Heterocyclic Letters
Vol. 10| No.2|221-229|Feb–April | 2020
ISSN : (print) 2231–3087 / (online) 2230-9632
CODEN: HLEEAI
http://heteroletters.org

DEVELOPMENT AND VALIDATION OF HPTLC METHOD FOR ESTIMATION OF GLIMEPIRIDE IN ACTIVE PHARMACEUTICAL INGREDIENT AND PHARMACEUTICAL FORMULATION

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Abstract:
The aim of the existing work was to develop a economical, simple, precise and accurate High performance thin layer chromatography (HPTLC) method for the estimation of Glimepiride in the single dosage tablet formulations and Active pharmaceutical ingredient. Chromatographic separation of Glimepiride was achieved on TLC aluminum plates pre-coated with silica gel 60 F254 using mobile phase as Acetone: Toluene (8:1 v/v). The detection of Glimepiride was completed at absorbance mode at 231 nm using Camag TLC Scanner. Glimepiride demonstrated Rf value at 0.73. The Method was validated in term of linearity (500-1750 ng/spot), Precision (% RSD for Repeatability 1.43%, % RSD for intra-day variation 1.66% and inter-day variation 0.60%), Accuracy in term of recovery was getting at three different level was 103.33%, 89.78% and 86.63% and Specificity. The limit of detection and limit of quantification for Glimepiride were found to be 250 ng/spot and 500 ng/spot correspondingly. It is concluded from the results that the estimated High performance thin layer chromatography is economical, simple, reproducible, precise and accurate and it is useful in daily analysis in quality control department for estimation of Glimepiride in Active pharmaceutical ingredient and Pharmaceutical dosage form. This method was validated as per ICH guideline Q2 (R1).

Keyword: HPTLC, Glimepiride, Validation, Toluene, Acetone, Estimation.

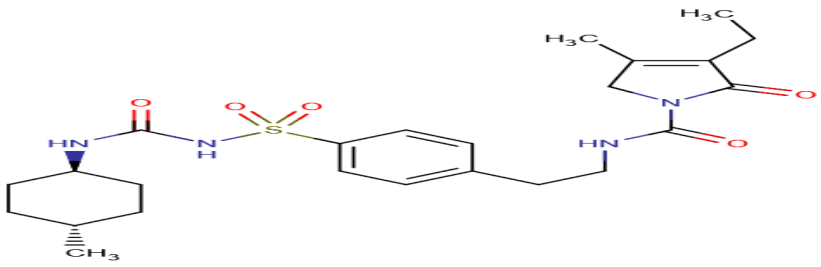
Introduction:

First presented in 1995, glimepiride is an individual from the second-age sulfonylurea (SU) medicate class utilized for the administration of type 2 diabetes mellitus (T2DM) to improve

glycemic controller. Type 2 diabetes is a metabolic issue with expanding prevalences around the world. It is portrayed by insulin obstruction as per dynamic β cell disappointment and long term microvascular and macrovascular inconveniences that lead to co-morbidities and mortalities. Sulfonylureas are one of the insulin secretagogues generally utilized for the administration of type 2 diabetes to bring down blood glucose levels. The fundamental impact of SUs is believed to be active when remaining pancreatic β-cells are available, as they work by animating the arrival of insulin from the pancreatic beta cells and they are likewise thought to apply extra-pancreatic impacts, for example, expanding the insulin-interceded fringe glucose take-up.

Glimepiride works by vivifying the emanation of insulin granules from pancreatic islet beta cells by blocking ATP-delicate potassium channels (KATP channels) and causing depolarization of the beta cells. It is now and again categorised a third-age SU on the grounds that it has bigger substitutions than other second-age SUs. Glimepiride was confirmed by the Food and Drug Administration (FDA) in the US in 1995 for the treatment of T2DM. It is regularly showcased under the brand name Amaryl as oral tablets and is ordinarily controlled once every day.

The IUPAC Name for Glimepiride is 3-ethyl-4-methyl-2-oxo-N-(2-{4-[({[1r,4r)-4-methylcyclohexyl]carbamoyl}amino)sulfonyl]phenyl}ethyl)-2,5-dihydro-1H-pyrrole-1-carboxamide and structure of the Glimepiride was shown in below **Fig.1**



Reviews of literature say to that a few method have been accounted for the estimation of Glimepiride which incorporates in EP and USP by High performance liquid chromatography, UV spectroscopic method, LCMS. In this analysis, an efficient HPTLC method has been developed for estimation of Glimepiride in Active pharmaceutical ingredient and pharmaceutical dosage form.

MATERIALS AND METHODS

1.0 Chemical and Reagent

Glimepiride Working standard was provided by Indoco remedies limited, Mumbai, India and tablets were procured from local market. The High purity grade Acetone and Toluene pureched from Merck India ltd.

2.0 Diluent: Methanol

2.1 Preparation of Standard stock solution:

Weighed accurately and transferred 10.0 mg of Glimepiride Working standard into a 10.0 ml volumetric flask and dissolved in diluent and made up the volume with the diluent. The concentration of the solution is 1000 µg/ml.

Preparation of Standard solution:
Further pipette 1.0 ml of the Standard stock solution in to a 10 ml volumetric flask and made up to the mark with diluent. The concentration of the solution is about 100 µg/ml of Glimepiride.

2.2 Preparation of Sample solution:
Weighed accurately twenty tablets and calculate average weight. These tablets was crushed and powdered in mortar pester.
These tablet powdered and an amount equivalent to 10.0 mg of Glimepiride in to a 10 ml volumetric flask and dissolved in diluent and made up the volume up to the mark with the diluent. This solution was filtered twice, first with 0.45 µm what man filter paper and later through 0.45 µm syringe filter in order to get a clear solution.
Further pipette 1.0 ml above filtered clear solution in to a 10 ml volumetric flask and made up the mark with diluent. Final concentration is 100 µg/ml of Glimepiride.

3.0 Optimization of the HPTLC method:
Initially method development was start using Hexane: Ethyl acetate in the ratio of (5:5) but spot was not observed. Then, Dichloromethane, Toluene, water, methanol, Acetonitrile, Hexane many polar and non-polar solvent was tried in different ratio with additives like Diethyl amine, Trifluroacetic acid, Glacial acetic acid, Formic acid and Ammonia on Pre-coated aluminum based silica gel 60 F254 TLC plates were used as stationary phase using Densitometry wavelength 231 nm, Finally the superior peak with excellent Rf valve was achieved by using Acetone: Toluene in the ratio of (8:1 v/v) as mobile phase with chamber saturation time of 20 minutes and the migration distance of 70 mm

4.0 Validation of Analytical method
The principle of validation of an analytical procedure is to demonstrate whether the procedure is suitable for its intended purpose. The projected method was validated for different parameters such as Specificity, Precision, Accuracy, Linearity & Range, Limit of Detection (LOD) and Limit of Quantitation (LOQ) according to ICH Q2 (R1) guidelines.

The specificity of the method was determined by analyzing standard drug and sample. The spot for drug in sample was confirmed by comparing the Rf with that of standard drug spot. The specificity of the method was also established by peak purity profiling studies by analyzing the spectrum at peak start, middle and at peak end.

The linearity was performed by spotting six different concentration of working standard solutions of Glimepiride. Calibration curve of peak area *v/s* Concentration was plotted and linear regression was performed. Regression equation and correlation coefficient were obtained. The range of solution has been decided according to statistical parameters of generated equation

The Determination of LOD and LOQ by Visual evaluation method.

The precision of the method was established by repeatability, intraday and interday precision studies. The Repeatability and Intermediate precision of the method was checked by analyzing same concentrations of Standard solutions of Glimepiride and relative standard deviation (%RSD) was calculated.

The accuracy of the method in term of recovery studies were conducted by over spotting standard drug solution to pre-analyzed sample solution at three different levels 80%, 100% and 120 %. The areas were noted after development of plate.

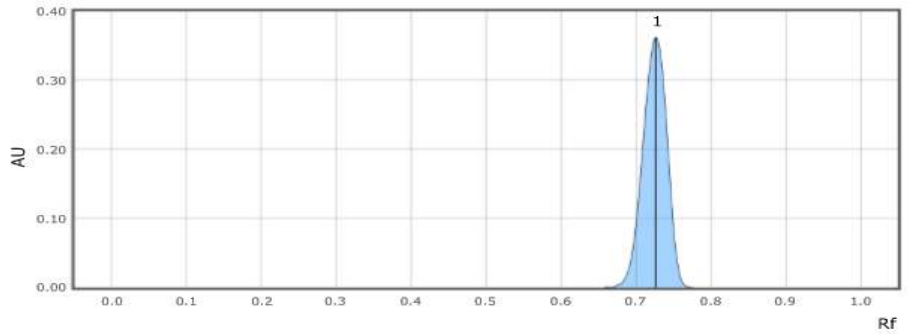
5.0 Results and Discussion:

5.1 Specificity:

Selection of wavelength for Detection

The working standard of Glimepiride in methanol was scanned by Camag TLC scanner 4 with UV visible detector over wavelength range 200 to 400 nm. Wavelength 231 nm was selected for detection of obtained spectrum.

The wavelength selection for Glimepiride was carried out by preparation of working standard solution of Glimepiride and the solution was scanned from 200 to 400 nm by using Camag TLC scanner 4 having UV-Visible detector. The detection wavelength selected for analysis of Glimepiride at 231 nm.



Peak #	Start		Max			End		Area		Manual peak	Substance Name
	Rf	H	Rf	H	%	Rf	H	A	%		
1	0.658	0.0000	0.727	0.3621	100.00	0.772	0.0004	0.01377	100.00	No	

Fig. 2. Densitogram of Working Standard solution of Glimepiride

The specificity of the method was determined by analyzing standard drug and sample. The spot for drug in sample was confirmed by comparing the Rf with that of standard drug spot. The Rf value for Standard drug found 0.73 and for Sample found 0.73

5.2 Linearity and Range:

The linearity of the method was established by spotting working standard solutions between 500-1750 ng/spot. The spectrums of these solutions were recorded and area was noted at wavelength 231 nm. Calibration curve of peak area *v/s* Concentration was plotted after suitable calculation and simple linear regression was performed. The Calibration curve was showing in below Fig. 3 and Linearity and range was reported in Table No.1.

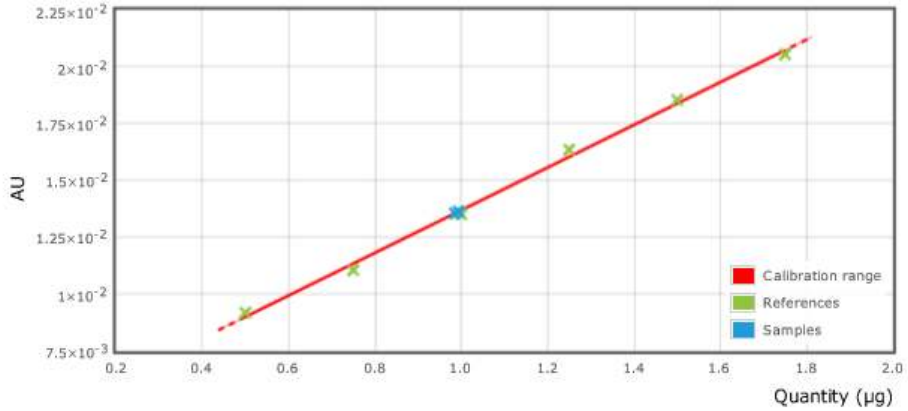


Fig. 3. Calibration curve of Standard solution for Glimepiride

Table No. 1: Result of Linearity and Range for Glimepiride

Track No.	Level	Injection volume (µl)	Concentration of Glimepiride (ng/spot)	Area of Glimepiride
1	50	1.0	500	0.00919
2	75	1.5	750	0.01106
3	100	2.00	1000	0.01352
4	125	2.50	1250	0.01635
5	150	3.00	1500	0.01854
6	175	3.50	1750	0.02054
Correlation Coefficient (r^2)			0.9984	
Slope			0.0093737	
Intercept			0.004321	
Standard deviation			0.6600	
Calibration function (y)			$y=9.377 \times 10^{-9} x + 4.318 \times 10^{-3}$	

5.3 Limit of Detection (LOD) and Limit of Quantitation (LOQ):

The Determination of LOD and LOQ by Visual evaluation method. The obtained results are shown in Table 2

Table 2: Result of LOD and LOQ of Glimepiride

Parameter	Glimepiride
LOD	250 ng/spot
LOQ	500 ng/spot

5.4 Precision:

Repeatability

The Repeatability of the method was proved by analyzing same concentrations of Standard solutions of Glimepiride (800 ng/spot). Area of each peak of these solutions was measured at 231 nm and Relative standard deviation (%RSD) was calculated in Table No.:3

Table 3: Result of Repeatability for Glimepiride

Concentration (ng/spot) (n=7)	**Average Area of Glimepiride**	**% RSD**
800	0.01190	1.43

Intermediate precision;

Intra-day precision:

The intra-day precision of the method was established by analyzing same concentrations of Standard solutions of Glimepiride (800 ng/spot). Area of each peak of these solutions was measured at 231 nm and Relative standard deviation (%RSD) was calculated in Table No.:4

Table 4: Result of Intra-day precision for Glimepiride

Concentration (ng/spot) (n=6)	**Average Area of Glimepiride**	**% RSD**
800	0.01235	1.66

Inter-day precision:
The inter-day precision of the method was established by analyzing same concentrations of Standard solutions of Glimepiride (800 ng/spot). Area of each peak of these solutions was measured at 231 nm and Relative standard deviation (%RSD) was calculated in Table No.:5
Table 5: Result of Inter-day precision for Glimepiride

Concentration (ng/spot) (n=6)	Average Area of Glimepiride	% RSD
800	0.01338	0.60

5.5 Accuracy:
Accuracy of the method was established in term of recovery and recovery studies were carried out by over spotting standard drug solution to pre-analyzed sample solution at three different levels 80%, 100% and 120 %. The concentration of sample was 400 ng/spot. The areas were noted after development of plate. The recovery result tabulated in Table No.: 6.
Table 6: Result of Accuracy (Recovery) for Glimepiride

% Level	Amount taken (ng/spot)	Amount added (ng/spot)	Total amount found (ng/spot)	Average peak area	% Recovery	Average % Recovery
80	400	320	720	0.00646	101.33	92.58
100	400	400	800	0.00668	89.78	
120	400	480	880	0.00713	86.63	

5.6 Analysis of Tablet Formulation
Result for the Tablet formulation reported in Table No.:7
Table 7: Result of Tablet Formulation of Glimepiride

Sr.No.	Labelled Claim	Amount found	Assay
1	1.000 mg/ml	1.031 mg/ml	103.1%

5.7 Conclusion:
The Proposed High performance thin layer chromatography method was economical, simple, reproducible, precise and accurate and it is useful in daily analysis in quality control department for estimation of Glimepiride in Active pharmaceutical ingredient and Pharmaceutical dosage form.

5.8 Acknowledgement:
The authors are thankful to Indoco remedies limited, Mumbai, India for providing API of Glimepiride as gift sample. Authors also thanks to Anchrom Enterprises Pvt. Ltd. Mumbai, India and Indoco remedies limited for required guidance and providing necessary facilities to complete this project.

5.9 References:
i. Mangarao Nakka*,Srinivas Nakka, Bhava Narayana Utpala, International Journal of Innovative Pharmaceutical Sciences and Research, 5,12: 76-87 (2017).
ii. Harrizul Riva[i1]*, Vera Wahyuni Adh[a2] and Fitra Fauzia[h2], Journal of Chemical and Pharmaceutical Research, 8, 3: 841-848 (2016).
iii. Ramesh Guguloth[1], Dr.Madhukar A.[2]*, Habeeba Sulthana[4,] G.Swetha[2],

Dr.C.H.Naveen Kumar, Raju Manda[5], Journal of Scientific Research in Pharmacy, 5, 5: 53-57 (2016).

iv. Srinivasa Rao Atla*, Baby Nalanda R., Natraj S.K., Word Journal of Pharmaceutical Sciences, 4(9): 433-441 (2016).

v. Abdul Aziz Ramadan[1*,] Hasna Mandil[2] ,SouadZeino , International Journal of Pharmacy and Pharmaceutical Sciences, 8, 6 (2016).

vi. Kinnari K.Patel, Vaishali V. Karkhanis and Mrs. Shital S. Gajjar , International Journal of Pharmaceutical Sciences and Research,6 (3) (2015): 1222-1229.

vii. Sawant Ramesh. L., Tanpure Kallyani D., Jadhav Kalyani*A , Indian Journal of Drugs,3, (4) (2015).

viii. Srikanth. Kallagunta* and Dr. SK.Abdul Rahaman, World Journal of Pharmaceutical Research, 4, 12: 881-891 (2015).

ix. Pragya Nand Badyala, Chetan Sharmaa, Ravi Shankarb , Ravindra K. Rawala, Asian Journal of Biomedical and Pharmaceutical Sciences, 5 (47): 23-29 (2015).

x. Kadam V.N.*, Yadav P.J, Mohite, S.K, Magdum C.S, International Journal of Pharmacy and Pharmaceutical Research, 1, (2): 10-21 (2014).

xi. Yuni Retnaningtyas, Lestyo Wulandari dangabriella F.Punu, JKTI, 16, 1:11-15 (2014).

xii. S.M. Sandhya*, U.Fathima Beevi, G. Babu, International Journal of Pharmacy, 4(3): 182-188 (2014).

xiii. Devi Ramesh1 and Mohamad Habibuddin2, International Scholarly Research Notices, 754695,8 (2014).

xiv. K. Neelima1*, Y.Rajendra Prasad2, Pharmaceutical Methods, 5,1 (2014).

xv. Abdul Bari Mohd[1], Krisna Sanka[2], Rakesh Gullapelly[2], Prakash V Diwan1 and Nalini Shastri[3*] , Journal of Analytical Science and Technology, 5:27 (2014).

xvi. Vania Maslarska, International Journal of Pharmaceutical Sciences and Research, 5 (8): 3195-3198 (2014).

xvii. Ashwini S. Deshpande*, NileshAhire, Shashikant B. Bagade, and Shirish S. Deshpande, Word Journal of Pharmacy and Pharmaceutical Sciences, 3, 3:1812-1823 (2014).

xviii. C Parthiba[n1]*, M Bhagavan Raj[u2], M Sudhaka[r1] and B Siddarth[a1], International Journal of Pharmacy and Pharmaceutical Sciences, 5,4 (2013).

xix. Seema M.Dhol[e1*], Pramod B. Khedeka[r2], Nikhil D. Amnerka[r1], Journal of the Chilean Chemical Society, 58, 2 (2013).

xx. M. Suchitr[a1*], D. Sunith[a2], C. Parthiba[n1], B.Siddarth[a1] and C. Madhavi, International Research Journal of Pharmacy, 4 (8) (2013).

xxi. [1]V Asha Ranjani, [*2]C.Abigna, [2]D Akhileshkumar, [2]K Prashanthi, [2]M Sindhuja, International Journal of Pharmacy and Analytical Research, 2, 4 (2013).

xxii. Gadapa Nirupa[1], and Upendra M. Tripathi[2], Journal of Chemistry, 726235, 8 (2012).

xxiii. Nahed M El-Enany[1*], Amina A Abdelal[1], Fathalla F Belal[1], Yoshinori l Itoh[2] and Mitsuhiro N Nakamura[2], Chemistry Central Journal, 6:9 (2012).

xxiv. M.S.V.Sakuntala*, S.V.U.M. Prasad, S.Sri Devi, S.Kishore Yadav, K.Srinivas Reddy, Journal of Chemical and Pharmaceutical Research, 4,(1): 154-159 (2012).

xxv. Dhirender Singh[*1], S.C.Dwivedi[1], Ashok Kushnoor[2], International Journal of Biomedical and Advance Research, 02 (09) (2011).

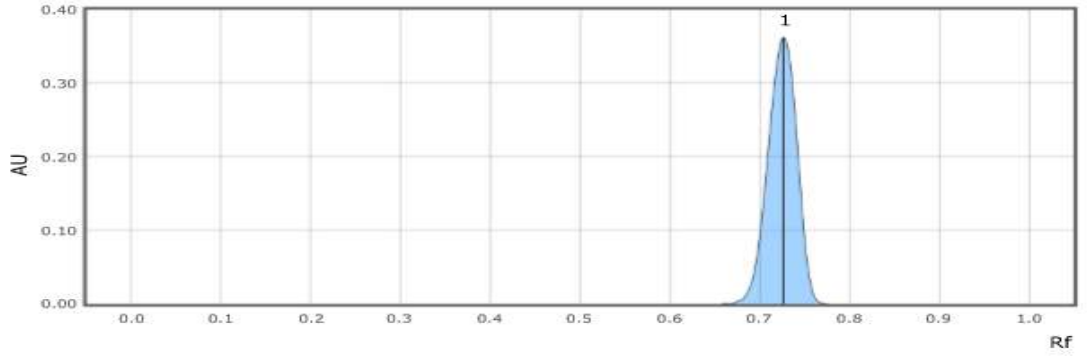
xxvi. Indrajeet Singhvi, Khushboo Mehta and NidhiKapadiya, Journal of Applied Pharmaceutical Science, 01 (06): 159-161 (2011).

xxvii. T.M. Kalyankar*, M.R. Badgujar[1], S.S. Mitkare[1] and R.B. Kakde[2,] Journal of Pharmacy Research, 3 (12): 3118-3120 (2010).

xxviii. Shraddha Pawar[1,2], Gangadhar Meshram[2], Rajendra Jadhav[1] and Yatish Bansal[1], Der Pharma Chemica, 2 (4): 157-168 (2010).
xxix. Y.Padmanabha Reddy, C. Sowmya*, M.Santhosh Raja, K.Raghu Pavan Kumar, B.Suresh and T. Chaithanya, Int. J. Chem.Sci, 7 (3), 1624-1628 (2009).
xxx. D.B.Wanjari* and N.J.Gaikwad, Indian Journal of Pharmaceutical Sciences, (2005).

Received on March11, 2020.

Graphical Files
5.1 Specificity:



Peak #	Start		Max			End		Area		Manual peak	Substance Name
	Rf	H	Rf	H	%	Rf	H	A	%		
1	0.658	0.0000	0.727	0.3621	100.00	0.772	0.0004	0.01377	100.00	No	

Fig. 2. Densitogram of Working Standard solution of Glimepiride

5.2 Linearity and Range:

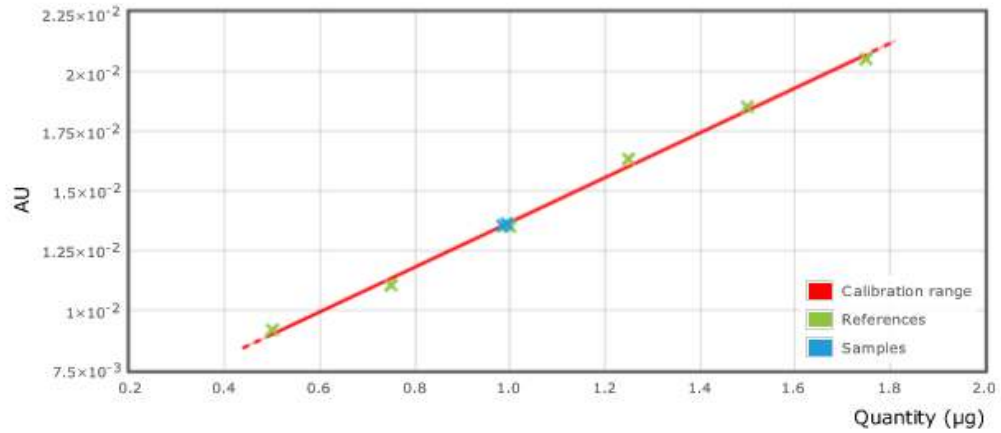


Fig. 3. Calibration curve of Standard solution for Glimepiride