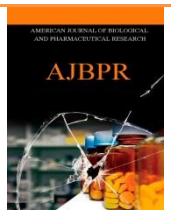
e-ISSN - 2348-2184
Print ISSN - 2348-2176

AMERICAN JOURNAL OF BIOLOGICAL AND PHARMACEUTICAL RESEARCH

Journal homepage: www.mcmed.us/journal/ajbpr



VALIDATED RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF METFORMIN HDROCHLORIDE AND DAPAGLIFLOZIN IN TABLET DOSAGE FORM

Shyamala*, Nidhi B, Kavitha M, Pooja and JVC Sharma

Joginpally B R Pharmacy College, Moinabad, Hyderabad, Telangana, India.

Article Info

Received 29/03/2015
Revised 16/04/2015
Accepted 29/04/2015

Key words: -UV Spectrophotometer, Dapagliflozin, Metformin HCL, High Performance Liquid Chromatography.

ABSTRACT

A new, precise, rapid, accurate RP-HPLC method was developed for the Simultaneous Estimation of dapagliflozin and metformin HCL in tablet dosage form. After optimization the good chromatographic separation was achieved by Isocratic mode with a mixture of Phosphate Buffer (pH 6.5):methanol:acetonitrile in the ratio of 50:30:20 v/v/v as the mobile phase with , column as stationary phase at flow rate of 1 mL/min and detection wavelength of 240 nm. The retention times metformin HCL and dapagliflozin found to be 2.475min and 3.647min respectively. The linearity of this method was found in the concentration range of 85 µg/mL to 510 µg/mL for metformin HCL and 0.5 µg/mL to 3 µg/mL for dapagliflozin. The correlation coefficient R^2 value is found to be 0.997 for metformin HCL and 0.9973 for dapagliflozin. The LOD and LOQ for metformin HCL were found to be 2.469 ppm and 2.468ppm respectively. The LOD and LOQ for dapagliflozin were found to be 3.650ppm and 3.649ppm respectively. This method was found to be good percentage recovery metformin HCL and dapagliflozin were found to be 100.67 and 99.54 respectively indicates that the proposed method is highly accurate. The specificity of the method shows good correlation between retention times of standard with the sample so, the method specifically determines the analyte in the sample without interference from excipients of tablet dosage form. The method was extensively validated according to ICH guidelines for Linearity, Range, Accuracy, Precision, specificity and Robustness.

INTRODUCTION

Dapagliflozin

Dapagliflozin is an antidiabetic drug. Its chemical name is (2S,3R,4R,5S,6R)-2-[4-chloro-3-(4-ethoxybenzy) phenyl]- 6- (hydroxymethyl)tetrahydro-2H-pyran-3,4,5-triol. It acts as SGLT-2 inhibitor. Inhibition of these enzyme system reduces the rate of digestion of carbohydrates. It is not official in any of the pharma copoeia. Literature survey revealed estimation of dapagliflozin by HPLC [1-4] indivually.

Metformin HCL

Metformin HCL is also an anti diabetic drug. Its Chemical Name is 1,1-Dimethyl biguanide. It is official in IP[3], USP[4] and other official books [5,6].

Metformin HCL decreases hyperglycemia by suppressing glucose production by the liver (hepatic gluconeogenesis). Literature survey revealed estimation of metformin by several techniques like HPLC [7-9].

Corresponding Author

Shyamala
Email:- shyamala.mudavath@gmail.com

In this present study an attempt was made to develop rapid and economical RP-HPLC method for estimation of Dapagliflozin and metformin combination and in pharmaceutical formulation with better sensitivity, precision and accuracy using BDS column.

MATERIALS AND METHODS

Instrumentation

The analysis was performed by using the analytical balance , pH meter (Globel Digitl pH meter), the HPLC used is of Waters with PDA detector. Column used in HPLC is of BDS (250 x 4.6 mm, packed with 5 micron) with a flow rate of 1.0 ml/min (isocratic).

Chemicals

The mobile phase consist of phosphate buffer: methanol: acetonitrile (50:30:20) are degassed in a sonicator for about 10min. the UV detection was at 240nm. Acetonitril HPLC grade and methanol HPLC grade were obtained from standard reagents Pvt.ltd. potassium di hydrogen phosphate was obtained from merk-HPLC grade.

Reagents and solutions

Preparation of stock solution

Weigh accurately 34mg of metformine HCL 2mg dapagliflozin into 10ml volumetric flask, shaken and sonicated to dissolve, made upto volume with 10ml of mobile phase.

Stock Preparation

Transferred 1 mL of solution A, 1 mL of solution B, into 10 mL volumetric flask and diluted with mobile phase.

PREPARATION OF MOBILE PHASE

Preparation of buffer

Dissolve 1.36gm of potassium di hydrogen phosphate and 0.45gm of di hydrogen phosphate in 100ml of HPLC grade water, adjusted to pH 4.5 using (dilute Orthophosphoric acid or dilute NaOH solution).

Preparation of mobile phase

Mixed 50ml of buffer, 30ml of Acetonitrile and 20ml methanol (50:30:20) filtered through 0.45 µm nylon filter paper and degassed.

Experimental Condition

Flow rate of the mobile phase was changed from 0.5 – 1.5 ml/min for optimum separation. A minimum flow rate as well as minimum run time gives the maximum saving on the usage of solvents. It was found from the experiments that 1.0 ml/min flow rate was ideal for the successful elution of the analyte. The HPLC system was hence operated using an isocratic mode at a flow rate of 1.0 ml/min. for analysis the most suitable mobile phase was found to be buffer: methanol: acetonitrile(50:30:20 v/v/v).detection was carried out at wave length of 240nm.

RESULTS & DISCUSSION

Method validation

The method was validated by determining linearity, precision, accuracy, specificity, ruggedness and robustness by analyzing dapagliflozin and metformin.

Optimized chromatographic conditions for assay

Linearity

The linear fit of the system was illustrated graphically. Least square regression analysis was carried out for the slope, intercept and correlation coefficient.

Precision

The precision of the assay was studied with respect to both repeatability and intermediate precision. Repeatability was calculated from six replicate injections of freshly prepared dapagliflozin and metformin HCL test solution in the equipment. Record the chromatogram.

Recovery

The recovery of the standard solutions was done by adding them to pre analyzed sample solution at different levels i.e. 50%, 100%, and 150% separately to study the accuracy of the above method. The corresponding results were recorded.

Specificity

Specificity was performed to exclude the possibility of interference with excipients in the region of elution of dapagliflozin and metformin HCL. The specificity and selectivity of the method was tested under normal conditions and the results of the tests proved that the components other than the drug did not produce a detectable signal at the retention place of dapagliflozin and metformin HCL.

Limit of detection (LOD) and limit of quantification (LOQ)

LOD and LOQ were determined from standard deviation of y-intercept of regression line and slope method as per ICH guidelines.

Robustness

Typical variations in liquid chromatography conditions were used to evaluate the robustness of the assay method. In this study, the chromatographic parameters monitored were retention time, area, capacity factor, tailing factor and theoretical plates.

Table 1. Optimized chromatographic conditions

Mobile phase	**Buffer : Methanol :ACN (50:30:20)v\v**
column	BDS
Flow rate	1.0ml/min
Column temperature	Room temperature(20-25^{o}C)
Sample temperature	Room temperature(20-25^{o}C)
Wave length	240nm
Run time	7min
Retention time	2.475 for metformin HCL and 3.647 for dapagliflozin

Table 2. Linearity of metformin HCL and Dapagliflozin

S.No	**Pipetted from stock (mL)**	**Volume of flask (mL)**	**Concentration in ppm(Met)**	**Concentration in ppm(dapa)**
1	0.25	10	85	0.5
2	0.5	10	170	1
3	0.75	10	255	1.5
4	1	10	340	2
5	0.25	10	425	2.5
6	0.50	10	510	3

Table 3. Regression analysis of the calibration curve

S. no	**Parameter**	**Metformin HCL**	**Dapagliflozin**
1	Correlation coefficient	0.997	0.997
2	Slope	37.41	81.83
3	Intercept	267.0	26.41

Table 4. Precision of Metformin HCL

Metformin HCL		
S. No.	**RT**	**Area**
1	2.451	382978
2	2.453	390758
3	2.456	382924
4	2.458	387255
5	2.465	394434
6	2.468	391366
Mean		388286
SD		4719.8
% RSD		1.22

Table 5. Dapagliflozin precision

Dapagliflozin		
S. No.	**RT**	**Area**
1	3.631	364475
2	3.632	362721
3	3.637	370615
4	3.637	369041
5	3.645	368377
6	3.646	362030
Mean		366210
SD		3599.2
% RSD		0.98

Table 6. Recovery of dapagliflozin and metformin HCL

S. no	Spiked level in %	Dapagliflozin amount recovered	Metformin HCL amount recovered	% recover of dapagliflozin	% recover of metformin HCL
1	50%	5.04	99.64	99.48	100.35
2	100%	6.04	122.01	99.59	100.62
3	150%	6.99	137.60	100.54	99.83

Table 7. LOD & LOQ of Metformin HCL and Dapagliflozin

S. no	Compound	Limit of Detection Concentration in ppm (mgL)	Limit of Quantitation Concentration in ppm(mgL)
1	Metformin HCL	2.469	2.468
2	Dapagliflozin	3.650	3.649

Table 8. Robustness of Dapagliflozin and Metformin

Proposed variations		Asymmetry factor of Dapagliflozin peak in standard	Asymmetry factor of Metformin peak in standard	Acceptance criteria
Variation in mobile phase composition	30% organic phase	1.35	1.571	In between 0.5 to 2.0
	40% organic phase	1.35	1.571	
Variant in flow rate	1.0ml/min	1.30	1.571	
	0.9ml/min	1.41	1.682	
Variation in temperature	25˚c	1.33	1.500	
	27˚c	1.34	1.571	

Table 9. Assay

Compound	Standard area	Sample area	Standard purity
Metformin HCL	382978	385248	99.8
Dapagliflozin	364475	362937	100.02

Figure 1. Structure of Dapagliflozin

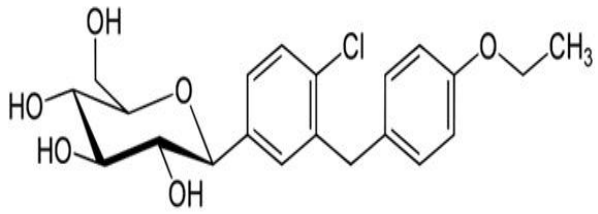


Figure 2. Structure of Metformin HCL

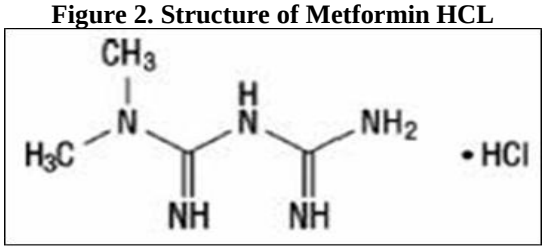


Figure 3. chromatograms of Metformin and Dapagliflozin assay

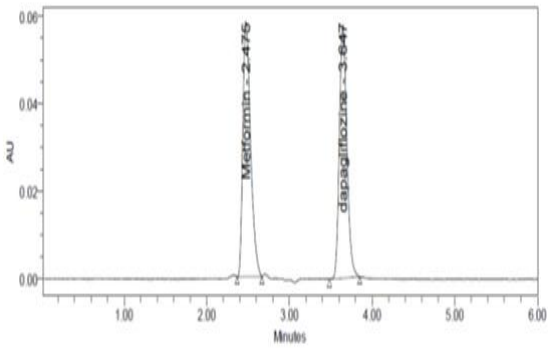


Figure 4. Calibration curve of Metformin HCL

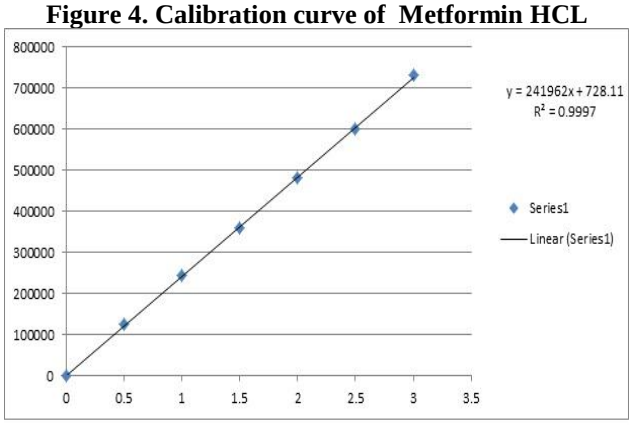
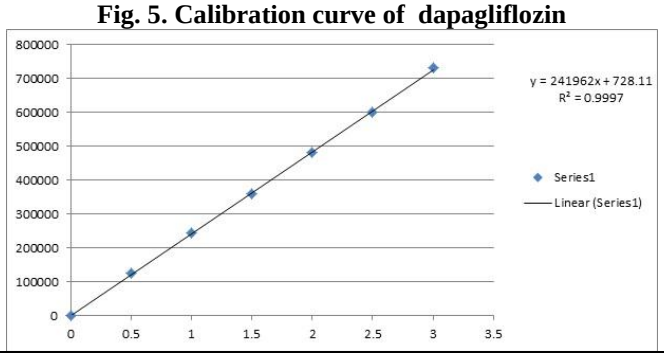


Fig. 5. Calibration curve of dapagliflozin



CONCLUSION

The developed method was validated and found to be simple, sensitive, accurate and precise. I was also proved to be convenient and effective for the determination of dapagliflozin and metformin HCL in the pharmaceutical dosage form. The percentage of recovery shows that the method is free from interference of the excipients used in formulation. Moreover, the lower solvent consumption along with the short analytical run time leads to cost effective chromatographic method. The mobile phase has no any type of salt buffers, then column works efficiently long time.

ACKNOWLEDGEMENT

The principal of Joginpally B.R Pharmacy College and Secretary of JB Educational Society, Telangana, for guidance, encouragement and providing laboratory facilities.

REFERENCES

1. Sanagapati Manasa, Dhanalakshmi K, Nagarjuna Reddy G, Sreenivasa S. (2014). Method Development and Validation of Dapagliflozin in API by RP-HPLC and UV-Spectroscopy. *IJPSDR*, 6(3), 250-252.
2. Aubry AF, Gu H, Magnier R, Morgan L, Xu X, Tirmenstein M et al. (2010). Dapagliflozin is an inhibitor of sodium-glucose co-transporter 2 (SGLT-2) in development for the treatment of Type 2 diabetes. *Bioanalysis*, 2(12), 2001-9.
3. Government of India. (2010). Ministry of health and family welfare, Indian Pharmacopoeia, Vol- I & II, The Controller of Publication, New Delhi, 1657-60.
4. The United States pharmacopoeia. (2011). USP 34 NF 29, Vol- III United States Pharmacopoeia Convention, lnc, Rockville, 3442.
5. The Merck Index. (2001). 14th Edn. Whitehouse Station, NJ: Merck & Co. Inc.
6. Martindale. (2002). The complete drug reference. 33th ed. London. Pharmaceutical press, 313-334.
7. Himal Paudel Chhetri, Panna Thapa. (2014). HPLC-UV method for the determination of metformin in human plasma. *Saudi Pharm J*, 22, 483-487.
8. Mousumi Kar and PK Choudhury. (2009). HPLC method has been developed for quantitative estimation of metformin hydrochloride from tablet dosage form and formulated microspheres. *Indian J Pharm Sci*, 71(3), 418-420
9. Madhukar A, A Prince, Vijay Kumar R, Sanjeeva. (2011). Simple and Sensitive Analytical Method Development And Validation Of Metformin Hydrochloride By Rp-Hplc. *IJPPS*, 3(2), 117-120.
10. International Conference of Harmonization Guidelines on validation of Analytical Procedures Definitions and Terminology. (1995). Federal Register, March 1, 7.
11. ICH Q2A. (1995). Guidelines on validation of analytical procedure; Definitions and terminology. Federal Register, 60, 11260.
12. ICH Q2B. (1996). Guidelines on validation of analytical procedure; Methodology. Federal Register, 60, 27464.