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Method Development and Validation of Anti-Diabetic Drug gliclazidein its pharmaceutical dosage form by HPLC with forced degradation Studies

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ABSTRACT:
A simple, rapid, and specific method for analysis of an anti-diabetic drug i.e., gliclazide by asensitive reverse phase high-performance liquid chromatographic method is described. HPLCis an essential tool which should be able to separate, quantify, detect the various drug, drugrelated impurity during the synthesis. The Gliclazide is an anti-diabetic drug which used for totreat of type 2 diabetes. It belongs to the sulfonylurea class of insulin secretagogues, which actby stimulating β cells of pancreas to release insulin. The developed method was validated asper of ICH guidelines. The chromatographic separation was achieved by using Agilent ZorbaxBonus-RP (250 x 4.6 mm, 5μ) column at ambient temperature using Phosphate Buffer: ACN(40: 60, % v/v) as mobile phase at flow rate of 1 ml/min and wavelength detection at 230 nm.Gliclazide shows the linearity over the concentration range of f 0.8-1.2 mg/ml with limit ofdetection of 2.86 ug/ml. The accuracy of the method was 99.49-101.08%. The proposedmethodwasvalidatedaspertheICHguidelineswithexcellentselectivity,linearity,sensitivity,precision, accuracy, inter-day and intra-day was applicable for the assay of given drug. Theproposedmethodcanbesuccessfullyappliedfortheestimationof Gliclazideinpharmaceuticaldosageforms.

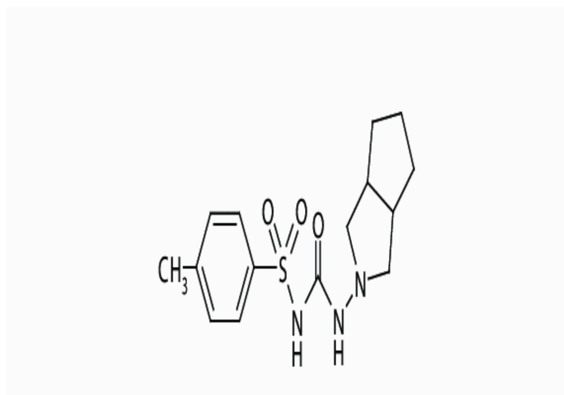
KEYWORDS: Anti-diabetic,insulinsecretagogues,ambienttemperature.

I. INTRODUCTION:

Gliclazide is an oral antihyperglycemic agent used for the treatment of non-insulin-dependentdiabetes mellitus (NIDDM). It has been classified differently according to its drug propertiesinwhichbasedonitschemicalstructure,gliclazideisconsideredafirst-generationsulfonylureadue to the structural presence of a sulfonamide group able to release a proton and the presenceofonearomaticgroup.[1]

Gliclazide belongs to the sulfonylurea class of insulin secretagogues, which act by stimulatingβ cells of the pancreas to release insulin. Sulfonylureas increase both basal insulin secretionand meal-stimulated insulin release. Sulfonylureas also increase peripheral glucose utilization,decrease hepatic gluconeogenesis and may increase the number and sensitivity of insulinreceptors. Due to their mechanism of action, sulfonylureas may cause hypoglycemia andrequire consistent food intake to decrease this risk. The risk of hypoglycemia is increased inelderly, debilitated and malnourished individuals. Gliclazide has been shown to decreasefasting plasma glucose, postprandial blood glucose and glycosylated hemoglobin (HbA1c)levels(reflectiveofthelast8-10weeksofglucosecontrol).Gliclazideisextensively metabolized by the liver; its metabolites are excreted in both urine (60-70%) and feces (10-20%).

Gliclazide binds to the β cell sulfonyl urea receptor (SUR1). This binding subsequently blockstheATPsensitivepotassiumchannels.Thebindingresultsinclosureofthechannelsandleadsto a resulting decrease in potassium efflux leads to depolarization of the β cells. This opensvoltage-dependent calcium channels in the β cell resulting in calmodulin activation, which inturnleads to exocytosis of insulin containingsecretortygranules.



Structureofgliclazide

CHEMICALSANDREAGENTS:

The tested pharmaceutical dosage Procter and Gamble Pharmaceuticals, Inc. Gliclazideworking standard pure drug form in powder form were purchased from Dhamtec pharma,Navi Mumbai. Acetonitrile, Phosphate Buffer were of HPLC grade from Loba chemicals,Mumbai. HPLC grade water was prepared using a Milli-Q purification system. All otherreagentswereof analytical grade.

INSRUMENTATION:

The HPLC system used for method development and validation consisted of an Agilent1200 Series UV Spectroscopy Shimadzu UV -1800 and Agilent Carry -60), SonicatorLabman1-SL-50HandLabmanPROB-250,WeighingBalanceshimadzuNo.D460020153,MagneticStirrer Remi equipment PVT.LTD.

- **Preparationofstockandworkingstandardsolutions**

➢ **PreparationofGliclazideStandardStockSolution(SSS-I):**

Weighaccurately10mgofGliclazide ina10mlvolumetricflaskanddilute itwithdiluentto makeup thevolume. (Conc. ofGliclazide =1000µg/ml)

➢ **PreparationofGliclazideStandardStockSolution(SSS-II):**

Pipetteout1mlofabovesolutionina10mlvolumetricflaskanddiluteitwithdiluentto makeup thevolume. (Conc. ofGliclazide =100µg/ml)

➢ **PreparationofGliclazideWorkingStandard(WS):**

Pipette out 1.0 ml of SSS-II in a 10 ml volumetric flask and dilute it with diluent tomakethevolume. (Conc.ofGliclazide =10 µg/ml)

- **DrugProductSamplePreparationforAssay:**

i. 10 tablets were weighed, and average weight was calculated. And tablets were crushed&mixed in mortarand pestle.
ii. Powder weight equivalent to 5 mg Gliclazide was weighed into 10 ml volumetric flask&add5mldiluent, sonicatefor5 minutes,andmakethevolumeto10 mlwithdiluent.(Conc.of Gliclazide =500 µg/ml).
iii. Further, pipette out 1.0 ml of above solution in 10 ml volumetric flask and add 5 mldiluent and vortex and make up the volume with diluent. (Conc. of Gliclazide = 50µg/ml).

- **SelectionofWavelength:**

The sample was scanned from 190-400 nm with DAD detector. The Wavelength selected foranalysischosenwas 230 nmforappropriate identification ofGliclazide.

- **Chromatographicconditions**

Thechromatographicseparationwasperformedusingisocraticelutionatambienttemperature (30 °C) on Agilent Zorbax Bonus-RP (150 x 4.6 mm, 5µ) with UV detection at230nm.ThemobilephasewascomposedPhosphateBuffer:ACN(40:60,%v/vTheflowratewasset at 1 mL/min. Theinjection volumewas 10 µLforeveryinjection.

- **Analyticalmethodvalidation:**

ThedevelopedRP-HPLCmethodwasvalidatedintermsofthefollowingparameters:specificity, linearity, sensitivity, precision, accuracy and stability of analytical solutions. Thevalidation was carried out according to International Conference on Harmonization (ICH)guidelinesfor validationofanalytical procedures.

a.Specificity&Assay:

iv. IndividualsampleofGliclazideworkingstandard&drugproductof50µg/mlwaspreparedandpeaks were foridentified from Retention Time.
v. Blankwasinjectedtoensurethereisnoblankpeakinterferingwiththemainanalytepeak.
vi. Assaywascalculated byusingfollowingformula.

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$$\%Assay = \frac{SampleArea}{StandardArea} x100$$

a. Repeatability&SystemSuitability:

vii. Asinglesamplewaspreparedasdescribedand6injectionsweremadefrom samesampleandchecked for system suitability.
viii. Systemsuitabilityparametersareasbelow:

1. RetentionTime,
2. Theoreticalplates,
3. Asymmetry(Tailingfactor),
4. Peakpurity.

b. Linearity&Range:

ix. 5samplesofvaryingconcentrationsrangingfrom80-120%weremade.
x. Theconcentrationsaregivenbelow.

%Level	GliclazideConc.(μg/ml)
80	40
90	45
100	50
110	55
120	60

xi. Thesamplepreparationsaregivenasbelow.
xii. XmlofGliclazidewasaddedto10mldiluenttomakeuptheconcentrationsgivenabove:

XmlofGSSS-I	Dilutedto
0.8	10ml
0.9	10ml
1.0	10ml
1.1	10ml
1.2	10ml

c. Accuracy:

xiii. Sampleswerepreparedof80%,100%and120%concentrationbyspikingthesameamountof concentrationgivenabovein table forbothGliclazide.
xiv. Sampleswereinjectedintriplicatetocalculate%RSD.
xv. %recoverywasalsocalculated.

d. LOD/LOQ:

xvi. Wascalculatedbyusing ANOVAtechnique.
xvii. Formula:

$$LOD = \frac{3.3 \times Std.ErrorofIntercept}{CoefficientsofXVariable1}$$

$$LOO = \frac{10 \times Std.ErrorofIntercept}{CoefficientsofXVariable1}$$

e. Robustness:

xviii. TheRobustnesswasperformed bychangingthecolumntemperatureby±2˚C.
xix. EachSamplewasinjected%Assaywascalculatedateachconditionwascalculated.

Condition	Increased	Normal	Decreased
ColumnOvenTemperature	32˚C	30˚C	28˚C

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f. Intra&Inter-dayPrecision:

xx. Singlemixtureworkingstandardanddrugproduct sampleswerepreparedandinjectedtwicein a dayat different timeintervals to evaluateintra-dayprecision.

xxi. Samemixtureworkingstandardanddrugproducts ampleswereanalysedon seconddaytoevaluatethe inter-dayprecision.

xxii. %Assaywascalculatedateachintervalands tabilityofsolutionswereestimated.

➢ **ForcedDegradationStudies**

Forceddegradationstudy(FD)studies(stresstesting)ar eanintrinsicpartofpharmaceuticalproduct development. It is procedure whereby the natural degradation rate of a product ormaterial is increased by the application of additional stress condition. It manifests chemicalbehavior of the molecule which helps in the development of formulation and packaging ofpharmaceuticaldevelopment.Itisnecessarytospecif ythespecificityofthestabilityindication methods and provide insight into degradation pathways and degradation productsofthe drugsubstanceandaid inan elucidationofthestructureofthe degradation products.

The Regulatory guidelines, the Various International guidelines are recommended for theforceddegradation studies[2]: -

a. ICHQ1A:StabilityTestingofNewDrugSubst ancesandProducts.

b. ICHQ1B:PhotostabilityTestingofNewDrug SubstancesandProducts.

c. ICHQ2B:ValidationofAnalyticalProcedure s:Methodology.

- **Variousdegradationconditions**

1. Hydrolysis: Over a wide range of pH most common degradation, chemical reactions areHydrolysis The decomposition of a chemical compound by reaction with water is calledHydrolysis. In acidic and basic hydrolysis, the catalysis of ionizable functional groupspresent in the molecule occurs. Forced degradation of a drug substance occurs when thedrug interacts with acid and base. It produces primary degradants in the desirable range.Dependingonthestabilityofthedrugsubstancet heclassandconcentrationsofacidorbasetakenshouldb edecided.Foracidhydrolysishydrochloricacidorsulp huricacids(0.1-1M)considered to be most suitable whereassodium hydroxide or potassium hydroxides (0.1-1M)forbasehydrolysisaresuggested[2,3].Co-solventscanbeusedifcompoundsarepoorlysoluble in water. Forced degradation started at room temperature and further temperatureincreasedif thereis no degradation[3].

2. Oxidation conditions: For oxidative forced degradation, hydrogen peroxide is broadlyused. Apart from this as metal ions, oxygen, and radical initiators: azobi- isobutyro-nitrile,AIBN can also be used. Drug structure will allow selecting concentration and condition ofoxidizing agents. An electron transfer mechanism occurs in the oxidative degradation ofdrugsubstance[4].

3. Photolyticconditions: Thelightexposuredoes notaffectthedrugsubstanceforthispurposephotostabil ityisconducted.Photostabilitystudiesareperformedto produceprimarydegradantsofdrugsubstancebyexpos uretoUVorfluorescentconditions.InICHguidelinesso merecommendedconditions forphotostabilitytestingaredescribedSamplesof drug substance and solid/liquid drug product should be exposed to a minimum of1.2millionlxhand200Wh/m2light,300-800nmis the mostcommonlyacceptedwavelength of light to cause the photolytic degradation. 6 million is the maximumillumination recommended. Photo oxidation can be caused by light stress conditions by thefree radical mechanism. Photosensitive groups are carbonyls, nitroaromatic, N-oxide,alkenes,arylchlorides, weakC–H and O–Hbonds, sulfides andpolyenes[5].

4. Thermal conditions: Thermal degradation (e.g., dry heat and wet heat) should be carriedoutatmorestrenuousconditionsthanrecomme ndedICHQ1Aacceleratedtestingconditions.

Samples of solid-state drug substances and drug products should beexposed todry and wet heat. Liquid drug products should be exposed to dry heat. Fora shorter period,studies may be conducted at higher temperatures. Through the Arrhenius equation theeffectoftemperatureonthermaldegradationofasub stancecan bestudied.

5. Humidity: Humidity is one of the effective factors in establishing the potentialdegradantsin the finished product and active pharmaceutical ingredient. Normally 90% humidity forthedurationofoneweekshallberecommendedforth eestablishmentofforceddegradationsamples[6].

II. RESULT:

The proposed method can be successfully

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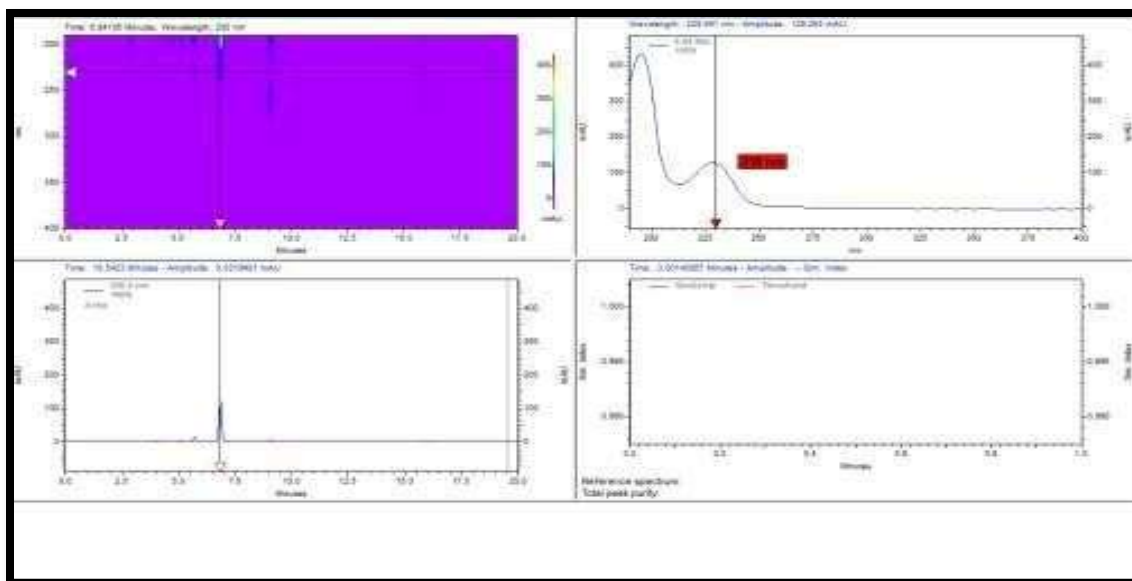
applied for the estimation of Gliclazide inpharmaceutical dosage forms. The RP-HPLC separation was developed on C18 columnunder isocratic condition with short retention time (6.75) acceptable resolution, use ofcost-effective solvents and ease of preparation. Quantitative analysis was achieved withhighchromatographicresponsepeak and optimum resolution.

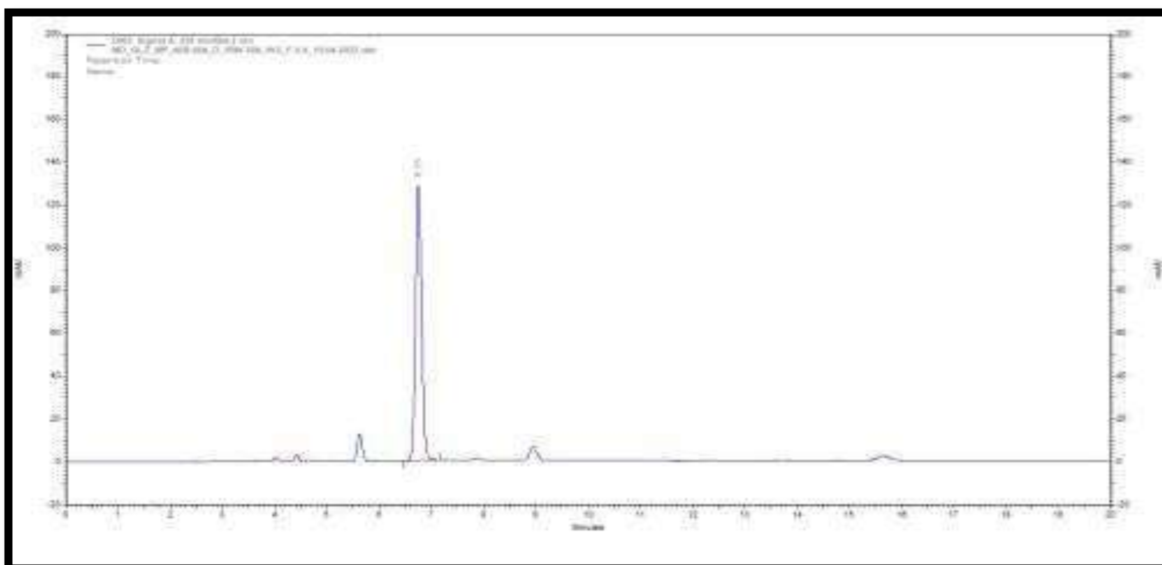


FigureNo.1-AbsorptionspectraofGliclazide

OptimizationofHPLCmethodDevelopment: TheoptimisedchromatographicconditionofGliclazideobtainedatafter5trails.Obtainedresultis as follows:

TableNo.1-OptimizationofChromatogram.

Chromatograph	HPLCSystem
Mobilephase	PhosphateBuffer:ACN(40:60,%v/v)
Column	AgilentZorbaxBonus-RP(150x4.6mm,5μ)
Flowrate	1.0ml/min
Wavelengthdetection	230nm
Injectionvolume	10μl
Temperature	30°C
Runtime	10min
Diluent	Water:ACN(50:50,%v/v)

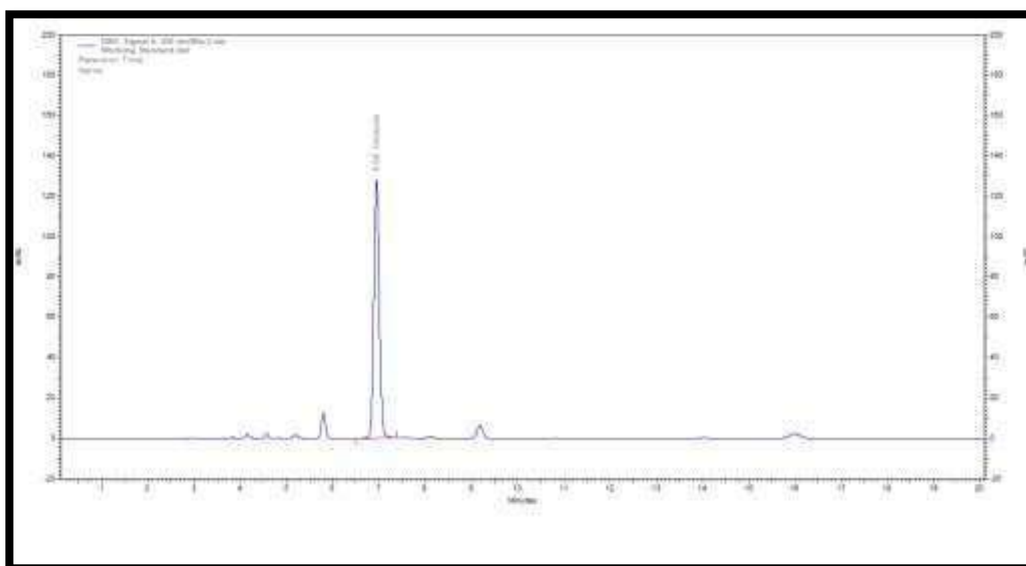


FigureNo.2-Chromatogramofoptimization

2.METHODVALIDATION:

1. Specificity:

Specificity was evaluated by comparing the chromatograms of mobile phase blank, standardsolution, and sample solution (Gliclazide 100 µg/ml). For this purpose, 10 µl from solutionsmobilephaseblank,standardsolution,andsamplesolutionwereinjectedintotheHPLCsystemseparately, and the chromatogram results are shown in Figures 4. It can be observed that peakfound at retention time at 6.75. Therefore-the result indicates that the peak of the analyte waspure, and this confirmedthe specificityof themethod.



Figureno.8-Chromatogramofworkingstandardofspecificity.

2. LinearityandRange.

Analytical method linearity is defined as the ability of the method to obtain test results thatare directly proportional to the analyte concentration, within a specific range. mean peakarea obtained from the HPLC was plotted against corresponding concentrations to obtainthecalibration graph.resultsoflinearitystudy(Figure5-6)gavelinearrelationshipovertheconcentration range

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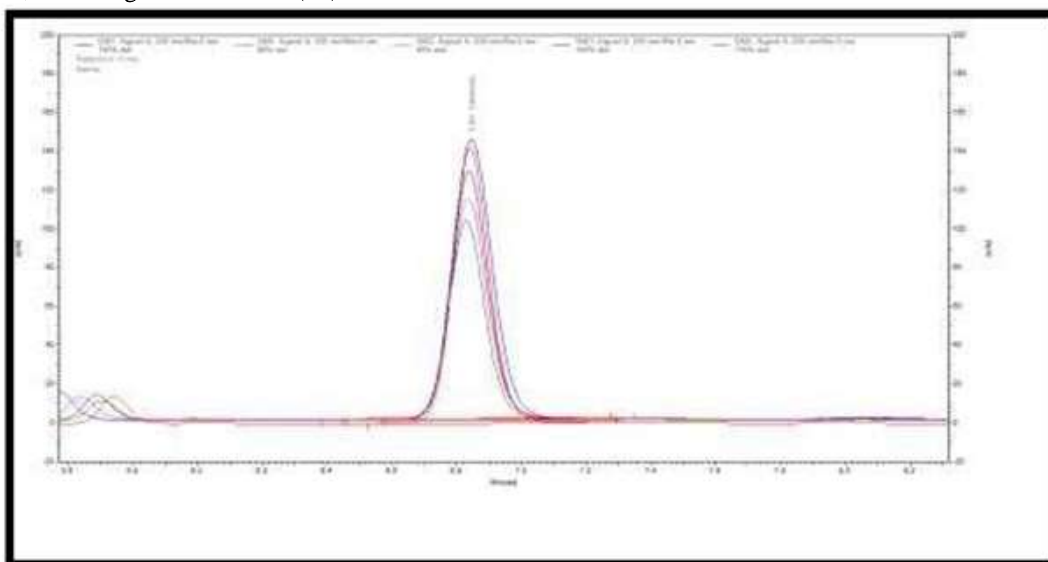
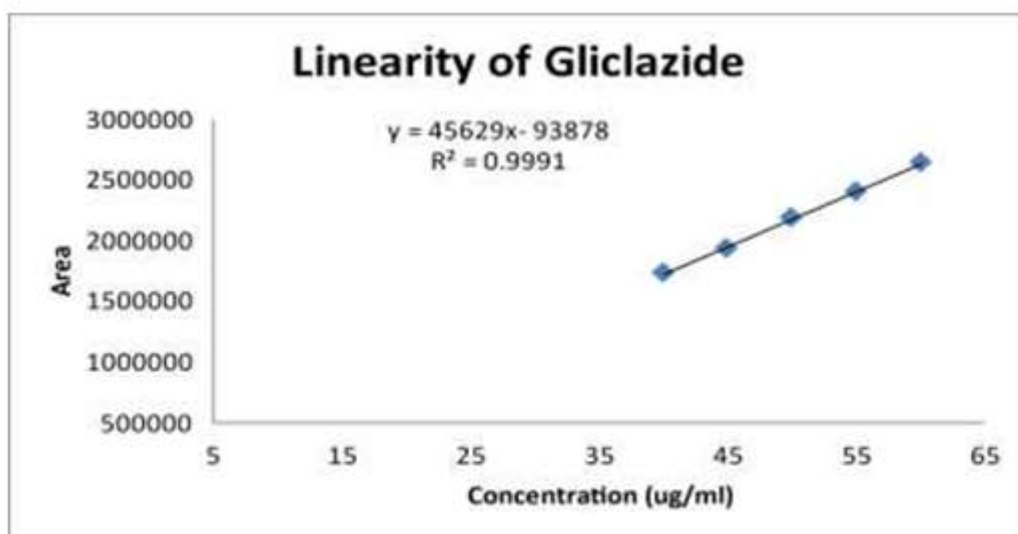
of 0.8-1.2µg/ml for Gliclazide. From the regression analysis, a linearequation was obtained: y = 45629x + 93878, and the goodness-of-fit (R^2) was found to be0.9991 indicating a linear relationship between the concentration of analyte and area underthepeak.



Figureno.4-LinearityoverlayofGliclazide



Figureno.5-LinearityPlotofGliclazide

3. LimitofDetectionandLimitofQuantification(LODand LOQ).

Limit of detection (LOD) is the lowest amount of analyte in a sample that can be detected, butnot necessarily quantitated, while the limit of quantification (LOQ) is the lowest amount ofanalyte in a sample that can be quantitatively determined with suitable precision. ResultsshowedanLODandLOQforGliclazideof2.86 and 8.67µg,respectively.

4. Accuracy.

Accuracyofananalyticalprocedureexpressestheclosenessofresultsobtainedbythat methodtothetruevalue.resultsofaccuracyshowedpercentagerecoveryatallthreelevelsintherangeof99.98–100.15%,andRDSvalueswereintherangeof0.03–0.02%as.i.eresultsofpercentage recovery and %RSD were within the accepted limits from 98.0% to 102.0% andnot more than 2.0%, respectively,

which indicates the Applicability of the method for routinedruganalysis.

5. Precision.

Precisionof the methodisdefinedas“theclosenessofagreementbetweena seriesofmeasurements obtained from multiple sampling of the same homogeneous sample under theprescribed conditions,” and it is normally expressed as the relative standard deviation . ¡eresults of both system and method precision showed that the method is precise within theacceptable limits. ¡e RSD, tailing factor, and number of theoretical plats were calculated forboth solutions; all the results are within limits. Acceptable precision was not more than 2.0%for the RSD and the tailing factor and not less than 1000 for number of plates, as shown inTables2 and 3.

TableNo.2-Intradayprecision

IntraDayprecision			
Day1	Sample ID	Nitrofurantoin	
		Area	Assay
Morning	WS	4085432	-
	DP	4074587	99.73
Evening	WS	4075412	-
	DP	4070054	99.87

TableNo.3-Interdayprecision.

Inter Dayprecision			
Day	Sample ID	Nitrofurantoin	
		Area	Assay
Day 2	WS	4068542	-
	DP	4061571	99.83

6. Robustness.

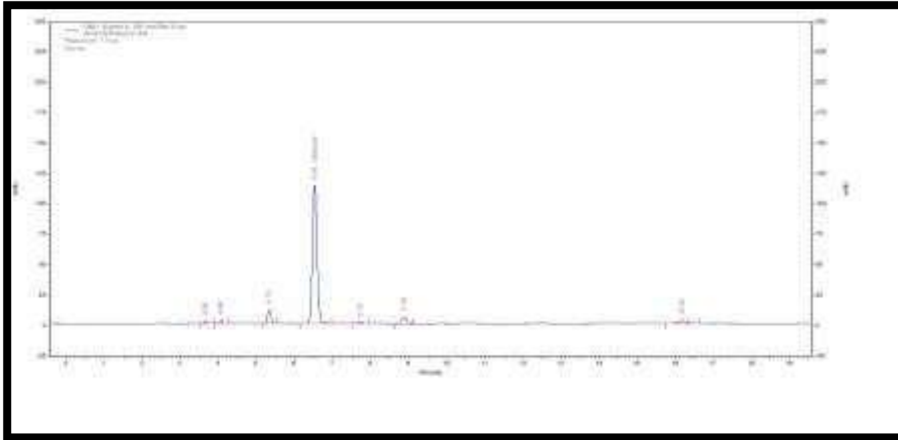
Analytical method robustness was tested by evaluating the influence of minor modificationsin HPLC conditions on system suitability parameters of the proposed method. Results ofrobustness testing showed that a minor change of method conditions, such as the compositionof the mobile phase, temperature, flow rate, and wavelength, is robust within the acceptablelimits.Results are summarized in Table 4. In all modifications, good separation of Gliclazidewas achieved, and it was observed that the percent of recovery was within acceptable limitsandthe%RSDiswithinlimit ofnotmorethan2.0%.Tailingfactors andnumberoftheoreticalplateswerefound within acceptablelimits as well.

TableNo.4-RobustnessdataoftheproposedHPLCmethod.

ColumnOvenTempChange				
Condition	Sample	Nitrofurantoin		
		RT	Area	Assay
28°C	WS	4.07	4078542	-
	DP	4.07	4069974	99.79
30°C	WS	4.07	4085432	-
	DP	4.07	4074587	99.73
32°C	WS	4.07	4080456	-
	DP	4.07	4075317	99.87

FORCEDDEGRADATIONSTUDYBYRP-HPLCMETHOD

1. AcidHydrolysisdegradationstudy

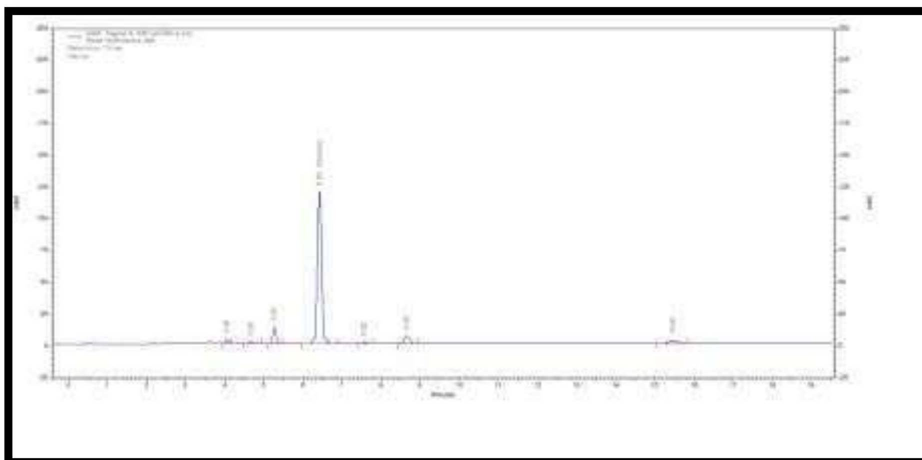


FigureNo.6–ChromatogramofAcidsample

Result- **TableNo.5-ResultofAcidHydrolysisdegradationstudy**

Sample	Area	Assay	%Degradation
AcidHydrolysis(1ml0.1 NHCl@RTfor10min)	218960 2	89.23	10.77

2. BaseHydrolysisdegradationstudy:



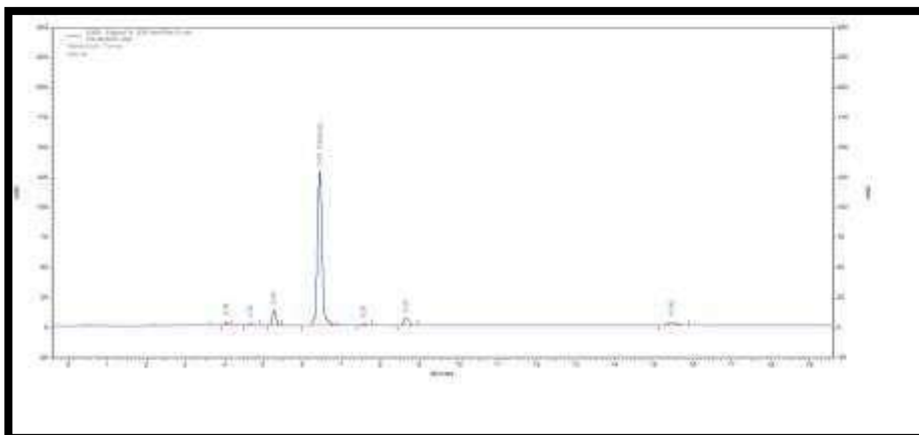
FigureNo.7–ChromatogramofBaseblank

Result-TableNo.6-ResultofBaseHydrolysisdegradationstudy

Sample	Area	Assay	% Degradation
BaseHydrolysis (1ml0.1N NaOH@RTfor10min)	2047 277	93.50	6.50

3. **Oxidationdegradationstudy:**

FigureNo.8–Chromatogramofoxidation

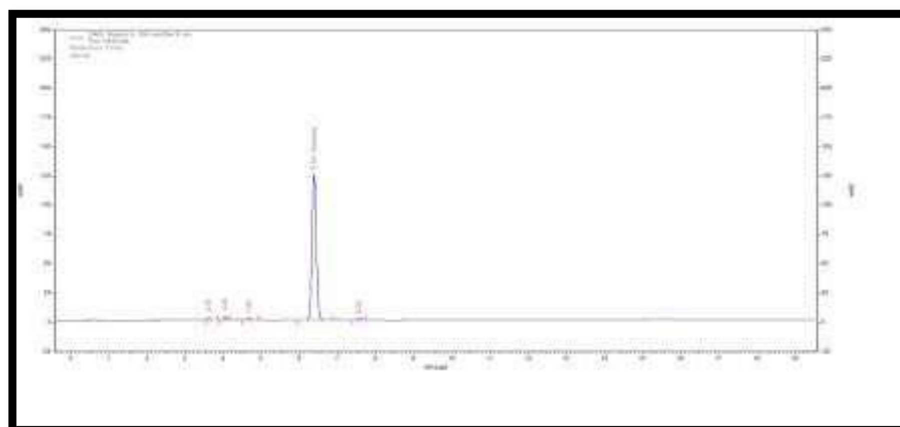


Result-TableNo.7–ResultsofOxidationdegradationstudy

Sample	Area	Assay	% Degradation
Oxidation(1mlof30%H2O2@RT for10min)	2069173	94.50	5.50

4. **DryHeatdegradationstudy**

FigureNo.9-Chromatogramofdryheat



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Result-TableNo.8–ResultofDryHeatdegradationstudy

Sample	Area	Assay	%Degradation
DryHeat(@60°Cfor5 hrs)	2108586	96.30	3.70

5. photolysisdegradationstudy:

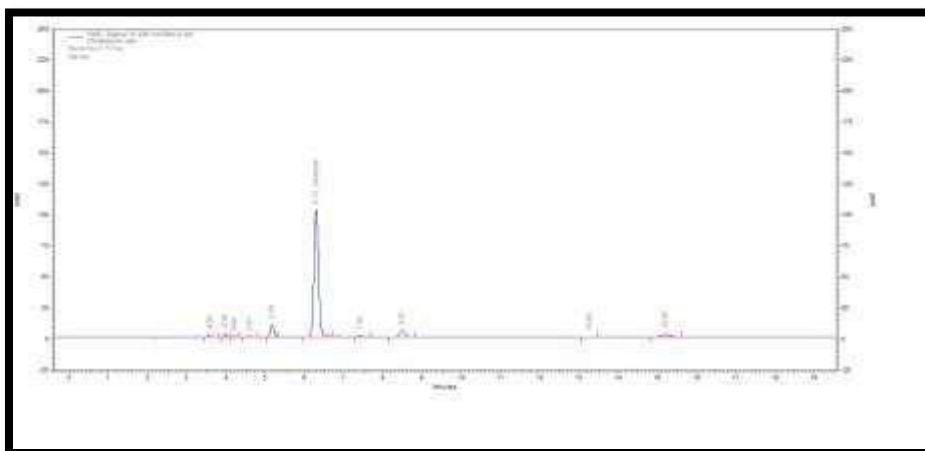

Figure No. 10- Chromatogram of photolysisResult-

TableNo9–Resultofphotolysisdegradationstudy

Sample	Area	Assay	%Degradation
UV(@254nmfor5hrs)	1885247	86.10	13.90

III. CONCLUSION:

In the present research, a fast, simple, accurate, precise, and linear HPLC method has beendeveloped and validated for nitrofurantoin, and hence it can be employed for routine qualitycontrol analysis.Analytical method conditions and the mobile phase solvents provided goodresolution for nitrofurantoin. In addition, the main features of the developed method are shortrun time and retention time around 6.75 min. Method was validated in accordance with ICHguidelines. Method is robust enough to reproduce accurate and precise results under differentchromatographicconditions.

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