IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
Volume 2, Issue 1, October 2022
Impact Factor: 6.252

Stability-Indicating RP-HPLC Method Development and Validation for Simultaneous Estimation of Gliclazde and Chromium Picolinate in Bulk and Formulations

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Abstract: *Simultaneous determination of Gliclazide and Chromium Picolinate two component methods has been developed in methanol. In this method, the overlapping spectra of Gliclazide and Chromium Picolinate were well resolved by making use of the zero order spectra of their direct absorption spectra. The method was based on the measurement of absorbance of Gliclazide and Chromium Picolinate at 272.00nm, 262.00 nm and 272.05nm respectively. This method obeyed Beer's law in the concentration range of 0 to 120 µg/mL for Gliclazide and Chromium Picolinate. The method was validated as per the ICH guideline and accuracy, precision are found to be within the acceptable limit. The limits of detection and quantitation were found to be 0.023 and 0.070 µg/ml, respectively for Gliclazide, and 0.021 and 0.063 µg/ml, respectively for Chromium Picolinate . A simple, specific, accurate and precise Simultaneous Equation Method for three Components Spectrophotometric methods was developed and validated for simultaneous estimation of Gliclazide and Chromium Picolinate in synthetic mixture. This method is validated as per ICH Q2R1Guidelines.*

Keywords: Simultaneous determination, Gliclazide and Chromium Picolinate

I. INTRODUCTION

Pharmaceutical analysis may be defined as a application of analytical procedures used to determine the purity, safety and quality of drug and chemicals .pharmaceutical analysis includes both quantitative and qualitative analysis of drug and pharmaceutical substances starts from bulk drug to the finished dosage forms

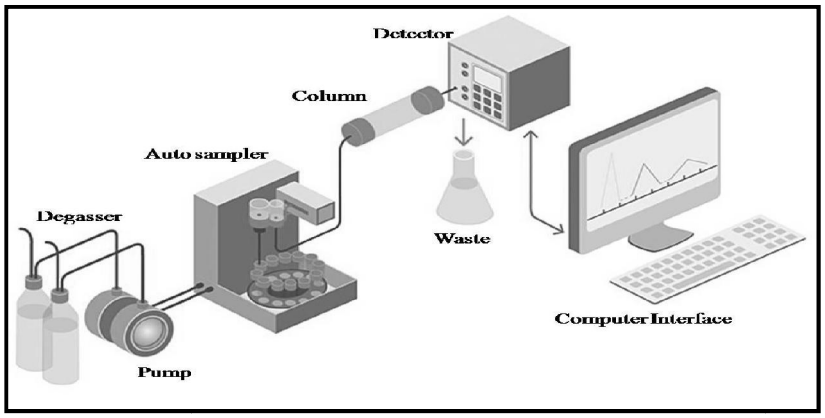

Figure 1: Instrumentation of HPLC

Chromatography is defined as a non- destructive procedure for resolving multi-component mixture of trace, minor, or major constituents into its individual fractions. In chromatography, the sample is dissolved in the mobile phase which may be a gas, liquid, or a supercritical fluid.

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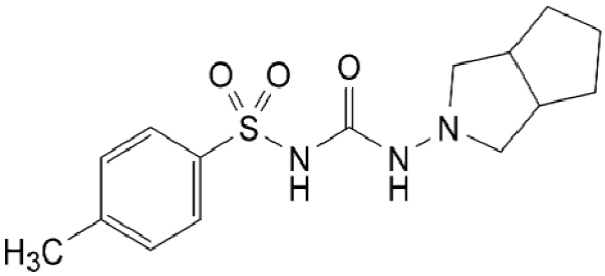
The principle involved in HPLC is that when a mixture containing different compounds is introduced into the mobile phase and allowed to flow over a stationary phase, the individual compounds travel at different speeds and get separated based on the relative affinities to the stationary phase and the mobile phase

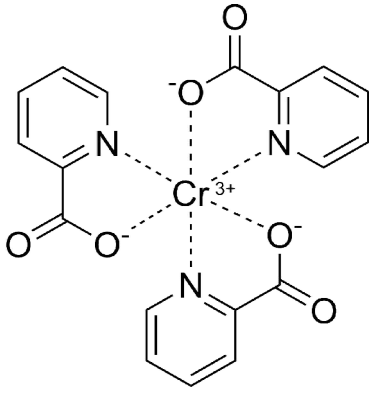
The compounds are separated based on the polarity of the stationary phase and the mobile phase.

DRUG PROFILE-GLICLAZIDE:



NAME	GLICLAZIDE
IUPAC NAME	1-(hexahydrocyclopenta[*c*]pyrrol-2(1*H*)-yl)-3-[(4-methylphenyl)sulphonyl]urea
Molecular Formula:	$C_{15}H_{21}N_3O_3S$
Molar mass	323.4 g/mol
Description	A white or almost white powder,
Solubility:	practically insoluble in water, freely soluble in methylene chloride, sparingly soluble in acetone, slightly soluble in alcohol.
Pharmacokinetic data	
Bioavailability:	88 % if taken orally
Metabolism:	Gastrointestinal and hepa
Biological half life	10.4 hours
Therapeutic uses:	**Therapeutic uses:** Gliclazide is used for control of hyperglycemia in gliclazide-responsive diabetes mellitus of stable, mild, non-ketosis prone, type 2 diabetes. It is used when diabetes cannot be controlled by proper dietary management and exercise or when insulin therapy is not appropriate. National Kidney Foundation (2012 Update) claims that Gliclazide does not require dosage uptitration even in end stage kidney disease.
TABLETS	**Gycor-C**

DRUG PROFILE-Chromium picolinate:



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IUPAC NAME	Tris(picolinate)chromium(III)
Formula:	$Cr(C_6H_4NO_2)_3$
Molar mass:	418.33 g/mol
Description:	Pinkish red crystalline compound,
Solubility	poorly soluble in water, freely soluble in methanol,
Pharmacokinetic data	
Metabolism	hepatic
Biological half-life:	**:** 6 days
Therapeutic uses:	There are claims that the picolinate form of chromium supplementation aids in reducing insulin resistance, particularly in type 2 diabetics, but a meta-analysis of chromium(III) supplementation studies showed no association between chromium and glucose or insulin concentrations for non-diabetics, and inconclusive results for diabetics.
Tablets	Gycor-C

II. MATERIALS AND METHOD

S.no	Material Name	Potency
1	Combination of Gliclazide and Chromium picolinate tablets(Glycor-C)	NA

2.1 Instruments

S.NO	INSTRUMENT	MAKE AND MODEL
1	HPLC	WATERS HPLC 2965 SYSTEM
2	UV-VIS spectrophotometer	T60 with special bandwidth of 2mm and 10mm
3	matched quartz	measuring absorbance
4	PH METER	METLER TOLEDO
5	sonicator	spectrochem
6	Analytical balance	Metler toledo

2.2 Reagents and Chemicals

S. No	Name of the material	Grade	Make
1	distilled water	Milli Q	MERCK
2	Acetonitrile	HPLC grade	MERCK
3	phosphate buffer	AR	Merck
4	ammonium acetate buffer	AR	Merck
5	glacial acetic acid	AR	Merck
6	methanol	HPLC	Merck
7	potassium dihydrogen phosphate buffer	AR	Merck
8	tetra hydrofuran	AR	Merck
9	tri ethyl amine	AR	Merck
10	ortho-phosphoric acid	AR	Merck

2.3 Aim and Objective

According to the literature survey it was found that few analytical methods on simultaneous estimation of Gliclazide and Chromium picolinate by using HPLC were reported. The objective of the proposed method is to develop simple and accurate method for the simultaneous estimation of Gliclazide and Chromium picolinate in pharmaceutical dosage forms by RP-HPLC.

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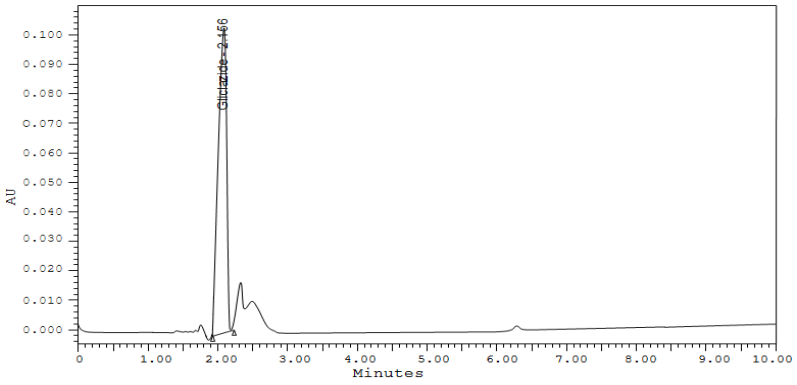
Method development by RP-HPLC method for Gliclazide and Chromium picolinate

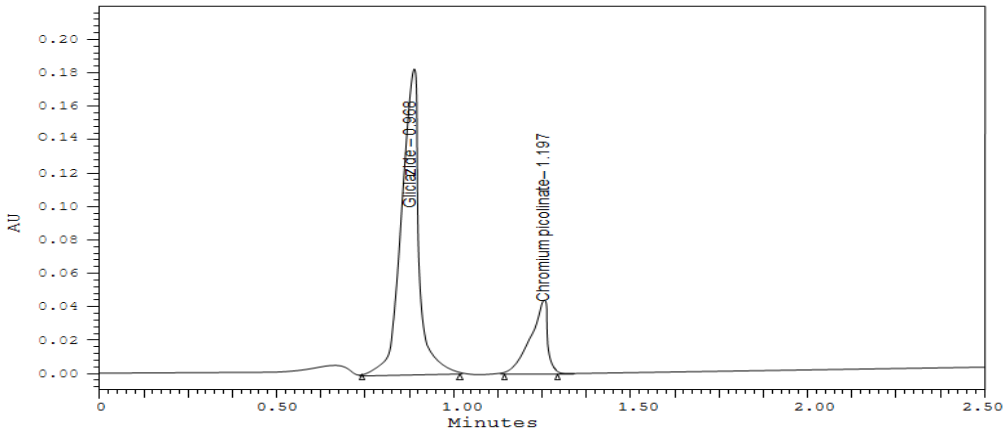
- Development of suitable mobile phase.
- Optimization of the chromatographic conditions.
- Selection of suitable detection wavelength.
- Preparation of standard calibration curve of Gliclazide and Chromium picolinate.
- Assay of pure mixed standards and formulation.
- Validation of the developed method.
- The parameters that will be validated are
- Linearity and Range
- System suitability
- Precision
- Accuracy
- Specificity
- LOD and LOQ
- Robustness
- Stability studies
- Ruggedness

2.4 Method Development Parameters

- **Method Development:** Many trials were done by changing columns and Mobile phases and were reported below:
- **Trial 1:** This trial was run through BDS 150 column with mobile phase composition of 60:40A Buffer and Acetonitrile, Flow rate set at 1ml/min. **Observation:** Chromium picolinate was not eluted.



- **Trial 2:** This trial was run through std Kromasil 150 column with mobile phase composition of 40:60 Buffer and Acetonitrile, Flow rate set at 1ml/min. **Observation:** Chromium picolinate was not eluted.



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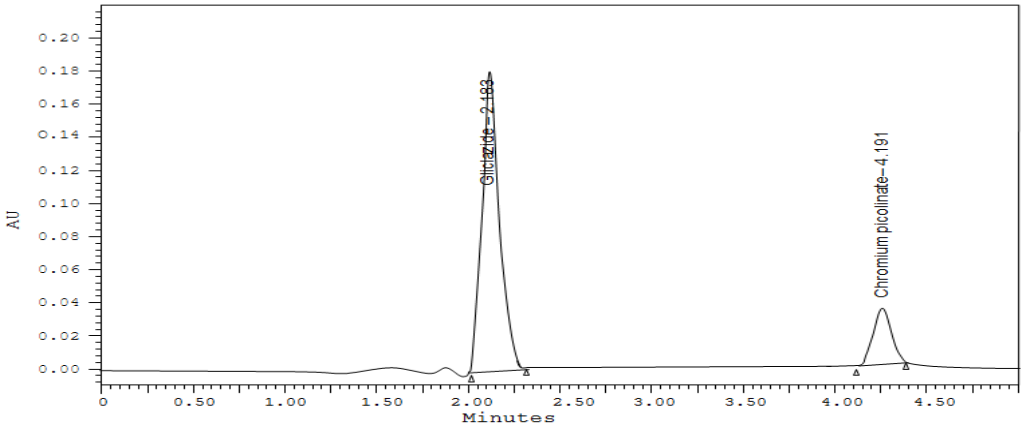
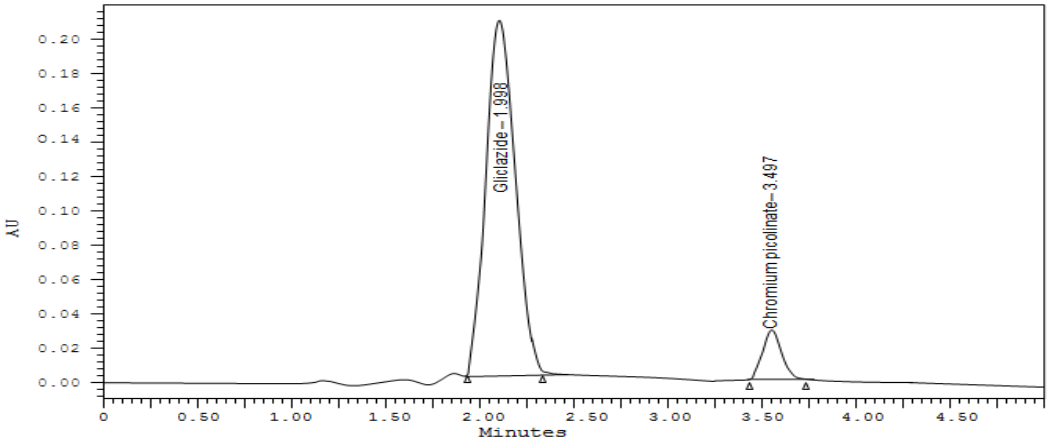
- **Trial 3:** This trial was run through Kromasil 150 column with mobile phase composition of 45:55A Buffer and Acetonitrile, Flow rate set at 1ml/min.



Observation: retention time needs to be decreased, so further trials are carried out

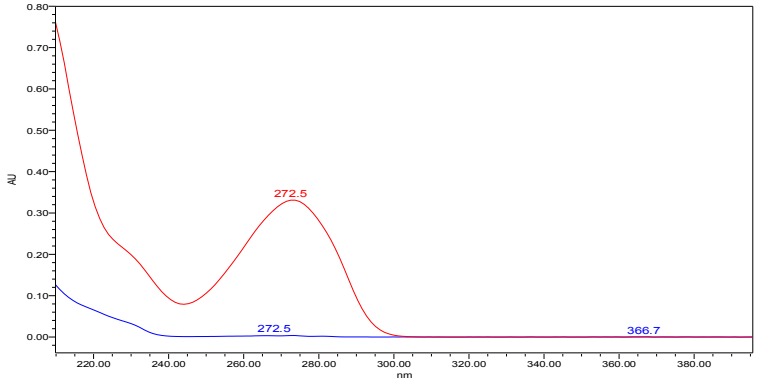
- **Trial 4:** This trial was run through Kromasil 150mm column with mobile phase composition of 50:50 Buffer and Acetonitrile, Flow rate set at 1ml/min.



Observation: Retention time need to decrease so further trials are carried out

III. SELECTION OF WAVELENGTH

- The standard & sample stock solutions were prepared separately by dissolving standard & sample in a solvent in mobile phase diluting with the same solvent.(After optimization of all conditions) for UV analysis.
- It scanned in the UV spectrum in the range of 200 to 400nm.
- The isobestic point was found to at 272.5 nm



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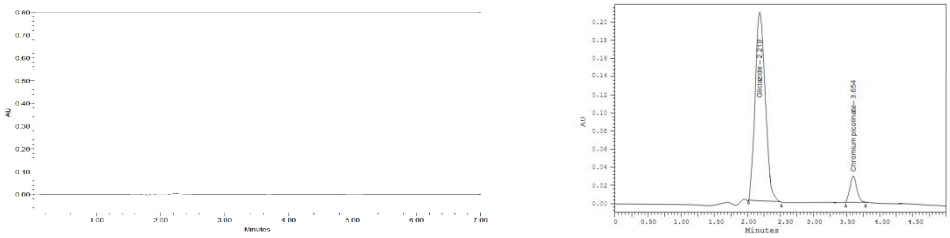
IV. RESULTS

- **System suitability:** All the system suitability parameters are within range and satisfactory as per ICH guidelines

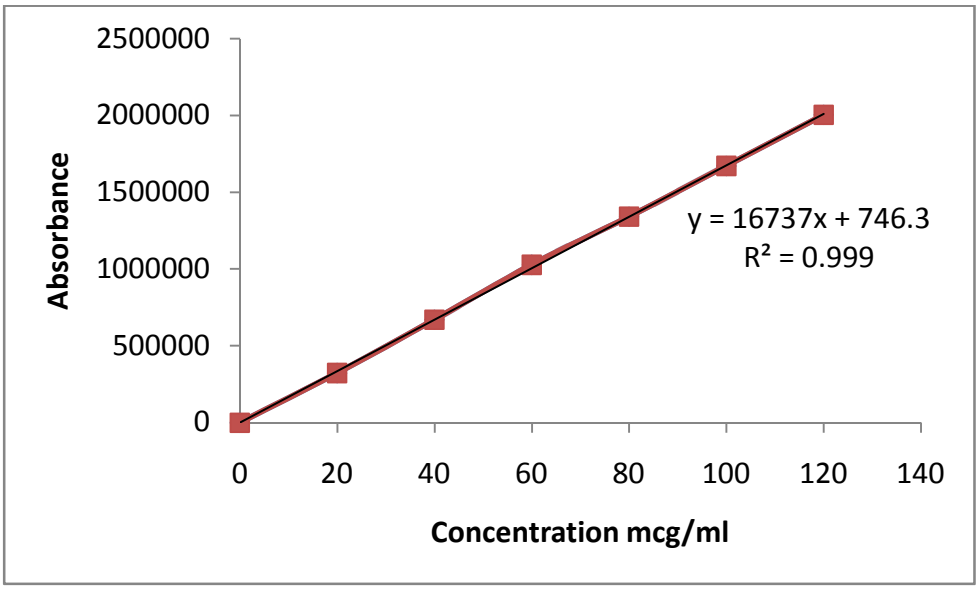
Property	Gliclazide	Chromium picolinate
Retention time (tR)	2.219 min	3.654 min
Theoretical plates (N)	7195 ± 63.48	7726 ± 63.48
Tailing factor (T)	1.11 ± 0.117	1.08± 0.117



Chromatogram of blank. Typical chromatogram of Gliclazide and Chromium picolinate

Linearity: Six Linear concentrations of Gliclazide (20-120μg/ml) and Chromium picolinate (2.5-15 μg/ml) are prepared and Injected. Regression equation of the Gliclazide and Chromium picolinate are found to be, y = 16737x + 746.3. and y = 28583x + 209.3. And regression co-efficient was 0.999

S.no	Concentration Gliclazide (µg/ml)	Response	Concentration Chromium picolinate (µg/ml)	Response
1	0	0	0	0
2	20	323146	2.5	69979
3	40	670379	5	142646
4	60	1027529	7.5	215116
5	80	1340660	10	291260
6	100	1670681	12.5	357075
7	120	2002526	15	426005



Calibration curve of Gliclazide

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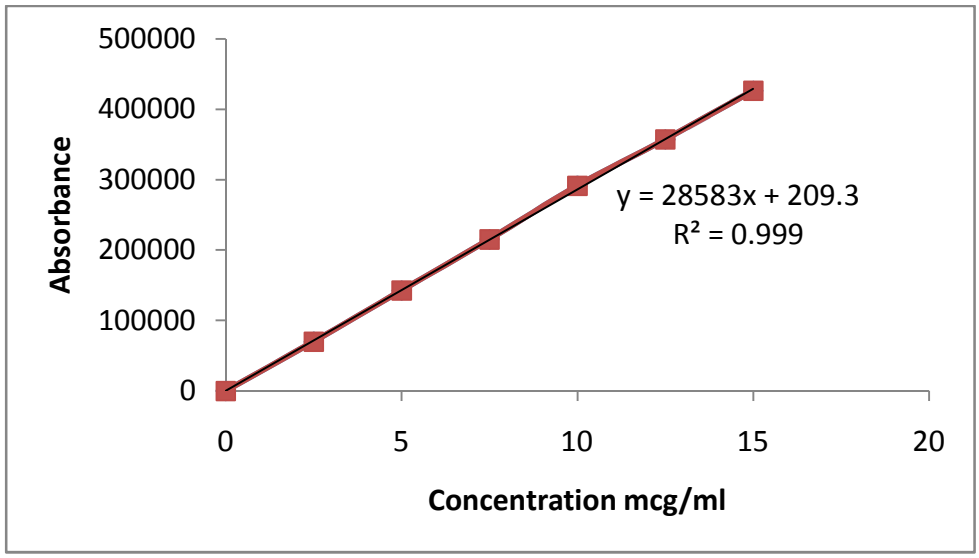
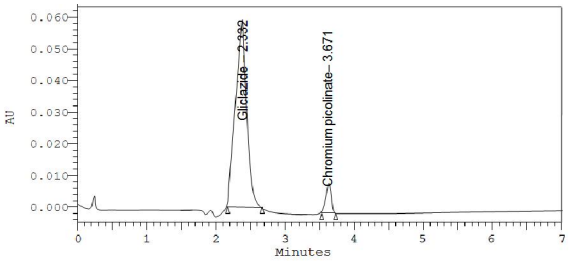
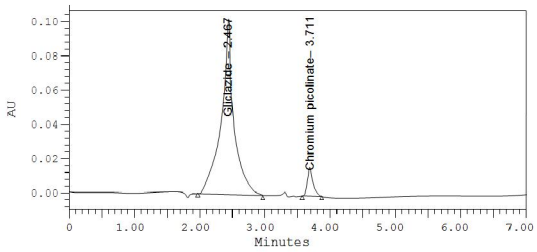
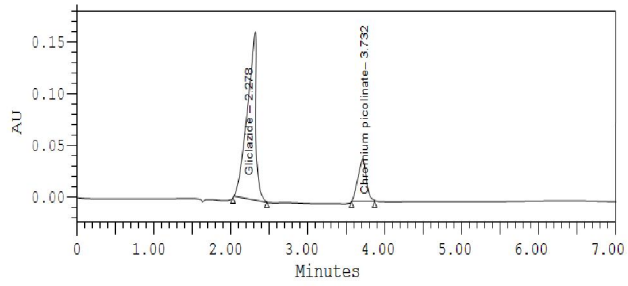


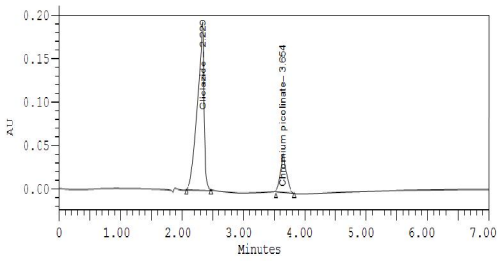
Calibration curve of Chromium picolinate



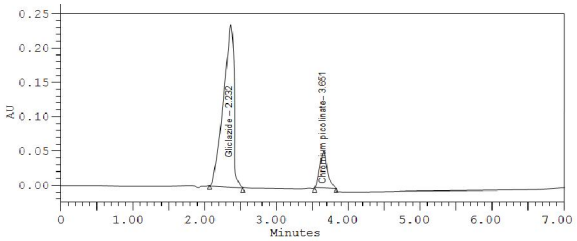


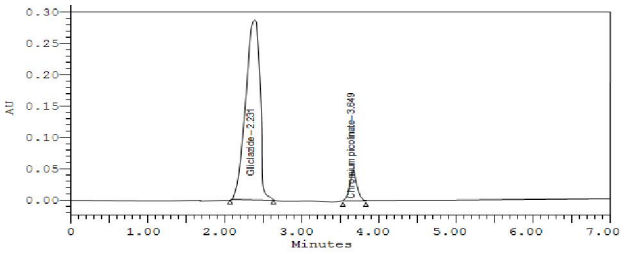
Linearity 25% Chromatogram of Gliclazide Linearity 50% Chromatogram of Gliclazide and Chromium picolinate and Chromium picolinate





Linearity 75% Chromatogram of Gliclazide Linearity 100% Chromatogram of Gliclazide and Chromium picolinate and Chromium picolinate





Linearity 125% Chromatogram of Gliclazide Linearity 150% Chromatogram of Gliclazide and Chromium picolinate and Chromium picolinate

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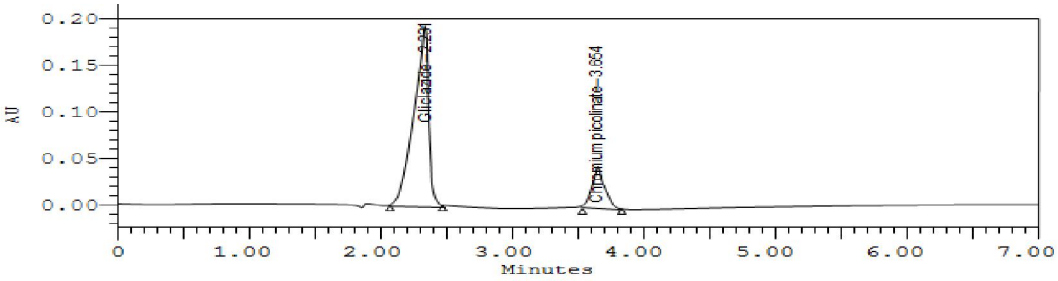
Intraday precision (Repeatability): Intraday Precision was performed and % RSD for Gliclazide and Chromium picolinate were found to be 0.69% and 0.93% respectively.

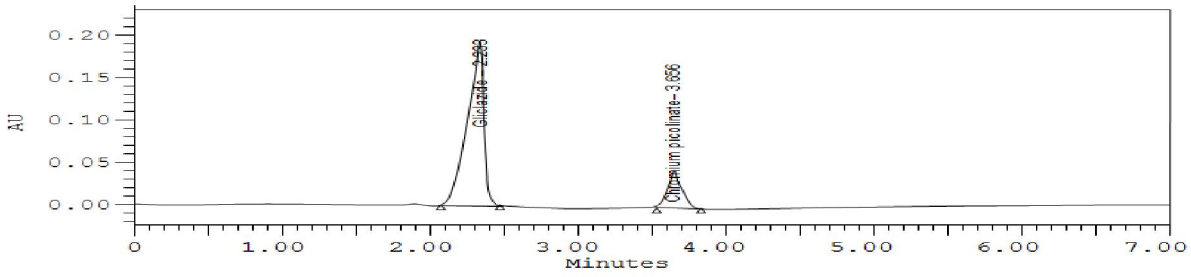
Sr. No.	Gliclazide	Chromium Picolinate
1	1231433	258600
2	1251013	261077
3	1249556	260998
4	1252836	260994
5	1242594	255872
6	1237103	256435
Mean	1244089	258996
Std. Dev.	8550.5	2400.2
%RSD	0.69	0.93



Repeatability Chromatogram of Gliclazide and Chromium picolinate

Precision: **Inter day precision:** Inter day precision was performed with 24 hrs time lag and the %RSD Obtained for Gliclazide and Chromium picolinate were 0.26% and 0.23%.

Sr. No.	Gliclazide	Chromium Picolinate
1	930350	904477
2	930036	907116
3	929207	906543
4	933876	909811
5	926248	905070
Mean	929443	903981
Std. Dev.	929860	906166.3
%RSD	2451	2152.153
%RSD	0.69	0.93



Inter Day precision Chromatogram of Gliclazide and Chromium picolinate.

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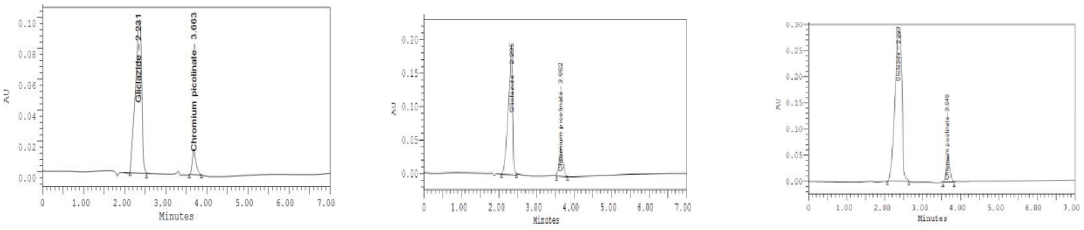
Accuracy:

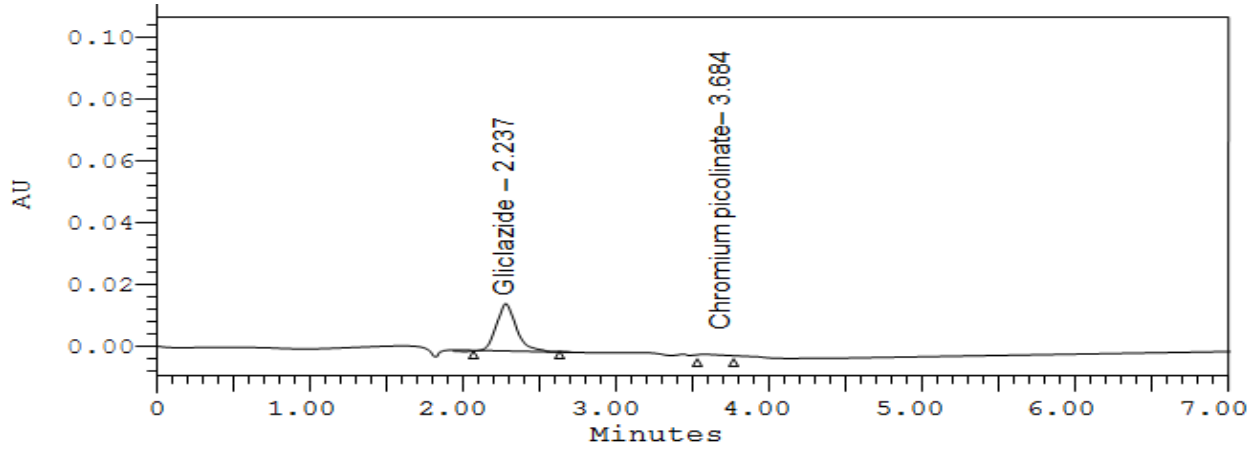
Sample	Amount added (µg/ml)	Amount Recovered (µg/ml)	Recovery (%)	% RSD
Gliclazide	40	40.08	100.22	0.75
	80	80.49	100.62	1.09
	120	121.33	101.18	0.23
Chromium picolinate	5	5.05	101.11	0.60
	10	10.12	101.29	0.83
	15	15.11	100.75	1.35

Accuracy Chromatograms of Gliclazide and Chromium picolinate



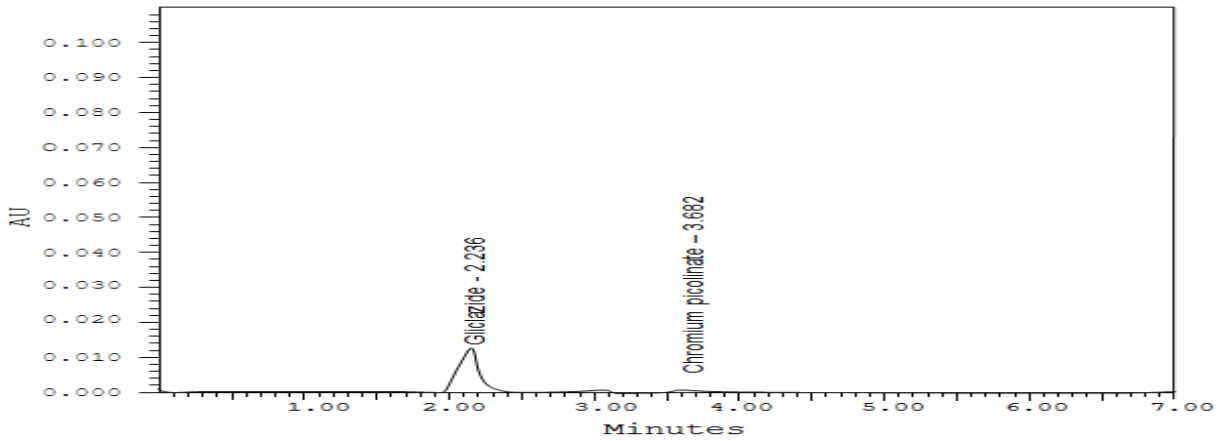
50% 100% 150%

LOD: Limit of ditection was calculated by Gliclazide and Chromium picolinate and LOD for Gliclazide and Chromium picolinate were found to be 0.03 and 0.05 respectively.



LOD Chromatogram of Gliclazide and Chromium picolinate

LOQ: Limit of Quantification was calculated for Gliclazide and Chromium picolinate and LOQ for Gliclazide and Chromium picolinate were found to be 0.10 and 0.16 respectively.



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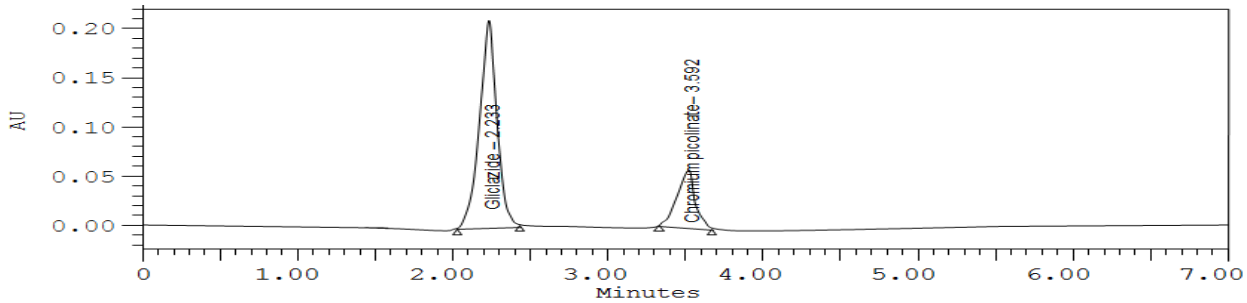
Robustness: Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guide lines.

S. NO	Robustness condition	Gliclazide %RSD	Chromium picolinate % RSD
1	Flow minus	0.6	1.4
2	Flow Plus	0.5	0.6
3	Mobile phase minus	0.8	0.4
4	Mobile phase Plus	1.3	0.6
5	Temperature minus	0.8	0.5
6	Temperature Plus	0.4	0.4

Robustness data of Gliclazide and Chromium picolinate

Assay: Standard preparations are made from the API and Sample Preparations are from Formulation. Both sample and standards are injected six homogeneous samples. Drug in the formulation (Glycor-c Tablet) was estimated by taking the standard as the reference. The Average %Assay was calculated and found to be 100.19% and 100.51% for Gliclazide and Chromium picolinate respectively.

S. No.	Gliclazide %Assay	Chromium picolinate %Assay
1	100.1641	100.3601
2	101.7567	101.3214
3	101.6382	101.2907
4	101.905	101.2892
5	101.0719	99.30136
6	100.6253	99.51986
AVG	101.19	100.51
STDEV	0.70	0.93
%RSD	0.69	0.93



Summary Table:

Parameters	Gliclazide	Chromium picolinate
Calibration range (mcg / ml)	20-120ppm	2.5-15ppm
Optimized wavelength	272nm	272nm
Retention time	2.232min	3.659min
Regression equation (Y*)	y = 16737x + 746.3	y = 28583x + 209.3
Correlation coefficient(r2)	0.999	0.999

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Precision (% RSD*)	0.69	0.93
% Recovery	101.19%	100.51%
Limit of Detection (mcg / ml)	0.03ppm	0.05ppm
Limit of Quantitation (mcg / ml)	0.10ppm	0.16ppm

V. CONCLUSION

A simple, Accurate, precise method was developed for the simultaneous estimation of the Gliclazide and Chromium picolinate in Tablet dosage form (Glycor-c Tablet). Retention time of Gliclazide and Chromium picolinate were found to be 2.232min and 3.659min. %RSD of the Gliclazide and Chromium picolinate were and found to be 0.69 and 0.93 respectively. %Recover was Obtained as 101.19% and 100.51% for Gliclazide and Chromium picolinate respectively. LOD, LOQ values are obtained from regression equations of Gliclazide and Chromium picolinate were 0.03ppm, 0.10ppm and 0.05ppm, 0.16ppm respectively. Regression equation of Gliclazide and Chromium picolinate y = 16737x + 746.3. And y = 28583x + 209.3. Retention times are decreased and that run time was decreased so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

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