



DEVELOPMENT AND VALIDATION OF A STABILITY-INDICATING RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF METFORMIN HCL AND DAPAGLIFLOZIN IN FIXED-DOSE COMBINATION TABLET

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Abstract

A fixed-dose combination of Metformin HCl (MET) and Dapagliflozin (DAPA) is widely used for the management of type 2 diabetes mellitus. A simple, precise, and economical analytical method is required for routine quality control of this combination.

To develop and validate a simple, accurate, and robust reversed-phase high-performance liquid chromatography (RP-HPLC) method for the simultaneous estimation of MET and DAPA in bulk and pharmaceutical tablet formulation.

Chromatographic separation was achieved on an Inertsil ODS-3V C18 column (250 mm × 4.6 mm, 5 µm particle size) using an isocratic mobile phase consisting of phosphate buffer (pH 3.5) and acetonitrile (50:50 v/v) at a flow rate of 1.0 mL/min. The detection wavelength was set at 233 nm (isobestic point). The method was validated according to ICH Q2(R1) guidelines for system suitability, linearity, accuracy, precision, ruggedness, robustness, LOD, and LOQ.

The retention times of MET and DAPA were 1.893 min and 5.262 min, respectively, with a total run time of 8.0 min. The method was linear over the concentration ranges of 10–20 µg/mL for MET and 32–64 µg/mL for DAPA, with correlation coefficients (r^2) of 0.999 for both drugs. Mean recoveries ranged from 99.06% to 101.58%, with %RSD <2%. Intra-day and inter-day precision (%RSD) were <1% for both drugs. The LOD and LOQ were 0.57 and 1.72 µg/mL for MET, and 1.22 and 3.72 µg/mL for DAPA, respectively. The method was robust to small changes in flow rate and wavelength, and ruggedness was confirmed by different analysts. Assay results for marketed tablets were 99.70% (MET) and 102.00% (DAPA) of label claim.

A simple, precise, and accurate RP-HPLC method was successfully developed and validated for the simultaneous estimation of MET and DAPA in bulk and tablet dosage form. The method is suitable for routine quality control analysis in pharmaceutical laboratories.

Keywords: Metformin HCl, Dapagliflozin, RP-HPLC, method validation, ICH guidelines, fixed-dose combination.

1. Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycaemia resulting from defects in insulin secretion, insulin action, or both. Type 2 diabetes mellitus (T2DM) accounts for approximately 90% of all diabetes cases and is often managed with oral antihyperglycaemic agents

[1,2]. Fixed-dose combinations (FDCs) are increasingly used to improve patient compliance by reducing pill burden and simplifying treatment regimens [3].

A newer FDC combines Metformin HCl (MET) and Dapagliflozin (DAPA). Metformin, a biguanide, reduces hepatic glucose production and improves peripheral insulin sensitivity [4]. Dapagliflozin, a sodium-glucose co-transporter-2 (SGLT-2) inhibitor, promotes renal glucose excretion, thereby lowering blood glucose levels independently of insulin [5]. The combination provides complementary mechanisms for glycaemic control.

For quality control of such FDCs, robust analytical methods are essential. A literature survey revealed that several analytical methods have been reported for MET and DAPA individually or in combination, including HPLC [6–9], UV spectrophotometry [10], and HPTLC [11]. However, some reported methods suffer from long run times, complex mobile phase compositions, or insufficient sensitivity. The aim of the present work was to develop a simple, rapid, and economical RP-HPLC method for the simultaneous estimation of MET and DAPA in bulk and tablet dosage form, and to validate the method according to ICH Q2(R1) guidelines [12].

2. Materials and Methods

2.1. Chemicals and Reagents

Working standards of Metformin HCl (purity 100%) and Dapagliflozin (purity 100%) were obtained as gift samples from Ipca Laboratories Pvt. Ltd., Aurangabad. The commercial tablet formulation (OXRAMET XR, AstraZeneca Pharmaceuticals) containing MET 500 mg and DAPA 10 mg per tablet was purchased from a local pharmacy.

HPLC-grade acetonitrile, analytical-grade potassium dihydrogen phosphate, ortho-phosphoric acid, and methanol were purchased from Merck and Fisher Scientific. High-purity water was obtained from a Milli-Q system.

2.2. Instrumentation and Chromatographic Conditions

The analysis was performed on a Shimadzu LC-2010 HPLC system equipped with a quaternary pump, an autosampler, a column oven, and a UV-Vis detector. Data acquisition and processing were carried out using Shimadzu LC-solution software.

Table 1: Optimized chromatographic conditions

Chromatographic Conditions	
Column	250x4.6 mm, 5 μ , ODS(Inertsil ODS 3V)
Flowrate	1.0 mL/min
Wavelength	233 nm
Injection volume	25 μ L
Column oven temperature	Ambient
Runtime	8.00 min
Mobile phase	Buffer(pH 3.5):ACN(50:50v/v)

2.3. Preparation of Solutions

Buffer (pH 3.5): 6.8 g of potassium dihydrogen phosphate was dissolved in 1000 mL of water, and the pH was adjusted to 3.5 with dilute ortho-phosphoric acid.

Mobile phase and diluent: A mixture of buffer and acetonitrile (50:50 v/v) was used as both mobile phase and diluent.

Standard stock solutions:

- MET: 25 mg was dissolved in 100 mL mobile phase to obtain 250 μ g/mL.
- DAPA: 20 mg was dissolved in 25 mL mobile phase to obtain 800 μ g/mL.

Working standard solution: 5 mL of MET stock and 5 mL of DAPA stock were mixed and diluted to 100 mL with mobile phase to give final concentrations of 12.5 µg/mL (MET) and 40 µg/mL (DAPA).

Sample solution: Five tablets were crushed. Powder equivalent to 500 mg MET and 10 mg DAPA was dissolved in mobile phase, sonicated, filtered through 0.45 µm filter, and suitably diluted to obtain the working concentration.

2.4. Method Validation

The method was validated according to ICH Q2(R1) guidelines for system suitability, linearity, accuracy (recovery), precision (intra-day and inter-day), ruggedness (intermediate precision), robustness, LOD, and LOQ [12].

LOD and LOQ were calculated from the calibration curve data using:

$$\text{LOD} = 3.3 \times \sigma/S,$$

$$\text{LOQ} = 10 \times \sigma/S,$$

where σ is the standard deviation of the intercept and S is the slope.

3. Results and Discussion

3.1. Method Development

The selection of the detection wavelength was based on overlain UV spectra of MET and DAPA in acetonitrile. Both drugs showed an isobestic point at 233 nm, which was selected as the detection wavelength (Figure 1).

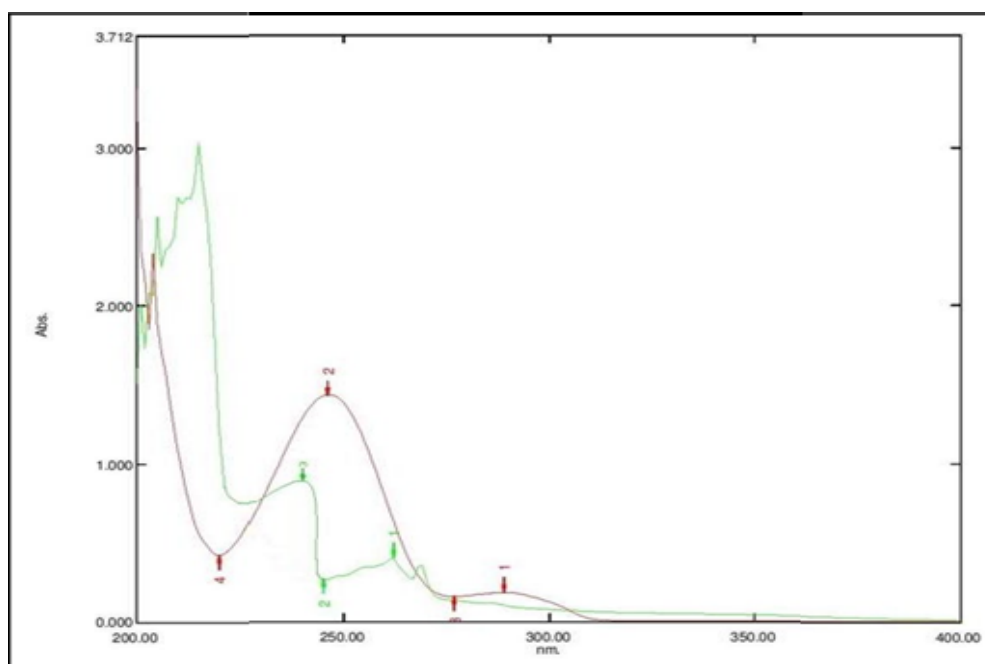


Figure 1. Overlain UV spectra of MET and DAPA showing isobestic point at 233 nm

Mobile phase composition was optimized by trial runs (Table 2). Initially, buffer pH 4.5:acetonitrile (70:30) gave poor peak purity. Buffer pH 4.5:acetonitrile (50:50) did not provide adequate resolution. Finally, buffer pH 3.5:acetonitrile (50:50) produced sharp, well-resolved peaks with acceptable system suitability parameters (Figure 2). The retention times were 1.893 min for MET and 5.262 min for DAPA, with a total run time of 8.0 min.

Table 2. Optimization of mobile phase composition.

Sr. No.	Mobile Phase Strength Buffer	Column	RT of MET + DAPA
1	Buffer(pH 4.5):ACN (70:30 v/v)	150 x4.6 mm , 5 μ ,BDS (Hypersil)	4.189 min and 5.013 min
2	Buffer(pH 4.5):ACN (50:50 v/v)	250 x4.6 mm , 5 μ ,ODS (InertsilODS3V)	2.024 min and 5.693 min
3	Buffer(pH 3.5):ACN (50:50 v/v)	250 x4.6 mm , 5 μ ,ODS (InertsilODS3V)	1.893 min and 5.262 min

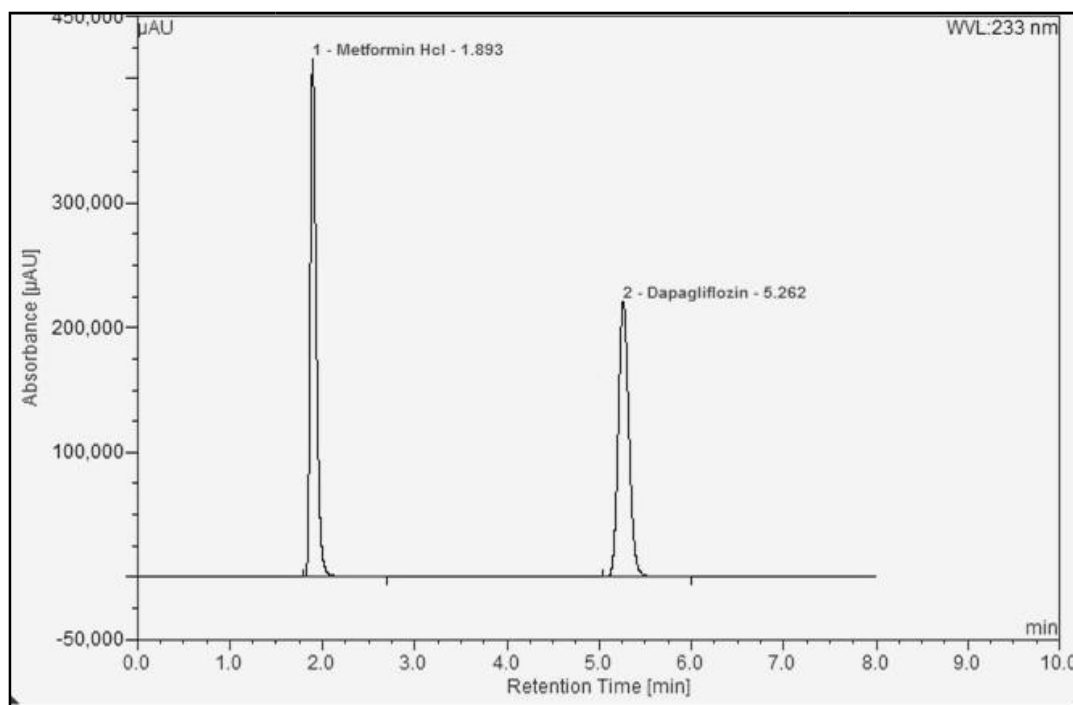


Figure 2. Optimized chromatogram of MET and DAPA

3.2. System Suitability

System suitability was assessed by injecting six replicates of the working standard solution. The results are summarised in **Table 3**. Theoretical plates were >4000, tailing factor <1.5, and %RSD of peak areas <0.05%, meeting the acceptance criteria.

Table 3. System suitability parameters

Parameter	MET	DAPA
Theoretical plates	4252	10138
Tailing factor	1.41	1.13
%RSD (area)	0.050	0.024

3.3. Linearity, LOD, and LOQ

Linearity was evaluated over five concentration levels: 10–20 μ g/mL for MET and 32–64 μ g/mL for DAPA. Calibration curves were constructed by plotting peak area vs. concentration (Figure 3 and Figure 4). Both drugs showed excellent linearity with $r^2 = 0.999$. Regression equations:

a. MET: $y = 2455x + 173.6$

b. DAPA: $y = 2454x + 345.9$

LOD and LOQ values are given in **Table 4**.

Table 4. Linearity, LOD, and LOQ data

Drug	Range (µg/mL)	r ²	LOD (µg/mL)	LOQ (µg/mL)
MET	10 – 20	0.999	0.57	1.72
DAPA	32 – 64	0.999	1.22	3.72

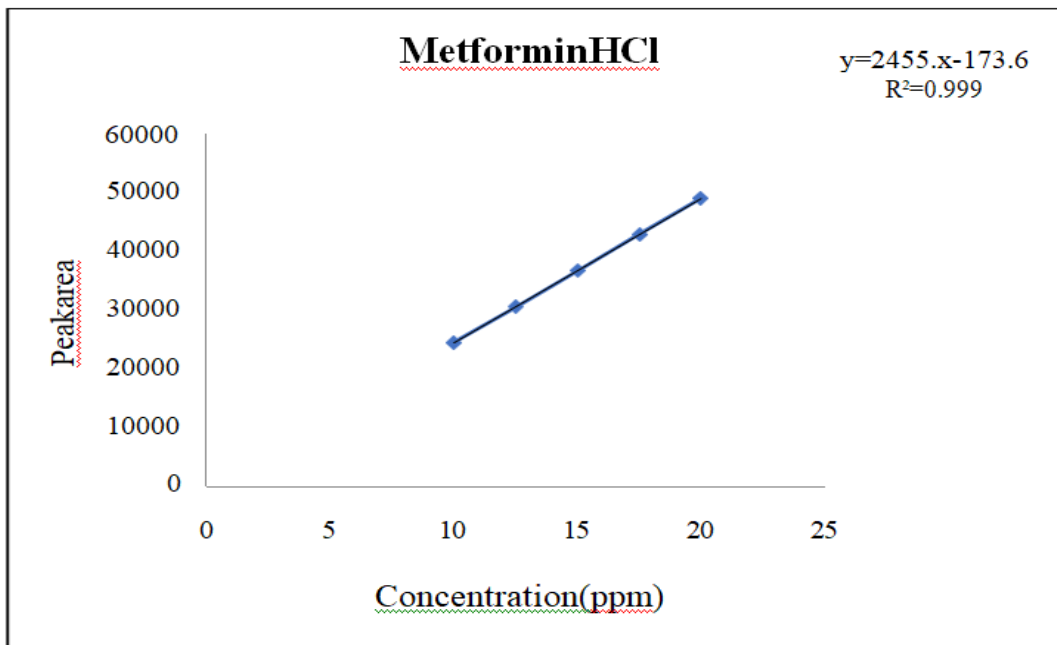


Figure 3. Calibration curve of Metformin HCl

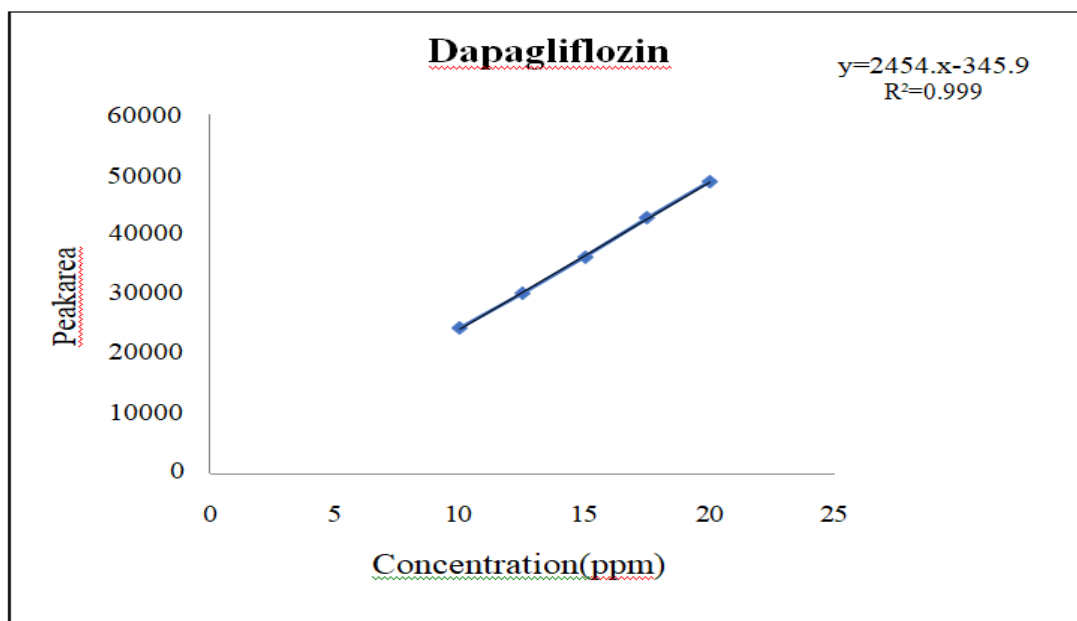


Figure 4. Calibration curve of Dapagliflozin

3.4. Accuracy (Recovery)

Accuracy was determined by spiking the formulation with known amounts of MET and DAPA at three levels (80%, 100%, and 120% of the test concentration). Each level was prepared in duplicate. The results (Table 5) showed mean recoveries between 99.06% and 101.58%, with %RSD <2%, indicating excellent accuracy and no interference from excipients.

Table 5: Result of mean recovery.

Drug	80% recovery (%)	100% recovery (%)	120% recovery (%)
MET	101.58	100.72	99.06
DAPA	100.11	101.51	100.85

3.5. Precision

Intra-day precision was assessed by injecting the standard solution three times in the morning and three times in the evening on the same day. Inter-day precision was assessed on two different days. The results are presented in **Table 6**. The %RSD values were <1% for both drugs, confirming good precision.

Table 6. Intra-day and inter-day precision

Drug	Intra-day %RSD	Inter-day %RSD
MET	0.87	0.82
DAPA	0.68	0.56

3.6. Ruggedness (Intermediate Precision)

Ruggedness was evaluated by having two different analysts perform the analysis on different days using the same instrument and column. The %RSD of assay results between analysts was <0.5% for both drugs (Table 7), demonstrating that the method is rugged.

Table 7. Ruggedness data (Analyst-I vs. Analyst-II) i)MET ii)DAPA

Parameters	Method Precision (Analyst-I)(±5%)	Intermediate Precision (Analyst-II) (± 5 %)
HPLC Instrument No.	Ins 002	Ins 002
HPLC column No.	Inertsil ODS 3V 250x4.6 mm 5μ	Inertsil ODS 3V 250x4.6 mm 5μ
Sample No.	% Assay	
1	99.70 ± 4.985	101.68 ± 5.084
2	99.76 ± 4.988	101.42 ± 5.071
3	99.88 ± 4.994	101.41 ± 5.070
4	99.96 ± 4.998	101.16 ± 5.058
5	99.81 ± 4.990	101.48 ± 5.074
6	99.92 ± 4.996	101.60 ± 5.08
Mean	99.83	101.46
%RSD	0.10	0.18

Parameters	Method Precision (Analyst-I) (±5%)	Intermediate Precision (Analyst-II) (±5 %)
HPLC Instrument No.	Ins002	Ins002
HPLC column No.	Inertsil ODS 3V 250 x4.6 mm 5μ	Inertsil ODS 3V 250 x4.6 mm 5μ
Sample No.	% Assay	
1	102.00 ± 5.1	102.86 ± 5.143
2	101.97 ± 5.098	101.75 ± 5.087
3	101.74 ± 5.087	101.86 ± 5.093
4	101.81 ± 5.090	101.57 ± 5.078
5	101.80 ± 5.09	101.76 ± 5.088
6	101.76 ± 5.088	101.75 ± 5.087
Mean	101.85	101.93
%RSD	0.11	0.46

3.7. Robustness

Robustness was tested by deliberately altering the flow rate (0.8 and 1.2 mL/min) and detection wavelength (231 and 235 nm). The system suitability parameters remained within acceptable limits, and the absolute difference in %assay was <0.5% (Table 8). Hence, the method is robust.

Table 8. Robustness data

Parameters	Values	Retention Time	Tailing factor	Theoretical plates	%RSD Of standard area	%Assay	Absolute difference
Control	1.00	1.893	1.41	4252	0.050	99.84	-
Flowrate (± 0.1 mL/min)	0.8	2.262	1.44	4559	1.13	99.89	0.17
	1.2	1.593	1.38	4040	0.156	99.82	0.11
Change in Wave length (±1 nm)	231	1.898	1.44	4403	1.437	99.82	0.12
	235	1.897	1.182	4395	1.44	99.69	0.45

Parameters	Values	Retention Time	Tailing factor	Theoretical plates	%RSD of standard area	%Assay	Absolute difference
Control	1.00	5.262	1.13	10138	0.02	101.85	-
Flowrate (± 0.1 mL/min)	0.8	6.606	1.13	11063	0.162	101.92	0.24
	1.2	4.468	1.11	9482	0.196	102.0	0.46
Change in Wavelength (± 1 nm)	231	5.318	1.12	10441	0.035	101.81	0.14
	235	5.314	1.13	10367	0.091	101.91	0.22

3.8. Assay of Marketed Formulation

The developed method was applied to the analysis of OXRAMET XR tablets (MET 500 mg + DAPA 10 mg). The %assay values were 99.70% for MET and 102.00% for DAPA (Table 9), which are within the acceptance limits (95–105%). Chromatograms of standard and sample solutions are shown in Figure 5 and Figure 6.

Table 9. Assay results of marketed formulation

Drug	Label claim (mg)	% Assay found
MET	500	99.70
DAPA	10	102.00

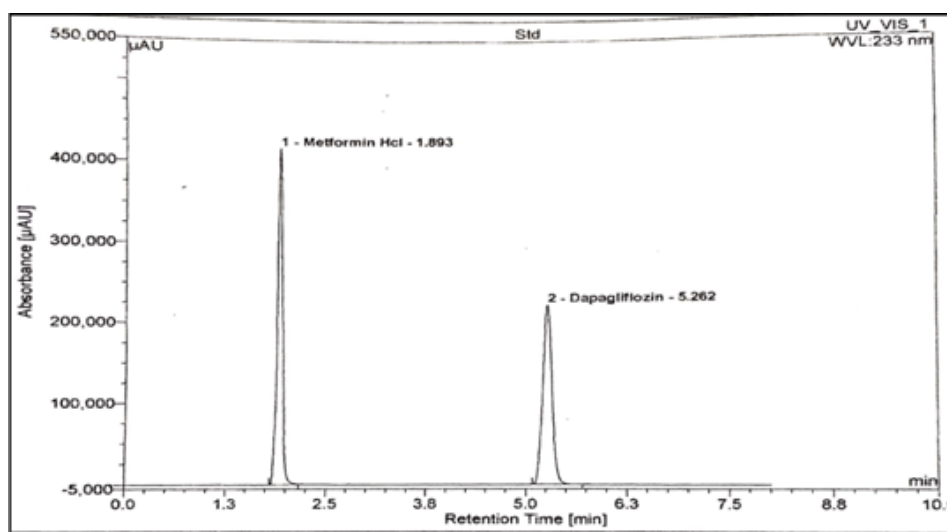


Figure 5. Chromatogram of standard solution

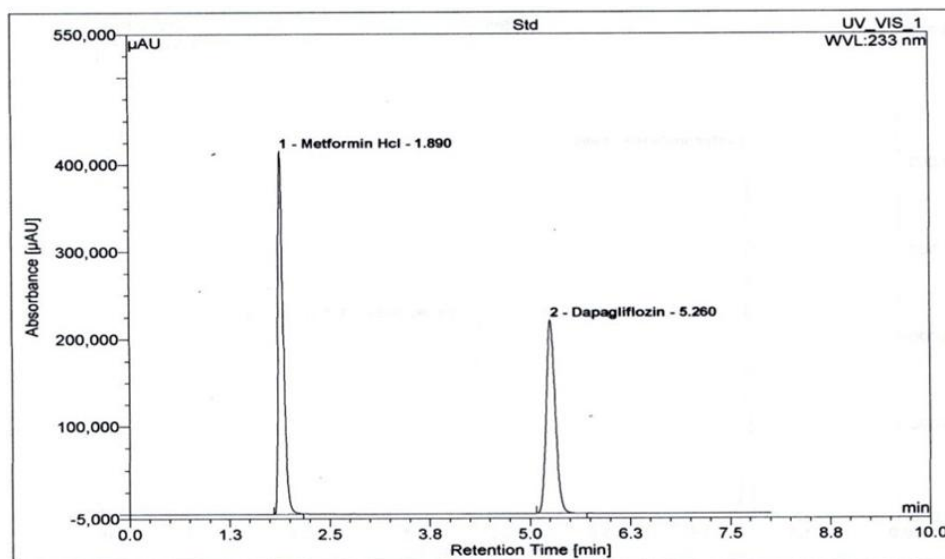


Figure 6. Chromatogram of sample solution

4. Conclusion

A simple, precise, accurate, and robust RP-HPLC method was successfully developed and validated for the simultaneous estimation of Metformin HCl and Dapagliflozin in bulk and tablet dosage form. The method uses an economical mobile phase (phosphate buffer:acetonitrile, 50:50 v/v), a short run time (8.0 min), and a single detection wavelength (233 nm). The method was validated according to ICH Q2(R1) guidelines and met all acceptance criteria for linearity, accuracy, precision, ruggedness, robustness, and sensitivity. The method can be readily applied for routine quality control analysis of MET and DAPA in pharmaceutical formulations.

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