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Development of a new UV/Visible Spectophotometric and HPLC methods for simultaneous estimation of Metformin and Sitagliptin

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A B S T R A C T

The main aim and objective of the present research work was to develop a new UV/VISIBLE spectophotometric method for simultaneous estimation of Metformin and Sitagliptin, a new HPLC method for simultaneous estimation of Metformin and Sitagliptin, a validated method according to ICH guidelines and to apply validated method for the estimation of Metformin and Sitagliptin in pharmaceutical formulation**.** A simple, Accurate, precise method was developed for the simultaneous estimation of the Metformin and Dapagliflozin in Tablet dosage form. Retention time of Metformin and Dapagliflozin were found to be 2.7min and 3.7min. %RSD of the Metformin and Dapagliflozin were and found to be 0.81 and 0.50 respectively. % Recover was Obtained as 99.74% and 100.53% for Metformin and Dapagliflozin respectively. LOD, LOQ values are obtained from regression equations of Metformin and Dapagliflozin were 1.30ppm, 0.43ppm and 3.95ppm, 1.31ppm respectively. Regression equation of Metformin is y = 20318x + 8024, and y = 12128x + 1591 of Dapagliflozin. 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.

Keywords: HPLC, ICH, simultaneous estimation, LOD, LOQ

A R T I C L E I N F O

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1. Introduction

A drug1 may be defined as a substance meant for diagnosis, cure, mitigation and prevention, treatment in human beings or animals, for altering any structure or function of the body of human beings or animals [1]. Pharmaceutical chemistry[2-6] is a science that makes use of general laws of chemistry to study drugs i.e., their preparation, chemical nature, composition, structure, influence on an organism, methods of quality control and the conditions of their storage etc. The family of drugs may be broadly classified as: pharmacodynamics agents and chemotherapeutic agents Pharmacodynamics agents refer to a group of drugs which stimulate or depress various functions of body so as to provide some relief to the body in case of body abnormalities without curing the diseases [3-5]. Chemotherapeutic agents are the agents which are selectively more toxic to the invading organisms without harmful effect to the host. Every country has legislation8 on bulk drugs and their pharmaceutical formulations that sets standards and obligatory quality indices for them. These regulations are presented in separate articles relating to individual drugs and are published in the form of book called "Pharmacopoeia" (eg. IP9: USP10: BP11 and Martindale Extra Pharmacopoeia, MEP12).

Pharmaceutical analysis deals not only with medicaments (drugs and their formulations) but also with their precursors i.e., with the raw material on which degree of purity and quality of medicament depends. The quality of a drug is determined after establishing its authenticity by testing its purity and the quality of pure substance in the drug and its formulations. Quality is important for every product or service but it is vital in medicine as it involves in life. Unlike ordinary consumer goods there can be no "second quality" in drugs. Quality control is a concept which strives to produce a perfect product by series of measures designed to prevent and eliminate errors at a different stages of production. Analytical chemistry may be defined as the science and art of determining the components of materials in terms of the elements or compound contained[6]. Analytical chemistry is important in all aspects of chemistry, for example, agricultural, clinical, environmental, forensic, manufacturing metallurgical and pharmaceutical chemistry. In general terms pharmaceutical analysis comprises, those procedures necessary to determine the identity, strength, quality and purity of drugs and chemicals.

Method of validation

"Doing thorough method validation can be tedious, but the consequences of not doing it right are wasted time, money, Metformin and Sitagliptines. Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics. Method validation is the process of demonstrating that analytical procedures are suitable for their intended use and that they support the identity, quality, purity, and potency of the drug substances and drug products. The real goal of validation process is to challenge the method and determine limits of allowed variability for the conditions needed to run the method. [7]

Type of analytical procedures to be validated

Validation of analytical procedures is directed to the four most common types of analytical procedures. Identification test, quantitative test for impurities content. Limit test for the control of impurities. Quantitative test of the active moiety in samples of drug substance on drug product on other selected components in the drug product. In our method of validation, we are following last type. Assay procedures are intended to measure the analyst present in given sample, assay represent a quantitative measurement of the major component(s) in the drug sample. Two steps are required to evaluate an analytical method: first determine the classification of the method and the second step is to consider the characteristics of the analytical method. For analytical method validation of pharmaceuticals, guidelines from the International Conference on Harmonization (ICH), United States Food and Drug Administration (US FDA), American Association of Official Analytical Chemists (AOAC), United States Pharmacopoeia (USP) and International Union of Pure and Applied Chemists (IUPAC) provide a framework for performing such validations in efficient and productive manner.

Reasons for method validation

There are two important reasons for validating assays in the pharmaceutical industry. The first, and by for the most important, is that assay validation is an integral part of the quality control system. The second is that current good manufacturing practice regulation requires assay validation.

Performance characteristics examined when carrying out method validation [8]: Specificity, linearity, range, accuracy, precision, repeatability, ruggdness, detection and quantitation limit, robustness and system suitability.

2. Materials and Methods

Materials:

Metformin and Sitagliptin, Combination Metformin and Sitagliptin SR tablets are purchased from local retail shops. Distilled water, acetonitrile, phosphate buffer, ammonium acetate buffer, glacial acitic acid, methanol, potassium dihydrogen phosphate buffer, tetra hydrofuran, tri ethyl amine, ortho-phosphoric acid etc all are supplied from institutional store and were of A.R and L.R grade.

Instrument:

HPLC instrument used was of WATERS HPLC 2965 SYSTEM with Auto Injector and PDA Detector. Software used is Empower 2. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz was be used for measuring absorbance for Metformin and Sitagliptin solutions.

Methods of Preparation of different solutions

Buffer (0.1%OPA buffer): In the preparation 0.1% OPA buffer 1ml of conc. Ortho phosphoric acid was diluted to 1000ml with HPLC grade water.

Mobile phase: Buffer and Acetonitrile taken in the ratio 50:50A

Standard Preparation:

Accurately Weighed and transferred 34mg of metformine and 2mg of dapagliflozine working Standards into 10ml and 100ml clean dry volumetric flasks, add 3/4 ml of diluent, sonicated for 5 minutes and make up to the final volume with diluents. 1ml from the above two stock solutions was taken into a 10ml volumetric flask and made up to 10ml.

Sample Preparation:

5 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to 5 tablets was transferred into a 250 mL volumetric flask, 200mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 0.2ml was pipeted out into a 10 ml volumetric flask and made upto 10ml with diluent.

Method Development [9-16]

Many trials were done by changing columns and Mobile phases and were reported below

Trial 1:

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

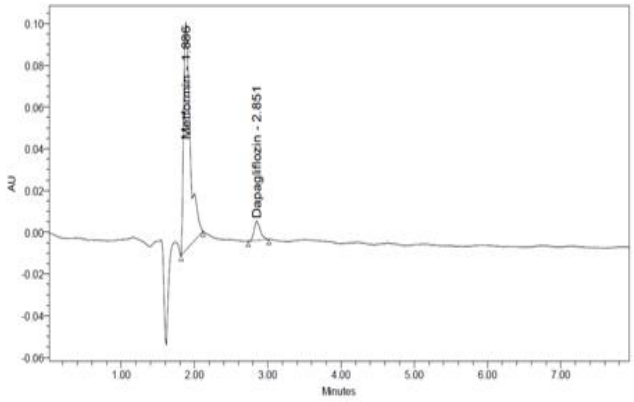

Figure 1: Trial chromatogram 1

Observation: Metformin eluted before 2mins.

Trial 2:

This trial was run through STD hiber 150mm column with mobile phase composition of 80:20A Buffer and Acetonitrile, Flow rate set at 1ml/min.

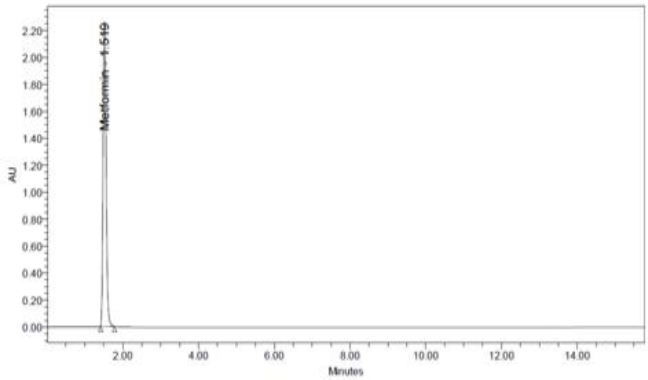

Figure 2: Trial chromatogram 2

Observation: Metformin eluted before 2minutes and Dapagliflozin was not found.

Trial 3:

This trial was run through std hiber 150mm column with mobile phase composition of 50:40 Buffer and Acetonitrile, Flow rate set at 1ml/min.

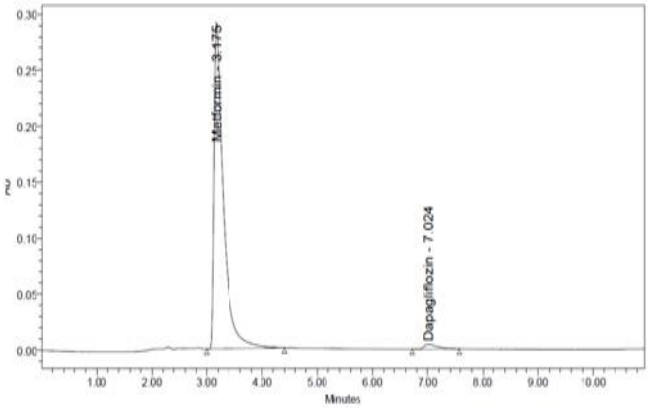

Figure 3: Trial chromatogram 3

Observation: Metformin and Dapagliflozin were eluted with good resolution, but retention time of Dapagliflozin was more and plate count of Dapagliflozin was less.

Trial 4:

This trial was run through BDS 250mm column with mobile phase composition of 55:45 Buffer and Acetonitrile, Flow rate set at 1ml/min.

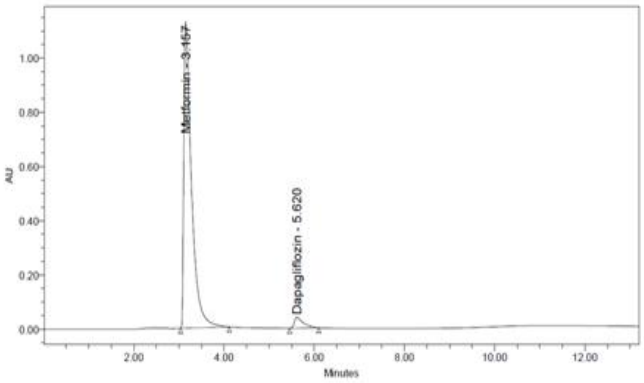

Figure 4: Trial chromatogram 4

Observation: Metformin and Dapagliflozin were eluted with good resolution, but retention time of Dapagliflozin was more than 4 and tailing was observed, still need to decrease the retention time of Dapagliflozin.

Optimized Method: Drugs were eluted with good retention time, resolution; all the system suitable parameters like Plate count and Tailing factor were within the limits.

Column Used	:	Std BDS (250mm 4.6mm, 5µ)
Buffer used	:	Ortho phosphoric acid
Mobile phase	:	Buffer: Acetonitrile (50:50A)
Flow rate	:	1ml/min
Diluent	:	methanol and buffer
Wavelength	:	240
Temperature	:	30°C
Injection Volume	:	10µl

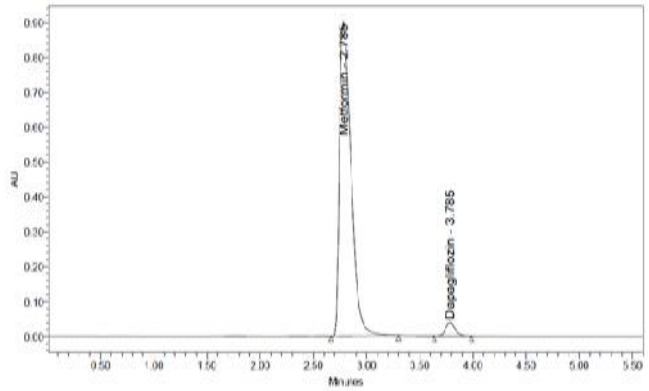

Figure 5: Optimized chromatogram of Metformin and Dapagliflozin

3. Results and Discussion

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

Table 1: System suitability studies of Metformin and Dapagliflozin method

Property	Metformin	Dapagliflozin
Retention time (tR)	2.77min	3.77min
Theoretical plates (N)	3072 ± 63.48	8846± 63.48
Tailing factor (T)	1.74 ± 0.117	1.20± 0.117

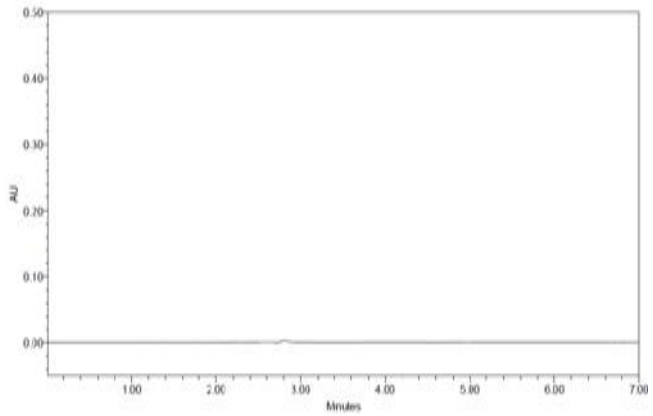

Figure 6: Chromatogram of blank

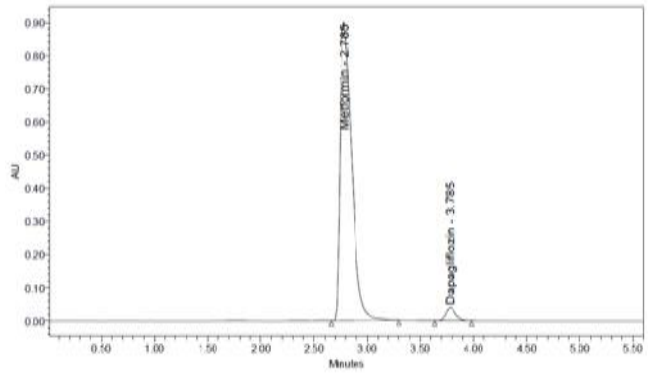

Figure 7: Typical chromatogram of Metformin and Dapagliflozin

Linearity: Six Linear concentrations of Metformin (85-510ppm) and Dapagliflozin (0.5-3ppm) are prepared and Injected. Regression equation of the the Metformin and Dapagliflozin are found to be, y = 20318x + 8024, y = 12128x + 1591.. And regression co-efficient was 0.999.

Table 2: Calibration data of Metformin and Dapagliflozin method.

S.no	Conc. Metformin (µg/ml)	Response	Conc. Dapagliflozin (µg/ml)	Response
1	0	0	0	0
2	85	1783407	0.5	66067
3	170	3458007	1	120870
4	255	5141377	1.5	184412
5	340	6911623	2	240506
6	425	8617109	2.5	307796
7	510	1041167	3	364952

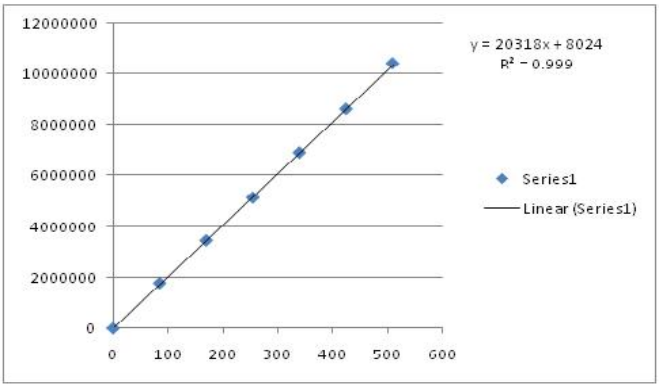

Figure 8: Calibration curve of Metformin

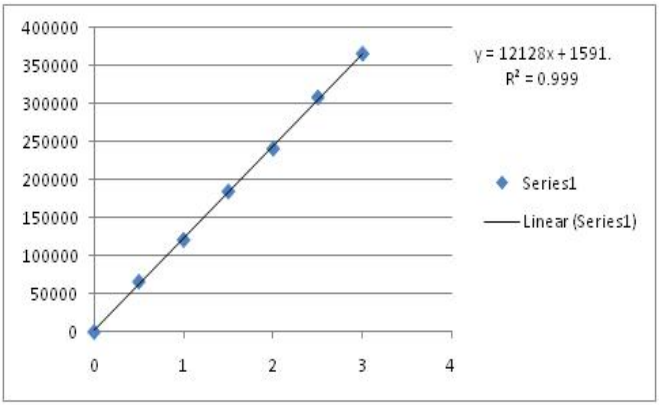

Figure 9: Calibration curve of Dapagliflozin

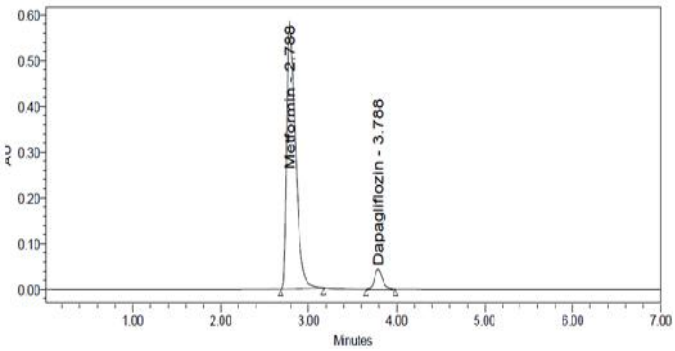

Figure 10: Linearity 25% Chromatogram of Metformin and Dapagliflozin method.

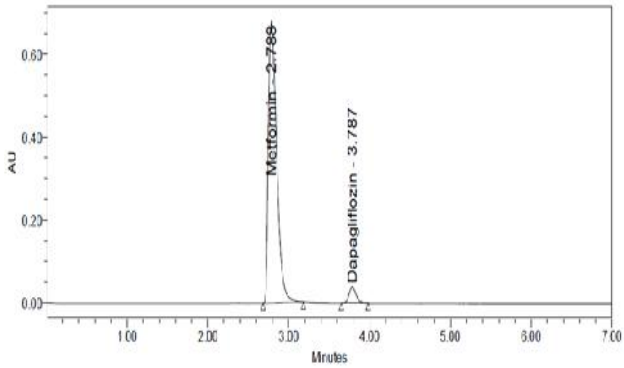

Figure 11: Linearity 50% Chromatogram of Metformin and Dapagliflozin method.

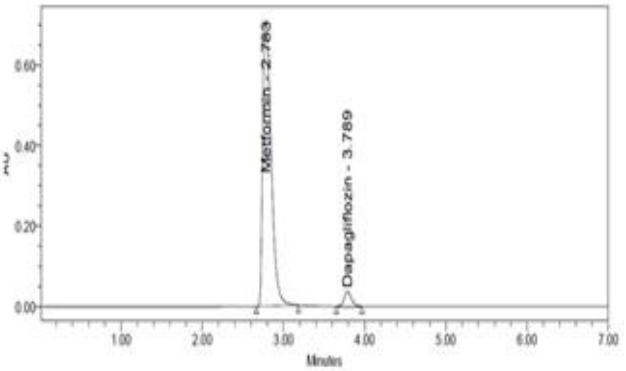

Figure 12: Linearity 75% Chromatogram of Metformin and Dapagliflozin method.

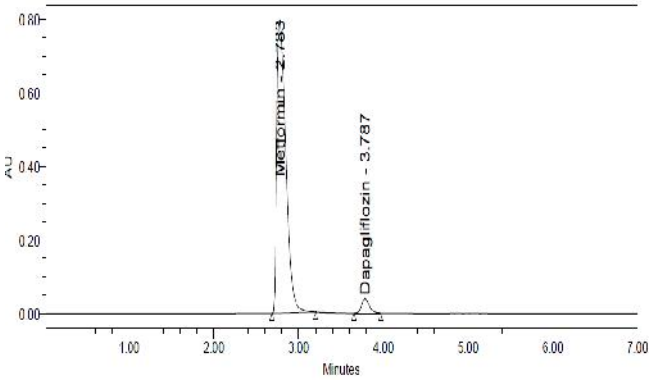

Figure 13: Linearity 100% Chromatogram of Metformin and Dapagliflozin method.

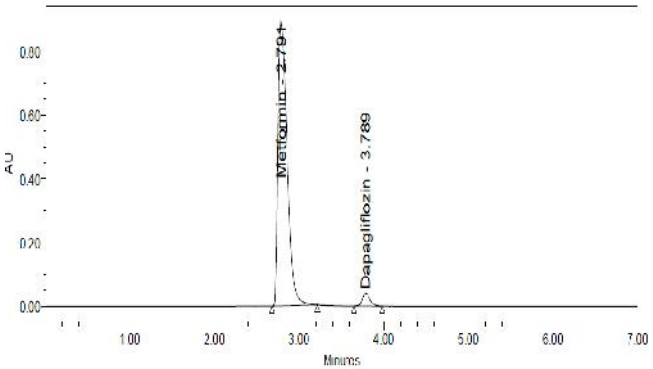

Figure 14: Linearity 125% Chromatogram of Metformin and Dapagliflozin method.

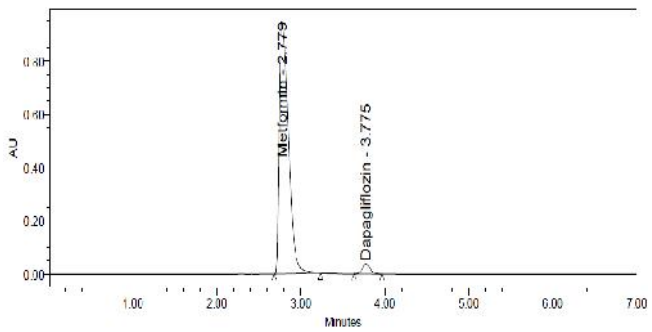

Figure 15: Linearity 150% Chromatogram of Metformin and Dapagliflozin method.

Precision: Intraday precision (Repeatability):
Intraday Precision was performed and % RSD for Metformin and Sitagliptin were found to be 0.91% and 1.18% respectively.

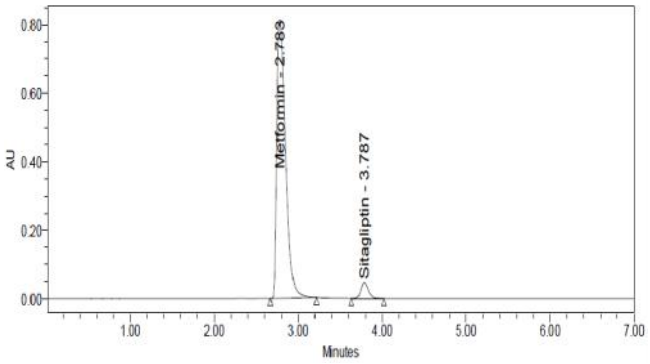

Figure 16: Precision

Table3: Repeatability results for Metformin and Sitagliptin.

S.NO	Metformin	Dapagliflozin
1	6828851	248709
2	6790139	250403
3	6824010	251370
4	6822242	247083
5	6750054	250348
6	6764584	246354
Mean	6796647	249045
Std.dev	33711.2	2007.6
%RSD	0.5	0.8

*Average of six determinations

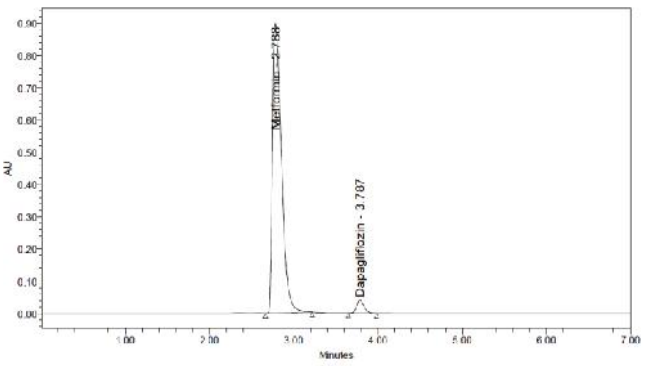

Figure 17: Repeatability Chromatogram of Metformin and Dapagliflozin method.

Inter day precision: Inter day precision was performed with 24 hrs time lag and the %RSD Obtained for Metformin and Dapagliflozin were 0.26% and 0.19%.

Table 4: Inter day precision results for Metformin and Dapagliflozin.

S.NO	Metformin	Dapagliflozin
1	6808042	249612
2	6828145	248441
3	6848111	250361
4	6826423	250150
5	6821525	249964
6	6826449	249706
Mean	14449.8	758.6
Std.dev	0.2	0.3
%RSD	6808042	249612

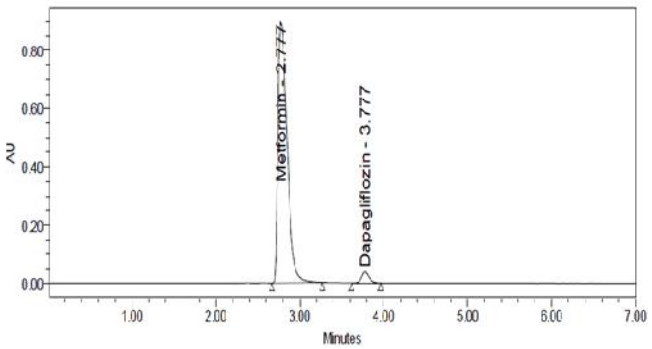

Figure 18: Inter Day precision Chromatogram of Metformin and Dapagliflozin method.

Accuracy: Three concentrations 50%, 100%, 150%, were injected in a triplicate manner and amount Recovered and % Recovery were displayed in Table 5.

Table 5: Accuracy results of Metformin and Dapagliflozin

Sample	Amount added (µg/ml)	Amount Recovered (µg/ml)	Recovery (%)	% RSD
Metformin	50	170.90	100.53	0.46
	100	169.72	99.84	0.32
	150	169.42	99.66	0.55
Dapagliflozin	50	0.99	99.61	0.67
	100	2.00	100.38	0.43
	150	3.00	100.13	1.03

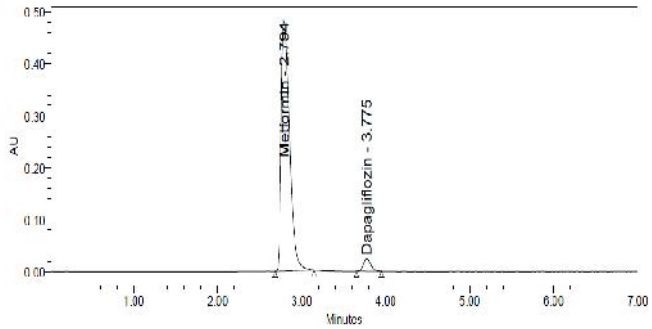

Figure 19: Accuracy 50% Chromatogram of Metformin and Dapagliflozin method.

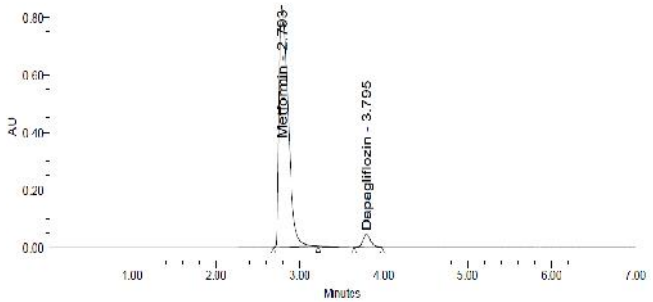

Figure 20: Accuracy 100% Chromatogram of Metformin and Dapagliflozin method

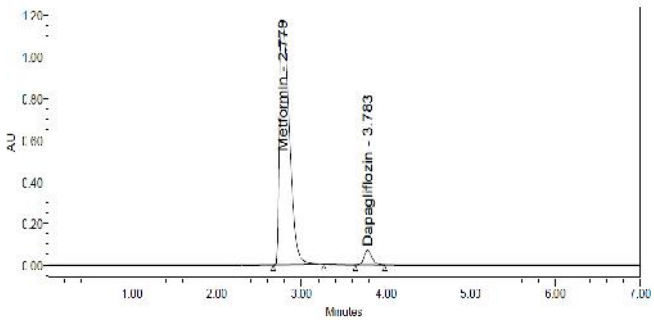

Figure 21: Accuracy 150% Chromatogram of Metformin and Dapagliflozin method.

LOD: Limit of ditection was calculated by Metformin and Dapagliflozinpt method and LOD for Metformin and Dapagliflozin were found to be 1.30 and 0.43 respectively.

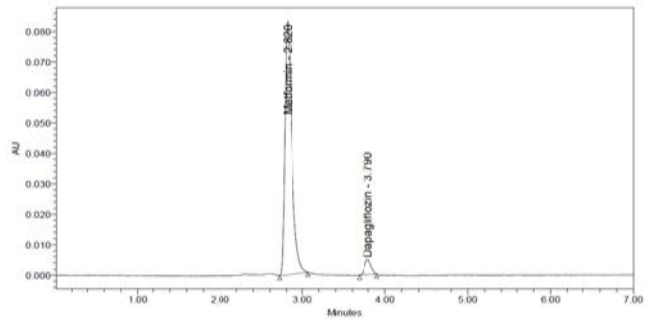

Figure 22: LOD Chromatogram of Metformin and Dapagliflozin method.

LOQ: Limit of Quantification was calculated by Metformin and Dapagliflozinpt method and LOQ for Metformin and Dapagliflozin were found to be 3.95and 1.31 respectively.

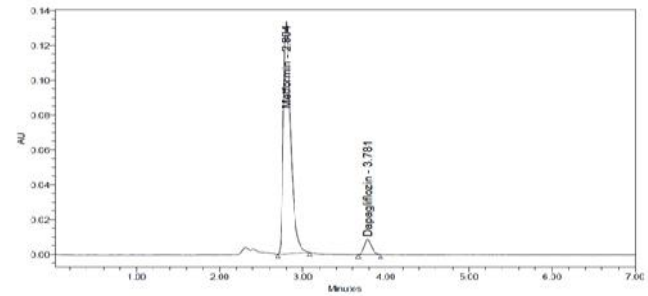

Figure 23: LOQ Chromatogram of of Metformin and Dapagliflozin method.

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.

Table 6: Robustness data of Metformin and Dapagliflozin method.

S.NO	Robustness condition	Metformin % RSD	Dapagliflozin % RSD
1	Flow minus	0.0	0.2
2	Flow Plus	0.5	0.6
3	Mobile phase minus	0.2	0.6
4	Mobile phase Plus	0.2	0.0
5	Temperature minus	0.2	0.7
6	Temperature Plus	0.1	0.1

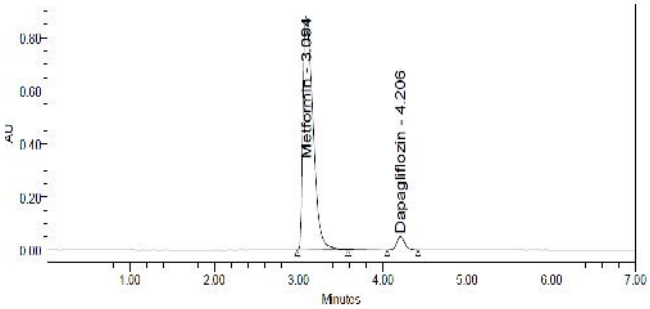

Figure 24: Flow minus Chromatogram of Metformin and Dapagliflozin method.

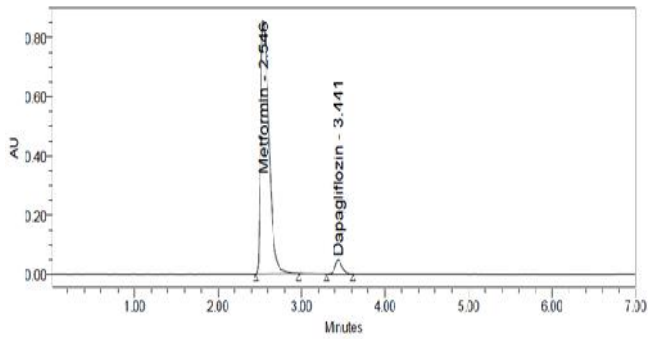

Figure 25: Flow plus Chromatogram of Metformin and Dapagliflozin method.

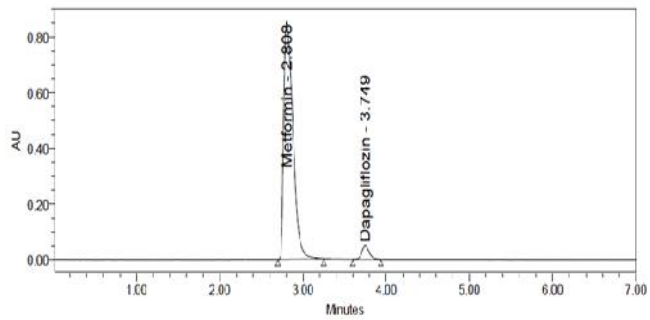

Figure 26: Mobile phase minus Chromatogram of Metformin and Dapagliflozin method.

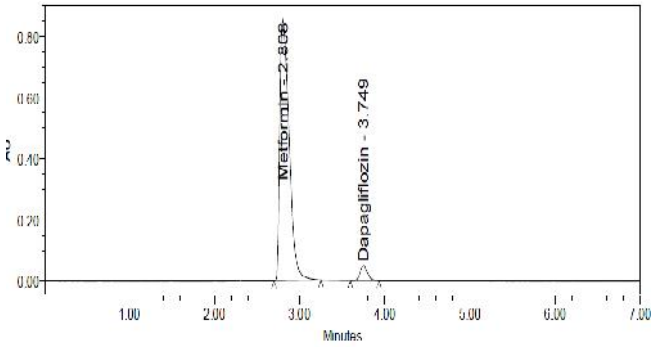

Figure 27: Mobile phase Plus Chromatogram of Metformin and Dapagliflozin method

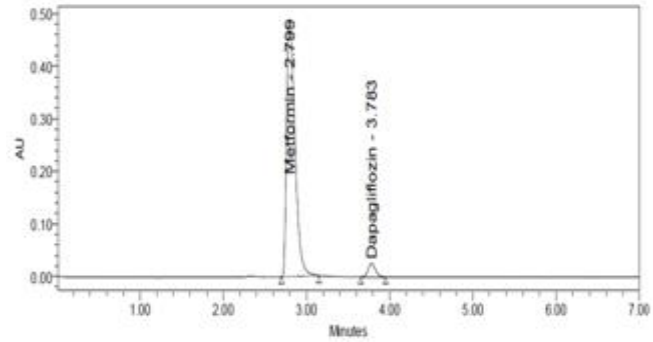

Figure 28: Temperature minus Chromatogram of Metformin and Dapagliflozin method.

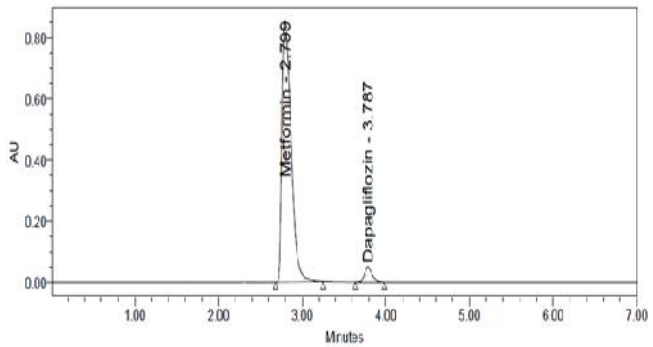

Figure 29: Temperature Plus Chromatogram of Metformin and Dapagliflozin method

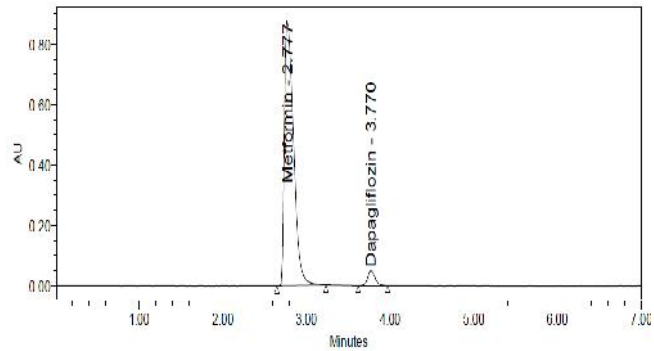

Figure 30: Assay of Tablet

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 was estimated by taking the standard as the reference. The Average %Assay was calculated and found to be 99.74% and 99.86% for Metformin and Dapagliflozin respectively.

Table 7: Assay of Tablet

S. No.	Metformin %Assay	Dapagliflozin % Assay
1	100.22	99.72
2	99.65	100.40
3	100.15	100.79
4	100.12	99.07
5	99.06	100.38
6	99.27	98.78
AVG	99.74	99.86
STDEV	0.49	0.80
% RSD	0.50	0.81

4. Conclusion

A simple, Accurate, precise method was developed for the simultaneous estimation of the Metformin and Dapagliflozin in Tablet dosage form. Retention time of Metformin and Dapagliflozin were found to be 2.7min and 3.7min. %RSD of the Metformin and Dapagliflozin were and found to be 0.81 and 0.50 respectively. %Recover was Obtained as 99.74% and 100.53% for Metformin and Dapagliflozin respectively. LOD, LOQ values are obtained from regression equations of Metformin and Dapagliflozin were 1.30ppm, 0.43ppm and 3.95ppm, 1.31ppm respectively. Regression equation of Metformin is y = 20318x + 8024, and y = 12128x + 1591 of Dapagliflozin. 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.

5. References

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