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ANAYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF DAPAGLIFLOZIN, GLICLAZIDE AND METFORMIN IN PHARMACEUTICAL DOSAGE FORM BY RP-HPLC

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Abstract:

The present study focuses on the development and validation of a simple, accurate, precise, and robust reverse-phase high-performance liquid chromatography (RP-HPLC) method for the simultaneous estimation of Dapagliflozin, Gliclazide, and Metformin in pharmaceutical dosage forms. These drugs are widely used in the management of type 2 diabetes mellitus and are available in various combination formulations. The proposed analytical method was developed by optimizing chromatographic conditions, including stationary phase, mobile phase composition, flow rate, detection wavelength, injection volume, and run time, to achieve satisfactory separation of the selected drugs. Chromatographic separation was achieved using a suitable reversed-phase C18 column with an optimized mobile phase under isocratic conditions. The developed method was validated according to International Council for Harmonization (ICH) guidelines with respect to specificity, linearity, accuracy, precision, robustness, limit of detection, and limit of quantification. The method demonstrated good linearity over the selected concentration ranges, with satisfactory recovery and precision. No significant interference was observed from excipients present in the pharmaceutical dosage form. The developed method was found to be rapid, economical, reproducible, and suitable for routine quality control analysis of Dapagliflozin, Gliclazide, and Metformin in combined pharmaceutical formulations. The method may also provide a reliable analytical approach for quality assessment during formulation development and stability studies.

Keywords: Dapagliflozin; Gliclazide; Metformin Hydrochloride; RP-HPLC; Analytical Method Development; Method Validation; Pharmaceutical Dosage Form; Simultaneous Estimation; Antidiabetic Drugs; ICH Guidelines; Quality Control

1. INTRODUCTION – DRUGS

Dapagliflozin is an orally active antidiabetic drug belonging to the sodium-glucose co-transporter-2 (SGLT-2) inhibitor class. It is primarily used in the management of type 2 diabetes mellitus and is also used in selected patients with heart failure and chronic kidney disease. Dapagliflozin selectively inhibits SGLT-2 present in the proximal renal tubules, thereby reducing glucose reabsorption and increasing urinary glucose excretion. Its molecular formula is $C_{21}H_{25}ClO_6$, with a molecular weight of approximately 408.88 g/mol. It is available mainly as oral tablets in strengths of 5 mg and 10 mg.

Gliclazide is an oral hypoglycemic agent belonging to the second-generation sulfonylurea class. It is used for the treatment of type 2 diabetes mellitus. Gliclazide acts mainly by stimulating insulin secretion from pancreatic β-cells. It binds to sulfonylurea receptors on pancreatic β-cells, resulting in closure of ATP-sensitive potassium channels, membrane depolarization, calcium influx, and subsequent release of insulin. Its molecular formula is $C_{15}H_{21}N_3O_3S$, and its molecular weight is approximately 323.41 g/mol. Gliclazide is commonly available as immediate-release and modified-release oral tablets.

Metformin hydrochloride is a widely used oral antidiabetic drug belonging to the biguanide class. It is considered a first-line pharmacological treatment for type 2 diabetes mellitus. Metformin primarily decreases hepatic glucose production and improves peripheral insulin sensitivity, thereby enhancing glucose uptake and utilization. It does not usually stimulate insulin secretion and therefore has a relatively low risk of hypoglycemia when used alone. Its molecular formula is $C_4H_{11}N_5 \cdot HCl$, with a molecular weight of approximately 165.63 g/mol. It is available in immediate-release and extended-release tablet formulations in different strengths.

The simultaneous presence of Dapagliflozin, Gliclazide, and Metformin in pharmaceutical formulations creates an important requirement for a reliable analytical method capable of separating and quantifying all three drugs accurately. Therefore, the development and validation of an RP-HPLC method provides a suitable approach for routine quality-control analysis of these drugs in combined pharmaceutical dosage forms.

2. DRUG PROFILE

Dapagliflozin

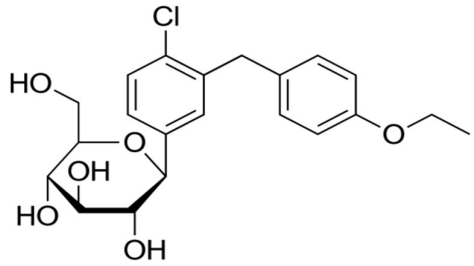

Figure : Molecular Structure of Dapagliflozin

Molecular formula : $C_{21}H_{25}ClO_6$ Molecular weight : 408.87 g/mol

Pharmacokinetic Data

Bioavailability : 78%

Protein binding : 91%

Metabolism : Hepatic

Excretion : 75% Renal, 21-25% Feces

Gliclazide

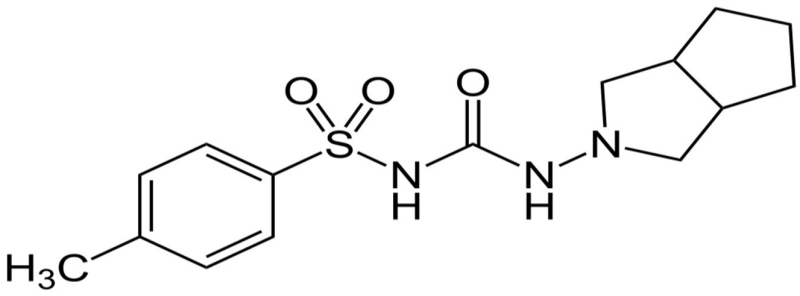

Figure : Molecular Structure of Gliclazide

IUPAC Name : 1-(3,3a,4,5,6,6a-hexahydro-1H-cyclopenta[c]pyrrol-2-yl)-3-(4-methylphenyl)sulfonylurea

Molecular formula : $C_{15}H_{21}N_3O_3S$ Molecular weight : 323.41 g/mol

Pharmacokinetic Data

Bioavailability : 79%

Protein binding : 95%

Metabolism : Hepatic

Excretion : 60-70% Renal, 10-20% Feces

Metformin

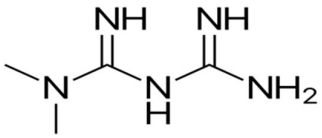

Figure: Molecular Structure of Metformin

IUPAC Name : 3-(diaminomethylidene)-1,1-dimethylguanidine

Molecular formula : $C_4H_{11}N_5$

Molecular weight : 129.16 g/mol

Pharmacokinetic Data

Bioavailability : 50-60%

Protein binding : 0-4%

Metabolism : Renal

Excretion : Renal

3. REVIEW OF LITERATURE

Sharma et al., (2024) have reported a robust and sensitive RP-HPLC method for simultaneous estimation of Dapagliflozin and Metformin in pharmaceutical dosage forms. The chromatographic separation was achieved on a C18 column using UV detection at 230 nm. The mobile phase composed of phosphate buffer (pH 3.0) and acetonitrile (55:45, v/v) delivered at

1.0 ml/min. Linearity was observed in the range of 2–20 µg/ml for Dapagliflozin and 10–150 µg/ml for Metformin (R^2 = 0.9998). The method was successfully applied for assay of commercial tablets without interference from excipients.[1]

Reddy et al., (2023) have developed a validated RP-HPLC method for the estimation of Gliclazide and Metformin in combined dosage forms. Separation was achieved using a C18 column with UV detection at 254 nm. The mobile phase consisted of methanol and phosphate buffer (pH 4.5) in the ratio 60:40 (v/v). Linearity was found to be 5–50 µg/ml for Gliclazide and 20–200 µg/ml for Metformin (R^2 = 0.9995). The method was accurate, precise and suitable for routine QC analysis.[2]

Patel et al., (2023) have reported a stability-indicating RP-HPLC method for quantitative estimation of Dapagliflozin in bulk drug. Chromatographic separation was performed using a C18 column with UV detection at 225 nm. The mobile phase comprised acetonitrile and water (70:30, v/v). Linearity was obtained between 1–40 µg/ml (R^2 = 0.9997). Forced degradation studies confirmed stability-indicating capability.[3]

Singh et al., (2022) have developed a precise RP-HPLC method for estimation of Gliclazide in tablet dosage form. Separation was carried out on a C18 column at 226 nm using a mobile phase of acetonitrile and water (50:50, v/v). Linearity was observed from 2–32 µg/ml (R^2 = 0.9994). The method was successfully applied for marketed product analysis.[4]

Mehta et al., (2022) have reported a validated RP-HPLC method for simultaneous analysis of Dapagliflozin, Gliclazide and Metformin. A C18 column with UV detection at 210 nm was used. The mobile phase consisted of phosphate buffer (pH 3.5) and methanol (45:55, v/v). Linearity was observed within 5–30 µg/ml for all analytes ($R^2 > 0.999$). The method proved suitable for triple-drug formulation analysis.[5]

Basha et al., (2021) have developed a simple RP-HPLC method for the estimation of Metformin in bulk drug using a C18 column and UV detection at 233 nm. The mobile phase used was phosphate buffer (pH 6.8) and acetonitrile (75:25, v/v). Linearity was found to be 10–150 µg/ml ($R^2 = 0.9993$). Excipients did not interfere with the assay.[6]

Choudhary et al., (2021) have reported an RP-HPLC method for simultaneous quantification of Dapagliflozin and Gliclazide. Separation was attained on a C18 column with mobile phase methanol:water (70:30, v/v) at 1.0 ml/min. Detection was carried out at 210 nm. Linearity was achieved between 2–25 µg/ml for both drugs ($R^2 > 0.999$).[7]

Jain et al., (2020) have reported a validated RP-HPLC method for analysis of Metformin and Dapagliflozin. A C18 column was used with UV detection at 240 nm. The mobile phase was ammonium acetate buffer and acetonitrile (50:50, v/v). Linearity was observed over 5–30 µg/ml for Metformin and 1–15 µg/ml for Dapagliflozin ($R^2 = 0.9996$).[8]

4. EXPERIMENTAL

Chemicals and Reagents

Table No.1: List of Chemicals and reagents

S.NO.	NAME	MANUFACTURER	GRADE
1.	Dapagliflozin, Gliclazide and Metformin	Eris Lifesciences Ltd	-
2.	Dummy pellets 16 # 20	Jackson	-
3.	Disodium hydrogen orthophosphate	Merck	GR
4.	Ortho phosphoric acid	Merck	GR
5.	Acetontrile	Merck	HPLC

6.	0.45 µm Nylon filter	Axivia	S0761009

Equipment/Instrument Details

Table No.2: List of Equipment/Instrument details

S.NO.	INSTRUMENT NAME	MODEL
1.	HPLC system	Agilent 1220 Infinity LC(G4288C)
2.	Analytical balance	Shimadzu
3.	pH Meter	Thermo electron corporation orion 2 star
4.	Sonicator	Ultrasonic cleaner power sonic 420

RESULTS AND DISCUSSION

S.No.	Parameter	Value
1	Column	Inertsil- ODS C18 (250mm×4.6mm, Particle size 5µm)
2	Mobile Phase	Water (PH 5.2 adjusted with sodium acetate) and methanol in the ratio of 600:400 (v/v)
3	Flow Rate	1.0 ml/min.
4	Diluent	Mobile phase
5	Column Temp.	25 ℃
6	PH	5.2
7	API Concentration	Dapagliflozin – 20µg/ml Glimepiride – 20µg/ml Metformin – 5µg/ml
8	Run Time	6 min.
9	Rentention Time	Dapagliflozin – 1.4 min. Glimepiride – 2.2 min. Metformin – 4.4 min.

10	Volume of Injection	10µL
11	Detection wave Length	247nm

Table 3: Optimized Chromatographic conditions

S.No.	Concentration (µg/mL)	Peak area	
1	10.00	1877189	Slope =37490 C.C = 0.99 (≈1.0)
2	15.00	2812563	
3	20.00	3747683	
4	25.00	4688354	
5	30.00	5621489	

Table 4: Linearity data of Dapagliflozin

S.No.	Concentration (µg/mL)	Peak area	
1	10.00	3099184	Slope =61968 C.C = 0.99 (≈1.0)
2	15.00	4642641	
3	20.00	6197340	
4	25.00	7744800	
5	30.00	9298119	

Table 5: Linearity data of Gliclazide

S.No.	Concentration (µg/mL)	Peak area	
1	2.5	1793210	Slope =35929 C.C = 0.99
2	3.75	2694269	

3	5.00	3593031	(≈1.0)
4	6.25	4491038	
5	7.5	5390636	

Table 6: Linearity data of Metformin Hydrochloride

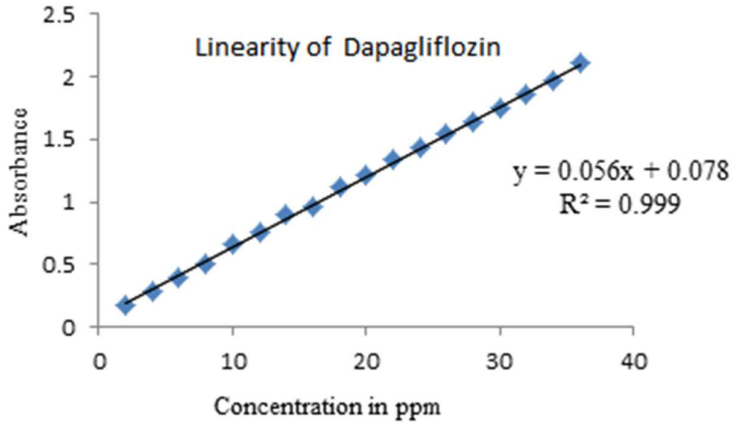

Fig. : Linearity Curve for Dapagliflozin

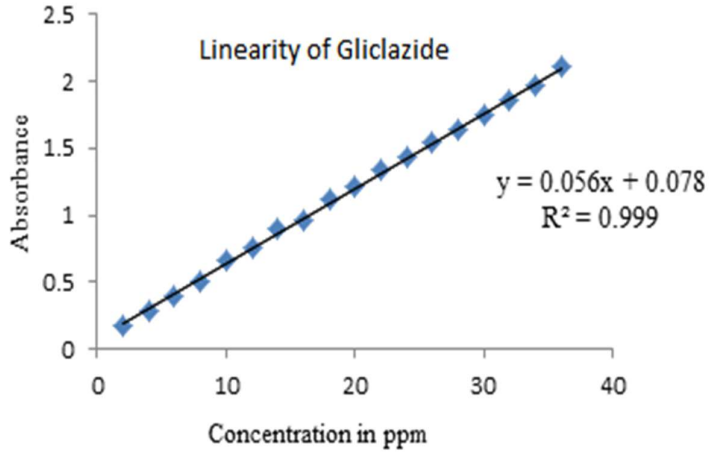

Fig. : Linearity Curve for Gliclazide

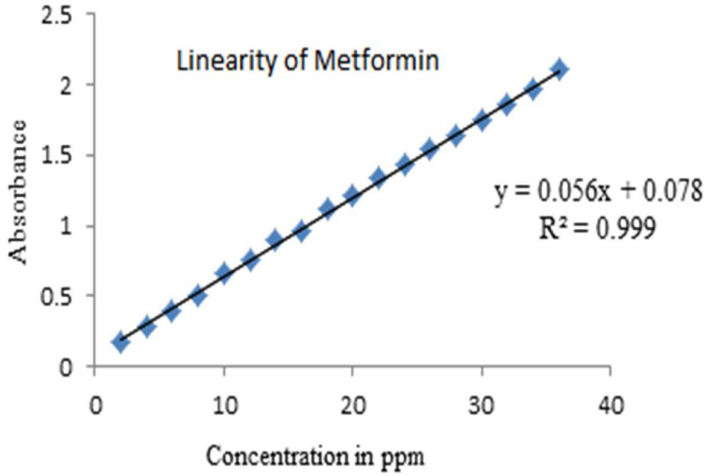

Fig. : Linearity Curve for Metformin

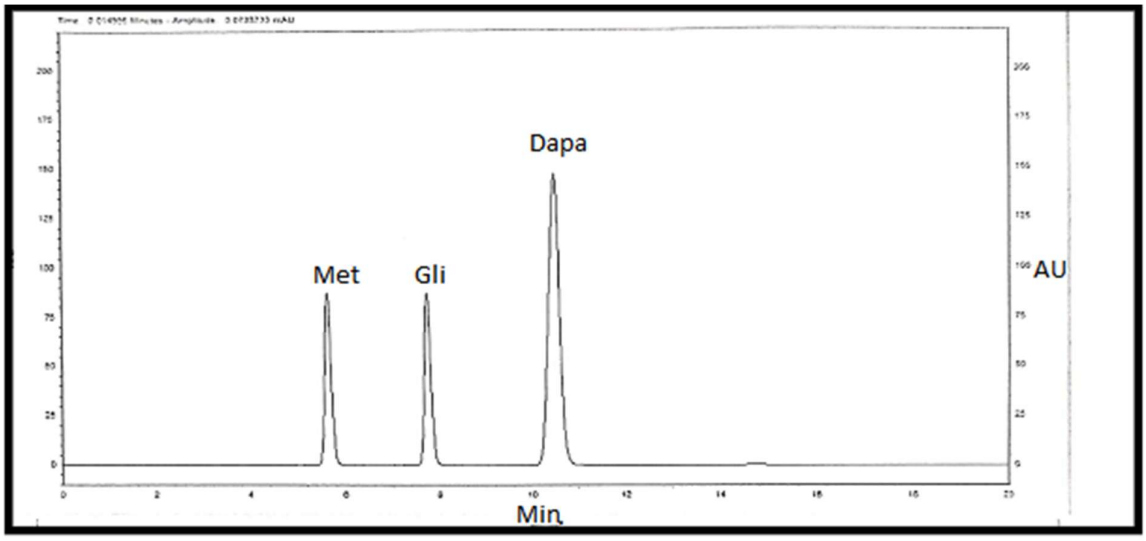

Fig.: Overlay chromatogram of Linearity for Dapagliflozin, Gliclazide and Metformin

S. No	Sample Weight (mg)	Dapagliflozin	Gliclazide	Metformin	%Assay (Dapagliflozin)	%Assay(Gliclazide)	% Assay(Metformin)
1	482.20	3746397	6199675	3595004	99	100	99

2	482.20	3746266	6195760	3592678	99	100	99
3	482.20	3741869	6197389	3590231	99	100	99
4	482.20	3740761	6199444	3591778	99	100	99
5	482.20	3740569	6195273	3599453	99	100	99
6	482.20	3749990	6195000	3593793	99	100	99
Average Assay					99	100	99
STD					0.10	0.03	0.09
% RSD					0.10	0.03	0.09

Table 8: Intra-day precision of Dapagliflozin, Gliclazide and Metformin

S. No	Sample Weight (mg)	Dapagliflozin	Gliclazide	Metformin	%Assay (Dapagliflozin)	%Assay(Gliclazide)	% Assay(Metformin)
1	482.20	3746548	6198328	3595456	99	100	99
2	482.20	3745985	6195284	3594689	99	100	99
3	482.20	3746892	6194983	3594867	99	100	99
4	482.20	3748657	6195749	3598746	99	100	99
5	482.20	3746829	6194862	3598743	99	100	99
6	482.20	3748219	6194168	3598749	99	100	99
Average					99	100	99

Assay							
STD					0.03	0.02	0.06
% RSD					0.03	0.02	0.06

Table 9: Inter-day precision of Dapagliflozin, Gliclazide and Metformin

Limit of detection and Limit of Quantification (LOD&LOQ) study:

LOD is the smallest concentration of the analyte which gives a measurable response. It is calculated by taking the concentration of the peak of interest divided by three times the signal to noise ratio (s/n). LOQ is the smallest concentration of the analyte, which gives response that can be absolutely quantified. It is determined by analyzing samples containing known quantities of the analyte and determining the lowest level at which acceptable degrees of accuracy and precision are attainable.

The limit of detection and limit of quantification were evaluated by serial dilutions of Dapagliflozin, Gliclazide and Metformin stock solutions by the proposed method in order to obtain signal to noise ratio of 3:1 for LOD and 10:1 for LOQ. The LOD value for Dapagliflozin, Gliclazide and Metformin were found to be 0.050, 0.0370 and 0.033 respectively and the LOQ value 0.167, 0.1233 and 0.109 respectively. Chromatograms of LOD and LOQ study were shown in Fig 6.10 & Fig 6.11.

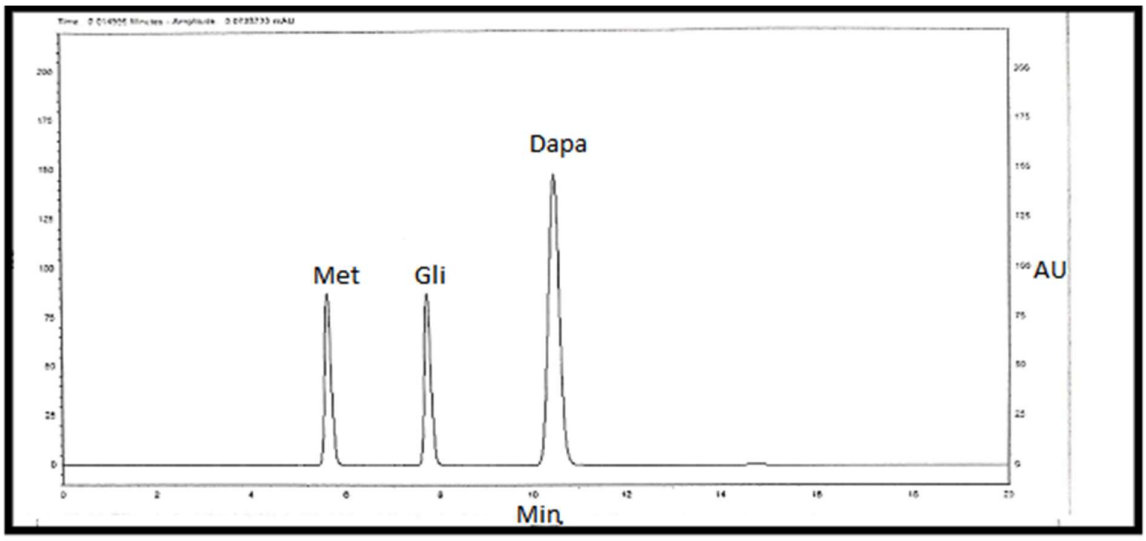

Fig. 6.10: Chromatogram of LOD study of Dapagliflozin, Gliclazide and Metformin

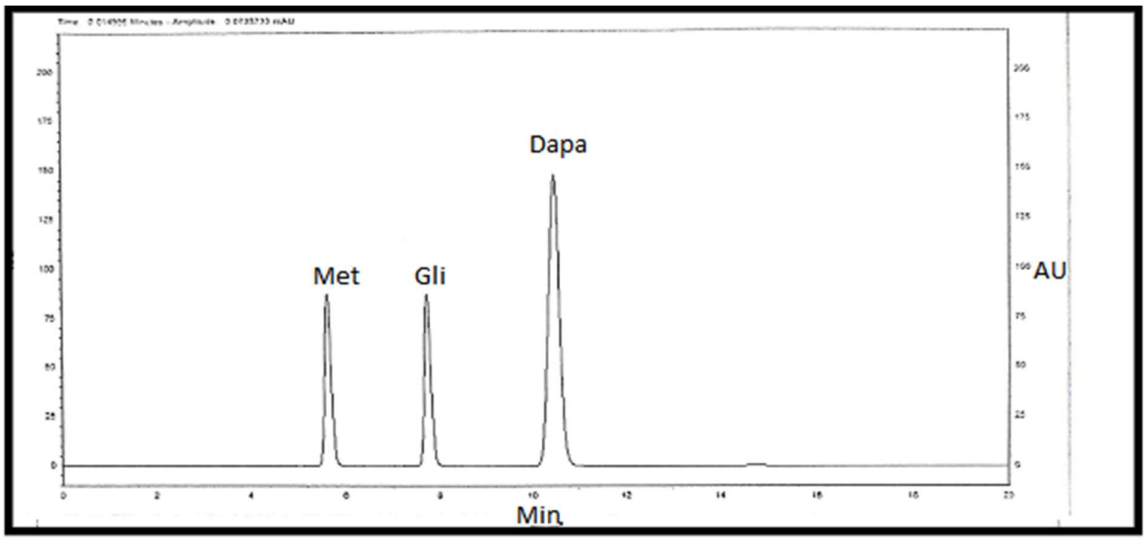

Fig.: Chromatogram of LOQ study of Dapagliflozin, Gliclazide and Metformin

LOD	0.050
LOQ	0.167

Table 13: LOD and LOQ of Dapagliflozin

LOD	0.050
LOQ	0.167

Table 14: LOD and LOQ of Gliclazide

LOD	0.050
LOQ	0.167

Table 15: LOD and LOQ of Metformin

5. CONCLUSION

Analytical Method Development and Validation for the Determination of Dapagliflozin, Gliclazide and Metformin in Tablet Dosage Form Using Rp HPLC Method was done. First it is discussed about drug of Dapagliflozin, Gliclazide and Metformin, second the materials and methods used in this work, the method development and the methodology of the validation parameters using HPLC, then third the results of system suitability, specificity, linearity, accuracy, precision, repeatability, intermediate precision and robustness.

The application of this method in routine analysis can be justified since easy sample preparation steps are involved and simple reagents, solvents were used experimentally. The method was validated as per ICH guidelines which demonstrated that the procedure is suitable for the intended purpose as it is linear, accurate, precise, rugged, robust, suitable and specific. It shows that the developed HPLC method could be conveniently adopted for the routine quality control analysis of Dapagliflozin, Gliclazide and Metformin form its pharmaceutical formulation and bulk drug.

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