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Research Article

DEVELOPMENT AND VALIDATION OF STABILITY INDICATING NEW RP-HPLC METHOD FOR THE DETERMINATION OF ATAZANAVIR SULFATE IN BULK AND CAPSULE DOSAGE FORM

Sanket Supare[1], Nitin Charbe[2,3], Laxmicant Barde[4], Ujwala Mahajan[1], Amol Warokar*[1]

[1]*Dadasaheb Balpande College of Pharmacy, Near Swami Samarth mandir, Besa, Nagpur, Maharashtra, India*

[2]*Departamento de Química Orgánica, Facultad de Química y de Farmacia, Pontificia Universidad Católica de Chile, Av. Vicuña McKenna 4860, 7820436, Macul, Santiago, Chile*

[3]*School of Medical and Allied Sciences, KR Mangalam University, Sohna Road, Gurgaon, Haryana, India*

[4]*P.R. Patil Institute of Pharmacy, Arvi Road, Talegaon(Sp), Tq. Ashti, Dist. Wardha*

**Corresponding author: amol_warokar@rediffmail.com, warokar.amol1@gmail.com*

ABSTRACT

A new stability indicating RP-HPLC method was developed and validated for the determination of Atazanavir sulfate in bulk and capsule dosage form on Zorbax Eclipse XDB C_{18} (150x 4.6mm) 3.5 µm column. Elution was carried using mobile phase consists of phosphate buffer pH 6.5: acetonitrile (40:60 v/v) at column temperature of 30°C. The flow rate of the mobile phase was maintained at 1 ml/min, and effluents were monitored by the PDA detector. Atazanavir sulfate was separated at the retention time of 3.9 min. The specificity of the method was determined by spiking major impurities like pyridinyl lactose acetal, 5-hydroxymethyl-2-furaldehyde, dealkyl atazanavir, and pyridinyl benzaldehyde into Atazanavir sulfate. Atazanavir sulfate was subjected to acid, base hydrolysis, peroxide oxidation, thermal and photolytic degradation. The stability studies indicated that Atazanavir sulfate was stable in acid, thermal, UV light while susceptible to alkaline hydrolysis and peroxide oxidation. Degraded products of peroxide and photolytic degradation coincide with the retention time of 5-hydroxymethyl-2-furaldehyde and pyridinyl benzaldehyde impurity, respectively. The new method is rapid, sensitive, linear, precise, accurate and without any interference of degraded products and excipients. Hence, the method can be successfully applied to the routine quality control of Atazanavir sulfate in bulk and capsule dosage form.

Keywords: Atazanavir sulfate, Stability-indicating assay, RP-HPLC, Specificity study.

1. INTRODUCTION

Chemically, Atazanavir sulfate is 2,5,6,10,13-Pentaa-zatetradecanedioic acid, 3-12-bis(1,1-dimethylethyl)-8-hydroxy-4,11-dioxo-9-(phenylmethyl)-6-[[-4-(2-pyridi-nyl)phenyl]methyl]-dimethyl ester-sulfate [1]. Atazanavir sulphate is a HIV protease inhibitor used in combination with the other antiretroviral agents for the treatment of HIV-1 infection [2-5].

Literature survey revealed that various methods were developed and validated for the estimation of the Atazanavir sulfate [6-12]. Total thirteen impurities of Atazanavir sulfate were determined by LC-MS; the most prominent among them are pyridinyl benzaldehyde lactose acetal (PBLA), 5-hydroxymethyl-2-furaldehyde (5-HMF), dealkyl atazanavir impurity (DA), and pyridinyl benzaldehyde (PB) [6].

International Conference on Harmonization (ICH) guidelines were used to interpret the stability attribute of the drug product under the stress conditions; acidic, alkaline, oxidative, UV, and photolytic degradation [13-18]. It was evident from the literature review that reported methods have not documented the interference of the major impurities of Atazanavir sulfate. Although few stability-indicating RP-HPLC methods were reported for Atazanavir sulfate; however, methods were not performed on the capsule dosage form [19-22]. Therefore, it was thought worthwhile to develop and validate stability, indicating a new RP-HPLC method for the estimation of Atazanavir sulfate in bulk and capsule dosage form. The molecular structure of Atazanavir sulfate was given in Fig. 1.

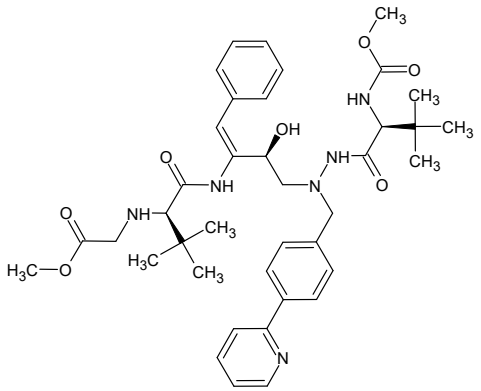

Fig 1: Structure of Atazanavir sulfate

2. MATERIAL AND METHODS

2.1. Instrumentation

Shimadzu HPLC system model 2010 AHT, connected with a PDA detector and operated by Lab solution software. The separation of atazanavir and other impurities was carried out on column Zorbax eclipse XDB C_{18} (150x4.6mm, 3.5µm).

2.2. Chemicals and Reagents

Pharmaceutical grade Atazanavir sulfate and Atazanavir sulfate capsule (500 mg) were generously gifted by Hetro Drugs Ltd., Hyderabad, India. Acetonitrile and water (HPLC grade) were purchased from Rankem, Mumbai. Orthophosphoric acid and triethylamine were purchased from Merck, Mumbai. Membrane filter (0.45 µ) and Teflon glass membrane filter were procured from MDI Pvt. Ltd., Mumbai.

2.3. Preparation of standard Atazanavir sulfate solution

Accurately weighed 50 mg of Atazanavir sulfate API was dissolved in 70 ml of diluent water: acetonitrile (10:90 v/v), and it was kept for sonication at room temperature. The solution was allowed to cool at room temperature and made up to the mark with the diluent. In a 50 ml volumetric flask, 4.5 ml of standard Atazanavir sulfate stock solution was transferred, and volume was made up to mark with diluent to obtain the concentration 45 µg/ml of the working solution.

2.4. Preparation of Atazanavir sulfate capsule solution

The weight equivalent to 50 mg of Atazanavir sulfate was dissolved in about 70 ml of diluent with intermittent swirling in between followed by 15 min stirring. It was allowed to cool and the volume was made up to the mark with diluent. The solution was filtered through 0.45µTeflon and glass membrane. In a 50 ml volumetric flask, 4.5 ml of filtrate from the stock was diluted up to the mark with the diluent to obtain working solution (45µg/ml).

2.5. Method development

Chromatographic separation was performed on Zorbax Eclipse XDB C_{18} (150x 4.6 mm, 3.5 µm) column. The composition of the mobile phase was optimized by trial and error method. The mobile phase comprises 10 mM triethylammonium phosphate buffer pH 6.5: acetonitrile (40:60 v/v) was optimized to resolve the chromatogram. The mobile phase was transferred by an isocratic mode at a flow rate of 1 ml/min into a column maintained at 30°C. The injection volume of atazanavir, was 5 µl. The effluent was sonicated, degassed, and transferred through 0.45 µ Teflon and glass membrane filter. The effluent was monitored by PDA detector. The desired peak was integrated to obtain its $\lambda_{max.}$ The chromatographic parameters like peak symmetry, tailing factor, and retention factor was measured as per USP guidelines [15, 23-26].

2.6. Method validation

The optimized method was validated for its accuracy, linearity, precision, detection limit, quantitation limit, robustness, and specificity. Method validation was carried out as per the ICH and USP guidelines [27-29].

2.6.1. Specificity

The specificity studies were performed by spiking the Atazanavir capsule sample with its known impurities. In 25 ml of the volumetric flask, 5 ml of Atazanavir capsule (45µg/ml) was spiked with 1.0 ml of each impurity solution of Pyridinyl benzaldehyde lactose acetal (PBLA) 0.8 µg/ml, 5-Hydroxymethyl-2-furaldehyde (5-HMF) 0.8 µg/ml, Dealkyl Atazanavir (DA) 4 µg/ml, Pyridinyl benzaldehyde (PB) 0.8µg/ml.The volume was made up to the mark with the diluent.

2.6.2. Linearity

Atazanavir sulfate working standard 22.5, 36.0, 45.0, 54.0, and 67.5µg/ml were analysed by RP-HPLC. A calibration curve was constructed using concentration (µg/ml) on X-axis and area under the curve (AUC) on Y axis. The linear regression equation and correlation coefficient (r^2) were calculated by the LC solution software.

2.6.3. Limit of detection (LOD) and Limit of quantitation (LOQ)

The limit of detection (LOD) and limit of quantitation (LOQ) were calculated by following formulae: LOD = 3.3(SD)/S and LOQ = 10(SD)/S

Where, SD= standard deviation of response (peak area) and S = average of the slope of the calibration curve.

2.6.4. Accuracy

The accuracy of the method was determined by recovery studies. The weight of placebo (excipients) equivalent to 45µg/ml was spiked by the addition of standard Atazanavir sulfate 25, 50, and 75 µg/ml, respectively.

2.6.5. Precision

The precision was determined to ensure the closeness of the data values to each other for the number of measurements under the same analytical conditions. The system and method precision determined by six replicate injections of a homogeneous sample of standard Atazanavir sulfate (45µg/ml) and Atazanavir capsule (45µg/ml), respectively. Intermediate precision was determined by the different analyst, days, and instruments.

2.6.6. Robustness

The standard solution of Atazanavir sulfate (45µg/ml) was injected six times for each varied conditions of flow rate (1±0.1 ml/min), column temperature(30±5℃), and wavelength (250±2 nm).

2.7. Forced degradation

Forced degradation studies of Atazanavir sulfate was accomplished in acid, base hydrolysis, peroxide oxidation, thermal and photolytic conditions. In a series of 100 ml of the volumetric flask, a quantity equivalent to 50.0 mg of Atazanavir capsule was dissolved in 60 ml of diluent with intermittent swirling for 15 min and the volume was made up to the mark with the diluent. In a series of 50 ml volumetric flasks, 4.5 ml (500µg/ml) stock solution was pipetted and treated with 5.0 ml each 5N HCl (acid degradation), 5N NaOH (base degradation), 30 % H_2O_2 (peroxide degradation). These stock solutions were placed in a water bath maintained at 60°C for 2 h. The mixture was allowed to cool. The acid and base stock solution was neutralized with 5.0 ml of 5N NaOH and 5N HCl, respectively. The volume was made up to mark with diluent to obtain working solution of the concentration 45 µg/ml. The photolytic degradation sample (45 µg/ml) was transferred in transparent, amber colour and flask covered with aluminum foil. The solution was exposed under UV and white light for 1.2 million lux h and integrated near the ultraviolet energy of not less than 200 w/sq. Thermal degradation studies was carried by exposing the solution of Atazanavir capsule (45µg/ml) at 60°C in a hot air oven for 2 h. The solution of the forced degradation studies was filtered through 0.45µ Teflon membrane filter. The initial volume of the filtrate was discarded to prevent minor adsorption of the analyte. The sample were analysed by optimized method.

3. RESULTS AND DISCUSSION

New stability indicating RP-HPLC method was developed and validated for the determination of Atazanavir sulfate in bulk and capsule dosage form.

3.1. Development and optimization of the method

RP-HPLC method was optimized in the mobile phase triethylammonium phosphate buffer (10 mM) pH 6.5: acetonitrile (40:60v/v). The t_R of standard Atazanavir sulfate showed at 3.730 min with optimum peak symmetry (Fig 2).

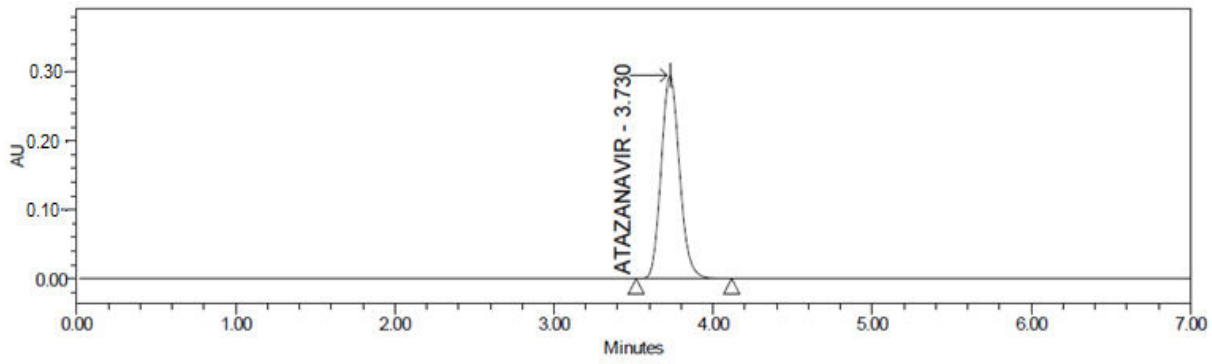

Fig 2: Chromatogram of Atazanavir capsule

The assay of Atazanavir sulfate was found to be 99.1±1.20%. The peak tailing was evident with an increase in acetonitrile concentration beyond 70 % v/v and splitting of the peak was observed as the concentration decreased beyond 30 % v/v.

Separation efficiency is enhanced at a lower value of HETP and a higher number of theoretical plates (N). In the proposed method, the HETP value was 25.056, N 29567, and k' 1.11, which was obtained by LC solution software. A high Capacity (k') factor indicates that the sample is highly retained and has spent a significant amount of time interacting with the stationary phase [30]. These optimization parameters complied with the ICH and USP guidelines.

3.2. Specificity

The specificity studies were performed by spiking the impurities (PBLA, 5-HMF, DA & PB) with the Atazanavir capsule. The chromatogram of specificity study has shown in (Fig. 3), which depicts the main peak has well resolved from its impurities.

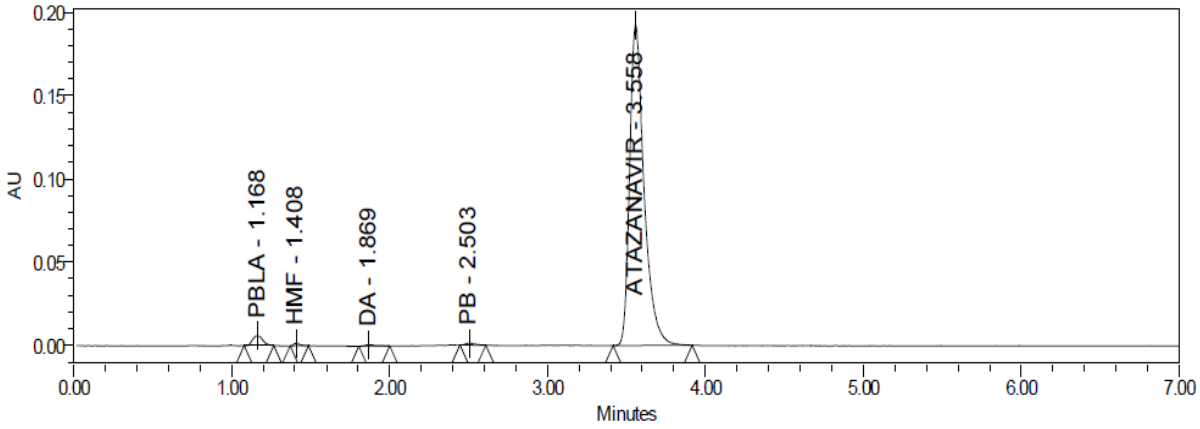

Fig 3: Chromatogram of Atazanavir capsule spiked with impurities

3.3. Linearity

The linearity study of standard Atazanavir sulfate was studied in the range of 22.5-67.5 µg/ml. The calibration function (AUC Vs Concentration) was linear with five-point calibration used for quantitation by linear regression analysis. The regression equation was y =5588.578x-15303.027; with coefficient of correlation (r^2) 0.9995. The LOD and LOQ for Atazanavir sulfate were 0.142 and 0.420 µg/ml, respectively.

3.4. Accuracy

Accuracy study was performed by standard addition of excipients into standard Atazanavir sulfate at three different levels 50, 100, and 150% (n=3). The recovery was found to be 101.3- 101.6 %, which indicates the accuracy of the method according to the acceptance criteria mean recovery in the range of 98.0 - 102.0%. The chromatographic analysis of the spiked sample has not shown any interfering peak at the retention time of Atazanavir sulfate (fig. 3). The results and statistical data of the system suitability parameters have shown in Table 1.

3.5. Precision

The method was found to be precised for six replicates of standard Atazanavir sulfate. The % RSD of the chromatographic determination was 0.19%, which was well within the acceptance criteria. The % RSD values of intra-day and intermediate precision (n=3) were found to be 0.36% for each determinant Table 1.

Table 1: Summary of validation and system suitability parameters

Parameter (Units)	Atazanavir sulfate
Linearity range (µg/ml)	22.5-67.5
Correlation coefficient	0.9995
LOD (µg/ml)	0.142
LOQ (µg/ml)	0.420
Recovery (%)	101.46
Precision (%RSD)	
System precision (n=6)	0.19
Interday (n=3)	0.36
Intraday (n=3)	0.36
Retention Time (t_R)	3.969
Tailing factor (asymmetry factor)	1.31

3.6. Robustness

The results of robustness studies were expressed relative to control (optimized parameter). The relative deviation caused by deliberate variation in flow rate (1± 0.1 mL/min), wavelength (250±5 nm), and column temperature (30±5°C) was within the acceptable criteria ≤2%. Hence, the proposed method was robust. The result of robustness study is summarised in Table 2.

Table 2: Robustness evaluation of method

Parameters	Condition	Mean±SD	Absolute difference
Control	Original	99.6±0.32	-
Flow rate (± 0.1 ml/min)	0.9	98.4±0.21	1.2
	1.1	99.2±0.25	0.4
Change in wavelength (± 5 nm)	245 nm	100.7±0.15	1.1
	255 nm	101.3±0.13	1.7
Column temperature (± 5℃)	25	99.6±0.27	0.0
	35	99.7±0.12	0.1

3.7. Forced degradation

The degradation of the drug was observed by a decrease in peak area and an additional peak of the degraded product when compared with the non-degraded drug (control). The degradation studies indicated that the Atazanavir capsule was susceptible to base hydrolysis, photolytic (transparent container) UV radiation, and peroxide oxidation. However, it was stable to acidic, thermal, and photolytic (amber colour& amber colour covered with aluminum foil) conditions.

Atazanavir sulfate was degraded in the stress condition of basic hydrolysis as well as peroxide oxidation. The maximum deviation of 18.2% was estimated in the basic hydrolysis compared to control. Chromatogram of base degradation showed t_R at 4.6 and 4.8 min.The deviation of 6.4% was estimated in the peroxide oxidation compared to control. A chromatogram of peroxide oxidation showed t_R at 1.4 coincide with the $t_R$of 5-HMF impurity; hence the degraded product may be 5-hydroxy-methyl-2-furaldehyde.The photolytic degrada-tion exposed to UV and white light to transparent medium has shown 11.4% deviation compared to control with three additional peak t_R at 1.2, 2.0 and peak at t_R2.6 min coincide with t_R of PB impurity hence it may be pyridinyl benzaldehyde. Summary of degradation studies of the Atazanavir capsule has shown in Table 3.

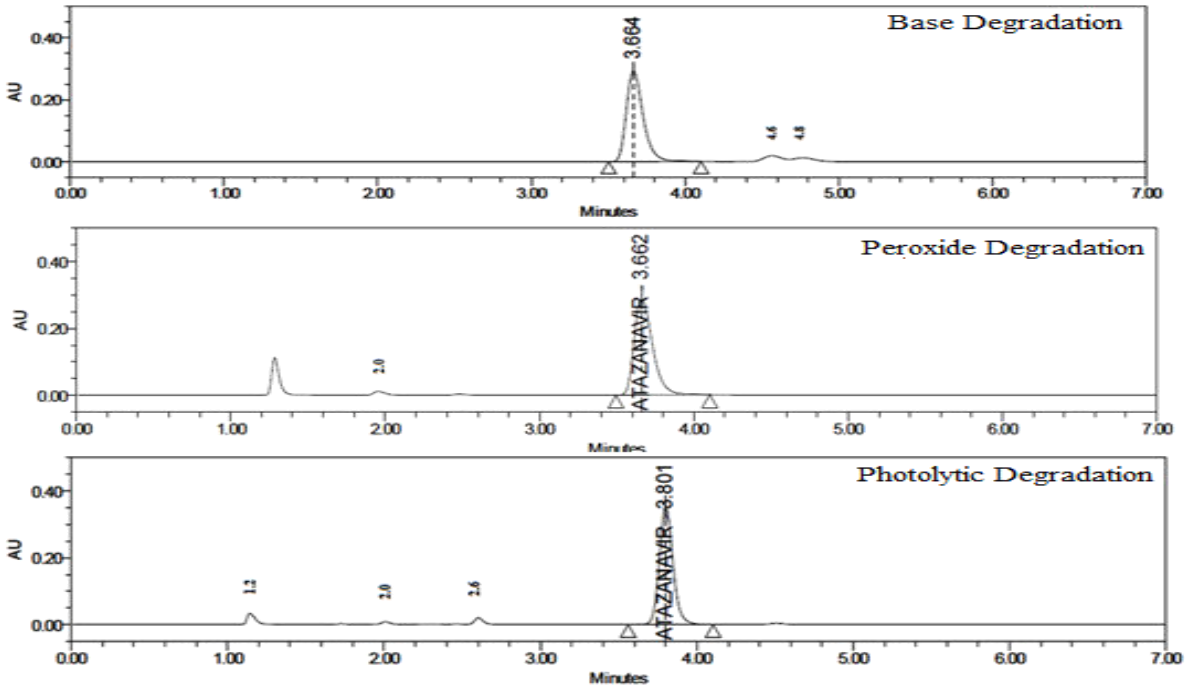

Fig 4: Chromatogram of depicting forced degradation studies

Table 3: Summary of degradation studies

Conditions	Time (h/day)	% Assay	Degradation obtained	t_R (min) of degradation products
Control (acid-base degradation)	-	99.2	-	-
Acid degradation at 60°C in water bath	2 h	96.3	2.9	ND
Base degradation at 60°C in water bath	2 h	81.0	18.2	4.6, 4.8
Peroxide degradation at 60°C in water bath	2 h	92.8	6.4	1.4, 2.0
Thermal degradation at 60°C in hot air oven	2 h	97.5	1.7	ND
Control	-	98.6	-	-
Photolytic (Ambered) UV and white light	11 days	97.4	1.2	ND
Photolytic (Ambered + foil) UV and white light	11 days	98.2	0.4	ND

4. CONCLUSION

The proposed stability-indicating assay method was simple, sensitive, accurate, precise, and repeatable for the determination of Atazanavir sulfate. The results indicated the method's suitability under various force degradation conditions. It may be employed for routine quality control analysis.

5. ACKNOWLEDGMENTS

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6. REFERENCES

1. Longstreet AR, Opalka SM, Campbell BS, Gupton BF, McQuade DT. *Beilstein journal of organic chemistry*, 2013; **9(1)**:2570-2578.
2. De Clercq E. *J ClinVirol*, 2004; **30(2)**:115-133.
3. Gallo RC, Montagnier L. *N Engl J Med*, 2003; **349(24)**:2283-2285.
4. Eisenstein M. *Nat Biotechnol*, 2015; **33(10)**:1014.
5. Cattaneo D, Baldelli S, Castoldi S, Charbe N, Cozzi V, et al. *AidsTher Drug Monit,* 2016; **38(3)**:407-413.
6. Dey S, Patro SS, Babu NS, Murthy PN, Panda SK. *J. Pharm. Anal,* 2017; **7(2)**:134-140.
7. Marzinke MA, Breaud A, Parsons TL, Cohen MS, Piwowar-Manning E, et al. *ClinicaChimicaActa,* 2014; **433**:157-168.
8. Koal T, Burhenne H, Römling R, Svoboda M, Resch K, et al. *An International Journal Devoted to the Rapid Dissemination of Up-to-the-Minute Research in Mass Spectrometry*, 2005; **19(21)**:2995-3001.
9. Gadhvi MP, Bhandari A, Suhagia BN, Desai UH. *Research J.* Pharm. and *Tech.*, 2013; **6(2)**:200-203.
10. Srinivasu K, Rao JV, Raju NA, Mukkanti K. *E- J. Chem.,* 2011; **8(1)**:453-456.
11. **Mondhe CC, Dabhade PS.** *World J Pharm PharmSci*, 2017; **6(8)**:1313-1327.
12. Konidala SK., Sujana K., Rani AP. *Der PharmaChem,* 2012; **4 (3)**:1305-1310
13. ICH Expert Working Group. ICH Guideline Q1A(R2) Stability Testing of New Drug Substances and Products, 2003.
14. ICHQ2AText on Validation of Analytical Procedures, 1995.
15. Walfish S. *BioPharm International,* 2006; **19(12)**:1-6.
16. ICH Q2B Validation of Analytical Procedures: Methodology, 1996.
17. ICHQ1A (R2) Stability Testing of New Drug Substances and Products, 2003.
18. ICH Q2 (R1) Validation of analytical procedures: text and methodology, 1996
19. Chitturi SR, Somannavar YS, Peruri BG, Nallapati S, Sharma HK, et al. *J Pharm Biomed Anal*, 2011; **28(1)**:31-47.
20. Seshachalam U, Rao DN, Haribabu B, Chandrasekhar KB. *Chromatographia*, 2007; **65(5)**:355-358.
21. Mantripragada MK, Rao SV, Nutulapati VV, Mantena BP. *J. Chromatogr. Sci.*, 2018; **56(3)**:270-284.
22. Chinnaiah P, Lanka AR, Pamidi S, Govada PP, Jillella VL. *J Compr Pharm.,* 2015; **2(3)**:71-83.
23. Snyder LR, Kirkland JJ, Dolan JW. Introduction to Modern Liquid Chromatography, 3rd Ed, John Wiley & Sons, Inc; 2010.
24. Kazakevich Y, LoBrutto R. HPLC for Pharmaceutical Scientists, John Wiley & Sons, Inc;2006
25. U.S. Food and Drug Administration (FDA). Q2B Validation of Analytical Procedures: Methodology. GuidInd 1997.
26. ICHQ2 (R1) International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use ICHHarmonised Tripartite Guideline Validation of

Analytical Procedures: Text and Methodology, 1997.

27. Sharma A, Sharma R, *Int Res J Pharm.,* 2012; **3(6)**:39-42.
28. Analytical Procedures and Methods Validation for Drugs and Biologics, Guidance for Industry, 2015.
29. Maggio RM, Vignaduzzo SE, Kaufman TS. *TrAC Trends in Analytical Chemistry*, 2013; **49:57**-70.
30. Skoog DA, Leary JJ, Principles of Instrumental Analysis; 4th ed. Saunders College Publishing, Orlando, USA,1992.