Forced Degradation Study For Assay Method Of Zolmitriptan Content In Zolmitriptan Pharmaceutical Drugs Product

Dr. M.V. Katariya[1] Mr. Sushil H Jaiswal[2]

1,2 Deparment of Chemistry, Deogiri college, Aurangabad.

Abstract

To determine the stability indicating nature[1] of newly developed reversed-phase high performance liquid chromatography (RP-HPLC)[2] method for the estimation of Zolmitriptan in pharmaceutical drugs dosage forms, a forced degradation study was performed. The separation was achieved on Inertsil C8 –3, (150 mm X 4.6 mm), 5µm using the gradient composition of phosphate buffer pH 7.5 as mobile phase A and mixture of methanol and buffer pH 7.5 in the ratio of 750:250 v/v as mobile phase B at flow rate 1.5mL per minute and detection wavelength 285 nm. The retention time of Zolmitriptan was about 7.2 min. The method for the quantitative determination of Zolmitriptan in Zolmitriptan tablets was validated. The validated method was evaluated for its specificity, and forced degradation parameter under the light of International Conference on Harmonization (ICH) guidelines[3 4 5] And for the statistical evaluation of results, standards guidelines were followed[6].

Key words: Zolmitriptan, RP-HPLC, Forced degradation, drug product.

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 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.

MATERIALS & METHODS

Instrumentation:
HPLC method development[7 8 9] was carried out by using Shimadzu HPLC, Series LC2010 autosampler system equipped with UV and UV/PDA detector and consisted Inertsil C-8 3 (150 mm X 4.6mm) column with 5µ particle.

Chemicals & Reagents

HPLC grade Sodium dihydrogen phosphate monohydrate (Merck India), Potassium Hydroxide (Merck India), Methanol (Rankem), Hydrochloric acid (Rankem), Sodium hydroxide (Thomas Baker), Hydrogen peroxide (Rankem) were used throughout as it is unless and until stated the experiment and was purchased from reliable commercial source.

Zolmitriptan Drug substances and Drug product Zolmitriptan tablet 5 mg, Zolmitriptan N-Oxide Impurity, Zolmitriptan Amino Alcohol, were kindly gifted by Macleods Pharmaceutical Ltd. India.

Standard Preparation

Weighed accurately and transferred about 50 mg of Zolmitriptan to a 100 ml volumetric flask. Added about 60 ml of diluent and sonicated to dissolve. Allowed to equilibrate to room temperature and diluted to volume with diluent. Further dilute 10 ml of this solution to 50 ml with diluent.

Sample preparation

Weighed 10 intact tablets and transfer to a dry 500mL volumetric flask. Added about 300mL of diluent and sonicated for 30 min with intermittent shaking. Allowed equilibrating to room temperature and diluted to volume with diluent. Filtered the solution through 0.45 µm nylon filter (25mm) discarding first few ml of the filtrate.

Impurity solutions (For specificity study)

N-oxide impurity preparation

Weighed accurately about 3.75 mg of N-oxide impurity to 50 ml volumetric flask added 30 ml of diluent, sonicated to dissolved equilibrated to room temperature and diluted to volume with diluent. Further diluted the 1 ml of the solution to 50 ml with diluent.

Amino Alcohol impurity preparation

Weighed accurately about 2.52 mg of Amino Alcohol impurity std to 500 ml volumetric flask and added 360 ml of diluent Sonicated to dissolve and equilibrated to room temperature and diluted to volume with diluent. Further diluted 2.5 ml of this solution to 25 ml with diluent.

Mobile phase:

Prepared mixture of potassium dihydrogen orthophosphate buffer pH 7.5 and methanol (750:250v/v) and degassed.

Chromatographic condition

The experimental condition further optimized to get the desired separation and sensitivity[10]. The final chromatographic conditions are as given in Table 1

Table 1 : Final chromatographic condition

Chromatographic Mode	Isocratic
Column	Inertsil C8 –3, (150 mm X 4.6 mm), 5µm
Wavelength	285 nm
Flow rate	1.5 mL / min.
Injection volume	10 µL
Column oven temperature	Ambient
Sample temperature	Ambient
Run Time	15 min

Result and Discussion

The validation[11] of any developed method ensures credibility of analysis. It demonstrates the scientific soundness of the measurement or characterization. The method was validated for Precision, Repeatability, Intermediate Precision Accuracy / Recovery Linearity, Range and Robustness In the present study, the validated method was studied for Specificity and Forced degradation study.

Specificity

The solutions prepared for identification of Zolmitriptan and each impurities solutions were injected. The Zolmitriptan eluted at 6.712 min. and well separated from impurity. Chromatogram of sample solution (Figure1) and impurities retention time point is as given in Table 2.

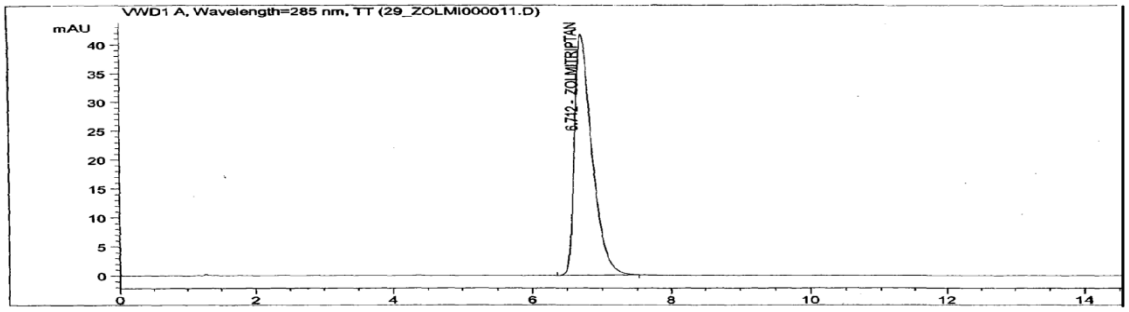

Figure 1 : Chromatogram of Sample solution of Zolmitriptan

Table 2: The retention time of impurity solution

Name of solution		Retention Time (minutes)
Blank		No interference
Placebo		No interference
Impurities of Zolmitriptan	N-oxide	4.370
	Amino alcohol	1.972

Forced degradation study

Thermal Degradation

Tablet powder was heated at 80°C for 24 hours and allowed to cool. 2 g of the exposed sample was weighed and transferred to a 500 mL volumetric flask. Add 300 mL of diluent was added and shaken mechanically for 5 minutes and sonicate for 30 minutes with occasion shaking. The mixture was allowed to equilibrate at room temperature and volume was made with diluent.

The solution was filtered through 0.45 µm nylon (25mm) filter by discarding first 5 mL of the filtrate and subsequent filtrate was used as such. Solution was analysed on HPLC

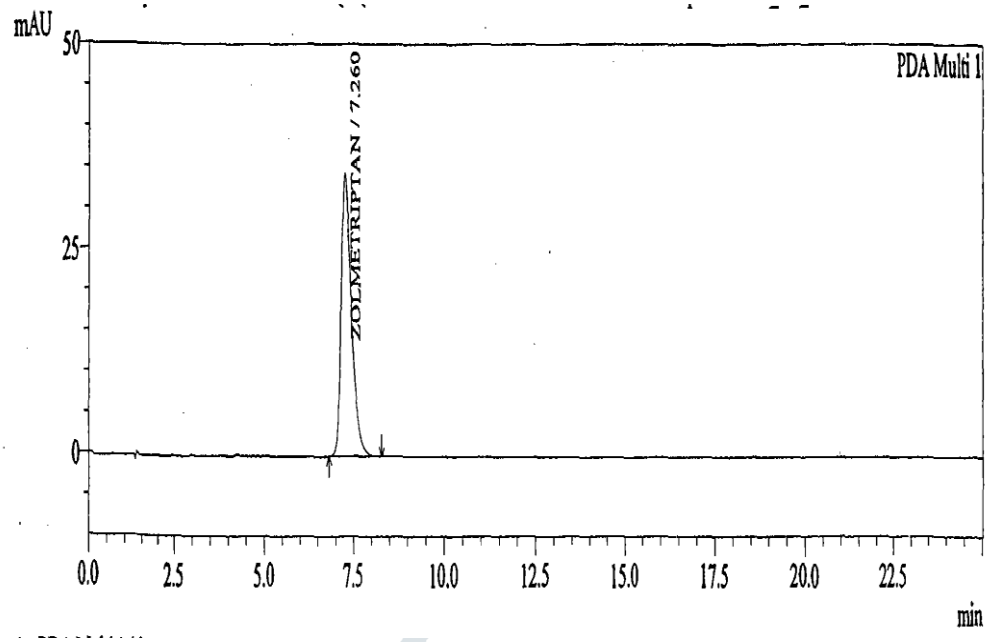

Figure 2 : Chromatogram of Thermal degradation exposed sample

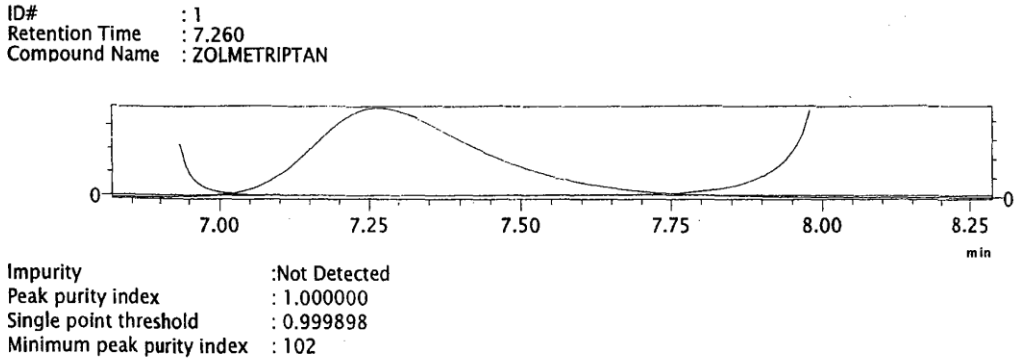

Figure 3: Peak purity Thermal degradation exposed sample

Photolytic degradation

Exposed Sample

Tablet powder (covered with aluminium foil) was exposed in the photo stability chamber, as per ICH guidelines (An overall illumination of not less than 1.2 million lux hours and an integrated near ultraviolet energy of not less than 200 watt hours / square meter).

2 g of the exposed sample was weighed and transferred to a 500 mL volumetric flask. 250 mL of diluent was added and shaken mechanically for 5 minutes and sonicate for 30 minutes with intermittent shaking. The mixture was allowed to equilibrate at room temperature and volume was made with diluent.

The solution was filtered through 0.45 µm nylon (25mm) filter by discarding first 5 mL of the filtrate and subsequent filtrate was used as such. Solution was analysed on HPLC

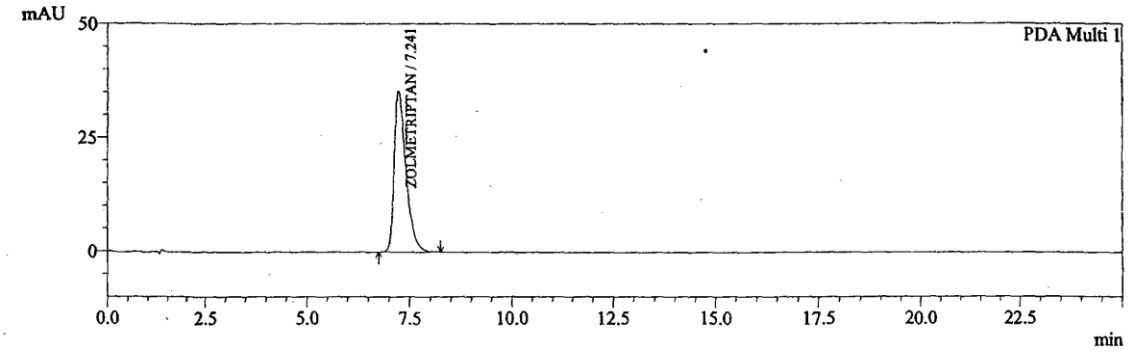

Figure 4: Chromatogram of Photolytic degradation exposed sample

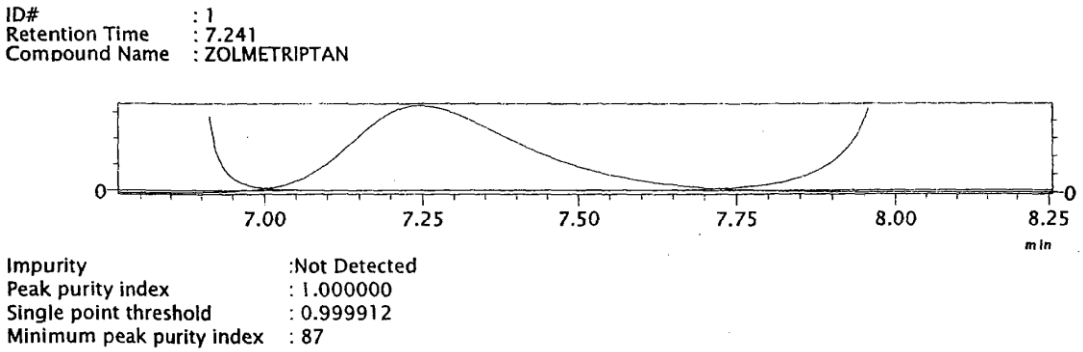

Figure 5: Peak purity chromatograms for photolytic degradation of exposed sample

Thermal and Humidity degradation

Tablet powder was exposed at 40°C / 75 % RH for 24 hours. 2 g (10 intact tablets) of the exposed sample was weighed and transferred to a 500 mL volumetric flask. 20 mL of diluent was added and shaken mechanically for 5 minutes and sonicate for 30 minutes with intermittent shaking. The mixture was allowed to equilibrate at room temperature and volume was made with diluent.

The solution was filtered through 0.45 µm nylon (25mm) filter by discarding first 5 mL of the filtrate and subsequent filtrate was used as such. Solution was analysed on HPLC

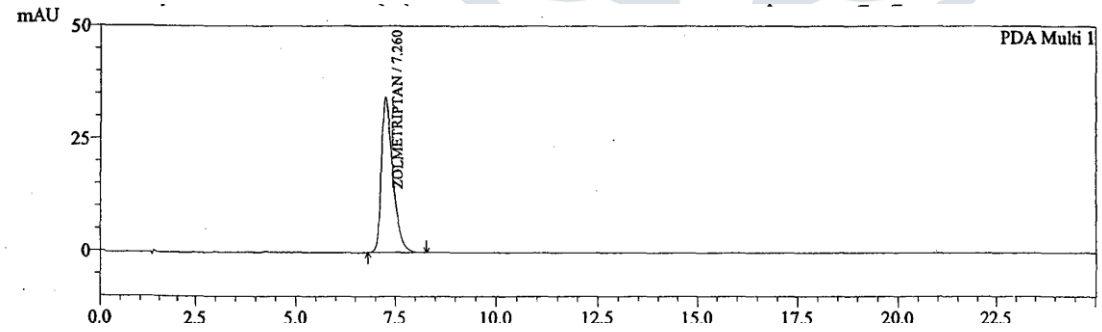

Figure 6 : Chromatogram of Thermal and Humidity degradation sample.

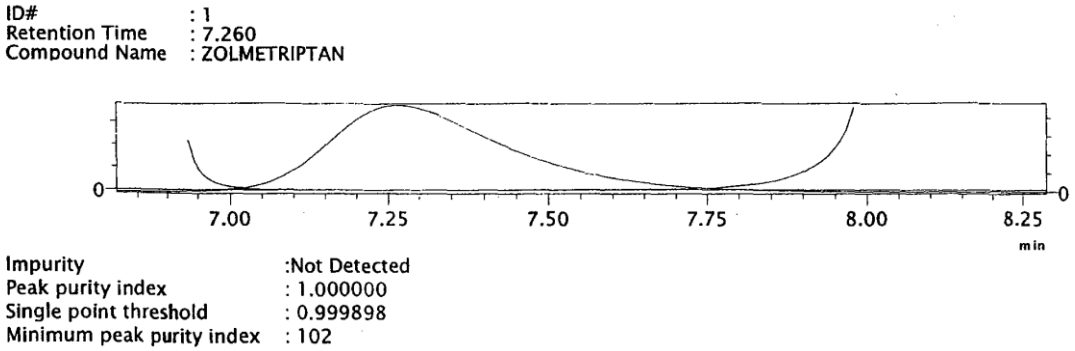

Figure 7: Peak purity chromatogram of Thermal and Humidity degradation exposed sample.

Oxidative Degradation

10 intact tablets (about 2 g) of the sample was weighed and transferred to a 500 mL volumetric flask. 20 mL of diluent was added and shaken mechanically for 5 minutes and sonicate for 30 minutes with intermittent shaking. 5 mL of 3 % H_2O_2 was added and exposed at 25°C for 20 minutes. Equilibrated at room temperature and diluted to volume with diluent.

The solution was filtered through 0.45 µm nylon (25mm) filter by discarding first 5 mL of the filtrate and subsequent filtrate was used as such. Solution was analysed on HPLC

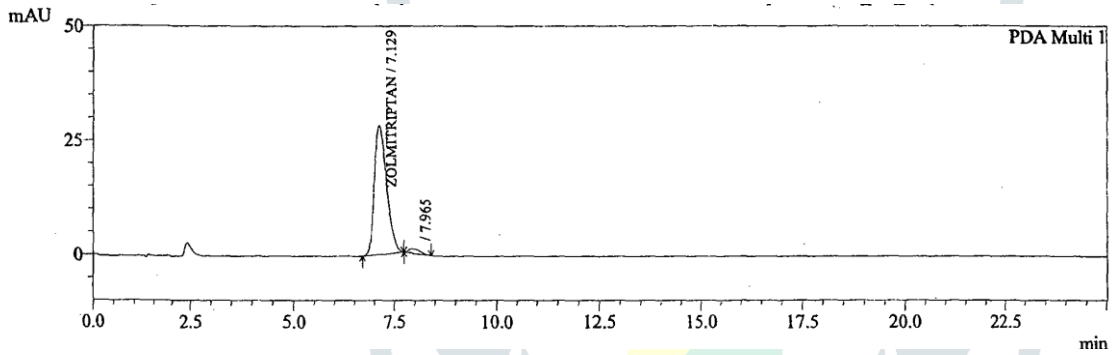

Figure 8: Chromatogram of oxidative degradation sample

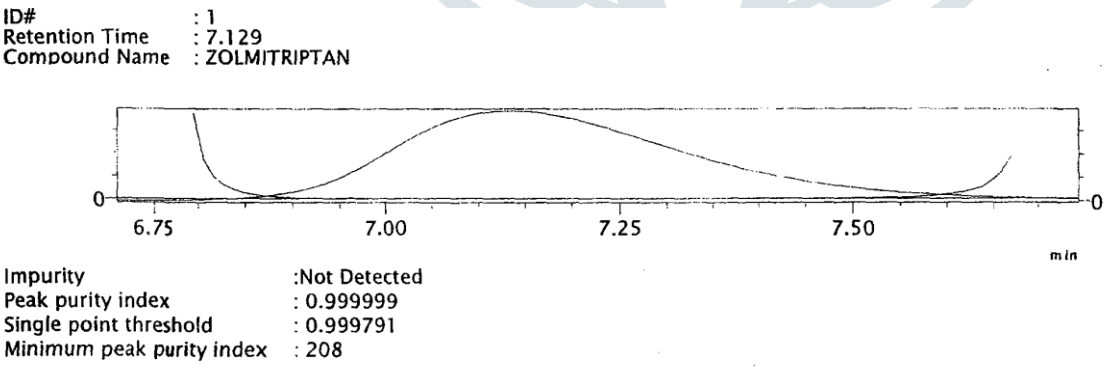

Figure 9 : Peak purity chromatogram of oxidative degradation sample

Acid Degradation

10 intact tablets (about 2 g) of the sample was weighed and transferred to a 500 mL volumetric flask. 20 mL of diluent was added and shaken mechanically for 5 minutes and sonicate for 30 minutes with intermittent shaking. 5.0 mL of 5.0 N HCl was added kept at 80°C for 5 hours on water bath. After 5 hours 5.0 mL of 5.0 N NaOH solution was added for neutralization and volume made up with diluent and mixed. It was equilibrated to room temperature and diluted the volume with diluent.

The solution was filtered through 0.45 µm nylon (25mm) filter by discarding first 5 mL of the filtrate and subsequent filtrate was used as such. Solution was analysed on HPLC

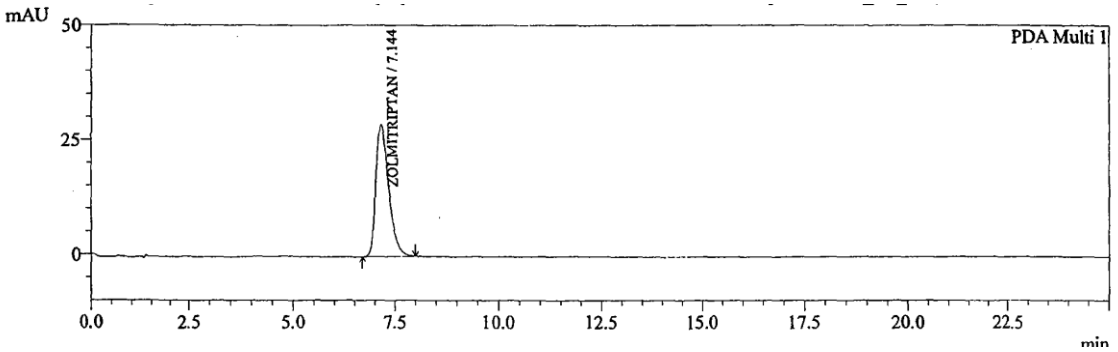

Figure 10: Chromatogram of acid degradation sample.

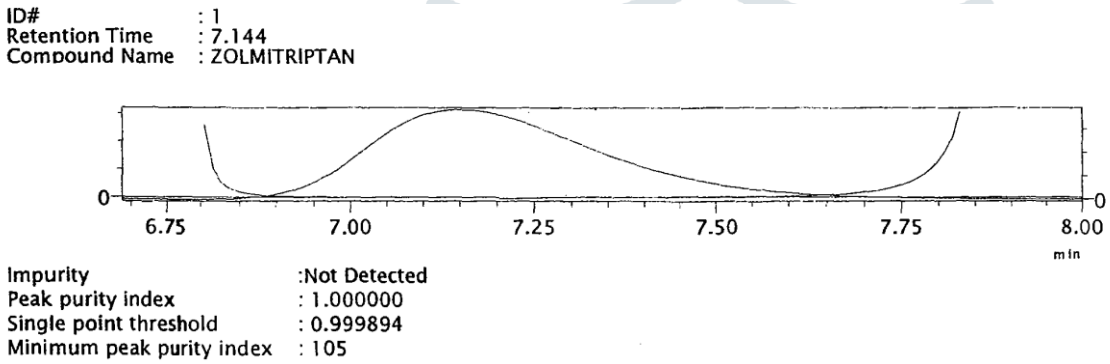

Figure 11 : Peak purity chromatogram of acid degradation

Base Degradation

10 intact tablets (about 2 g) of the sample was weighed and transferred to a 500 mL volumetric flask. 20 mL of diluent was added and shaken mechanically for 5 minutes and sonicate for 30 minutes with intermittent shaking. 5.0 ml of 5N NaOH was added and exposed at 80°C for 60 minutes on water bath. Further 5.0 mL of 5N HCl solution was added for neutralization. It was equilibrated to room temperature and diluted the volume with diluent.

The solution was filtered through 0.45 µm nylon (25mm) filter by discarding first 5 mL of the filtrate and subsequent filtrate was used as such. Solution was analysed on HPLC

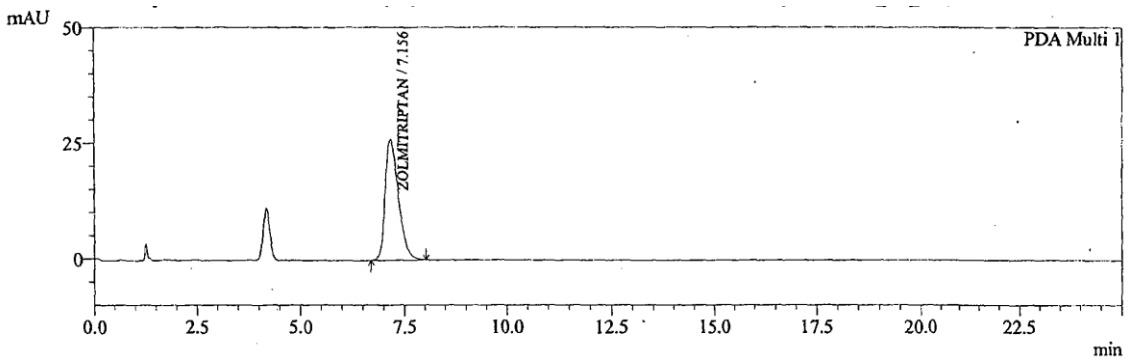

Figure 12 : Chromatogram of base degradation sample.

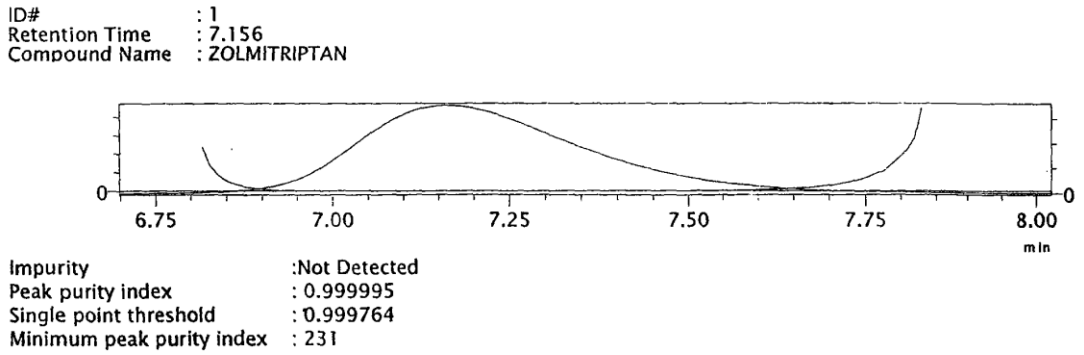

Figure 13: Peak purity chromatogram of base degradation sample

Conclusion of forced degradation study

The peak due to Zolmitrptan was found spectrally pure in all the degradation conditions, indicating that there is no co-elution with main peak.

Based on the above results it is concluded that the method for determination of assay of Zolmitrptan in Zolmitriptan OD tablets 5 mg is specific and stability indicating method.

Table 3 : Summary of forced degradation

Force Degradation condition	% Degradation	Peak Purity
	ZOLMITRIPTAN	
Initial	--	1.000
Exposed at 25°C in oven for 24 hours (Thermal Degradation)	No Degradation	1.000
Photolytic degradation (Control sample)	No Degradation	1.000
Photolytic degradation (Exposed sample)	No Degradation	1.000
Thermal and Humidity at 40°C/75% RH for 24 hours	0.5	1.000
5.0 mL of 3 % H_2O_2 -	14.0	1.000
5.0 mL of 5 M HCl kept at 80°C for 5 hour on water bath.	7.8	1.000
5.0 mL of 5 M NaOH kept at room temperature for two and a half hour.	17.9	1.000

References

1 D.W. Reynolds, K.L. Facchine, J.F. Mullaney, *et al.* Available guidance and best practices for conducting forced degradation studies.Pharm. Technol., 26 (2) (2002), pp. 48–56

2 Reversed-phase high-performance liquid chromatography: theory, practice, and biomedical applications, Ante M. Krstulović, Phyllis R. Brown, 1982,Wiley Publication.

3 International Conference on the Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). Validation of Analytical Procedures:Text and Methodology Q2 (R1). (2005). Available from http://www.ich.org

4 International Conference on Harmonization (ICH) Q8(R2): Pharmaceutical Development (August 2009).

5 International Conference on Harmonization (ICH) Q11: Development and Manufacture of Drug Substances (Chemical Entities and Biotechnological/Biological Entities) (May 2011)

6 United States Pharmacopeia/National Formulary

General Chapter <621> Chromatography

General Chapter <1010> Analytical Data-Interpretation and Treatment

General Chapter <1224> Transfer of Analytical Procedures

General Chapter <1225> Validation of Compendial Procedures

General Chapter <1226> Verification of Compendial Procedures

7 Soven P., et al, Assay method development and validation of Ibuprofen tablets by HPLC, Pelagia Research Library 2013, 4(4):91-96

8 Satinder Ahuja Henrik Rasmussen, HPLC Method Development for Pharmaceuticals, Vol. 8.

9 A. K Gupta, P.K Patel, Analytical Method Validation of Stability-Indicating HPLC Method for Determination of Assay of Carbamazepine CR Tablets, Global Research Analysis Volume : 2 Issue : 11 Nov 2013

10 Sun H, Qin X, Ge X, Wang L., Effective separation and sensitive determination of cyanuric acid, melamine and cyromazine in environmental water by reversed phase high-performance liquid chromatography, Pub Med. gov, 2011 Feb-Mar;32(3-4):317-23.

11 Gohil K., Spectrophotometric analysis of Amlodipine besylate in bulk and in tablet dosage forms. Indian J.Pharm. Sci., 2005; 67(3): 376.