Est. 1984

ORIENTAL JOURNAL OF CHEMISTRY
An International Open Free Access, Peer Reviewed Research Journal

www.orientjchem.org

ISSN: 0970-020 X
CODEN: OJCHEG
2017, Vol. 33, No. (5):
Pg. 2608-2615

Estimation of Related Substances in Tigecycline by rp-hplc Method

BAVIREDDI MOHAN[1], R. S. K. SHARMA[1], S. V. MURALI MOHAN RAO[1]* and N. V. S. VENU GOPAL[1]*

[1]Department of Chemistry, GITAMUniversity, Gandhi Nag[illegible] Rush[illegible]onda, Visakhapatnam-530045, Andhra Prade[illegible]dia.
*lCorresponding author E-mail: bavirddim[illegible]@[illegible]mail.com

http://dx.doi.org/10.13005/oj[illegible]0558

(Received: June 14, 2017; Ac[illegible]epted: July 18, 2017)

A[illegible]ST[illegible]CT

Estimation of related substanc[illegible]y usin[illegible]-performance liquid chromatographic method was developed and validated f[illegible]the d[illegible]mination of Tigecycline in the present work. Reversed-phase chromatography was per[illegible]hed o[illegible]aters 2489 UV 2695 pump, Waters 2998 PDA 2695 pump Software Empower[2] photodi[illegible]array detector using Zorbax Eclipse plus C18 (100 mm × 4.6 mm, 1.8 µm particle siz[illegible]mn w[illegible]eluent-A: pH 6.50 buffer: acetonitrile: DMSO (90:5:5 %v/v/v) and eluent-B: pH 6.5[illegible]buff[illegible]: acetonitrile: DMSO (71:24:5 %v/v/v) as mobile phase at a flow rate of 1.0 mL/m[illegible]n UV detect[illegible]n at 270 nm.Linearity was observed in the concentration range of Tigecycline LOQ–[illegible]13% ([illegible]= 1.000), the concentration range of di-MA-TIG impurity 0.04–0.23% (R2 =[illegible]000) [illegible]oncentration range of CMI 0.05-0.23% (R2 = 0.999). The limit of quantitation (LOQ) and lim[illegible]detection (LOD) were found to be di-MA-TIG impurity 0.0001 and 0.0004mg/mL, CMI impurity [illegible]001and 0.0004µg/mL, Tigecycline 0.0001and 0.0005mg/mL respectively. The method was validate[illegible]as per ICH guidelines. The %RSD precision was found to be less than 1.0 %. The percentage recovery was in good agreement with the labeled amount in the pharmaceutical formulations and the method is simple, specific, precise and accurate for the determination of Tigecycline in pharmaceutical formulations.

Keywords: Tigecycline, Estimation of related substances, validation and reverse phase-liquid chromatography,

INTRODUCTION

Tigecycline [TIG] is (4S,4aS,5aR,12aS)-9-[2-(tert-butylamino)acetamido]-4,7-bis (dimethylamino)-1,4,4a, 5, 5a, 6, 11, 12 a-octahydro 3,10,12,12atetrahydroxy-1,11-dioxo-2-naphthacenecarboxamide. Tigecycline is a new glycylcycline with an expanded broad spectrum antibiotic, including inhibition of Gram positive, anaerobic and antibiotic resistant organisms. Studies have demonstrated that Tigecycline is superior to the treatment of complicated skin infections as well as complicated intra-abdominal infections. Tigecycline is only available as an intravenous injection[1].

There are only limited reports regarding determination of Tigecycline in pharmaceutical

dosage forms and biological fluids such as spectrophotometric[2-4] and HPLC methods[5-7] to determine Tigecycline in pharmaceutical dosage forms. The assay of Tigecycline in the human bone is also reported by LC-MS method[8].

Tigecycline is not official in any pharmacopoeia and there is no monograph containing methods to characterize or quantify Tigecycline. Such methods could offer official parameters to guarantee the validity of the assay. Hence, there is a need for simple, rapid and reproducible method for the routine analysis of Tigecycline in pharmaceutical dosage forms.There is not even a single method estimation of impurities in TIG by using RP-liquid chromatographic method in pharmaceutical dosage forms. In the present work a simple estimation of impurities in TIG reverse phase liquid chromatographic method has been developed for the determination of TIG and validated as per ICH guidelines[9-11].

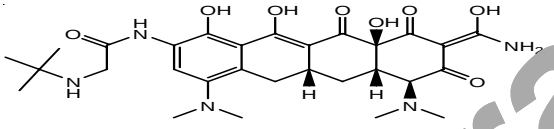

Fig.1.1 Chemical Structure of Tigecycline (TIG).

Related substance structures:

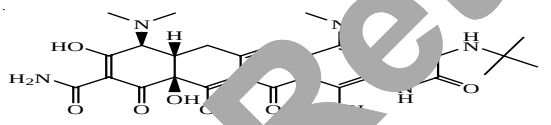

Fig. 1.2 Chemical structure of TIG-6-ene.

(i) TIG-6-ene=(4S,4aS,5aR,12aS)-9-(2-(tert-butylamino)acetamido)-4,7-bis(dimethylamino)-1,4,4a,5,11,12a-hexahydro-3,10,12,12a-tetrahydroxy-1,11-dioxo-2-naphthacenecarboxamide.

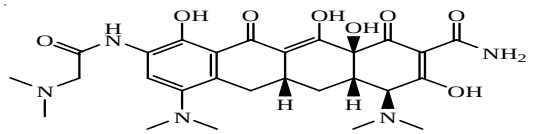

Fig. 1.3 Chemical Structure of di-MA-TIG.

(ii) di-MA-TIG=(4S,4aS,5aR,12aS)-9-(2-(dimethylamino)acetamido-4,7-bis(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,10,12,12a-tetrahydroxy-1,11-dioxo-2-naphthacenecarboxamide.

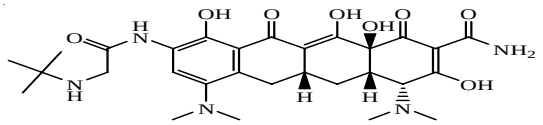

Fig. 1.4 Chemical Structure of epi-TIG.

(iii) epi-TIG=(4R,4aS,5aR,12aS)-9-(2-(tert-butylamino)acetamido)-4,7-bis(dimethylamino)-,4,4a,5,5a,6,11,12a-octahydro-3,10,12,12a-tetrahydrox-1,11-dioxo-2-aphthacenecarboxamide.

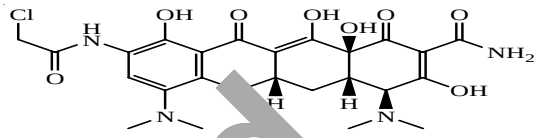

Fig..1.5 Chemical Structure of CMI.

(iv) CMI=(4S,4aS,5aR,12aS)-9-chloroacetamido-4,7-bis(dimethylamino)-1,4,4a,5,5a,6,11,12aoctahydro-3,10,12,12a-tetrahydroxy-1,11-dioxo-2-naphthacenecarboxamide.

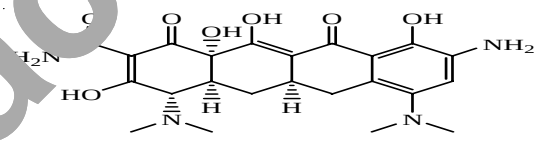

Fig. 1.6 Chemical Structure of AMC.

(v) **AMC=**(4S,4aS,5aR,12aS)-9-amino-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide.

EXPERIMANTAL

Reagents and Chemicals

Ammonium acetate, Acetonitrile (HPLC grade), Dimethyl sulfoxide (DMSO) and Ethylenediamine tetra acetic acid disodium dihydrate (EDTA $Na_2.2H_2O$) were obtained from Merck (India). All chemicals were of an analytical grade and used as received.

Preparation of pH 6.50 buffer

1.54 g of Ammonium acetate, 3.7224 g of Ethylenediaminetetraacetic acid, disodium dihydrate in 1000mL HPLC grade water sonicated to dissolve then adjusted pH 6.50 with 25% aqueous ammonia solution. Filtered through 0.45 µmembrane filter paper and degassed.

Preparation of buffer

1.54g of Ammonium acetate, 3.72g of Ethylenediaminetetraacetic acid, disodium dihydrate and 0.66gms of Sodium sulfitein 1000ml HPLC grade water sonicated to dissolve then adjusted top H 6.50 with 25% aqueous ammonia solution. Filtered through 0.45µmembrane filter paper and degassed. Transferred above buffer, acetonitrile and of dimethyl sulfoxide in the ratio of (90:5:5 %v/v/v).Filtered through 0.45µmembrane filter paper and degassed.

Chromatographic conditions

Chromatographic separation was achieved by using a Waters 2489 UV 2695 pump, Waters 2998 PDA 2695 pump Software Empower^2 photodiode array detector using Zorbax Eclipse plus C18 (100 mm×4.6 mm, 1.8µm particle size) column with eluent-A: pH 6.50 buffer: acetonitrile:DMSO (90:5:5 %v/v/v) and eluent-B: pH 6.50 buffer: acetonitrile: DMSO (71:24:5 %v/v/v) as mobile phase at a flow rate of 1.0 mL/min. with UV detection at 270 nm. Column maintained at temperature 30 ºC, sample temperature 2-5ºC.Th overall run time was 26 min. and the flow rate w s 1.0 mL/min. 10µl of sample was injected in the HPLC system. Retention times of im urities were 13.50 min for di-MA-TIG impurity, 16.7 in for MI and 14.35min for Tigecycline.

Method validation

System suitability

The system suit s performed by analyzing the referen solution three times. Calculate % RSD for rep te injections of each component from reference solution.Preparations of Tigecycline, di-MA-TIG and CMI standard at concentrations:28.500×10^{-3}mg/ml(0.7%), 5.0516×0^{-3} mg /ml 0.15% and 5.0516×10^{-3} mg /ml 0.15% of the nominal concentration of sample required by the method were analyzed in triplicate for each solution according to the method. The details of summary of system suitability from reference solution were incorporated in the Table 1.1.

Table 1.1: Summary of system suitability from reference solution.

Injection No	Tigecycline	di-MA-TIG	CMI
1	484950	78622	110620
2	486317	78114	109323
3	485113	78903	109471
Mean	485460	78546	109805
%RSD	0.2	0.5	0.6

Specificity

Solutions of TIG-6-ene impurity, di-MA-TIG impurity, epi-TIG impurity, CMI impurity, AMC impurity and Tigecycline each were prepared and analysed individually. A spiked solution of each potential impurity to the Tigecycline drug substance was preapred and analyzed. Performed the analysis using PDA detector and the peak purity was determined.Th study showed that all the known impurities of cycline are adequately resolved and the details of retention time and relative retention time for known impurities were presented in the Table 1.2. Therefore the method is selective for the determination of related substances in Tigecycline.

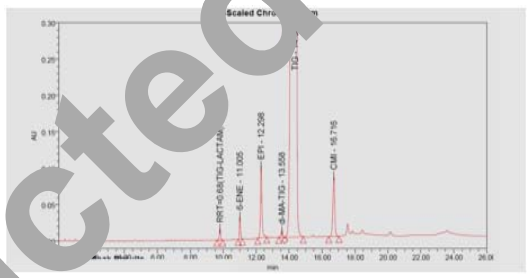

Fig 1.7. Specificity chromatogram of Spiked Solution.

Table 1.2: Summary of retention time, and relative retention time for known impurities

Peak Name	Retention Time	Relative retention time(RRT)
TIG-Lactam	9.804	0.68
TIG-6-ene	11.005	0.77
Epi-TIG	12.298	0.85
di-MA-TIG	13.558	0.94
Tigecycline	14.366	1.00
CMI	16.716	1.16

Limit of Detection

A solution containing 0.8152×10^{-3} mg/ml of Tigecycline standard (0.02% of the nominal concentration of a sample), 0.8152×10^{-3} mg/ml of di-MA-TIG standard (0.02% of the nominal

concentration of a sample) and 0.8368×10^{-3} mg / ml of CMI standard (0.02% of the nominal concentration of a sample), was injected three times. The worst found signal to noise ratio for each peak was greater than 3 in each injection. All the peaks were detected in all the three injections. Results of LOD for Tigecycline and impurities were shown in Table 1.3. The limit of detection values obtained for each impurity and Tigecycline are within the acceptance criteria.

Table: 1.3 Limit of detection (LOD) for Tigecycline and impurities.

S.No	Tigecycline		di-MA-TIG		CMI	
	Area	conc.mg/ml	Area	conc.mg/ml	Area	conc.mg/ml
1	10448	0.00015664	7908	0.000126917	10977	0.00014494
2	10629	0.00015930	7967	0.000127856	10965	0.00014479
3	10436	0.00015647	8045	0.000129114	11018	0.00014542
Mean	10504		7973		10986	
%RSD	1.0		0.9		0.	

Linearty and Range

The linearity is determined by injecting the solutions in duplicate containing known impurities and Tigecycline ranging from 0.05 to 1.13% and impurities ranging om 05% to 0.22% of the specified limit. Regr sion nalysis was performed and the correl coe ent and residual sum of squares wer de ined. The response factor for each urit it respect to Tigecycline was determin Linearity range as the range for de ining e impurities were reported. Results o ained are incorporated in Table1.5- Table 1.7

Table: 1.4 Limit of Quantitati r Tig cycline and impurities.

S.No	Tigecycline			di-MA-TIG			CMI		
	Area	conc. mg/mL	%Accuracy	Area	conc. mg/mL	%Accuracy	Area	conc. mg/mL	%Accuracy
1	34479	0.00051693	101 7	2	0.000418825	99.59	36225	0.0004783	104.25
2	35075	0.00052568	1 3.4	26290	0.000421925	100.32	36183	0.0004778	104.14
3	34440	0.0005 5	1 6	26549	0.000426075	101.31	36358	0.0004799	104.6
4	34045	0.00 105	100	26208	0.0004206	100.01	35953	0.00047508	103.55
5	34432	0. 516	101.63	26251	0.0004213	100.18	35746	0.0004726	103.01
6	34849	0.00 35	102.84	26281	0.000421775	100.29	35582	0.00047063	102.58
Mean	34553			26279			36008		
%RSD	1.0			0.6			0.8		

Limit of Quantitation

Asolutioncontaining2.0317×10^{-3}mg/ml of Tigecyclinestandard (0.05% of the nominal concentrationofasample),1.6822×10^{-3}mg/ml of di-MA-TIG standard(0.04% of the nominal concentrationofasample) and 1.8352×10^{-3}mg/mlofCMI standard (0.05%of the nominal concentrationofasample),wasinjectedsixtimes. The RSD of areas, deviation so feach six replicates from the line arregression curveandaverage deviation for each standard were calculated. Theresults of LOQ for Tigecycline and its impurities were presentedinTable 1.4. The limit of quantitation values obtained for each impurity and Tigecycline are within the acceptance criteria.

Table 1.5: Linearity of Tigecycline.

% of Tigecycline	Concentration (mg/mL)	Average Peak Area
0.05	0.00051	34479
0.10	0.00102	67287
0.15	0.00152	103231
0.20	0.00204	138278
0.25	0.00256	172143
0.50	0.00512	348385
0.70	0.00713	485950
1.13	0.01126	764512

Table 1.6. Linearty of di-MA-TIG.

% di-MA-TIG	Concentration (mg/ml)	AveragePeak Area
0.04	0.000421	26097
0.08	0.000842	53002
0.13	0.001263	78622
0.17	0.001682	104520
0.22	0.002220	138911

Table 1.7: Linearity of CMI.

% of CMI	Concentration (mg/mL)	AveragePeak Area
0.05	0.00046	36225
0.09	0.00091	70502
0.14	0.00136	110620
0.18	0.00184	148152
0.24	0.00238	197098

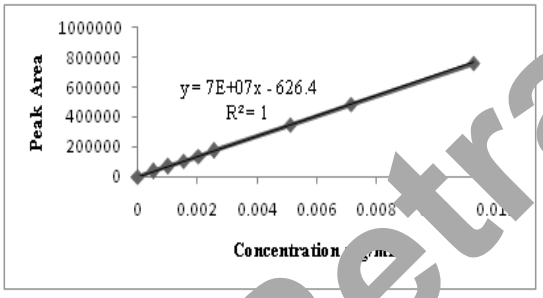

Fig 1.8. Linearity graph of Tigecycline.

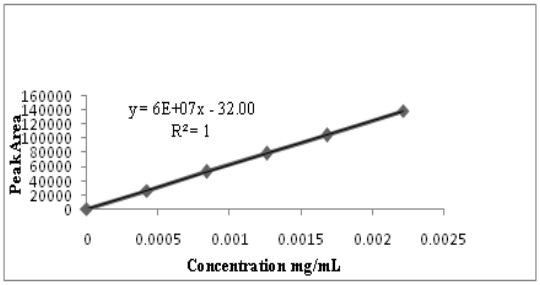

Fig 1.10. Linearity graph of CMI.

and figures 1.8-1.10 and showed the line of best fit for peak area versus concentration for each impurity. The linearity results for Tigecycline and all the impurities in the specified concentration range are found satisfactory, with correlation coefficient greater than 0.99.

Accuracy

Tigecycline solution spiked with a known amount of each impurity at five levels each in triplicate (in total 15 determinations) was prepared and analyzed as per the method.Summary of % recoveries for Tigecycline and impurities such as di-MA-TIG and CMI were presented in the tables from Table 1.8-1.10.The percentage recovery values obtained for each impurity are in the range of about 99.6-104.3, which are within the specified criteria. The relative standard deviation values of recoveries obtained for all impurities are in the range of 0.04%-0.23.

Table 1.8: Summary of % recoveries for Tigecycline.

% of Tigecycline	Theoretical conc. (mg/mL.)	Measured conc.(mg/mL)	% Recovery	Avg.
0.05	0.0020317	0.0020677	101.77	102.3
	0.0020317	0.0021027	103.49	
	0.0020317	0.0020654	101.66	
0.10	0.0040714	0.0039961	98.15	99.0
	0.0040714	0.0040461	99.38	
	0.0040714	0.0040476	99.41	
0.70	0.0285000	0.028605	100.37	100.30
	0.0285000	0.028627	100.44	
	0.0285000	0.028556	100.20	
1.12	0.0450480	0.044979	99.85	100.0
	0.0450480	0.045024	99.95	
	0.0450480	0.045099	100.11	

Precision

System precision

The analysis of reference solution six times was performed and determined the percentage relative standard deviation of peak area of replicate injections of each impurity and Tigecycline. Datails of results were mentioned in the Table 1.11.

Method precision

The precision of the method is determined by analyzing a sample of Tigecycline solution spiked with impurities at 100% of the specification limit. The precision of the method for Tigecycline and its impurities were shown in the Table 1.12. The relative standard deviation observed for Tigecycline and

Table 1.9: Summary of % recoveries for di-MA-TIG.

% of di-MA-TIG	Theoretical conc. (mg/mL.)	Measured conc.(mg/mL)	% Recovery	Avg.
0.04	0.0016822	0.0016753	99.59	100.4
	0.0016822	0.0016877	100.32	
	0.0016822	0.0017043	101.31	
0.12	0.0050516	0.0050387	99.74	99.6
	0.0050516	0.0050062	99.10	
	0.0050516	0.0050567	100.10	
0.22	0.0088780	0.0088899	100.23	100.1
	0.0088780	0.0088848	100.08	
	0.0088780	0.0088690	99.89	

Table 1.10: Summary of % recoveries for CMI

% of CMI	Theoretical	Measured conc.(mg/mL)	%Recovery	Avg.
0.04	0.0018352	0.0019132	104.25	104.30
	0.0018352	0.0019112	104.14	
	0.0018352	0.0019196	104.60	
0.13	0.0054521	0.0054682	100.29	99.60
	0.0054521	0.0054062	99.16	
	0.0054521	0.0054133	99.29	
0.23	0.0095319	0.0096005	100.72	100.70
	0.0095319	0.0096142	100.86	
	0.0095319	0.0095904	100.61	

Table 1.11: Summary of peak areas of the Tigecycline and its impurities.

Injection No	Tigecycline	di-MA-TIG	CMI
1	484950	78622	110620
2	486317	78114	109323
3	485113	78903	109471
Mean area	485460	78546	109805
%RSD	0.2	0.5	0.6

Table 1.12: Summary of results for precision of the method.

Inj. No	% of di-MA-TIG Impurity	% of CMI	Unknown1 (RRT0.86)	Unknown2 (RRT1.37)
1	0.103	ND	0.041	0.042
2	0.105	ND	0.044	0.042
3	0.105	ND	0.042	0.042
4	0.103	ND	0.044	0.042
5	0.103	ND	0.042	0.042
6	0.103	ND	0.046	0.041
Mean (%)	0.10	N/A	0.04	0.04
% RSD	0.09	N/A	0.0	0.0

impurities are less than 10%.The results comply with the acceptance criteria and indicate acceptable precision of the system.

RESULTS AND DISCUSSION

A simple, economic, accurate and precise HPLC method was successfully developed by using Zorbax Eclipse plus C18 (100 mm × 4.6 mm, 1.8 µm particlesize). Injection volume of 10µl is injected and eluted with the mobile phase eluent-A: pH 6.50 buffer: acetonitrile: DMSO (90:5:5 %v/v/v) and eluent-B: pH 6.50 buffer: acetonitrile: DMSO (71:24:5 %v/v/v), which is pumped at a flow rate of 1.0 ml/min. Detection, was carried out at 270 nm. The results obtained were accurate and reproducible.The method developed was statistically validated in terms of Selectivity, accuracy, linearity, precision, robustness, and stability of solution.

For Selectivity, the chromatograms were recorded for standard and sample solutions of Tigecycline and its related substances. Selectivit studies reveal that the peak is well separated fr h each other. Therefore the method is selecti e for the determination of related su stan s in Tigecycline. The limit of detection (LO nd lim of quantitation (LOQ) for di-MA-TIG impurit .0001 and 0.0004mg/ml, CMI impurity .0001 and 0.0004µg/ml, Tigecyclin .000 nd .0005 mg/ml respectively. Usi the optimized chromatographic co ic tention times of impurities were 13.50 fo -MA-TIG impurity, 16.75 for CMI, and 14.35 for Ti cycline. The linearity results for Tigecycline and all the impurities in the specified concentration range are found satisfactory, with a correlation coefficient greater than 0.99.Calibration curve was plotted and correlation co-efficient for Tigecycline and its impurities found to be 1.000, 1.000, and 0.9999 respectively.

The accuracy studies were shown as % recovery for Tigecycline and its impurities at specification level . The limit of % recovered shown is in the range of 90 and 110% and the results obtained were found to be within the limits. Hence the method was found to be accurate. The accuracy studies showed % recovery of the Tigecycline and its related substances in the range 99.6 to104.30 respectively.For Precision studies six replicate injections were perfo ed. % RSD was determined from the peak areas of cycline and its impurities. The acceptance lim shou be not more than 10, and the results w e fo nd to be within the acceptance li its.

NCLUSIONS

A simple and precise RP-HPLC method h been eveloped by the author for the estimation f re d impurities present in the Tigecycline and as observed that the chromatographic method eveloped for Tigecycline and its related substances are rapid, sensitive, precise, and accurate. Therefore, the proposed method can be successfully applied for the routine analysis of the active pharmaceutical ingredients for assurance of its quality during its formulation.

ACKNOWLEDGEMENTS

The author was thankful to the Head, Department of Chemistry and the authorities of Gitam University, Vishakhapatnam for providing necessary scientific support and help.

REFERENCES

1. Lucelia, M.S.;Herida,R.N.S.Tigecycline: A review of properties application and analytical methods, *Ther. drug monitoring*, **2010**, *32(3)*,282-287.
2. Aparna,B.C.;Dipti,B.P.AUC spectrophotometric method for the determination of Tigecycline in pharmaceutical formulations, *J. of Pharm. Sci. and Bio scientific Res.*, **2012**,*2(2),* 88-91.
3. Silva, L.M.; Salgado, H.R.N.Development and validation of spectrophotometric method for the assay of Tigecycline in lyophilized powder, *International congress of Pharmaceutical sciences*, CIFARP-2011, **2011**.
4. Lucelia,M.S.;Adelia,E.A.;Herida,R.N.S.

Thermal analysis and validation of UV spectrophotometric methods for the determination of new antibiotic Tigecycline in pharmaceutical products, *Adv. in analytical.chem*, **2012**, *2(1)*, 10-15.

5. Srikanth,K.;Reddy,D.R.;Venkatesan,C.S. Development and validation of RP-HPLC pre-column derivatisation for the trace level determinatrion of ter-butyl amine in Tigecycline drug substance,*Int. j. of pharm. and Biological sci*, **2013**,*4(2)*, 522-531.
6. Chonghuva, L.;Christina, A.S. Quantification of Tigecycline: A novel glycylcycline by liquid chromatography,*J. chromatography. B*, **2004**, *811*,225-229.
7. Allena, J.J.; James, P.S.A sensitive human bone assay for the quatification of Tigecycline using LC/MS/MS,*J. of Pharm. and Biomed.Anal*, **2008**,*48*,866-875.
8. Patel, D.; Parikh, K.S. Stability indicating RP-HPLC method development and its validation for the quatification of Tigecycline in lyophilized parental preparation, *Int. j.Chemtech Applications*, **2013**,*2*(1), 77-86.
9. ICH Guidance on analytical method validation, international convention on quality for the pharmaceutical industry, Toronto, Canada, **2002**.
10. ICH Q1B IFPMA, Stability testing: photo-stability testing of new drug substances and products, International Conference on Harmonization, Geneva, Switzerland, **1996**.
11. ICH of technical requirements for the registration of pharmaceutical for the human use, validation of analytical procedures: text and methodology, ICH, Q2 (R1), **2005**.