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Analytical method for simultaneous estimation of metformin and Dapagliflozin by RP HPLC method

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Abstract
Background: The present investigation reported in the thesis was aimed to develop a new method development and validation for the simultaneous estimation of Metformin and Dapagliflozin by RP-HPLC method. Literature reveals that there are no analytical methods reported for the simultaneous estimation Metformin and Dapagliflozin by RP-HPLC method.
Objective: The analytical method for the simultaneous estimation of Metformin and Dapagliflozin will be developed by RP-HPLC method by optimizing the chromatographic conditions.
The developed method is validated according to ICH guidelines for various parameters specified in ICH guidelines, Q2 (R1).
Materials and Methods: The detection wavelength was selected by dissolving the drug in mobile phase to get a concentration of 10µg/ml for individual and mixed standards. The resulting solution was scanned in U.V range from 200-400nm. The overlay spectrum of Metformin and Dapagliflozin was obtained and the isobestic point of Metformin and Dapagliflozin showed absorbance's maxima at 240 nm.
Results: The chromatographic method development for the simultaneous estimation of Metformin and Dapagliflozin were optimized by several trials for various parameters as different column, flow rate and mobile phase, finally the following chromatographic method was selected for the separation and quantification of Metformin and Dapagliflozin in API and pharmaceutical dosage form by RP-HPLC method.
Conclusion: It can be concluded that the proposed Reverse Phase High Performance Liquid Chromatographic (RPHPLC) method is accurate, precise, sensitive, specific, robust and reproducible for the simultaneous analysis of Metformin and Dapagliflozin. The results are summarized on evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±0.2ml/min. The method is robust only in less flow condition.

Key words: Metformin, Dapagliflozin RP-HPLC

Introduction
Metformin decreases blood glucose levels by decreasing hepatic glucose production, decreasing intestinal absorption of glucose, and improving insulin sensitivity by increasing peripheral glucose uptake and utilization. These effects are mediated by the initial activation by metformin of AMP-activated protein kinase (AMPK), a liver enzyme that plays an important role in insulin signaling, whole body energy balance, and the metabolism of glucose and fats. Activation of AMPK is required for metformin's inhibitory effect on the production of glucose by liver cells. Increased peripheral utilization of glucose may be due to improved insulin binding to insulin receptors. Metformin administration also increases AMPK activity in skeletal muscle. AMPK is known to cause GLUT4 deployment to the plasma membrane, resulting in insulin-independent glucose uptake. The rare side effect, lactic acidosis, is thought to be caused by decreased liver uptake of serum lactate, one of the substrates of gluconeogenesis. In those with healthy renal function, the slight excess is simply cleared. However, those with severe renal impairment may accumulate clinically significant serum lactic acid levels. Other conditions that may precipitate lactic acidosis include severe hepatic disease and acute/decompensated heart failure.

Dapagliflozin is indicated for the management of diabetes mellitus type 2, and functions to improve glycemic control in adults when combined with diet and exercise. Dapagliflozin is a sodium-glucose cotransporter 2 inhibitor, which prevents glucose reabsorption in the kidney. Using Dapagliflozin leads to heavy glycosuria (glucose excretion in the urine), which can lead to weight loss and tiredness.

Chemical and Reagents

Water, Methanol, Acetonitrile, Ortho phosphoric acid, KH_2PO_4, K_2HPO_4, 0. 22µ Nylon filter, 0.45µ filter paper, Tancodep-2, Metformin and Dapagliflozin were collected from local market.

Instrumentation: HPLC-auto sampler-UV detector, U.V double beam spectrometer, Digital weighing balance (sensitivity 5mg), pH meter, Sonicator.

Chromatographic Condition

The mobile phase 70:30 methanol: Sodium acetate buffer was found to resolve Metformin and Dapagliflozin. Ortho phosphoric acid was pre-owned for pH adjustment of buffer to 3. The mobile phase was strained through 0.45 µm membrane filter and degassed by sonication. The flow rate was set to 1.0 ml/min. The drug showed good absorbance at 240 nm, and this was selected as wavelength for further analysis.

Preparation of mobile phase

Mix a mixture of above buffer 30 ml (30%) and 70 ml of ACN (HPLC grade-70%) and degassed in ultrasonic water bath for 5 minutes. Filter through 0.22 µ filter under vacuum filtration.

Preparation of standard solution

10 mg metformin and 10 mg Dapagliflozin working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and add about 1 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 1ml of the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

Preparation of sample solution

10 mg of metformin and 10 mg Dapagliflozin tablet powder were accurately weighed and transferred into a 10 ml clean dry volumetric flask, add about 1 ml of diluent and sonicate to dissolve it completely and making volume up to the mark with the same solvent(Stock solution). Further pipette 10ml of the above stock solution into a 100ml volumetric flask and was diluted up to the mark with diluents.

Method development

The detection wavelength was selected by dissolving the drug in mobile phase to get a concentration of 10µg/ml for individual and mixed standards. The resulting solution was scanned in U.V range from 200-400nm. The overlay spectrum of Metformin and Dapagliflozin was obtained and the isobestic point of Metformin and Dapagliflozin showed absorbance's maxima at 240 nm.

The chromatographic method development for the simultaneous estimation of Metformin and Dapagliflozin were optimized by several trials for various parameters as different column, flow rate and mobile phase, finally the following chromatographic method was selected for the separation and quantification of Metformin and Dapagliflozin in API and pharmaceutical dosage form by RP-HPLC method. Several systematic trials were performed to optimize the mobile phase. The trial showing Dapagliflozin peak in the chromatogram, so more trials were required for obtaining peaks. The separation was good, peak shape was good, so we conclude that there is no required for reduce the retention times of peaks, so it is taken as final method. The retention time of Metformin and Dapagliflozin was found to be 2.467mins and 3.762mins respectively. The system suitability parameters for Metformin and Dapagliflozin such as theoretical plates and tailing factor were found to be 5358, 1.2 and 7597, 1.1. Resolution was 8.85. The% purity Metformin and Dapagliflozin in pharmaceutical dosage form was found to be 98.11 and 103.26% respectively.

Results

Validation Report

Specificity

The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The study was performed by injecting blank.

The specificity test was performed for Metformin and Dapagliflozin. It was found that there was no interference of impurities in retention time of analytical peak.

Linearity

The linearity study was performed for the concentration of 50 ppm metformin to 250 ppm Dapagliflozin and 5ppm metformin to 25 ppm Dapagliflozin level. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient.

Accuracy

The accuracy study was performed for 50%, 100% and 150% for Metformin and Dapagliflozin. Each level was injected in triplicate into chromatographic system. The area of each level was used for calculation of% recovery.

The accuracy study was performed for% recovery of Metformin and Dapagliflozin. The% recovery was found to be 99.56% and 99.47% respectively (NLT 98% and NMT 102%)

Precision

Repeatability

The precision study was performed for five injections of Metformin and Dapagliflozin. Each standard injection was injected into chromatographic system. The area of each Standard injection was used for calculation of% RSD. The Method precision study was performed for the%RSD of Metformin and Dapagliflozin was found to be 0. 3 and 0.3 (NMT 2).

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD was performed for Metformin and Dapagliflozin which was estimated to be 2.17 and 0.0372, respectively. The LOQ was performed for Metformin and Dapagliflozin was found to be 6.60 and 0.112 respectively.

Robustness

The robustness was performed for the flow rate variations from 0.8 ml/min to 1.2 ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Metformin and Dapagliflozin. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%.

The results are summarized on evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±0.2ml/min. The method is robust only in less flow condition.

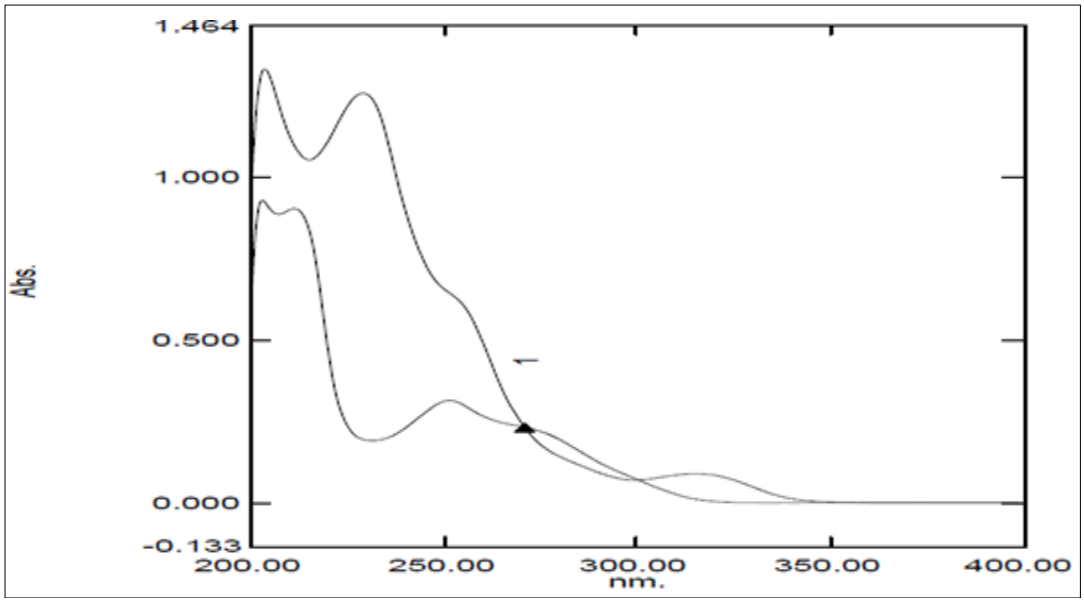

Fig 1: Spectrum showing overlapping spectrum of MET and DAPA

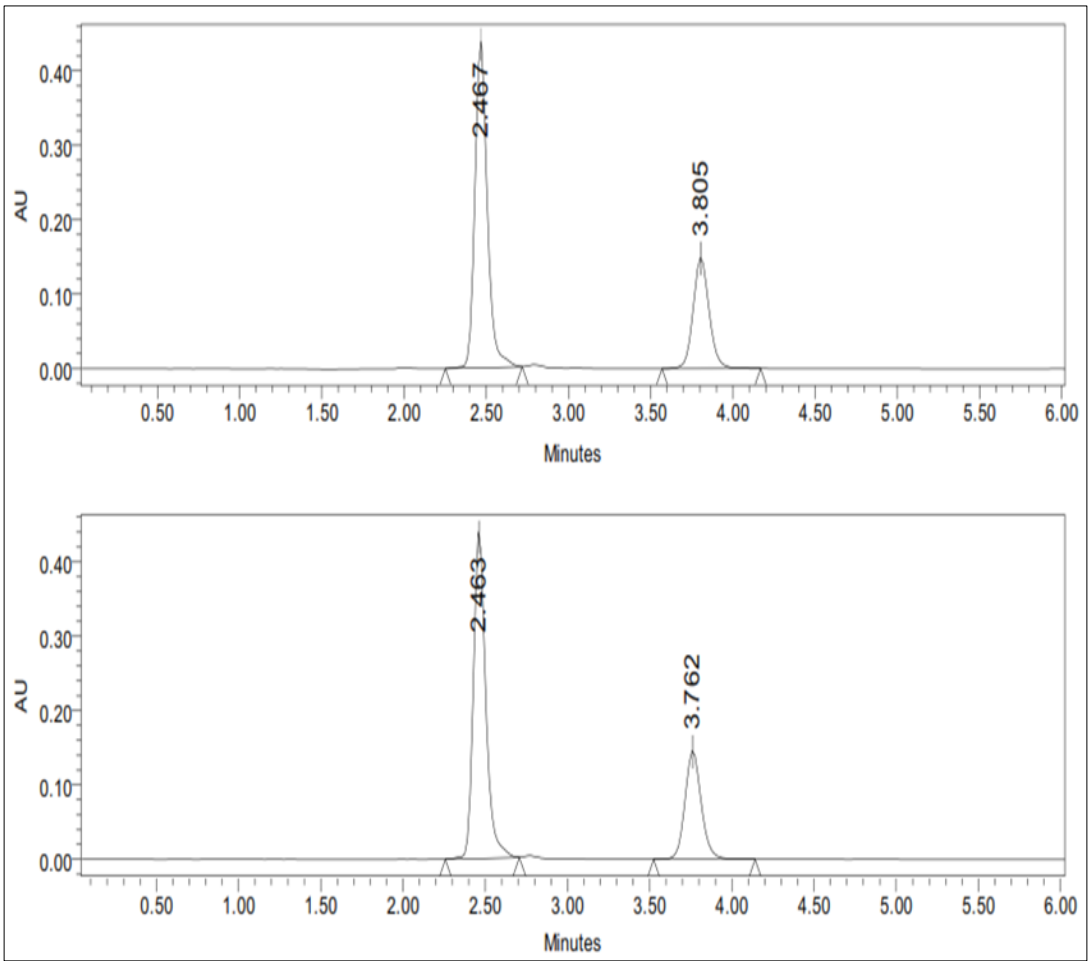

Fig 2: Chromatogram showing standard of sample injection -1, 2, 3

Table 1: Showing assay results

SL. No	Name of compound	Amount taken(mg)	%purity
1	Metformin	729.5	98.11
2	Dapagliflozin	725.5	103.26

Table 2: Linearity Results for Metformin

SL. No	Leniarity level	Concentration	Area
1	I	5 ppm	339009
2	II	10ppm	689527
3	III	15ppm	994963
4	IV	20ppm	1385006
5	V	25ppm	1766425
Correlation Coefficient			0.999

Table 3: Linearity Results for Dapagliflozin

SL. No	Leniarity level	Concentration	Area
1	I	5 ppm	339009
2	II	10ppm	689527
3	III	15ppm	994963
4	IV	20ppm	1385006
5	V	25ppm	1766425
Correlation Coefficient			0.999

Table 4: Showing accuracy results for metformin

%Concentration (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean Recovery
50%	11842004	5	4.96	99.91%	99.56%
100%	2121872	10	9.98	99.18%	
150%	3525766	15	15.02	99.60%	

Table 5: Showing accuracy results for Dapagliflozin

%Concentration (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean Recovery
50%	522218	0.5	0.99	99.53%	99.47%
100%	939319	1.0	1.05	99.38%	
150%	1576651	1.5	1.495	99.52%	

Table 6: Showing results for intermediate precision of Metformin and Dapagliflozin

Peak Name : Metformin				
	Peak Name	RT	Area (uV*sec)	Height (uV)
1	Metformin	2.463	2285660	435548
2	Metformin	2.462	2286224	433860
3	Metformin	2.463	2292264	438537
4	Metformin	2.465	2301304	437911
5	Metformin	2.463	2355896	437895
Mean			2304269.6	
Std. Dev.			29539.0	
% RSD			1.3	

Peak Name : Dapagliflozin				
	Peak Name	RT	Area (uV*sec)	Height (uV)
1	Dapagliflozin	3.751	1005320	145777
2	Dapagliflozin	3.753	1006642	145438
3	Dapagliflozin	3.757	1007252	145816
4	Dapagliflozin	3.745	1011606	146569
5	Dapagliflozin	3.741	1014319	147558
Mean			1009027.9	
Std. Dev.			3782.8	
% RSD			0.4	

The intermediate precision was performed for % RSD of Metformin and Dapagliflozin was found to be 1. 3 and 0.4 respectively (NMT 2)

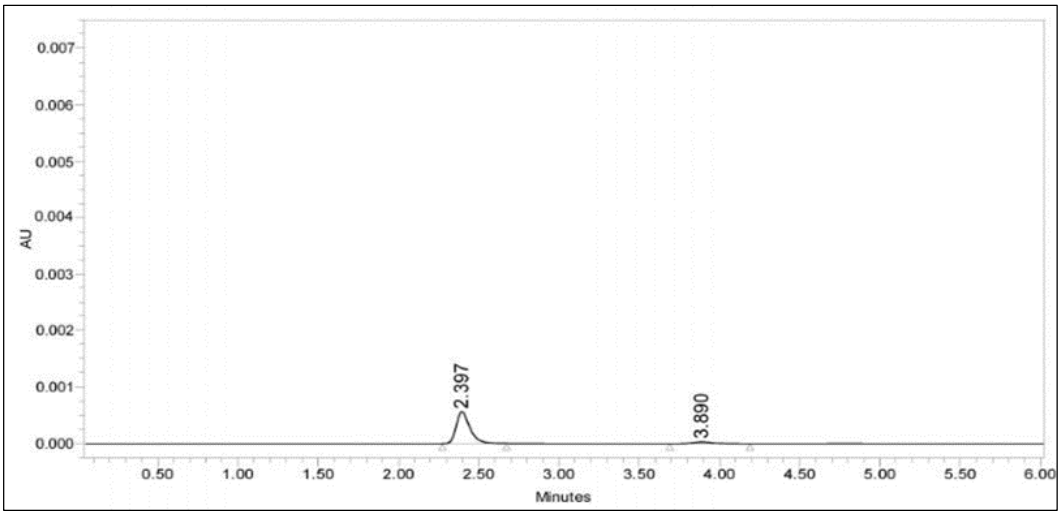

Table 7: Showing results for Limit of Detection

Drug name	Standard deviation(σ)	Slope(s)	LOD(µg)
Metformin	371827.90	563365963	2.17
Dapagliflozin	5401.60	479884400	0.0372

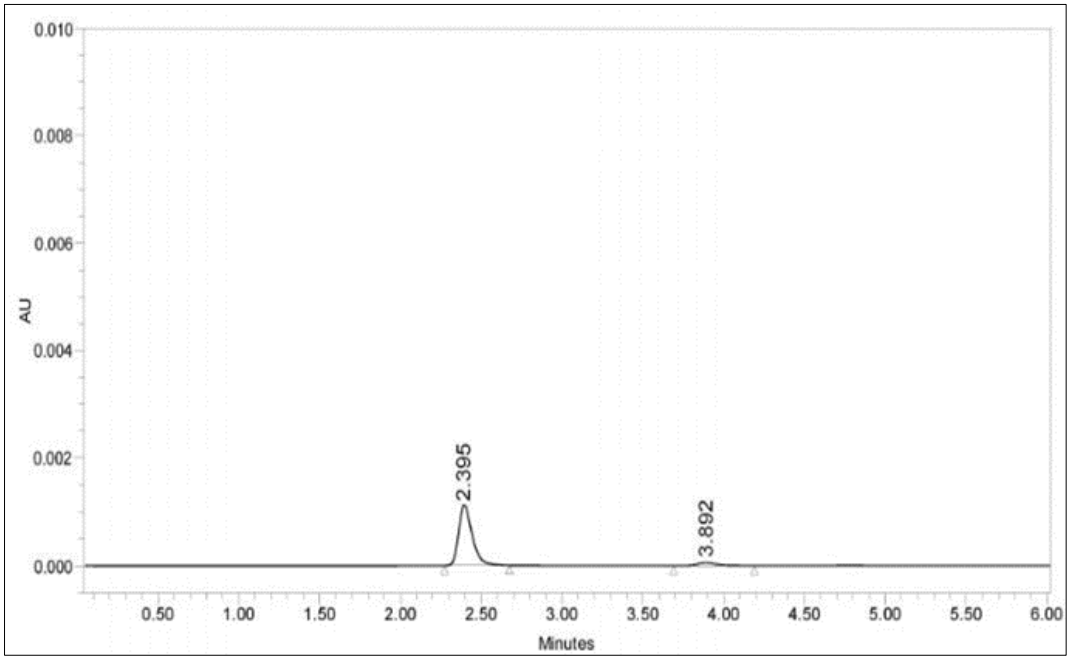

Table 8: Showing results for Limit of Quantitation

Drug name	Standard deviation(σ)	Slope(s)	LOQ(µg)
Metformin	371827.90	563365963	6.60
Dapagliflozin	5401.60	479884400	0.112

Table 9: Showing system suitability results for metformin

SL. No	Change in organic composition in the mobile phase	System suitability results	
		USP Plate Count	USP Tailing
1	5% less	4573	1.2
2	*Actual	4567	1.27
3	5% more	5889	1.2

Table 10: Showing system suitability results for Dapagliflozin

SL. No	Change in organic composition in the mobile phase	System suitability results	
		USP Plate Count	USP Tailing
1	5% less	6255	1.1
2	*Actual	6579	1.1
3	5% more	8583	1.1

Conclusion

It can be concluded that the proposed RPHPLC method is accurate, precise, sensitive, specific, robust and reproducible for the simultaneous analysis of Metformin and Dapagliflozin. Flow rate was 1.1 ml/min. Both the samples were in the range of 200 to 400 nm and maximum wavelength was identified at 240 nm.

Discussion

The analysis method developed for separation of Metformin and Dapagliflozin has shown good resolved peaks. Since the RT is short, it indicates that in a shorter duration more samples could be completed and developed method will be easy for analyzing larger samples. The values of LOD and LOQ for these both drugs were significantly low; hence, this method is appropriate for detecting and quantifying the fairly low concentrations of these drugs. Results of statistical analysis, lower % RSD values confirm the ability of the analytical assay. The analytical to ICH guidelines (ICH, Q2 (R1). A result is simple, reliable, précised, accurate, linear and reproducible, hence can be applied for drug delivery analysis. This method could be even suitable in active pharmaceutical preparations for quality control analysis.

Consent and ethical approval

It is not applicable

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Competing interest

Authors have declared that no completing interests exists

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