INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

ULTRAVIOLET-SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF DAPAGLIFLOZIN PROPANEDIOL AND METFORMIN HYDROCHLORIDE

Minal Harde *[1], Sameer Lakade [2] , Valmik Gite [3], Vitthal Chopade [1], Anuja Khomne [1]
[1]P. E. Society's Modern College of Pharmacy Yamunanagar, Nigdi, Pune, Maharashtra, India
[2]Rasiklal M. Dhariwal Institute of Pharmaceutical Education and Research, Chinchwadgaon, Pune, Maharashtra, India
[3]Adv. Manoharrao Nanasaheb Deshmukh ACS College, Rajur, Ahmednagar, Maharashtra, India
*Corresponding Author Email: minaltharde@yahoo.com

Article Received on: 04/12/18 Approved for publication: 12/03/19

DOI: 10.7897/2230-8407.1004130

ABSTRACT

Two simple, precise and economical UV spectrophotometric methods have been developed for the simultaneous estimation of Dapagliflozin and Metformin in bulk drug and marketed formulation. In simultaneous equation method, 223 nm (λ_{max} of DAPA) and 233 nm (λ_{max} of MET) analytical wavelengths were selected and analysis were carried out. In area under curve method, 220-229 nm (λ_{max} of DAPA) and 229-236 nm (λ_{max} of MET) wavelengths were selected and analysis were carried out. The method was validated using various parameters according to ICH guidelines. The low relative standard deviation values indicate good precision and high recovery values indicate accuracy of the proposed method. Assay results were in good agreement with label claim.

Keywords: Metformin HCl, Dapagliflozin Propanediol, Simultaneous estimation, Area under curve, Validation.

INTRODUCTION

Metformin Hydrochloride (MET) is a chemically 1, 1 dimethyl biguanide hydrochloride[1] (Fig 1) member of the biguanide class of oral antidiabetic drug, improves glucose tolerance in type 2 diabetic patients, lowering both basal and postprandial plasma glucose. MET minimize hepatic glucose production, reduces intestinal absorption of glucose and improves Insulin sensitivity by enhancing peripheral glucose uptake and utilization[2]. It has a molecular formula $C_4H_{22}CLN_5$ with molecular weight 165.625 g/mol and it is a white crystalline powder and freely soluble in water, ethanol, and methanol. I.P. recommends non-aqueous titration method for raw material and UV spectrometric method for tablets[3-5].

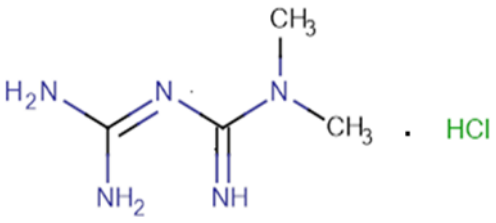

Figure 1: Metformin Hydrochloride

Dapagliflozin Propanediol (DAPA) is a chemically (1s)-1, 5-anhydro-1-C-[4-chloro-3-[(4-ethoxyphenyl) methyl] phenyl]-D-glucitol[6,7] (Fig 2). Dapagliflozin propanediol blocking$SGLT_2$ transporter mechanism causes blood glucose to be eliminated through the urine and maintained the insulin concentration in blood, at least 90% of the glucose to be eliminated through urine [8]. It has a molecular formula $C_{24}H_{33}ClO_8$ with molecular weight 408.873 g/mol. Dapagliflozinpropanediol is a white to off white crystalline powder which is soluble in water, ethanol,methanol and dimethyl formamide [9,10].

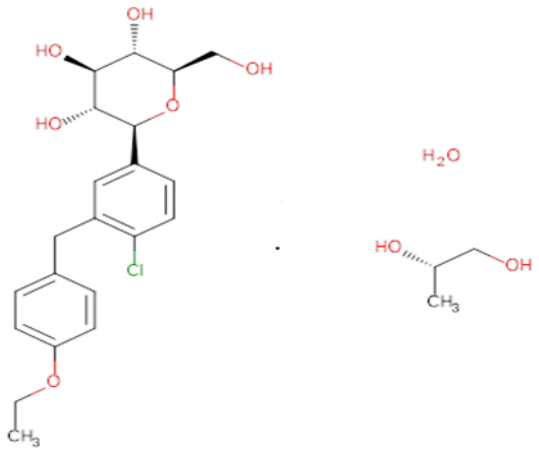

Figure 2: Dapagliflozin Propanediol

Literature survey reveals various methods for estimation of MET and DAPA in formulations, either alone or in combination with other drugs. Literature survey also revealed single RP-HPLC method for the binary mixture in API and tablet dosage form[11]. Three methods have been reported for estimation of the studied drugs in combine formulation by simultaneous equation method [12], Q-Absorbance ratio method [12] and first derivative spectrophotometric method [13]. In present study, an effort has been successfully made to determine MET And DAPA in bulk and tablet dosage form by simultaneous equation method and area under curve method. The proposed method has been optimized as

per the International conference on harmonization (ICH) guidelines 2005[14, 15].

MATERIALS AND METHODS

Materials and Reagents

Working standards of pharmaceutical grade MET and DAPA was obtained as a generous gift sample from Neon Laboratories Ltd, Mumbai and Sun Parma. Pvt. Ltd., Mumbai respectively. Xigduo® XR tablets were purchased from local pharmacy shop labeled to contain 500 mg MET and 10 mg DAPA. Analytical grade methanol and water were purchased from Merck specialties Pvt. Ltd., Mumbai.

Instruments

JASCO Double beam UV-Vis Spectrophotometer (Model V-630) with 1 cm matched quartz cuvettes, was used for all spectra measurement. Sonication of samples was carried out using ultra sonicator (Biomedica, India), analytical balance (Schimadzu AUX 220, Japan) was used for weighing purpose and auto pipettes (Eppendorf, Hamburg, Germany) were used. Calibrated volumetric glassware (Borosil®) was used for the validation study.

Preparation of Standard Solutions and Selection of Wavelength

For Method I (Simultaneous equation method), MET (10mg) and DAPA (10mg) were accurately weighed and transferred to two separate 100 ml volumetric flask and dissolved in 10 ml of methanol and diluted with double distilled water up to mark to obtain standard working solution of concentration 100 µg/ml of MET and DAPA respectively were prepared separately in 100 ml volumetric flask and standard stock solutions were further diluted with double distilled water to obtain concentration ranges from 3-15 µg/ml and 2-30 µg/ml respectively.

For Method II (Area under curve method), MET (10mg) and DAPA (10mg) were accurately weighed and transferred to two separate 100 ml volumetric flask and dissolved in 10 ml of methanol and diluted with double distilled water up to mark to obtain standard working solution of concentration 100 µg/ml of MET and DAPA respectively were prepared separately in 100 ml volumetric flask and standard stock solutions were further diluted with double distilled water to obtain concentration ranges from 3-15 µg/ml and 2-30 µg/ml respectively.

For Method I: 10 µg/ml of MET and 10 µg/ml of DAPA were scanned separately in the range of 200-400nm. MET and DAPA showed maximum absorbance at 233 nm and 223nm respectively.

For Method II: 10 µg/ml of MET and 10 µg/ml of DAPA were scanned separately in the range of 200-400nm. MET and DAPA showed maximum absorbance at 229-236 nm and 220-229nm respectively.

Method I: Simultaneous Equation Method (Vierodt's Method)

If a sample contains two drugs with reasonably dissimilar λmax, each of which exhibits absorbance at the λmax of other, then it is possible to determine the drugs by simultaneous equation method (Vierodt's Method). Two equations are constructed based on the fact that the absorbance at a particular λmax is sum of individual absorbance of two components.The scanning spectra of 10µg/ml solution of DAPA and MET show clear peaks at 223nm and 233nm respectively (Fig.3, 4).The λmax of each drug was selected for analysis.

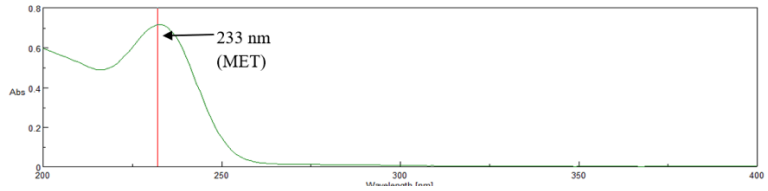

Figure 3: UV Spectrum of Metformin HCl at 233 nm

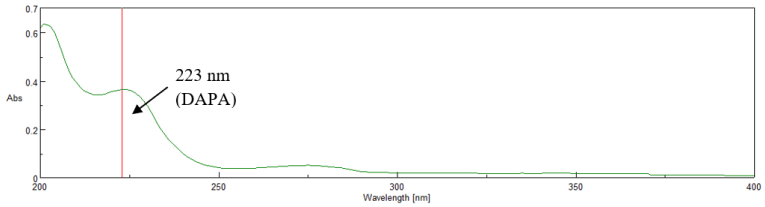

Figure 4: UV Spectrum of Dapagliflozin propanediol at 223nm

The standard stock solution of DAPA and MET was diluted with double distilled water and calibration curve was obtained between 2-30 µg/ml for DAPA and 3-15 µg/ml for MET. Calibration curves were plotted to verify the Beer's law and the absorptivity values were calculated at the respective wavelengths for both the drugs. Absorbances of prepared solutions were measured at 223 nm and 233 nm. Two simultaneous equations are given below were formed using absorptivity values, A(1%, 1cm)(Table 1).

$$C_x = (A_2 . ay_1 - A_2 . ay_2) / (ax_2 . ay_1 - ax_1 . ay_2) \quad \text{.....(1)}$$

$$C_Y = (A_1 . ax_2 - A_2 . ax_1) / (ax_2 . ay_1 - ax_1 . ay_2) \quad \text{.....(2)}$$

Where,
Cx and Cy are concentrations of MET and DAPA respectively in µg/ml of sample solutions.
A_1 and A_2 are absorbances of sample solutions at 233 nm 223 nm.
ax_1 and ax_2 absorptivity of MET at 223nm and 233 nm.
ay_1 and ay_2 are the absorptivity of DAPA at 223 and 233 nm.

The absorbances (A1 and A2) of the sample solutions were recorded at 223 and 233nm, respectively and concentration of both components were calculated using above mentioned equations (1 and 2).

Method II: Area Under Curve Method

Area under curve involves the calculation of integrated value of absorbance with respect to the wavelength between two selected wavelengths λ_1-λ_2 and λ_3-λ_4. This wavelength range is selected on the basis of repeated observation of the linear concentrations so as to get linearity between area vs concentration. The solutions of drugs were scanned in the range of 200-400 nm. For Area under Curve method, the sampling wavelength ranges selected for estimation of Metformin HCl and Dapagliflozin propanediol were 229-236 nm (λ_1-λ_2) and 220-229 nm (λ_3-λ_4) respectively. For determining the concentration of drugs by AUC method, the following equation was used. Amount of each drug was calculated using following formula.

A = absorbance of sample
a = molar absorptivity of sample
B = path length
C= concentration of sample
a=A/bc

By substituting mean absorptivity values in the equation given below,

a_1 = ax1+ ay1
a_2 = ax2+ ay2

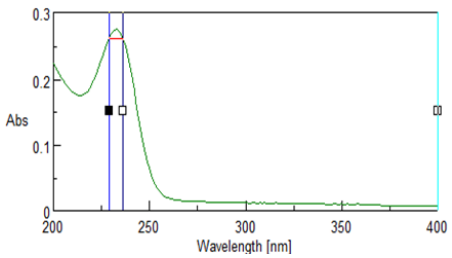

Figure 5: UV Spectrum of Metformin HCl at 229-236 nm

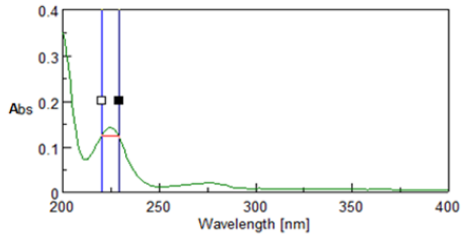

Figure 6: UV Spectrum of Dapagliflozin Propanediol at 220-229 nm

Application of The Proposed Methods for Determination of Met and Dapa in Tablet Dosage Form

Twenty tablets (Xidguo® XR, label claim MET 500 mg and DAPA 10 mg) were weighed and finely powdered. The powder equivalent to about 500 mg MET was weighed accurately transferred to a 100 ml volumetric flask and suspended in 10 ml methanol and the mixture was sonicated for 15 min and the volume was made to the mark with double distilled water. The mixture was filtered through Whatmann No. 41 filter paper. Aliquot portion of the filtrate was diluted with water to achieve the final concentration. The absorbance was noted at respective wavelength to determine the concentration of MET and DAPA by the Simultaneous equation method and Area under curve method.

Validation of Analytical Method

The developed method was validated using parameters such as Accuracy, Linearity, precision, LOD and LOQ and sensitivity.

Accuracy

Accuracy may often be expressed as % Recovery by the assay of known, added amount of analyte. It is a measurement of the exactness of the analytical method. Recovery studies carried out for both the methods by standard addition method at three different level i. e. 80%, 100% and 120%.

Linearity

The linearity of measurement was evaluated by analyzing different concentration of the standard solution of DAPA and MET. For both the method, the Beer-Lambert's concentration range was obtained between 2-30 µg/ml for DAPA and 3-15 µg/ml for MET.

Precision

The reproducibility of the proposed method was determined by performing tablet assay at different time intervals (morning, afternoon and evening) on same day (Intra-day assay precision) and on three different days (Inter-day precision). Result of intra-day and inter-day precision is expressed in % RSD.

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

The limit of detection (LOD) and limit of quantification (LOQ) were calculated by using the equations LOD = 3.3xσ/ S and LOQ = 10xσ/S, where σ is the standard deviation of intercept, S is the slope.

RESULT AND DISCUSSION

Method Development

An attempt was carried out to develop cheap, accurate and sensitive analytical method for simultaneous estimation of MET and DAPA in combined dosage form. In simultaneous equation method, wavelengths selected for analysis were 223 nm for DAPA and 233 nm for MET. In area under curve method, the area under curve in the range of 220-229 nm (for DAPA) and 229-236 nm (for MET) were selected for the analysis. The overlain is shown in Fig 7.

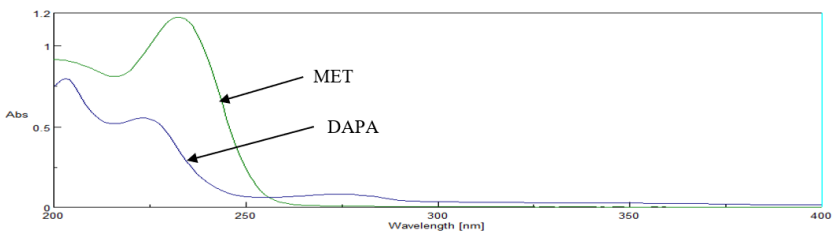

Figure 7: Overlain Spectra of MET and DAPA

Table 1: Result of Analysis of Formulation

	Drug	Label Claim (mg/tablet)	Amount estimated (mg/tablet)	% Label claim	%*RSD
DAPA	**Method I**	**10**	9.04	90.4	1.48
	Method II	**10**	8.98	89.83	1.39
MET	**Method I**	**500**	482.93	96.58	0.93
	Method II	**500**	475.76	95.15	1.15

* mean of six observations

Table 2: Results of Recovery study

Parameters		DAPA		MET	
	% level	Method I	Method II	Method I	Method II
% Recovery	**80**	100.87	87.68	101	1.345
	100	100.4	92.50	99.9	1.91
	120	98.5	98.57	99.97	1.651
% RSD*	**80**	1.81	0.9860	90.70	1.28
	100	1.499	1.902	87.34	0.6401
	120	1.639	0.5035	86.86	0.2160

* mean of six observations

Table 3: Results of Linearity Study

Parameters	DAPA		MET	
	Method I	Method II	Method I	Method II
Beers law limit (µg/ml)	2-30	2-30	3-15	3-15
λmax (nm)	223	220-229	223	229-236
Regression value				
i. Slope	0.059	0.0095	0.075	0.0143
ii. Intercept	0.0040	0.0012	0.047	0.0148
Regression coefficient (r^2)	0.999	0.9996	0.999	0.9983

Table 4: Results of Precision study

Drug	Concentration (µg/ ml)	Intraday precision % *RSD		Interday precision % *RSD	
		Method I	Method II	Method I	Method II
DAPA	**3**	0.309	1.5456	0.309	0.874
	6	0.163	0.9797	0.141	0.474
	9	0.121	1.3208	0.061	1.89
MET	**3**	0.33	0.2548	0.140	0.65012
	6	0.138	0.7497	0.143	0.0671
	9	0.02	1.4599	0.0369	0.7935

* mean of six observations

Table 5: Results of LOD and LOQ

Drug	LOD (µg/ml)		LOQ (µg/ml)	
	Method I	Method II	Method I	Method II
DAPA	0.6559	0.7746	1.98	2.34
MET	0.6522	0.749	1.97	2.27

* mean of six observations

Analysis of Formulation

The total amount of DAPA and MET present in formulation was calculated using the simultaneous equation method and Area under curve method. The results are presented in Table 1.

Validation of Analytical Method

Accuracy Study

To check the accuracy of the developed method and to study the interference of formulation additives, analytical recovery experiments were carried out by the standard addition method. The recovery studies were carried out at three different levels i.e. 80%, 100% and 120% level. The percentage recovery values were shown in Table 2.

Linearity Study

Linearity was established by least squares linear regression analysis of the calibration curve. The constructed calibration curves were observed linear over the concentration range of 2-30µg/ml for DAPA and 3-15µg/ml for MET (Figure 6-7). Absorbance were plotted versus their respective concentrations and linear regression analysis performed. Linearity study is presented in Table 3.

Precision

Intra-day precision was obtained by analyzing the three different concentration 3 µg/ml, 6 µg/ml, 9 µg/ml containing MET and DAPA, for three times in a day. Inter-day variability using the above mentioned three concentrations analyzed on three different days, over a period of one week. The repeatability of MET and

DAPA also performed using the same concentration of drugs in a repeated manner. The % RSD should be less than 2% as per ICH Guideline 2005. The results are represented in Table 4.

LOD and LOQ

Limit of detection and limit of quantitation was estimated using formula 3.3* σ/S and 10* σ/S, respectively, where σ is the standard deviation of the response (y-intercept) and S is the slope of the linearity plot. The result of sensitivity study is represented in Table 5.

CONCLUSION

The rapid, sensitive, cost effective and accurate simultaneous equation method and Area under curve method has been successfully developed and applied for determination of MET and DAPA in bulk and marketed tablet dosage form. The proposed method was validated successfully. Recovery studies indicated that there was no interference of the excipients during experiment, so the presented methods can be suitable for routine quality control analysis of MET and DAPA.

ACKNOWLEDGEMENT

Authors are thankful to Principal, Modern College of Pharmacy, Nigdi, Pune for providing instrumental and infrastructure facility to carry out the research work. Thanks, are also extended to Neon Laboratories Ltd, Mumbai and Sun Parma. Pvt. Ltd., Mumbai for providing gift samples of the pure drugs for research work.

REFERENCES

1. Klip A, Leiter LA. Cellular Mechanism of Action of Metformin. Diabetes care 1990; 13(6): 696-704.
2. Metformin HCl. http://www.drugbank.ca/drugs/DB00331 [Cited on 2017 15th Oct]
3. Indian Pharmacopoeia. Vol. 2 Govt. of India, Ministry of Health and Family welfare. The Indian Pharmacopoeia commission, Ghaziabad 2010:1657-1659
4. United States Pharmacopoeia. 34th edition, The United States Pharmacopoeia Convention Inc. Rockville, MD, 200: 3442-3443
5. British Pharmacopeia. Vol. 2. The Department of Health, British Pharmacopeia Commission, London; 2009: 1410-1412
6. Dapagliflozin Propanediol. http://www.drugbank.ca/drugs/DB06292 [cited on 2017 15th Oct].
7. Mante GV, Gupta KR, Hemk AT. Estimation of Dapagliflozin from its Tablet Formulation by UV-Spectrophotometry. Pharma Methods 2017; 8(2): 102-107.
8. Sanagapati M, Dhanlakshmi K, Reddy NG, Kavitha B. Method Development and Validation of Dapagliflozin API by UV Spectroscopy. Int J Pharm Sci Rev Res 2014; 24(1): 270-276.
9. Tatarkiewicz K, Polizzi C, Villescaz C, D'Souza LJ, Wang Y, Janssen S. Combined Antidiabetic Benefits of Exenatide and Dapagliflozin in Diabetic Mice. Diabetes Obes Metab 2014; 16(4): 376-80.
10. Lambers Heerspink HJ, de Zeeuw D, Wie L, Leslie B, List J. Dapagliflozin a Glucose-Regulating Drug with Diuretic Properties in Subjects with Type 2 Diabetes. Diabetes Obes Metab 2013; 15(9): 853-62.
11. Mohammad Y, Gowri SD. Validated Stability Indicating High Performance Liquid Chromatographic Method for Simultaneous Determination of Metformin HCl and Dapagliflozin Propanediol in Bulk Drug and Tablet Dosage Form. Asian J Pharm Clin Res 2015; 8(3): 320-326.
12. Jani BR, Shah KV, Kapupara PP. Development and Validation of UV Spectroscopic Method for Simultaneous Estimation of Dapagliflozin and Metformin HCl in Synthetic Mixture. Int. J. Res. Dev. Pharm. L. Sci 2015; 4(3): 1569-1576.
13. Jani BR, Shah KV, Kapupara PP. Development and Validation of UV Spectroscopic First Derivative Method for Simultaneous Estimation of Dapagliflozin and Metformin Hydrochloride in Synthetic Mixture. J Bioequiv. Studies 2015; 1(1): 1-8.
14. ICH guidelines: Validation of Analytical Procedures: Q2B, 2005.
15. ICH guidelines: Validation of Analytical Procedures: Q2A, 2005.

Cite this article as:

Minal Harde *et al*. Ultraviolet-spectrophotometric method for simultaneous estimation of dapagliflozin propanediol and metformin hydrochloride. Int. Res. J. Pharm. 2019;10(4):90-94 http://dx.doi.org/10.7897/2230-8407.1004130

Source of support: Nil, Conflict of interest: None Declared