International Journal of Research and Development in Pharmacy and Life Sciences
Available online at http//www.ijrdpl.com
April - May, 2015, Vol. 4, No.3, pp 1569-1576
ISSN (P): 2393-932X, ISSN (E): 2278-0238

Research Article

DEVELOPMENT AND VALIDATION OF UV SPECTROSCOPIC METHOD FOR SIMULTANEOUS ESTIMATION OF DAPAGLIFLOZIN AND METFORMIN HYDROCHLORIDE IN SYNTHETIC MIXTURE

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(Received: February 09, 2015; **Accepted:** March 27, 2015**)**

ABSTRACT

The objective of the work is to develop UV spectroscopic method for simultaneous estimation of Dapagliflozin (DAPA) and Metformin hydrochloride (MET). This method involve solving of simultaneous equations based on measurement of absorbance at two wavelengths 225 nm and 237 nm. Both the drugs obey the Beer's law in the concentration ranges employed for this method. Results of the methods were validated statistically. Novel, simple, sensitive, rapid, accurate and economical spectrophotometric methods have been developed for simultaneous estimation of Dapagliflozin and Metformin hydrochloride. The method can be used to estimate the amount of Dapagliflozin and Metformin hydrochloride in mixture containing Dapagliflozin and Metformin hydrochloride.

Keywords: Dapagliflozin, Metformin hydrochloride, Antidiabetic, Spectrophotometric analysis, Simultaneous equation method.

INTRODUCTION

Dapagliflozin is supplied as a crystalline solid. Dapagliflozin is inhibiting renal glucose reabsorption through the solid-glucose cotranspoter (SGLT) offers an insulin-independent alternative to controlling blood glucose concentrations in patients with type 2 diabetes. Dapagliflozin is a first generation, selective SGLT inhibitor that blocks glucose transport with about 100-fold selective for SGLT2 over SGLT1[1].

Metformin (originally sold as Glucophage) is an oral anti-diabetic drug in the biguanide class. It is the first-line drug of choice for the treatment of type 2 diabetes, particularly in overweight and obese people. Evidence is also mounting for its efficacy in gestational diabetes, although safety concerns still preclude its widespread use in this setting. It activates the AMP-activated protein kinase (AMPK). It is also used in the treatment of polycystic ovary syndrome and has been investigated for other diseases where insulin resistance may be an important factor [2].

Literature survey reveals that few HPLC and LC MS methods have been reported for determination of Metformin hydrochloride in bulk, in formulations [3,4,5,6,7,8,9,10].. There is no any single UV method reported for simultaneous analysis of Dapagliflozin and Metformin hydrochloride. A successful attempt has been made to estimate two drugs simultaneously by spectrophotometric analysis. The objective of the investigation is to develop and validate an analytical method for the estimation of Dapagliflozin and Metformin hydrochloride in a combined mixture by simultaneous UV spectroscopic method.

MATERIALS AND METHODS

A double beam UV/Visible spectrophotometer (Labtronic-LT2900) was employed with spectral bandwidth of 1 nm and wavelength accuracy of ± 0.3 nm with automatic wavelength correction with a pair of 10 mm quartz cells. A Shimadzu electronic analytical balance (BL – 220H) was used for weighing the sample. Dapagliflozin (Alembic Pharmaceuticals, Baroda), Metformin hydrochloride (Aarti Drugs, Mumbai), Methanol were used in the study.

Preparation of Calibration Curve:

Preparation of Standard Calibration Curve of Dapagliflozin in Methanol Solution:

Accurately weighed 100 mg of Dapagliflozin was dissolved in 100 ml of Methanol solution (stock solution).Then 0.5, 1, 1.5, 2 and 2.5 ml of above solution was transferred in a 100 ml volumetric flask and volume was made up to the mark with methanol solution to make 0.5, 1, 1.5, 2 and 2.5 ml concentration. The absorbance of each of these solutions were measured at the selected wavelengths (i.e. 225 nm and 237 nm) using UV spectrophotometer and plotted against concentration. The concentration range over which the drugs obeyed beer's law was chosen. The range was found to be 0.5-2.5 µg/ml at both the wavelength.

Preparation of Standard Calibration Curve of Metformin HCl in Methanol Solution:

Accurately weighed 100 mg of Metformin HCl was dissolved in 100 ml of Methanol Solution (Stock solution).Then 25, 50, 75, 100 and 125 ml of above solution was transferred in a 100 ml volumetric flask and volume was made up to the mark with methanol solution to make 25, 50, 75, 100 and 125 ml concentration. The absorbance of each of these solutions were measured at the selected wavelengths (i.e., 225 nm and 237 nm) using UV spectrophotometer and plotted against concentration. The concentration range over which the drugs obeyed beer's law was chosen. The range was found to be 25-125 µg/ml at both the wavelength.

Development of Simultaneous Equation for Dapagliflozin and Metformin HCl:

Absorptivity from all the concentration was calculated for both the drugs in methanol Solution and used for the development of simultaneous equation [11,12].

$$Cx = \frac{(A2*ay1 - A1*ay2)}{ax2*ay1 - ax1*ay2} \qquad (1)$$

$$Cy = \frac{(A1*ax2 - A2*ax1)}{(ax2*ay1 - ax1*ay2)} \qquad (2)$$

The concentration of CDAPA and CMET can be obtained by solving equation (3) and (4).

$$C_{DAPA} = \frac{A1 \times 1617.45 - A2 \times 2530.96}{9.69 \times 104 \times 1617.45 - 1.89 \times 105 \times 2530.96} \qquad (3)$$

$$C_{MET} = \frac{A2 \times 9.69 \times 10 - A1 \times 1.89 \times 105}{9.69 \times 104 \times 1617.45 - 1.89 \times 105 \times 2530.96} \qquad (4)$$

Where,

[1] 1.89×105 and 9.69×104 are absorptivity of Dapagliflozin at λ1 (225) and λ2 (237) respectively.

[2] 1617.45 and 2530.96 are absorptivity of Metformin HCl at λ1 (225) and λ2 (237) respectively.

[3] A1 and A2 are absorbance of mixture at λ1 (225) and λ2 (237) respectively.

[4] CDAPA and CMET are concentration in gm/liter.

Standardization of the method by analysis of powder mixture of known composition:

The mixture of Dapagliflozin and Metformin HCl having concentration of 1 µg/ml of DAPA and 50 µg/ml of MET were analyzed by preparing a solution of suitable dilution in Methanol solution. The absorbance of the solution at 225 nm and 237 nm for both drugs were measured. The values were substituted in equation (3) and (4) to get a concentration of Dapagliflozin and Metformin HCl respectively in Methanol solution.

The results of the analysis of powder mixture are reported in Table 4 and data for statistical validation are given in Table 5.

Procedure for Precision:

In intraday precision sample having concentration of 1 µg/ml of DAPA and 50 µg/ml of MET were scanned six times at different time interval in the same day. Interday precision was obtained by the assay of six sample sets on different days. The results are shown in Table.6 and 7.

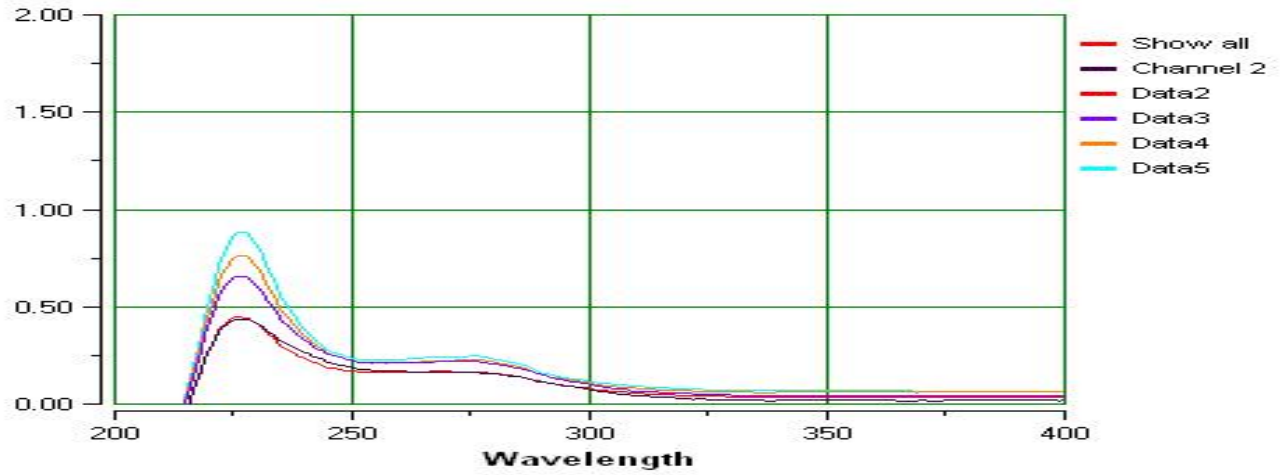

Figure 1: UV Spectrum of Dapagliflozin in methanol solution, Dapagliflozin showed λ_{max} at 225 nm

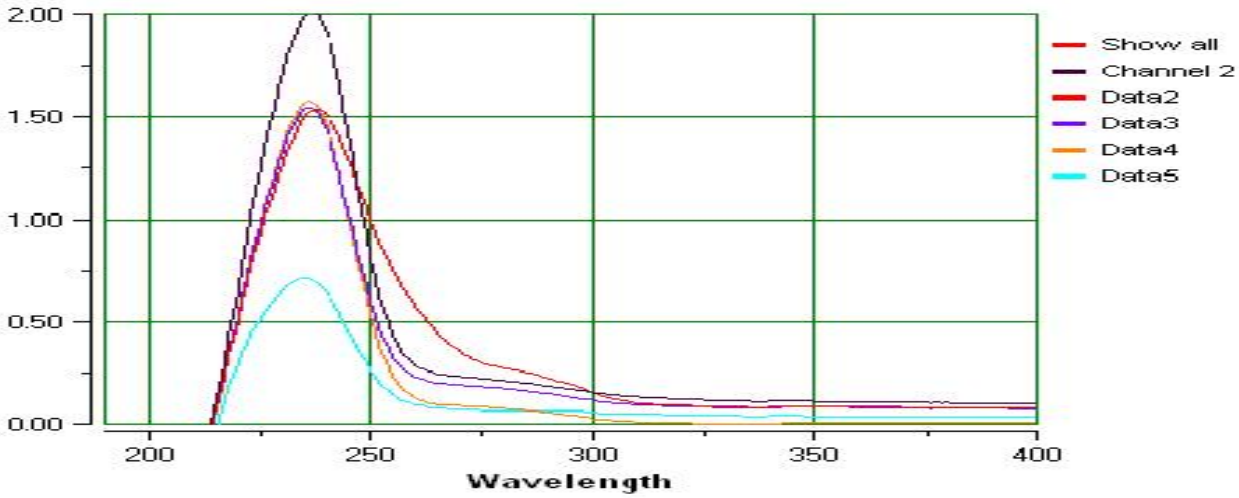

Figure 2: UV Spectrum of Metformin HCl in methanol solution, Metformin HCl showed λ_{max} at 237 nm in methanol solution

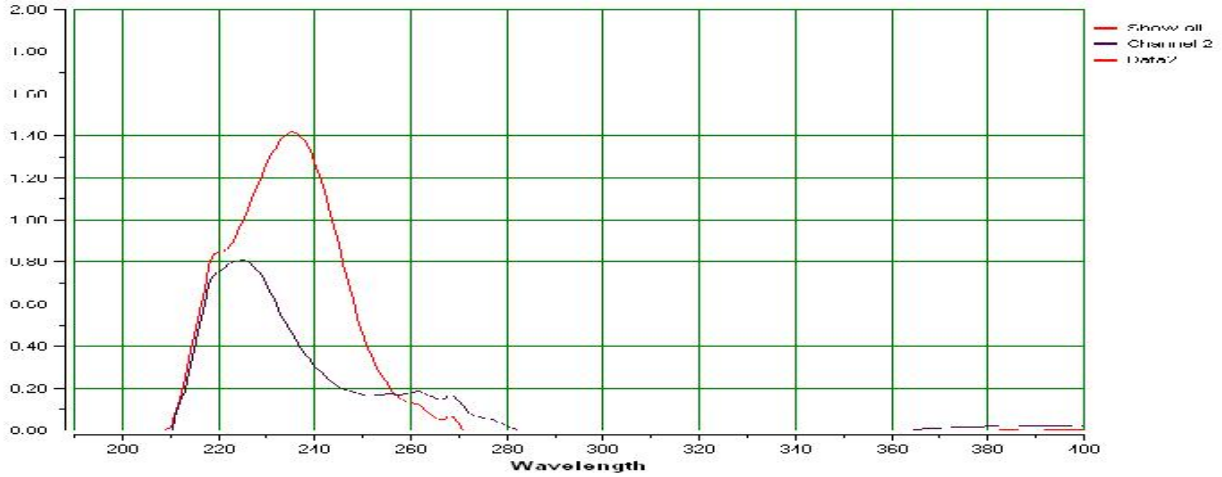

Figure 3: UV spectrum of Overlay of Dapagliflozin and Metformin HCl

Table 1: Data for Standard Calibration Curve of Dapagliflozin at 225 nm in methanol solution

Sr. No.	Concentration (µg /ml)	Absorbance at 225 nm	Absorbance at 237 nm
1	0.5	0.386	0.310
2	1	0.454	0.325
3	1.5	0.601	0.369
4	2	0.708	0.439
5	2.5	0.816	0.558

*= mean absorbance of three absorbances

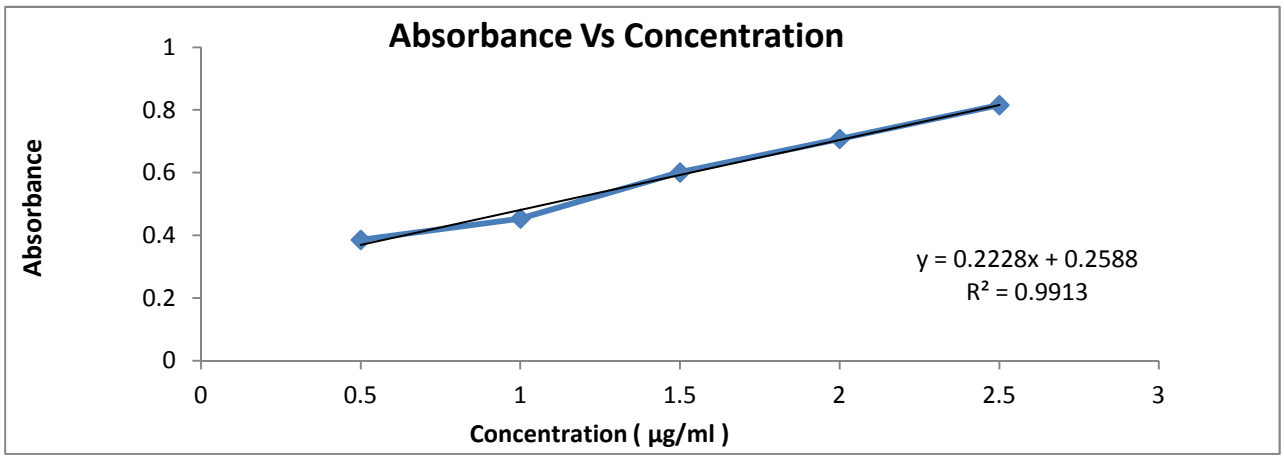

Figure 4: Calibration Curve of Dapagliflozin at 225 nm in Methanol Solution within the range of 0.5 to 2.5 µg/ml the drug obeyed Beer's law.

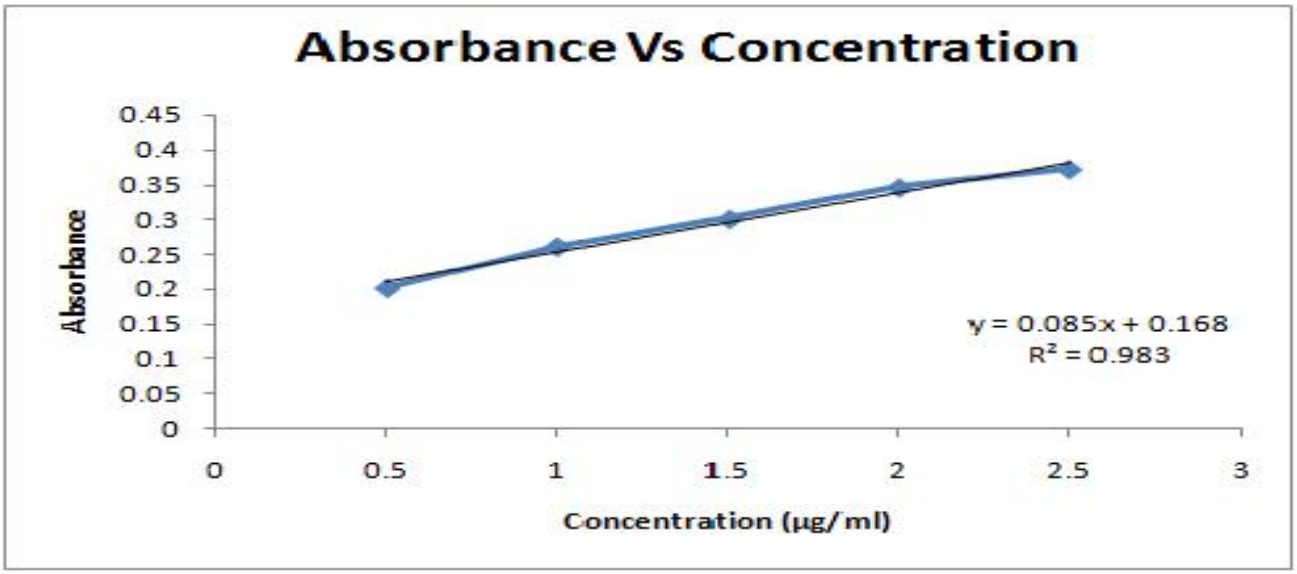

Figure 4: Calibration Curve of Dapagliflozin at 237 nm in methanol within the range of 0.5 to 2.5 µg/ml the drug obeyed Beer's law.

Table 2: Data for Standard Calibration Curve of Metformin HCl at 237 nm in methanol Solution

Sr. No.	Concentration (µg /ml)	Absorbance at 225 nm	Absorbance at 237 nm
1	25	0.399	0.67
2	50	0.691	1.02
3	75	0.857	1.42
4	100	1.1	1.66
5	125	1.29	1.89

*= mean absorbance of 3 absorbance

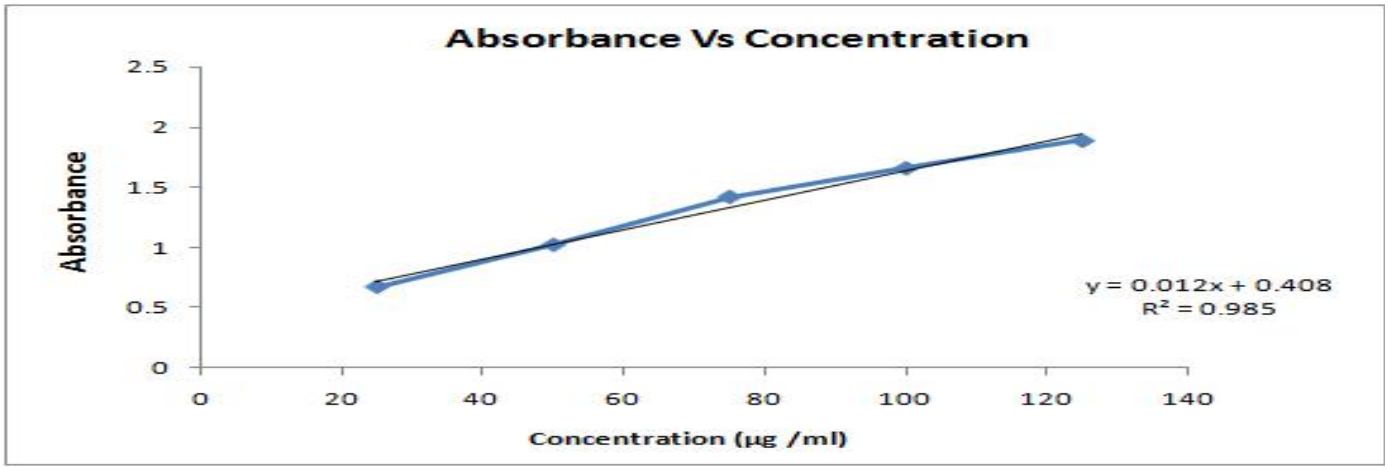

Figure 5: Calibration Curve of Metformin HCl at 237 nm in Methanol Solution, within the range of 25 to 125 µg/ml the drug obeyed Beer's law

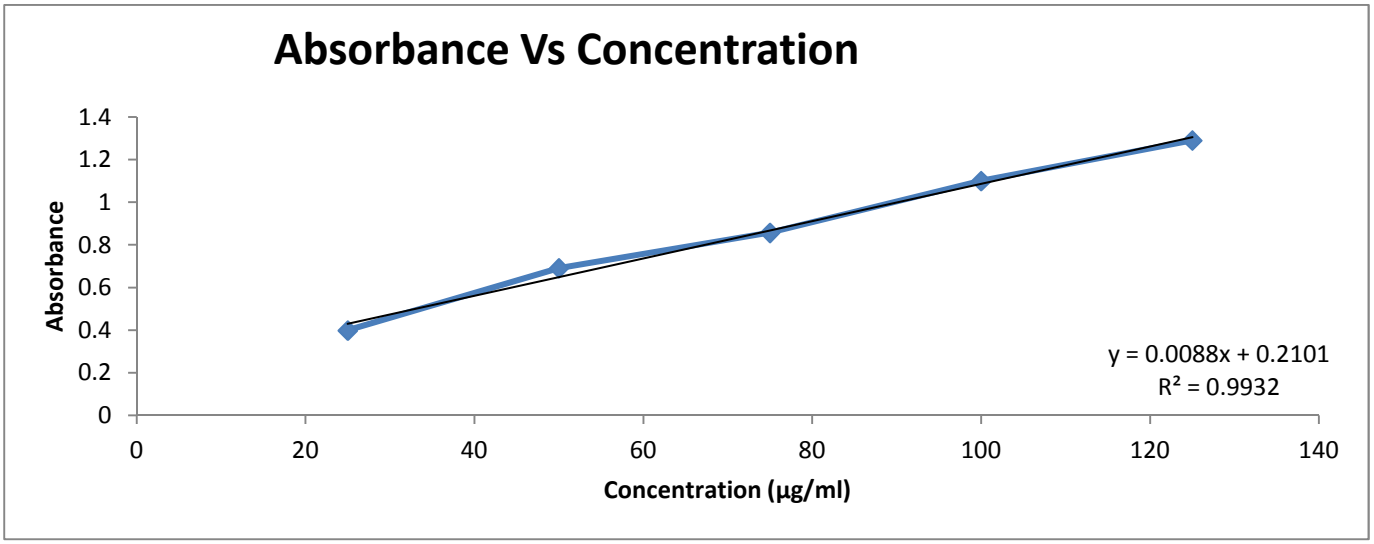

Figure 5: Calibration Curve of Metformin HCl at 225 nm in methanol solution within the range of 25 to 125 µg/ml the drug obeyed Beer's law

Table 3: Regression and Optical Characteristics of Dapagliflozin and Metformin HCl

Parameters	Dapagliflozin		Metformin HCl	
Working λ	225	237	225	237
Beer's Law range	0.5-2.5 µg/ml	0.5-2.5 µg/ml	25-125 µg/ml	25-125 µg/ml
Molar absorptivity (l / mole.cm)	1.89×10^5	9.69×10^5	1617.45	2530.96
Regression Values:				
i. Slope	0.222	0.085	0.008	0.012
ii. Intercept	0.258	0.168	0.210	0.408
iii. Regression coefficient (r^2)	0.991	0.983	0.993	0.985

Table 4: Data for Powder Mixture Analysis

Sr. No.	Amount present in µg/ml		Amount found in µg/ml		Amount found in %	
	DAPA	MET	DAPA	MET	DAPA	MET
1.	1	50	1.000	50.24	100.00	100.48
2.	1	50	1.021	51.65	102.10	102.70
3.	1	50	1.023	51.68	102.30	102.96
4.	1	50	0.991	50.28	99.10	100.56
5.	1	50	1.024	51.72	102.40	101.42
6.	1	50	1.015	51.87	101.50	102.61

* n=6

Table 5: Statistical validation of Powder mixture

Name of Component	Amount Present (µg/ml)	Mean*	Standard Deviation	% Co-efficient of Variation	Standard Error of Mean
DAPA	1	101.23	1.371	1.353	0.554
MET	50	101.78	1.117	1.102	0.455

* n=6

The %R.S.D. is less than 2% as required by USP and ICH guidelines.

Table 6: Intra – day Precision

Drug	% Mean*	S.D*	% R.S.D.*	S.E.*
DAPA	100.44	0.585	0.582	0.262
MET	101.32	0.494	0.484	0.221

* n=6

The standard deviation (S.D.), relative standard deviation (%R.S.D.) and standard error (S.E.) calculated are low, indicating high degree of precision of the method. The %R.S.D. is less than 2% as required by USP and ICH guidelines.

Table 7: Inter – day precision

Drug	% Mean*	S.D.*	% R.S.D.*	S.E.*
DAPA	100.36	0.553	0.551	0.226
MET	101.77	0.664	0.641	0.271

* n=6

The standard deviation (S.D.), relative standard deviation (%R.S.D.) and standard error (S.E.) calculated are low, indicating high degree of precision of the method. The %R.S.D. is less than 2% as required by USP and ICH guidelines.

DISCUSSION

Proposed method for simultaneous estimation of Dapagliflozin and Metformin HCl in combined sample solutions was found to be simple, accurate and reproducible. Table.3 shows data for optical characteristics. Data for validation and precision studies are given in Table. 5, 6 and 7. Once the equations are determined, analysis required only the measuring of the absorbances of the sample solution at the two wavelengths selected, followed by a few simple calculations.

The standard deviation (S.D.), relative standard deviation (%R.S.D.) and standard error (S.E.) calculated are low, indicating high degree of precision of the method. The %R.S.D. is less than 2% as required by USP and ICH guidelines complies in our method.

CONCLUSION

The method was successfully used to estimate the amount of Dapagliflozin and Metformin hydrochloride in synthetic mixture containing 1 mg of Dapagliflozin and 50 mg of Metformin hydrochloride.

By observing validation parameters, method was found to be specific, accurate, precise, repeatable and reproducible. This method is simple in calculation, hence can be employed for routine analysis of synthetic mixture as well as dissolution testing.

ACKNOWLEDGEMENTS

The authors thank Alembic Pharmaceuticals, Baroda and Aarti Drugs, Mumbai for supplying gift samples of Dapagliflozin and Pioglitazone hydrochloride respectively to carry out this work.

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