Estimation And Forced Degradation Study Of Thiazole Derivative By HPLC Technique

D.B.Kamkhede, P.R.Solanki.

ABSTRACT: The development and validation of thiazole derivatives under various stress conditions ,as acid/base hydrolysis, oxidative stress degradations. As carried out in this process.the simultaneous forced degradation study of thiazole derivatives, using gradient pump system the mobile phase water: methanol 30:70 was selected to achieve maximum detection, sensitivity at ambient temperature using phenomax c-18 column (250 mm × 4.5 mm,5µl) flow rate 1.0 ml/min , at 238 nm.the proposed method was found to be rapid, accurate and consistent.

KEYWORDS: HPLC Method Validation, Force degradation of thiazole derivative

INTRODUCTION

Forced degradation[2], studies are used to identify reactions which may occur to degrade a processed product[3]. Usually conducted before final formulation, forced degradation uses external stresses to rapidly screen material stabilities. Forced degradation is the process of subjecting compounds to extreme chemical environmental conditions to determine product breakdown levels[4]. preliminary degradation to identify degrading species. In which stability indicating methods to monitor the drug product's stability profiles. Therefore, method development validation[5] significantly impact the drug development process. Forced degradation studies are used to facilitate the development of analytical methodology. as Validated Quantitative analytical method that an detect the change with time in the chemical, physical or microbiological properties of the drug substance , that are specific so that the content of active ingredient, degradation can be accurately measured.Forced degradation studies also provide invaluable insight in investigating degradation products pathways of drug substances[17].Thiazoles are releated to azoles the thiazole moiety is a crucial part of vitamin $B_1$and epothilone,benzothiazoles are important thiazoles example eluciferin. Thiazole derivatives have possessed versatile biological activity [1]. The major action of antioxidants in living organism they cause damage to the normal cells. Quinazolin-4(3H)-ones1-5 are classes of fused heterocycles that are of considerable interest because of the diverse range of their biological activities[9] such as, anti-microbial, anti-cancer, anticonvulsant, anti-tubercular etc.

- *D.B.Kamkhede, P.R.Solanki.*
  Department of Chemistry, Vidyabharti Mahavidyalaya, C. K Naidu Road, Camp, Amravati – 444602 (M.S.) India
  Email: dbkamkhede@gmail.com

Materials & Methods

High performance liquid chromatography system with LC solution data handling system (SHIMADZU-LCAT) was used for analysis. The system was controlled and data was recorded with spinchrome (RF software). The assays were performed on LC system consisting of SHIMADZU-LC 20 AT pump and SHIMADZU-UV detector. The injection volume was 20µl and it is injected in rhenodyne injector system. The detector was set at 238 nm and peak area was integrated automatically by computer using spinchrome CRF software. Detection was carried out by using phenomenex C_{18} column (250mm × 4.6mm, 5µ (micron)) at ambient temperature all the calculation consisting quantitative analysis were perform with external standardization by the measurement of peak area.

Chemicals:

(3-(2,4-dichlorophenyl)-3,3a5,6-tetrahydro-5,6-diphenyl-2H-pyrazolo[3,4-d]thiazole was synthesized in our research laboratory, Vidyabharti Mahavidyalaya, C. K Naidu Road, Camp, Amravati – 444602 (M.S.) India HPLC grade methanol water (Qualigen fine chemicals) of analytical grade.

Preparation of mobile phase:

Water and Methanol solution are used as mobile phase in the ratio 30:70 respectively. The mobile phase was filter through 0.45 µ filter paper and degassed.

Prepration of Standard Solution:

Standard Stock solution of thiazole derivative was prepared by dissolving 1mg/ml thiazole sample in mobile phase. This stock solution is used to prepared the seven solution of various concentration i.e.0.010-0.040mg by dilution of suitable aliquots of mobile phase.

Preparation of sample solution:

Solutions of the sample thiazole derivative were prepared and centrifuged at appropriate rpm then filtered through 0.25µ membrane filter and degassed. All solutions were freshly prepared before the analysis.

RESULT AND DISCUSSION

Method optimization:

HPLC analysis was performed by isocratic elution with a series of mobile phase containing Water:Methanol. The

method had been developed on SHIMADZU LC-20 at dual pump system. The system was controlled and data analysis were performed with spinchrom CFR software .The assay were performed on LC system consisting of SIMADZU LC-20 shimadzu UV detector. Sample were injected with a Rheodyne injector system with a 20µl sample loop . The mobile phase Water:Methanol 30:70 was selected and to achieve maximum detection sensitivity was carried out at ambient temperature using phenomax c18 column (250 mm × 4.5 mm ,5 µl) flow rate 1.0 ml/min, it was filtered through 0.45 µ & 0.25 µ filter & sonicate for 5min in ultrasonic bath. samples were analyze at 238 nm at an injection volume 20µl . The chromatographic run time was 12 minutes and column void volume 10.133 minute.(fig-1). The developed chromatographic method was validated for precision, accuracy, LOD,LOQ, Linearity, Robustness, Solution Stability, Specificity as per ICH guidelines. Table.No.01

Precision:
The precision of the method was demonstrated by inter day & intra day variation studies. In the intra day studies, six repeated injections of sample solutions were made the response factor of drug peaks % RSD were calculated**.** The result shown in Table No.06

Accuracy:
Accuracy of the method was determined by recovery experiments. The recovery studies were carried out six times. The accuracy define in terms of % deviation. The calculated concentration from the actual concentration is listed in table No.02

LOD&LOQ:
limit of detection as the smallest level of analyte that gives a measurable response &limit of quantification as the smallest concentration of analyte which gives response that can be accurate quantified. for thiazole derivative was calculated as 0.00217mg/ml 0.6706 mg/ml,respectively.

Robustness:
Robustness studies are also used to establish system suitability parameters to make sure the validity of entire system, including both instrument and method maintained throughout implementation and use. No.03

Linearity:
To establish the linearity of the method 10-40 µg/ml seven solution of desired concentration were prepared by diluting stock solution to the required concentration of seven different levels. The linearity graph was drawn with concentration of solution on x-axis mean area count on y-axis. The slope y-intercept bias and correlation coefficient of the calibration were calculated as shown in table No.04. The slope intercept value for curve is Y=56.54x+2.5853 which shows the regression equation correlation coefficient $(r)^2$ RSD value of slope intercept for sample compound. Reactant linearity is found for sample compound between the peak area concentration of 10-40. µg/ml with r^2=0.9989617 fig (2) shows the calibration curve of standard solutions of different concentrations.

Specificity:
The specificity of the optimized method was performed by injecting stock solution of individual impurities to check among methanol and drug substance under chromatographic conditions. result show in Table No.05

FORCE DEGRADATION STUDIES

The forced degradation studies are important part of the validation of the stability indicating method. The method validation process for analytical procedure beings with the planned and systematic collection of validation data to support analytical procedure. In forced degradation studies, samples are stored under extreme conditions (acid, base, peroxide, heat, light, humidity etc) stress studies are performed according to ICH guidelines.

Alkali Hydrolysis - At Room Temprature:
1mg/ml of thiazole derivative solution was added to 0.1 M NaOH solution to Perform base hydrolysis equal volume of reactant reagent transfer to round bottom flask was stored at room temperature for 24hrs.The reaction mixture was neutralized diuted with mobile phase 30:70 Water:Methanol. Then sonicated filtered and 20µl solution was injected in HPLC system three times.thus chromatograms were analyzed for percent of degradation.Table.No.7

Alkali Hydrolysis - At high Temp:
A solution of 1 ml thiazole derivative in 0.1 M NaOH solution was refluxed for 30 minutes in RB flask, After cooling reaction mixture was diluted with appropriate mobile phase and 20µl of the solution was injected 3 times to HPLC system to get chromatogram and result were observed and analyzed. Table.No.7

Acid Hydrolysis - At Room Temperature:
To the 10ml vol.flask containing 1mg/ml thiazole derivative solution was added 0.1 M HCL solution to perform acid hydrolysis equal volume of reactant and reagent transferred to round bottom flask was stored at room temperature for 24hrs,after the reaction mixture neutralized by NaOH solution was diluted with mobile phase 70:30 methanol: water . Resulting solution was filtered through 0.25 µ membrane and 20 µl of it was injected three times to HPLC system. The chromatogram obtained were analyzed to get percent degradation of the sample as table No.08

Acid Hydrolysis - At high temperature :
1mg/ml thiazole derivative solution was refluxed with 1ml,0.1M HCL solution. at 80^0_C for 30 minute in a round bootom flask. After cooling the reaction mixture diluted with appropriate mobile phase filter sonicate it. 20 ul diluted solution was injected three times to HPLC system.The chromatogram obtained were analyzed to get present degradation of the sample as table No.05triplicate to observe degradation at high temperature. Table.No.8

Oxidative Stress degradation

A**t room temperature:**
To the 10ml vol.flask 1mg/ml thiazole derivative solution was added in 3% H_2O_2 solution transfer it to round bottom flask solution kept at room temperature.1ml sample solution was withdrawn after 24hrs diluted to 10 ml with mobile

phase. Same procedure is repeated for 5%H_2O_2 solution. Table.No.9

At high Temprature:
1mg/ml thiazole derivative solution was added in 3% H_2O_2 then transferd in to a round bottom flask reflux for 30 min. After cooling the solution transfer in vol. flask diluted, filtered injected to HPLC in triplate.obtained chromatogram were analyzed for present oxidative degradation of sample. Table.No.9

Conclusion

The method development and validation are continuous integrated process that are conducted throughout the drug development process. RP-HPLC method was successfully developed for the determination of stability of thiazole derivative. The developed method is selective precise, accurate and linear. Forced degradation data proved that the method is specific for analysis and free from interference of blank unknown degradation products. The result indicate the prevent stability of compound under various stages conditions. The method is suitable for the analysis forced degradation study of thiazole derivatives. Table.No.10

ACKNOWLEDGEMENT

We are very grateful to the Principal, and management, Vidya Bharti Mahavidyalaya, Amravati, (M.S.)India Vidyabharti Mahavidyalaya, C. K Naidu Road, Camp, Amravati – 444602 (M.S.) India for providing necessary laboratory facilities to perform the research work.

REFERENCES:

[1] Alagarsamy V, Rajasolomon V, Meena R Ramseshu KV. Synthesis, analgesic, anti-inflammatory anti-bacterial activities of some novel 2-butyl 3-substituted quinazolin- 4(3H)-ones. Biol. Pharm. Bull., 2005; 28(6): 1091-1094.

[2] Galanopoulou.O.,Rozou.S..E.A.HPLC analysis,isolation,and Identification of a new degradation product in carvediol tabalets,Journal of pharmaceutical and Biomedical Analysis,48(2008)70-77.

[3] Tamaro.T.Aprile.S.Glovenzana.,G.Grosa,Development and validation of a stability-indicating HPLC-CV method for the determination of alizapride and its degradation products.Journal of Pharmaceuticale and Biomedical Analysis 51(2010)1024-1031.

[4] Dick.L.,Dooley.N.Elliort.,M.A.,Ford.S.J.,Gordon.M.R .,Halbert.G.W.et al and related impurities:HPLC analysis,stability profile and degradation pathways,Journal of pharmaceutical and Biomedical Analysis 56(2011)633-636.

[5] Kasawar.G.B.Farooqui.M. Development and validation of stability indicating RP-HPLC method for the simultaneous determinationof related substances of albuterol

[6] Kant P. Synthesis anti-microbial activities of some new 2- substituted 3(1'-aryl-4'-nitrophenyl imidazol-5'-yl) amino quinazolin-4-ones. Indian J. Heterocycl. Chem., 2006; 15: 221- 224.

[7] El-Hiti GA, Abdel-Megeed MF Zied TMM. Synthesis reaction of some 3-aryl 2-thioxo quinazolin-4(3H)-ones. Indian J. Chem., 2002; 41B: 1519-1522.

[8] Alagarsamy V, Thangathirupathi A, Mal SC, Rajasekaran S, Vijayakumar S, Revathi R, Anburaj J, Arunkumar S Rajesh S. Pharmacological evaluation of 2-substituted (1,3,4)-thiadiazolo quinazolines. Indian J. Pharm. Sci., 2006; 68(1): 108-111.

[9] Ny P, Vishalakshi MT Bhat AR. Synthesis antitubercular activity of mannich bases of 2-methyl 3Hquinazolin- 4-ones. Indian J. Heterocycl. Chem., 2006; 15: 293- 294.

[10] Mahajan NS, Pattan SR, Jadhav RL, Pimpodkar NV Manikrao AM. Synthesis of some thiazole compounds of biological interest containing mercapto group. Int. J. Chem. Sci., 2008; 6(2): 800-806.

[11] Srivastava SD Sen JP. Synthesis of biological evaluation of 2-aminibenzothiazole derivatives. Indian J. Chem., 2008; 47B:1583-1586.

[12] Chowki AA, Magdum CS, Ladda PL Mohite SK. Synthesis antitubercular activity of 6-nitro-2-[4-formyl-3- (substituted phenyl) pyrazol-1-yl] benzothiazoles. Int. J. Chem. Sci., 2008; 6(3): 1600-1605.

[13] Souren A, Jack M Gustav JM. Synthesis microbiological properties of (2-benzothienyl) aminopropionic acid. J. Am. Chem., 1948; 70(9): 3075-3076.

[14] Kristina S, Marijeta K, Ivo P, Lidija S, Kresimir P Grace KZ. Synthesis, photochemical synthesis, DNA binding antitumor evaluation of novel cyano-amidino-substituted derivatives of naphtha-furans, naptho-thiophenes, thienobenzofurans, benzo-thiophenes their acyclic precursors. Eur. J. Med. Chem., 2006; 41: 925-939.

[15] Krzysztof K, Yijun Z, Damon P, Jeffrey D, Peter PR Vinay KP. New HIV-1 reverse transcriptase inhibitors based on a tricyclic benzothiophene scaffold: Synthesis, resolution, inhibitory activity. Bioorganic Med. Chem., 2006; 16: 3034-3038.

[16] Finkel T Holbrook NJ. Oxidants, oxidative stress the biology of ageing. Nature, 2000; 408: 239–247.

[17] Devasagayam TPA, Tilak JC, Boloor KK, Sane KS, Ghaskadbi SS Lele RD. Free radicals antioxidants

in human health: current status future prospects. J.Assoc.Phys. India. 2004; 52: 794–804.

[18] Miller AL. Antioxidant flavonoids: Structure, function clinical usage. Alt. Med.Rev., 1996; 1: 103–111.

[19] Rajasekaran S, GopalKrishna R, Sanjay Pai PN Gurpreet Singh S. Synthesis, Antibacterial invitro Antioxidant Activity2,3-Substituted Quinazolin-4(3H)-ones. J. Chem. Pharm. Res., 2010; 2(1): 482-488.

[20] Geronikaki A, Hadjipavlou-Litina D, Chatziopoulos C Soloupis G. Synthesis Biological Evaluation of New 4,5- Disubstituted-Thiazolyl Amides, Derivatives of 4-Hydroxy- Piperidine or of 4-N-Methyl Piperazine. Molecules, 2003; 8: 472-479.

[21] Shewiy, D. H, Kaale, E,Risha,P. Dejaegher, G. B. Verbeke, J. S. Heyden, Y. V. Pharmaceut. J. Biomed. Anal 2012, 66, 11–23.

[22] Rockville, M. D. General Tests, Chapter 621 – Chromatography System Suitability, United States Pharmacopeial Convention (USP), USP 31 (2009)

[23] Julia T, Mena AJ, Aucoin MG, Kamen AA. Development validation of a HPLC method for the quantification of baculovirus particles. J Chromatogr B 2011; 879: 61–68.

[24] Khan MC, Reddy NK, Ravindra G, Reddy KVSRK, Dubey PK. Development validation of a stability indicating HPLC method for simultaneous determination of four novel fluoroquinolone dimers as potential antibacterial agents. J Pharmaceut Biomed Anal 2012; 59:162–16., detect, quantify the various drug-related that can form on storage or ma

[25] Blanchet B, Sabourea C, Benichou AS, Billemont B, Taieb SR, Alain Dauphin. Development validation of an HPLC-Uvvisible method for sunitinib quantification in human plasma. Clin Chim Acta2009; 404:134–139.a. www.agilent.com/chem/store (Accessed on 18/5/2013)e APIs that could form dur

[26] ISO/IEC (2007). Guide 99: International vocabulary of metrology – Basic general concepts associated terms (VIM). First edition, Geneva: International Organization for Stardizationods shouldent FDA ICH guidance recommends inclusion of the results, including chromatograms of stressed 8

[27] Fukutsu, N., Kawasaki, T., Saito, K., Nakazawa, H. 2006. Application of high-performance liquid chromatography hyphenated techniques for identification of degradation products of cefpodoxime proxetil. *Journal of Chromatography A,* 1129:153-159 to be included in the filing. The experimental protocol for performing forced degradation studies will depend on the active Skoug, J. W. 1996. Strategy for development validation of dissolution test for solid Dosage Forms. *Pharm Tech*, 20, 5: 58-60

TABLES

Table:-1
Method optimization

Parameter	Optimum condition
Chromatographic column	SHIMADZU-HPLC, phenomenex C_{18}, 250×4.6mm, 5µ
Mobile phase	Water : Methanol(30:70)
Flow rate	1ml/min
Detection	238nm
Injection volume	20 µl
Temperature	Ambient
Retention time Thiazole Darivative	10.133 min

Table – 2
Accuracy developed method

Compound	***Spiked Concentration mg/ml***	***Measured Concentration mg/ml***	***% Deviation***
Thiazole	*0.020 mg/ml*	*0.0205 mg/ml*	*20.56 %*
Thiazole	*0.025mg/ml*	*0.0249 mg/ml*	*24.99 %*
Thiazole	*0.040mg/ml*	*0.0239 mg/ml*	*39.37 %*

Table:-3

Linearity result, limit of detection (LOD) and limit of quantification (LOQ)

Compound	Value	Equation	R2	LOQ	LOD
Thiazole	238nm	Y=56.54x+2.5853	0.9989617	0.00217	0.6706

Table:-4

The recovery studies were carried out six times.

Compound	Spiked Concentration mg/ml	Measured Concentration mg/ml	% Deviation
Thiazole	0.020 mg/ml	0.0205 mg/ml	20.56 %
Thiazole	0.025mg/ml	0.0249 mg/ml	24.99 %
Thiazole	0.040mg/ml	0.0239 mg/ml	39.37 %

Table:- 5

Robustness of method

Time	0.9ml/min	1ml/min	1.1ml/min
Mean	10.120	10.130	10.140
S.D	0.018	0.006	0.017
%RSD	0.0592	0.197	0.0552

Table:- 6

Day to day variability according to area

Date	4 April	5 April	6 April
Compound	Thiazole	Thiazole	Thiazole
Area	78.862	78.853	78.858
S.D.	0.0576	0.0568	0.0570
R.S.D %	0.00731	0.00679	0.00710

Table:-7

Base Hydrolysis

Sr.No	Reaction Condition	RetentionTime	λmax	Peak Area	Concentration	Degradation
4	**RT24hrsBase**	10.480	238	13.758	6.6494	83.37 %

5	**TP-80^{0}Base.**	10.703	238	42.243	9.2500	47.98 %

Table:-8

Acid Hydrolysis

Sr. No.	Reaction Condition	RetentionTime	λmax	Peak Area	Concentration	Degradation
2	**TP-a-RT.24hrsAcid**	10.589	238	19.139	9.2500	76.87 %
3	**TP-a-80^{0}Acid**	10.861	238	30.530	0.0147	63.25 %

Table:-9

Oxidative Degradation

Sr.No.	*Reaction Condition*	*Retention Time*	*λmax*	*Peak Area*	*Concentration*	*Degradation*
6	***TP-3% H_2 O_2 80^0***	*10.316*	*238*	*13.758*	*0.0350*	*12.5 %*
7	***TP-5% H_2 O_2 80^0***	*10.555*	*238*	*16.625*	*8.518*	*78.75 %*

Table:- N0.10

Quantification of Thiazole Derivative Under Various Stress Conditions

Sr. No.	Reaction Condition	% Recovery	% Degradation
1	**Acid Hydrolysis**		
	RT-24hrs	23.124	76.87
	Hyd-80^0	36.75	63.25
2	**Base Hydrolysis**		
	RT-24hrs	16.623	83.37
	Hyd-80^0	51.02	47.98
3	**Peroxide Oxidation**		
	3% H_2 O_2	87.5	12.5
	5 % H_2 O_2	21.25	78.75

Figure:-1

Standard Chromatogram

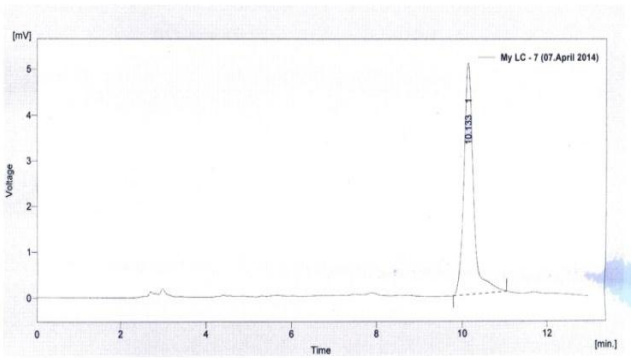
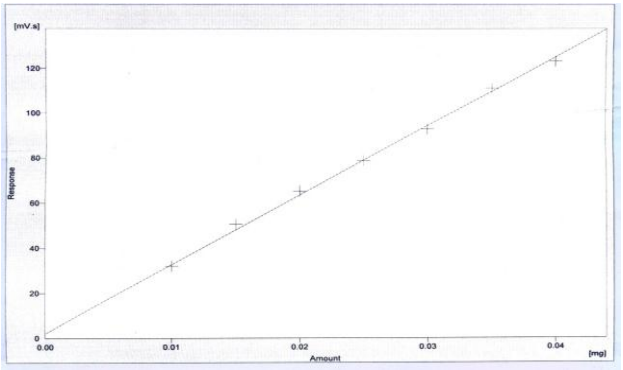


Figure:-2

Standard Calibration Curve


[mV.s]
120
100
80
60
40
20
0
Response
0.00
0.01
0.02
0.03
0.04
Amount
[mg]