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Original Article

A Simple and Validated Rp-Hplc Method for the Simultaneous Estimation of Metformin and Dapagliflozin in Bulk and Pharmaceutical Dosage Forms

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Abstract

A simple, rapid, specific, accurate and precise reverse phase high performance liquid chromatographic method was developed for the simultaneous estimation of Metformin and Dapagliflozin in tablet dosage form. An Inertsil ODS C18 column having 150 x 4.6mm id in Isocratic mode with mobile phase containing Acetonitrile : phosphate buffer (70:30 %v/v pH: 3.0) was used. The flow rate was 1.0ml/min and effluents were monitored at 240nm.) The retention time of Metformin and Dapagliflozin was 2.463min and 3.760min respectively. The concentration curves of Metformin and Dapagliflozin were linear in the concentration range of 50µg/ml-250 µg/ml and 5µg/ml-25µg/ml of Metformin and Dapagliflozin respectively. The developed method was validated for specificity, precision, linearity, accuracy, LOD, LOQ, robustness. Recovery of Metformin and Dapagliflozin in formulations was found to be in the range of 97.0% - 98.0% and 100%-103% respectively confirms the non-interferences of the excipients in the formulation. Due to its simplicity, rapidness and high precision, the proposed HPLC method may be used for the simultaneous determination of these two drugs in pharmaceutical dosage forms.

Keywords: RP-HPLC; Metformin and Dapagliflozin.

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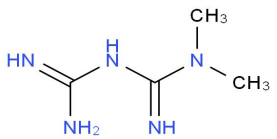
Introduction

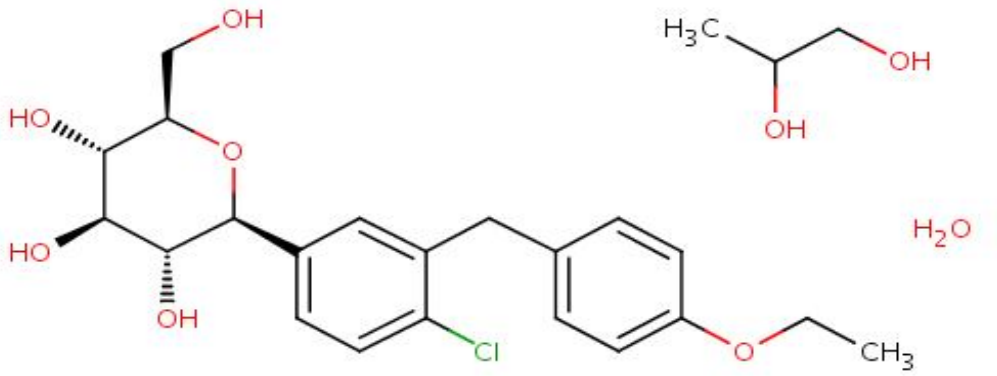
Metformin is chemically 1-carbamimidamido-N, N-dimethylmethanimidamide. It decreases blood glucose levels by decreasing hepatic glucose production, decreasing intestinal absorption of glucose, and improving insulin sensitivity by increasing peripheral glucose uptake and utilization.

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 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 [1].



Metformin

Dapagliflozin: Dapagliflozin(2S,3R,4R,5S,6R)-2-{4-chloro-3-[(4-ethoxyphenyl)methyl]phenyl}-6-(hydroxymethyl)oxane-3,4,5-triol propane-1,2-diol hydrate. 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 re-absorption in the kidney. Using dapagliflozin leads to heavy glycosuria (glucose excretion in the urine), which can lead to weight loss and tiredness [2].



Dapagliflozin

Several analytical procedures have been proposed for the quantitative estimation of Metformin and Dapagliflozin separately and in combination with other drugs. High performance liquid chromatography methods for estimation of metformin [3] alone and in combination with glibenclamide and pioglitazone are also available. Dapagliflozin [4] is estimated by UV and HPLC have also been reported.

To our knowledge simple and economical analytical method for simultaneous determination of Metformin and Dapagliflozin has not been reported so far. So attempt was taken to develop and validate an economic, rapid reversed-phase high performance liquid chromatographic method for the quality control of Metformin and Dapagliflozin in pharmaceutical preparations with lower solvent consumption along with the short analytical run time that leads to an environmentally friendly chromatographic procedure and will allow the analysis of a large number of samples in a short period of time. The method was validated and found to be accurate, precise and reproducible.

Materials and Methods

Apparatus

Waters e2695Alliance HPLC system connected with PDA Detector 2998 and Empower2 Software. The drug analysis data were acquired and processed using Empower2 software running under Windows XP on a Pentium PC.

Other Apparatus:: Electronic balance, Sonicator, 0.45µ membrane filter

Reagents and Chemicals

Pharmaceutical grade Metformin and Dapagliflozin were kindly supplied as a gift sample by Astra zenaca Pharma, India. Acetonitrile was of HPLC grade and collected from E. Merck, Darmstadt, Germany. Orthophosphoric acid was of analytical reagent grade supplied by Fischer Scientific Chemicals. Water HPLC grade was obtained from a Milli-QRO water purification system.

Commercial Formulation

Metformin and Dapagliflozin Tablets available in the market as XIGDUO XR in composition of Metformin and Dapagliflozin. The samples were properly checked for their manufacturing license numbers, batch numbers, production, expiry dates and stored properly.

Procedure

Preparation of phosphate buffer

2.95 grams of potassium dihydrogen phosphate and 5.45 grams of potassium hydrogen phosphate was weighed and taken into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water and pH was adjusted to 3 with ortho phosphoric acid. The resulting solution was sonicated and filtered.

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.

Diluents preparation

Mobile phase was used as the diluent.

Preparation of the individual metformin standard preparation

10 mg of metformin working standard was accurately weighed and transferred into a 10 ml 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 1.0 ml from the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent.

Preparation of the individual Dapagliflozin standard preparation

10 mg of Dapagliflozin working standard was accurately weighed and transferred into a 10 ml 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 1.0 ml from the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent.

Preparation of the Metformin and Dapagliflozin standard and sample solution

Sample solution preparation

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 diluent.

Standard solution preparation

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.

Development and Validation of Hplc Method

Present study was conducted to obtain a new, affordable, cost-effective and convenient method for HPLC determination of metformin and Dapagliflozin in tablet dosage form. The experiment was carried out according to the official specifications of USP–30, ICH- 1996 and Global Quality Guidelines-2002. The method was validated for the parameters like system suitability , selectivity, linearity, accuracy, precision, LOD, LOQ, and robustness [5,6,7].

System Suitability

System suitability study of the method was carried out by six replicate analysis of solution containing 100% target concentration of metformin and Dapagliflozin. Various chromatographic parameters such as retention time, peak area tailing factor, theoretical plates (Tangent) of the column and resolution between the peaks were determined and the method was evaluated by analyzing these parameters.

Selectivity

Selectivity test determines the effect of excipients on the assay result. To determine the selectivity of the method, standard sample of metformin and of Dapagliflozin were injected first. Then commercial product, blank and excipients solution were run in the instrument one after another.

Linearity

Linearity of the method was determined by constructing calibration curves. The linearity study was performed for the concentration of 50 ppm to 250 ppm metformin and 5ppm to 25ppm Dapagliflozin. Each measurement was carried out in six replicates and the peak areas of the chromatograms were plotted against the concentrations to obtain the calibration curves and correlation coefficients.

Accuracy (Recovery Studies)

To check the degree of accuracy of the method, recovery studies were performed in triplicate by standard addition method at 50%, 100% and 150%. Known amounts of standard metformin and

Dapagliflozin were added to pre-analyzed samples and were subjected to the proposed HPLC method.

Precision

Precision was evaluated by carrying out six independent sample preparation of a single lot of formulation. The sample solution was prepared in the same manner as described in sample preparation. Percentage relative standard deviation (%RSD) was found to be less than 2% for within a day and day to day variations, which proves that method is precise.

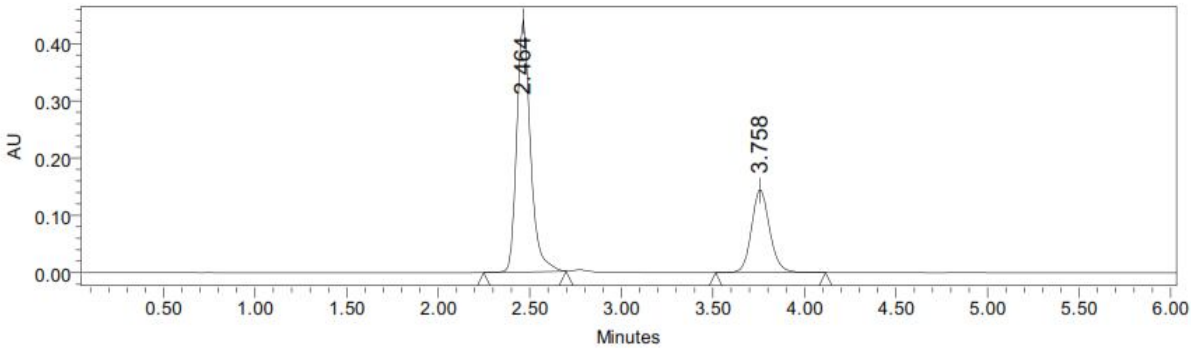
Robustness of Method

To evaluate the robustness of the developed RP-HPLC method, small deliberate variations in the optimized method parameters were done. The effect of change in flow rate, temperature, on the retention time and tailing factor were studied. The method was found to be unaffected by flow and temperature variation.

Results and Discussion

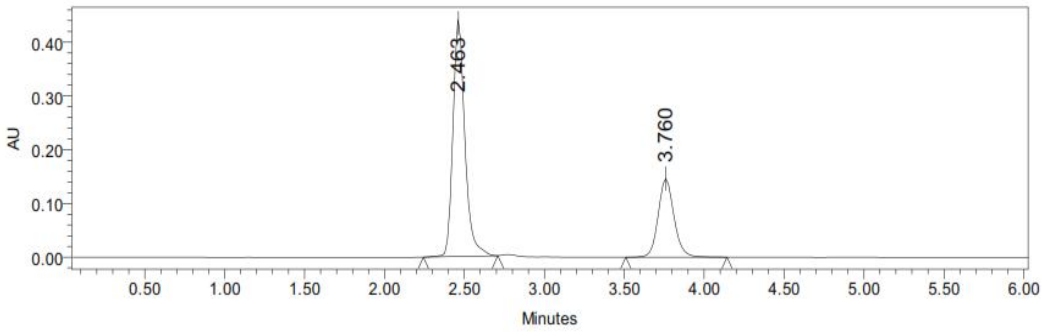
Results of system suitability study are summarized in Table 1. Six consecutive injections of the standard solution showed uniform retention time, theoretical plate count, tailing factor and resolution for both the drugs which indicate a good system for analysis.

Fig. 1: Typical chromatogram of metformin and dapagliflozin in standard preparation.



	Name	Retention Time	Area	Height	USP Tailing	USP Plate Count
1	Metformin	2.464	2288873	435418	1.2	3124.1
2	Dapagliflozin	3.758	1003783	144483	1.1	2366.2

Fig. 2: Typical chromatogram of metformin and dapagliflozin in marketed formulation.



	Name	Retention Time	Area	Height	USP Tailing	USP Plate Count
1	Metformin	2.463	2288873	435418	1.3	3137.6
2	Dapagliflozin	3.760	1006018	145772	1.1	2367.9

Table 1: Result of system suitability tests of Metformin and Dapagliflozin

Column	:	Inertsil RP C18 (4.5×150 mm) 5.0 µm
Column temperature	:	Ambient
Wavelength	:	240 nm
Mobile phase ratio	:	70:30 Acetonitrile : phosphate buffer
Flow rate	:	1 ml/min
Auto sampler temperature	:	Ambient
Injection volume	:	10µl
Run time	:	10.0 minutes

Chromatograms shown in figure 1 and figure 2 explain that retention time for standard sample and commercial product of Metformin and Dapagliflozin are same. This proves that, excipients have no effect on the analytical method. On the other hand, blank peak did not overlap drug peak. So the method is highly selective. A linear relationship between peak areas (average peak areas of six replicates) versus concentrations was observed for Metformin and Dapagliflozin in the range of 50 µg / ml to 250 µg / ml for Metformin and 5 µg / ml to 25 µg / ml for Dapagliflozin concentrations. Correlation coefficient was 0.999 for both the drugs which prove that the method is linear.

Precision was evaluated by carrying out six independent sample preparation of a single lot of formulation. The intermediate precision performed for %RSD of Metformin and Dapagliflozin was found to be 0.63 and 0.92 respectively. The method is highly precise as % RSD of peak area was less than 2% in all tests.

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. Results are tabulated in Table 2 and Table 3.

Table 2: Showing accuracy results for Metformin

%Concentration (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	3955152	5	4.96	99.91%	99.56%
100%	7893687	10	9.98	99.18%	
150%	12057868	15	15.02	99.60%	

Table 3: Showing accuracy results for Dapagliflozin

%Concentration (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	15532	0.5	0.99	99.53%	
100%	30089	1.0	1.05	99.38%	99.47 %
150%	46635	1.5	1.495	99.52%	

Results of accuracy study are presented in table 2 and Table 3. The measured value was obtained by recovery test. Percentage concentration of both the drugs was compared against the recovery amount. % Recovery was 99.56% for Metformin and 99.47% for Dapaglifloxin. All the results indicate that the method is highly accurate.

The results of robustness of the present method showed that small changes were made in the flow rate and temperature did not produce significant changes in analytical results which are presented in Table 4 and Table 5 . As the changes are not significant we can say that the method is robust.

Table 4: Results for robustness test of Metformin

S. No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.8	2590	1.39
2	1	2294	1.27
3	1.2	2146	1.26

Table 5: Results for robustness test of Dapagliflozin

S. No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.8	5435	1.04
2	1	4891	1.03
3	1.2	4781	1.04

Conclusion

The new HPLC method developed and validated for simultaneous determination of Metformin and Dapagliflozin pharmaceutical dosage forms and assured the satisfactory precision and accuracy and also determining lower concentration of each drug in its solid combined dosage form by RP-HPLC method. The method was found to be simple, accurate, economical and rapid and they can be applied for routine analysis in laboratories and is suitable for the quality control of the raw materials, formulations, dissolution studies and can be employed for bioequivalence studies for the same formulation.

References

1. Drug bank : dapaglifloxin (DBO6292)
2. Drug bank : metformin (DB00331)
3. Chhetri H P, Thapa P,Schepdael A V, Simple Method of Extraction and Determination of metformin in Human Plasma by High – Performance Liquid Chromatography using UV detection,2002; 27(5): 841-4.
4. Manasa. Sanagapati *, K. Dhanalakshmi , G. Nagarjunareddy and S. Sreenivasa, Development And Validation Of A Rp-Hplc Method For The Estimation Of Dapagliflozin In Api, International Journal of Pharmaceutical Sciences And Research,27:270-2.
5. Beckett A H and Stanlake J B , practical pharmaceutical chemistry, 4th edition CBS publicaters and distributers, New Delhi, part two 281-307.
6. Nash R A and Wachter A H, Pharmaceutical process validation, An international 3rd edition,Revised and Expanded,129, Marcel Dekker Inc.,New York ,507-522.
7. Green J M, A practical guide to analytical method validation, anal chem. News Feat 305A-309A.