Base Degradation

Figure 6 Chromatogram of Base Degradation



A base degradation studies revels that both the drugs are also capable of undergoing the strong degradation under basic medium (0.1 N NaOH). Since a stronger degradation peaks were reported at retention times of 3.05 & 4.28 min also both the drugs are strong basic in nature owing to higher pKa values.

Table 6 Overview of Base Degradation

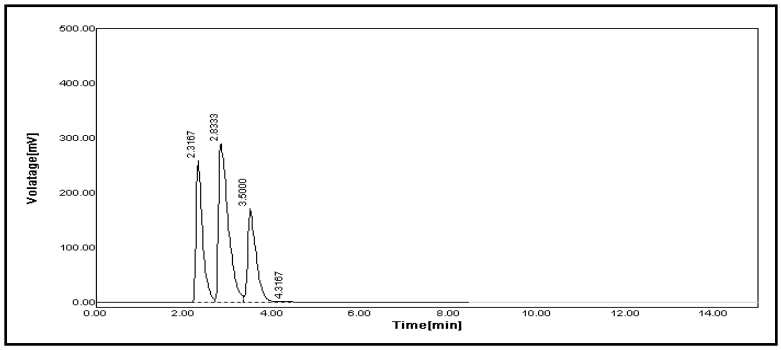
Base Degradation (0.1N NaOH)			
Name	**Retention Time**	**Area**	**% Area**
Atenolol	2.35	4961	68.27
Chlorthalidone	3.51	2076	28.58
Deg-I	3.05	9.63	0.13
Deg-II	4.28	219	3.02

Table 7 Summary of Base Degradation

Base Degradation			
Stress Condition	**Drugs**	**% Degradation**	**% Assay After Degradation**
0.1 N NaOH Reflux in water bath at 70^0C for 1 hr.	ATN	5.05	94.95
	CTN	5	95

3.4.3 Oxidative Degradation

Figure 7: Chromatogram of Oxidative Degradation



Atenolol undergoes strong oxidative degradation (3% H_20_2) since the atenolol is light sensitive in nature.

Table 8: Overview of Oxidative Degradation

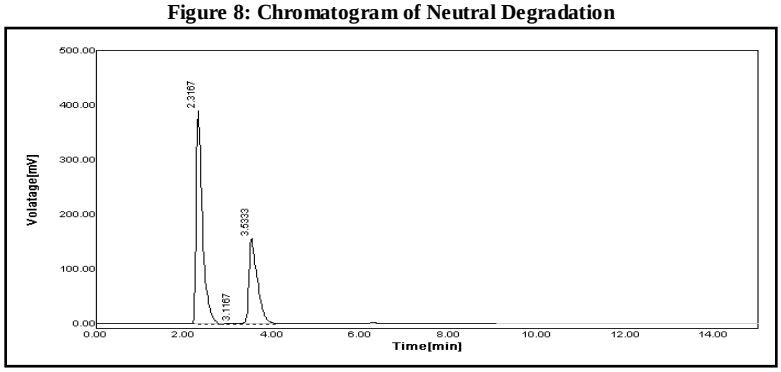
Oxidative Degradation (3% H_2O_2)			
Name	**Retention Time**	**Area**	**% Area**
Atenolol	2.31	2635	28.21
Chlorthalidone	3.5	2225	23.83
Deg-I	2.83	4462	47.78
Deg-II	4.31	16.95	0.18

Table 9: Summary of Oxidative Degradation

Oxidative Degradation			
Stress Condition	**Drugs**	**% Degradation**	**% Assay After Degradation**
3% H_2O_2 Reflux in water bath At 70^0C for 1 hr	ATN	9.8	90.2
	CTN	5	95

3.4.4 Neutral Degradation

Figure 8: Chromatogram of Neutral Degradation



A neutral degradation studies revels that the atenolol is capable of undergoing the neutral degradation (Water) owing to the water solubility of atenolol.

Table 10: Overview of Neutral Degradation

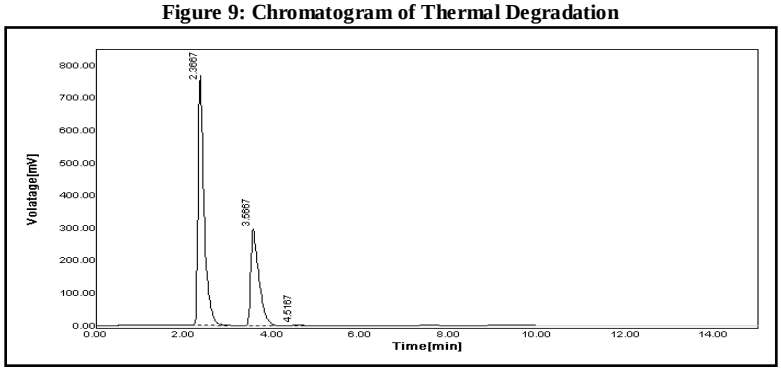
Neutral Degradation			
Name	**Retention Time**	**Area**	**% Area**
Atenolol	2.31	3901	64.59
Chlorthalidone	3.53	2098	34.74
Deg-I	3.11	40.03	0.66

Table 11 Summary of Neutral Degradation

Neutral Degradation (Water)			
Stress Condition	**Drugs**	**% Degradation**	**% Assay After Degradation**
(Water) reflux in water bath At 70^{0}C for 1 hr	ATN	2.7	97.3
	CTN	2	98

3.4.5 Thermal Degradation

Figure 9: Chromatogram of Thermal Degradation



A thermal degradation studies reveled that both the drugs are also capable of undergoing degradation under elevated temperatures. Owing to thermal instability of both drugs.

Table 12: Overview of Thermal Degradation

Thermal Degradation			
Name	**Retention Time**	**Area**	**% Area**
Atenolol	2.36	7230	65.65
Chlorthalidone	3.56	3746	34.02
Deg-I	4.51	36.08	0.33

Table 13 Summary of Thermal Degradation

Thermal Degradation			
Stress Condition	**Drugs**	**% Degradation**	**% Assay After Degradation**
Heat at 70^{0}C on heating mantle for 1 hr	ATN	1.7	98.3
	CTN	1.3	98.7

4. Conclusion

A force degradation studies were conducted as per the ICH Q1A (R2) guideline. Selection of stress conditions was primarily based on literature review and drug profile and results of the stress studies were undergo full agreement with literature review and drug profile. The degraded products were well resolved on under optimized a chromatographic condition which indicates selectivity of the developed method. Also the results of marketed formulation analysis indicate the specificity of the developed method hence the developed method could be employed for the stability studies on pharmaceutical preparations within pharmaceutical industry.

References

1. Vibha gupta *et al*. Development and validation of HPLC method- a review. *Int. Res. J Pharm. App Sci* 2012; 2(4): 17-25.
2. International Conferences on Harmonization Q2 (R1), Validation of Analytical Procedures: Text and Methodology, 2005, pp. 101-109.
3. ICH-Q1A (R2): Stability Testing of New Drug Substances and Products (Second Revision), FDA, Vol. 68, 2003, pp. 225, 657-678.
4. ICH-Q1B: Photostability Testing of New Drug Sub-stances and Products, FDA, Vol. 62, No. 95, 1997, pp.27115-27122.
5. Indian Pharmacopoeia, Government of India, Ministry of Health and Family Welfare. New Delhi; Published by the Controller of Publications; (2010), Vol.2, pp. 129.
6. Indian Pharmacopoeia, Government of India, Ministry of Health and Family Welfare. New Delhi; Published by the Controller of Publications; (2010), Vol.2, pp. 310.
7. Belal F *et al*. Stability-indicating HPLC Method for the Determination of Atenolol in Pharmaceutical Preparations. *J Chromat Separation Techniq* 2013; 4(1): 1-7.
8. Youssef RM *et al*. Validated Stability Indicating Methods for the Simultaneous determination of Amloride Hydrochloride, Atenolol and Chlorthalidone Using HPTLC and HPLC with Photodiode Array Detector. *J AOAC Int* 2013; 96(2): 313-323.
9. Chetta N. *et al*. Development and Validation of a Stability Indicating High Performance Liquid Chromatographic (HPLC) Method for Atenolol and Hydrochlorothiazide in Bulk Drug and Tablet Formulation. *Int J Chem Tech Re*s 2013; 1(3): 654-662.
10. Kumar GS *et al*. Development and Validation of RP-HPLC Method for Simultaneous Estimation of Atenolol and Chlorthalidone in Bulk and Dosage Form. *Int Res j Pharma* 2013; 3(10): 215-19.
11. Abdelwahab S. *et al*. Determination of Atenolol, Chlorthalidone and their degradation products by TLC-Densitometric and Chemometric Method, *J Adv Chem Sci* 2010; 4(1): 200-210.
12. Nivedita G. *et al*. Simultaneous Estimation of Atenolol and Chlorthalidone as Bulk and in Tablet Dosage form by Uv-Spectrophotometry. *IOSR Journal of Pharmacy and Biological Science*s 2012; 1(4):20-23.
13. Pawar PY.*et al*. Simultaneous Estimation of Amlodipine Besylate and Atenolol in Combined Dosage Form by Vierodt's Method Using U.V. Spectroscopy. *Scholars Research Library* 2013; 5(2): 97-102.
14. Tulija Rani G *et al*. A Validated RP-HPLC method for the Simultaneous Estimation of Atenolol and Indapamide in Pharmaceutical Formulations. *Journal of Chemistry* 2011; 8: 1238-1245.
15. Brijesh Singh *et al*. Development of RP-HPLC Method for the Determination of Chlorthalidone in Pharmaceutical Dosage Form. *International Journal of Pharmacy and Pharmaceutical Sciences* 2009; 1: 43-45.
16. Raval HV *et al*. Estimation of Metoprolol Tartarate and Chlorthalidone in Combine Dosage Form by UV Spectrophotometric methods. *Research Journal of Pharmacy and Technology* 2011; 04: 1132.
17. Jitendra Kumar *et al*. Recent Approaches for Impurity Profiling of Pharmaceuticals. *International Journal of Advances in Pharmaceutics* 2013; 2(3): 25-34.
18. Abhijeet Welankiwar *et al*. Photostability Testing of Pharmaceutical Products. *Int Res J Pharm* 2013; 4(9): 11-15.
19. www.drugbank.ca/DB00335/Atenolol.
20. www.drugbank.ca/DB00310/Chlorthalidone.