IJMPR 2025, 9(1), 69-75

International Journal of Modern Pharmaceutical Research
www.ijmpronline.com

ISSN: 2319-5878
IJMPR
Research Article
SJIF Impact Factor: 6.669

APPLICATION OF HYDROTROPIC SOLUBILIZATION IN SPECTROPHOTOMETRIC ESTIMATION OF GLIMEPIRIDE TABLETS

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Article Received on: 27/10/2024
Article Revised on: 17/11/2024
Article Accepted on: 07/12/2024

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ABSTRACT

Background: Solubility is the major problem for drugs in pharmaceutical industry. Various organic solvents such as methanol, chloroform, dimethyl formamide and acetonitrile have been employed for solubilization of poorly water-soluble drugs to carry out spectrophotometric analysis. Drawbacks of organic solvents include their higher cost, toxicity and pollution. Hydrotropy is a unique solubilization technique in which certain chemical compounds termed as hydrotropes can be used to affect a several fold increase in the aqueous solubility of sparingly soluble solutes under normal conditions due to the formation of organized assemblies of hydrotrope molecules at critical concentrations. Hydrotropic solubilization may be a proper choice to preclude the use of organic solvents. **Method:** A novel, simple, fast and reproducible UV spectrophotometric method was developed using 2.5M urea solution as hydrotropic solubilizing agent for the estimation of poorly water soluble drug glimepiride in bulk and in pharmaceutical dosage form. Glimepiride exhibits absorption maximum at 235 nm. Urea did not show any absorbance above 225 nm and thus no interference in the estimation of drug was seen. **Results:** The results of analysis have been validated as per ICH guidelines. Beer's law was found to be obeyed in the concentration range of 5-40µg/ml. In this method, there is no interference from any common pharmaceutical additives and diluents. The correlation co-efficient (' *r* ' value) for glimepiride was found to be 0.99983. The percentage recovery obtained for glimepiride ranges from 99.30 % to 99.99%. The developed method is accurate, precise and economical.

KEYWORDS: Glimepiride; Hydrotropy; Estimation; Solubility; Urea.

INTRODUCTION

Solubility enhancement processes are widely used in pharmaceutical industry to improve the dissolution and bioavailability of poorly water soluble drugs. Hydrotropy is a unique solubilization technique, however many hydrotropes do not seem to self-aggregate at all, unless a solubilizing agent has been added. In order to select suitable hydrotropes for various poorly water-soluble drugs, an approximate solubility determination method is used. This is a modified form of the method used by Simamora et al.[1] The hydrotropic solution was taken in a glass bottle and gross weight was noted. Then, few mg of fine powder of drug is transferred to the bottle. The bottle was shaken vigorously. When drug gets dissolved, more drug was transferred to the bottle and again the bottle was shaken vigorously and then operation was repeated till some excess drug remained undissolved. Then, again gross weight was noted. From the difference in two readings of weight, an approximate solubility was determined and solubility enhancement ratios are calculated. Earlier history of spectrophotometric estimation of water insoluble drugs using hydrotropic solubilization methods used concentrated hydrotropic aqueous solutions of sodium benzoate, sodium salicylate, urea, nicotinamide, sodium citrate and sodium acetate to increase the water solubility of drugs. Maheshwari et al employed sodium benzoate as a hydrotropic agent to quantify drugs with poor aqueous solubility like norfloxacin, nalidixic acid, metronidazole, and tinidazole by spectrophotometric analysis. Among the drugs, those have poor aqueous solubility, ibuprofen, flurbiprofen and naproxen. A 2M sodium benzoate solution was used as a solubilizing agent and the solubilities of ibuprofen, flurbiprofen and naproxen increased by more than 80, 110 and 120 times compared with the solubilities in distilled water, respectively.[6-13] Glimepiride is an anti-diabetic drug which is used for the treatment of diabetes. It is chemically, 1-{(p-[2-(3-ethyl-4-methyl2-oxo-3-pyrroline-1-carboxamide) ethyl] phenyl) sulfonyl}-3-(trans-4-methylcyclohexyl) urea[2-6], is a 3rd generation sulfonylurea. From the literature review it is observed that there are reported spectrophotometric methods

which uses organic solvents and isocratic reverse phase HPLC methods for the quantitative estimation of Glimepiride in tablet dosage form. There is a better possibility to develop simple and precise method by using hydrotropic solubilization for the spectrophotometric estimation of glimepiride. The present study, is new, simple, accurate, cost effective, and sensitive spectrophotometric method for the estimation of glimepiride in tablets .High cost, instability and toxicity are the main disadvantages of organic solvents.[14-22]

AIM AND OBJECTIVE

The aim of the present study was to preclude the drawbacks of organic solvents and inaccuracy in spectrophotometric estimations due to their volatility.

MATERIALS AND METHOD

Instruments

1. Infra-red analysis was carried out by using Perkin Elmer Spectrum II FT-IR Spectrometer with attenuated total reflection (ATR) contact sampling method
2. Spectrophotometric analysis was carried out by using a double beam UV-visible Spectrophotometer(Shimadzu model UV-1700, Japan) with 1cm matched quartz cells.

Reagents and Chemicals

1. Urea analytical grade was supplied from Astron Chemicals, Ahmedabad. Water was purified by using glass distillation apparatus.
2. Glimepiride was a gift sample from Blue Cross Labs. Ltd. (Nashik), India.
3. Commercially available Pharmaceutical dosage form of Glimepiride tablets-(Semiamaryl -0.5mg Batch no -9MAD 003, Mfg.date 04/2021, Exp.date 03/2023.) manufactured by Zentiva Pvt.Ltd. Gujarat and marketed by Sanofi India Ltd.Mumbai.

Methodology adopted

1. Preliminary solubility study of glimepiride
2. Preparation of standard solution
3. Study of spectral characteristics of glimepiride in 2.5M Urea
4. Calibration curve of Glimepiride R.S in 2.5M Urea solution
5. Selection & optimisation of wave length
6. Statistical evaluation of calibration plot
7. Stability profile
8. Validation of the method
   - Accuracy
   - Precision
   - Detection limit (LOD)
   - Quantitation limit (LOQ)
   - Linearity
   - Range
9. Estimation of Glimepiride in dosage form
10. Recovery studies

1. Preliminary solubility study and Infra red spectrum of Glimepiride

Solubility of Glimepiride was determined at 28±1°C. An excess amount of the drug was added to screw capped glass vials of 40 ml capacity containing Urea solutions, Sodium acetate and Sodium benzoate solutions of varying molarity (1.0M, 1.5M, 2.0M and 2.5 M), the vials were shaken mechanically for 12 hr at 28±1° C in a mechanical shaker. These solutions were allowed to equilibrate for the next 24 hours and then centrifuged for 5 minutes at 2000 rpm. The supernatant of each vial was filtered through Whatmann No. 41 filter paper. The filtrates were diluted suitably and analyzed spectrophotometrically against corresponding solvent blank.

2. Preparation of standard solution

The glimepiride R.S stock solution of concentration of 1mg/ml (solution A) was prepared and furhur dilutions were prepared using distilled water as solvent to get concentrations of 20 µg/ml, 50 µg /ml, and 90 µg/ml respectively.

3. Study of spectral characteristics of glimepiride in 2.5 M Urea

The spectral characteristics were studied using Glimepiride R.S in 2.5M Urea solution in the wavelength range of 200-400 nm after enabling blank correction in the above region.

4. Selection & Optimisation of wave length

For the fixing absorption maximum wavelength repeated the spectral scan using Glimepiride stock solution in three different concentrations by shortening the wavelength range from 200-300 nm after enabling blank correction in the above region.

5. Calibration curve of Glimepiride R.S

Aliquots from solution A was diluted with distilled water to get the drug concentrations ranging from 5-40 µg /ml. The absorbance of each solution was measured at 235 nm with blank.

6. Statistical evaluation of calibration plot

The data in table (1) was used to derive a regression equation of the absorbance (Y) on the concentration (X) by the principle of least squares. The equation is as follows

$$\mathbf{Y = a\,x + b}$$

7. Stability Profile

The period over which absorbance value at 235 nm, of glimepiride in Urea remained stable was investigated using three different concentrations of 10, 20, and 30 µg/ml. The absorbance values were measured at 15 min intervals for a period of 1 hour.

8. Validation of the proposed Method[23]

a. Accuracy

Accuracy was evaluated by carrying out a recovery study and the method was found to be accurate.

b. Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements from multiple sampling of the same homogenous sample under prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.

Repeatability expresses the precision under the same operating conditions over a short interval of time. The precision of an analytical procedure is usually expressed as the standard deviation of a series of measurements. The reproducibility of the method was studied using three different concentrations of Glimepiride (10, 20 and 30µg/ml) which were prepared from stock solution A. The absorbance was measured at 235 nm using distilled water as blank. The absorbance was measured two more times for each concentration and their mean values were calculated.

The intraday and interday precision study of glimepiride was carried out by estimating the corresponding responses three times on the same day and on three different days (1st, 2nd and 5th day) for three different concentrations of glimepiride (10, 20 and 30 µg/ml) and the results are reported in terms of relative standard deviation.

c. Detection limit (LOD)

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

d. Quantitation Limit (LOQ)

The quantitation limit of an individual analytical procedure is the lowest concentration of analyte in a sample, which can be quantitatively determined with a suitable level of precision and accuracy.

e. Linearity

The linearity of an analytical procedure is its ability, within a given range to obtain test results that are directly proportional to the concentration of analyte in the sample.

f. Range

The range of an analytical procedure is the interval between smallest and largest concentration that maintains a linear relationship between the concentration and the response of the method.

9. Estimation of Glimepiride in dosage forms

60 tablets were accurately weighed and finely powdered in a glass mortar. A weight equivalent to 25 mg was accurately weighed out and transferred to a 25ml stoppered tube. 5ml of 2.5M urea was added and swirled gently for a period of 10 minutes. Allowed to settle, the clear supernatant solution was then transferred into a 25ml standard flask through a Whatmann No.40 filter paper. The residue was further extracted twice, with 2.5M urea solution and passed through the same filter paper and the volume was finally made up to 25ml with urea to get concentration of 1mg/ml The absorbance of solutions had a concentration of 20µg/ml and 30 µg /ml were measured at 235nm using distilled water as blank.

Accuracy of analysis was determined by performing recovery studies by spiking different concentrations of pure drug (Glimepiride RS) in the pre analyzed tablet sample. This parameter was evaluated by the recovery studies at concentration levels of 50%, 100%, and 150% of drug which consisted of adding known amounts of Glimepiride reference materials to the samples.

RESULTS

The chemical structure and the Infrared spectrum of glimepiride R.S is furnished in "Fig.1" and "Fig .2" respectively.

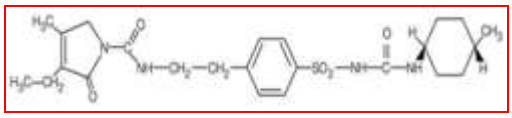

Fig. 1: Chemical structure of Glimepiride.

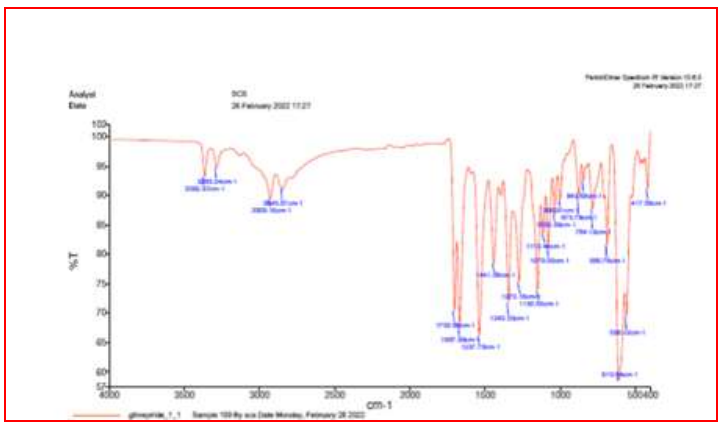

Fig.2: FTIR spectrum of Glimepiride.

Over lay UV spectrum of glimepiride R.S in 2.5M Urea solution is shown in figure.3. An absorption band ranging from 200-400 nm was observed with maximum absorption at 235 nm. From the spectra it was found that the 2.5M urea used does not interfere with the sampling wavelength. Therefore 2.5M Urea is used for the solubilization of drug. The three absorption bands ranging from 200-300 nm was observed with maximum absorption at 235 nm("Fig.3").

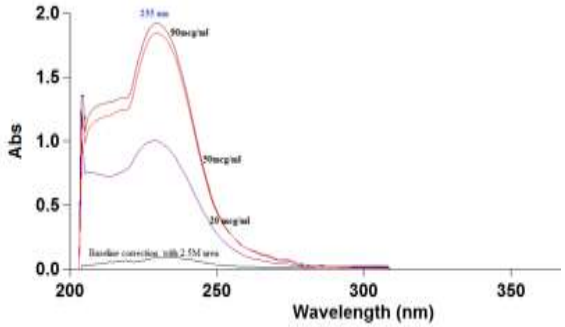

Fig. 3: Over lay UV absorption spectrum of glimepiride.

The Calibration data of Glimepiride R.S in table. 1 showed that Beer's law is obeyed from 5-40µg/ml ("Fig.4").

Table I: Calibration data of glimepiride R.S.

SI No:	Volume of Glimepiride Solution A (ml)	Concentration of Glimepiride in final solution (µg/ml)	Absorbance at 235 nm
1	0.5	5	0.594
2	1.0	10	0.675
3	1.5	15	0.745
4	2.0	20	0.825
5	2.5	25	0.906
6	3.0	30	0.986
7	3.5	35	1.07
8	4.0	40	1.15

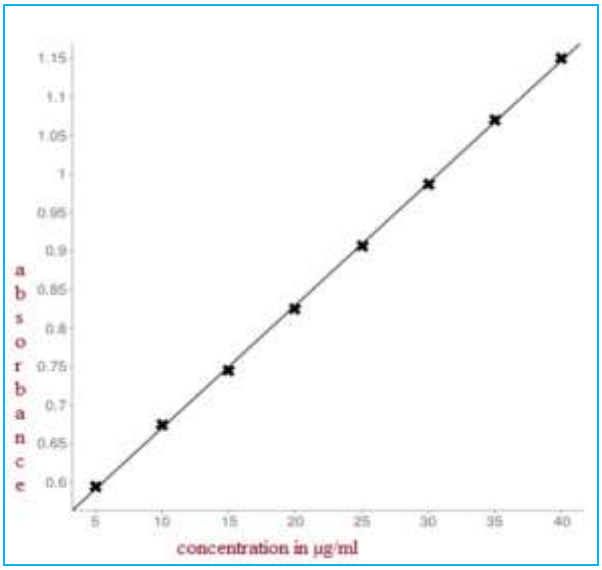

Fig. 4: Calibration plot of glimepiride R.S.

The data in table. 1 was used to derive a regression equation of the absorbance (Y) on the concentration (X) by the principle of least squares.

The equation is as follows

$Y = a x + b$

$Y=0.0158833x+0.5115$

Correlation coefficient was found to be 0.99983.

The optical characteristics of Glimepiride for the developed method is furnished in table.2

Table 2: Optical Characteristics of glimepiride for the developed method.

No	Parameters	
1	Beers law limit	5-40µg/ml
2	Correlation coefficient	0.99983
3	Y= ax + b	Y= 0.01588333x + 0.5115
4	Molar absorptivity	$1.65333X\ 10^4$l/mol.cm.

The absorbance values were measured at 15 min intervals for a period of 1 hour. Data obtained is furnished in table.3 and graph is shown in "Fig.5".

Table 3: Data of stability study of Glimepiride.

Sl:No	Concentration of Glimepiride (µg/ml)	Absorbance at 235 nm in 15minutes intervals				
		0	15	30	45	60
1	10	0.675	0.674	0.673	0.672	0.672
2	20	0.825	0.823	0.824	0.821	0.822
3	30	0.984	0.983	0.984	0.983	0.980

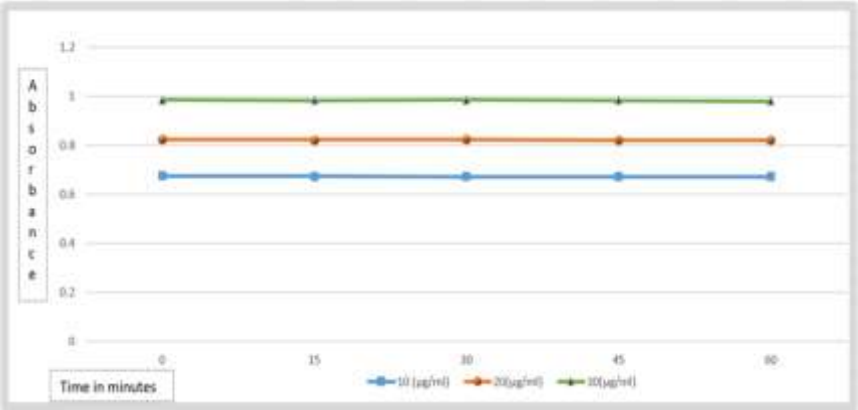

Fig. 5: Stability profile of the absorbance of Glimepiride.

The result of reproducibility study is given in table.4.

Table 4: Result of reproducibility study.

SI: No	Concentration (µg/ml)	Absorbance at 235nm	Mean value	Standard deviation
1	10	0.673 0.673 0.670	0.672	0.00141
2	20	0.823 0.821 0.823	0.822	0.00094
3	30	0.983 0.982 0.984	0.983	0.00082

The results of intra-day and inter-day precision study of glimepiride were reported in terms of relative standard deviation in table.5 and table.6.

Table 5: Results of intraday precision study.

SI No.	Concentration (µg/ml)	Absorbance at 235 nm			RSD,%
		0 hr	1.5 hr	3hr	
1	10	0.673	0.670	0.668	0.48
2	20	0.825	0.820	0.823	0.55
3	30	0.982	0.979	0.980	0.20

Table 6: Results of inter-day precision study.

SI No.	Concentration (µg/ml)	Absorbance at 235 nm			RSD,%
		1st day	2nd day	5th day	
1	10	0.670	0.665	0.664	0.38
2	20	0.824	0.819	0.815	0.31
3	30	0.983	0.981	0.979	0.16

The limit of detection, LOD of Glimepiride by the proposed method was found to be 3µg/ml.

The limit of quantitation, LOQ of Glimepiride by the proposed method was found to be 5µg/ml.

The calibration curve of Glimepiride was linear over the range of 5-40 µg /ml.

The results of estimation of Glimepiride tablets obtained are furnished in table .7.

Table 7: Results of estimation of Glimepiride tablets.

Sl. No	Conc. of Glimepiride (µg/ml)	Standard Absorbance at 235nm	Sample Absorbance at 235nm	Label claim	Active content per tablet (mg)	Average content per tablet (mg)
1	20	0.820	0.818	99.51	0.497	0.497
2	30	0.918	0.916	99.62	0.498	

Result of recovery studies are given in Table. 8

Table 8: Results of recovery studies.

Sl No	Brand name	GLP R.S added /spiked	Total concentration found	% recovery of pure drug * (Mean±S.D) (n=3)	%RSD
1	Semi amaryl 0.5mg	0.5mg	0.999	99.90±0.439	0.48
		1.0mg	1.49	99.33±0.099	0.36
2		1.5mg	1.99	99.99±0.385	0.28

DISCUSSION

By proper choice of hydrotropic agents, the use of organic solvents in analysis may be discouraged to a large extent. It is evident that there is good agreement between the amounts estimated and those claimed by the manufacturer. The mean percentage label claim 99.45 was found for Semi amaryl which is very close to 100 with low values of standard deviation which confirms the accuracy of the proposed method. The reproducibility and precision of the proposed method were further confirmed by the mean percentage recovery values (99.30% to 99.99%), which were close to 100 with low values of standard deviation. The proposed method for determination of Glimepiride showed molar absorptivity of 1.65333X 10^4 l/mol.cm.

CONCLUSIONS

In Summary, the method developed for the estimation of Glimepiride is simple, sensitive, fast, reproducible, pollution free and applicable to the routine analysis of glimepiride in raw drug and in dosage forms.

ACKNOWLEDGEMENT

The authors thank Blue Cross Labs. Ltd. (Nashik), India for the gift sample of glimepiride.

REFERENCES

1. Simamora P, Alvarez JM, Yalkowsky SH. Solubilization of rapamycin. Int J Pharm., 2001; 213: 25–29.
2. Rosskamp R. Safety aspects of oral hypoglycaemic agents. Diabetologia, 1996; 39: 1668-72.
3. Rosskamp R, Wernicke-Panten K, Draeger E. Clinical profile of the novel sulfonylurea glimepiride. Diabetes Res Clin Pract, 1996; 31: S33-42.
4. Schneider J. An overview of the safety and tolerance of glimepiride. Horm Metab Res., 1996; 28: 413-8.
5. Zimmerman BR. Sulfonylureas. Current Therapies for Diabetes, 1997; 26(3): 511-22.
6. Once-daily glimepiride in type 2 diabetes mellitus: possible tolerability advantages. Drug & Therapy Perspective, 1998; 12(2): 1-5.
7. Pandey S, MaheshwariRK. A novel spectrophotometric method for the estimation of ketoprofen in tablet dosage form using hydrotropic solubilization phenomenon. J Sci Res., 2010; 6(3): 209-212.
8. Maheshwari RK, Lakkadwala S, Vyas R, Ghode, P. Spectrophotometric determination of naproxen tablets using niacinamide as hydrotropic solubilizing additive. J Cur Pharm Res., 2010; 04: 11-14.
9. Maheshwari RK, Prasad S, PandeyP, WanareG. Novel Spectrophotometric analysis of Piroxicam Tablets Using Ibuprofen Sodium as Hydrotropic Solubilizing Agents. Int. J. Pharma. Sci. Drug Res., 2010; 2(3): 210-212. 27)
10. Sable PN, Chaulang GM, Bhosale AV, Chaudhari PD. Novel Spectrophotometric Estimation of Olanzapine Using Hydrotropic Solubilizing Agent. Research J. Pharm. and Tech, 2009; 2(2): 297-300.
11. Shyni Bernard, Molly Mathew and K. L. Senthilkumar. Spectrophotometric method of estimation of Amlodipine besylate using hydrotropic solubilization. J Appl Pharm Sci., 2011; 01(09): 177-180.
12. Banerjee T, Banerjee B, Jain P. Spectrophotometric Estimation of Lornoxicam and Paracetamol Tablet dosage form using Hydrotropic Solubilizing Agent. Int J of ChemTech Res., 2012; 4(1): 232-239.
13. Shyni Bernard 1, Molly Mathew, K.L. Senthilkumar and K.N. Girija. Simultaneous estimation of atorvastatin calcium and amlodipine besylate by UV spectrophotometric method using hydrotropic solubilization. Hygeia.J D Med., 2013; 5(1): 105-112.

14. Ravindra N, Singhvi I. “Spectrophotometric estimation of glimepiride from pharmaceutical dosage forms”. Asian Journal of Chemistry, Aug. 20, 2008; 20(6): 4379.
15. Bonfilio R, Araújo MB, Salgado H. “Development and validation of an UV-derivative spectrophotometric method for determination of glimepiride in tablets”. Journal of the Brazilian Chemical Society, Feb. 2011; 22(2): 292.
16. Samala, Sujatha, Sandhya Rani Tatipamula, and Ciddi Veeresham. "Determination of glimepiride in rat serum by RP-HPLC method." A J AnalChem., 2011; 2.2: 152.
17. Bhargavi S, Suryasagar G, Sowmya DK, Ashok K, Nama S. “UV spectrophotometric method for determination of glimepiride in pharmaceutical dosage forms”. Int J of PharmSci Re and Res., Jul. 2013; 21(2): 131-4.
18. Sakala BhargavSakala Bhargavi, Gopisetty Suryasagar, Dantu Krishna Sowmya & Kota Ashok. “UV spectrophotometric method for determination of glimepiride in pharmaceutical dosage forms”.Int J Phar Sci Re and Res., 2014; 23: 131-133.
19. Ramadan AA, Mandil H, Zeino S. “Development and validation of spectrophotometric determination of glimepiride in pure and tablet dosage forms through ion-pair complex formation using bromothymol blue”. Int J of Pharm and Pharm Sci., 2016; 8(6): 217-21.
20. Ramadan, A. A., & Zeino, S. “Direct spectrophotometric determination of glimepiride in pure form and pharmaceutical formulations using bromocresol purple”. J A Chem., 2018; 15(2): 6186–6198.
21. Aseem Kumar, Anil Kumar Sharma, Rohit Dutt.”Reverse-phase high-performance liquid chromatography method development and validation for estimation of glimepiride in bulk and tablet dosage form.” IJPQA, 2020; 11(2): 296-302.
22. Chafle DM. “Study on the development and validation of direct spectrophotometric method for the estimation of glimepiride”. Cur Asp in Pharm Res and Deve, Nov. 2021; 2(4): 1-11.
23. International Conference on Harmonization. ICH. Topic Q2B, Validation of Analytical Procedures: Methodology, Proceedings of the Commission of the European Communities. Rockville, 1996; 1-10.