Journal of Bioequivalence Studies
Volume 1 | Issue 1
ISSN: 2575-551X

Research Article Open Access

Development and Validation of UV Spectroscopic First Derivative Method for Simultaneous Estimation of Dapagliflozin and Metformin Hydrochloride in Synthetic Mixture

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Citation: Jani BR, Shah KV, Kapupara PP (2015) Development and Validation of UV Spectroscopic First Derivative Method for Simultaneous Estimation of Dapagliflozin and Metformin Hydrochloride in Synthetic Mixture. J Bioequiv 1(1): 102. doi: 10.15744/2575-551X.1.102

Received Date: February 19, 2015 **Accepted Date:** September 02, 2015 **Published Date:** September 04, 2015

Abstract

The Novel, simple, sensitive, rapid, accurate and economical and reliable First derivative spectroscopic method has been developed for synthetic mixture of Dapagliflozin (DAPA) and Metformin hydrochloride (MET). This method involve solving of first derivative method based on measurement of absorbance at two wavelengths 235 nm and 272 nm using UV visible spectrophotometer with 1cm matched quartz cells and methanol solvent were employed in this method. The Developed method obeyed Beer's-Lambert's law in the concentration range of 0.5-2.5 µg/ml, having correlation coefficient for Dapagliflozin was 0.984 and 25-125 µg/ml, having correlation coefficient for Metformin hydrochloride was 0.982. A derivative spectrum shows better resolution of overlapping bands than the fundamental spectrum. Different validation parameters like, precision (intra-day and inter-day studies), limit of detection, limit of quantitation were studied and were found to be within the limit. Results of the methods were validated statistically.The validation results showed that the proposed method was sensitive, economical and simple and it could be successfully applied for evaluation and to estimate the amount of synthetic mixture containing Dapagliflozin and Metformin hydrochloride.

Keywords: Dapagliflozin; Metformin hydrochloride; Antidiabetic; UV spectroscopy; First derivative method

Introduction

Dapagliflozin is 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 $SGLT_2$ over $SGLT_1$ [1].

Metformin 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].

The first derivative spectrum is a plot of the rate of change of absorbance with wavelength against wavelength, i.e. a plot of the slope of the fundamental spectrum against wavelength or a plot of $dA/d\lambda$ vs λ. The first derivative derivative spectrum of an absorption band is characterized by a maximum, a minimum and a cross over point at the λ_{max} of the absorption band.

These spectral transformations confer two principle advantages on derivative spectrophotometry. Firstly, an even order spectrum is of narrower spectral bandwidth than its fundamental spectrum. A derivative spectrtum shows better resolution of overlapping bands than the fundamental spectrum. Secondly, this discriminates in favor of substances of narrow spectral bandwidth against broad bandwidth substances. Substances of narrow spectral bandwidth display larger derivative amplitudes than those of broad bandwidth substances [3].

Literature survey reveals that few HPLC [4-6], LC MS methods [6-8], Spectrophotometry [9,10], First derivative methods [11,12] have been reported for determination of Metformin hydrochloride in formulations and LC-MS method reported for Dapagliflozin in rat plasma [13] and UV spectroscopy method [14]. 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 first derivative spectroscopy. 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 first derivative spectroscopic method.

Materials and Methods

Chemicals and Reagent

Dapagliflozin was obtained as a gift sample from Manus akkteva, Ahmedabad and Metformin hydrochloride was obtained as a gift sample Aarti Drugs, Mumbai. Methanol was purchased from Merck laboratories.

Instrumentation

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.

Preparation of stock solution and Calibration Curve

10 mg each of Dapagliflozin and Metformin hydrochloride were weighed separately and transferred in two different 100 ml volumetric flasks. Both the drugs were dissolved in 50 ml of methanol by vigorous shaking and then volume was made upto the mark with methanol to obtain final concentration of 100 µg/ml of each component.

Selection of Analytical Wavelength

Using appropriate dilutions of the standard stock solution, the solutions were scanned separately in the wavelength region of 400-190 nm. The absorbance spectra, thus obtained were derivatized to remove the interference of absorbing species. The two wavelengths selected should be such that at each wavelength the absorbance difference between the components should be as large as possible. From the examination of the overlay first derivative spectra of DAPA and MET, 235 nm (λ_1) and 272 nm (λ_2) (Table 1,2) were selected as working wavelengths for the first derivative spectroscopy (Figure 1, 2 and 3), as at 235.0 nm DAPA is exhibited zero absorbance and at 272.0 nm MET showed zero absorbance.

Sr. No.	Concentration (µg /ml)	First derivative at 235 nm
1	0.5	-0.003
2	1	-0.006
3	1.5	-0.01
4	2	-0.012
5	2.5	-0.014

*= mean absorbance of three absorbance

Table 1: Data for Standard Calibration Curve of Dapagliflozin at 235 nm

Sr. No.	Concentration (µg /ml)	First derivative at 272 nm
1	25	-0.005
2	50	-0.007
3	75	-0.011
4	100	-0.013
5	125	-0.015

*= mean absorbance of 3 absorbance

Table 2: Data for Standard Calibration Curve of Metformin HCl at 272 nm in methanol Solution

Selection of Analytical Concentration Range

For each drug appropriate aliquots were pipetted out from the standard stock solutions of DAPA into series of 10 ml volumetric flasks. The volume was made upto the mark with methanol to get a set of solutions having the concentration 0.5, 1, 1.5, 2, 2.5 µg/ml for Dapagliflozin and 25, 50, 75, 100, 125 µg/ml for Metformin hydrochloride. The absorbance of each of these solutions were measured at the selected wavelengths (for DAPA at 235.0 nm and for MET at 272.0 nm) and plotted against concentration. The concentration range over which the drugs obeyed Beer's law was chosen (Figure 4, 5 and 6). The range was found to be 0.5 to 2.5 µg/ml for Dapagliflozin and 25 to 125 µg/ml for Metformin hydrochloride. The working curve equation was found to be y = -0.0004x + 0.0004 with a correlation coefficient (r^2) value of 0.984 for DAPA and y = -0.0001x - 0.0024 with a correlation coefficient (r^2) value of 0.982 for MET.

Development of First derivative method for Dapagliflozin and Metformin HCl

The concentration of C_{DAPA} and C_{MET} can be obtained from calibration curve.

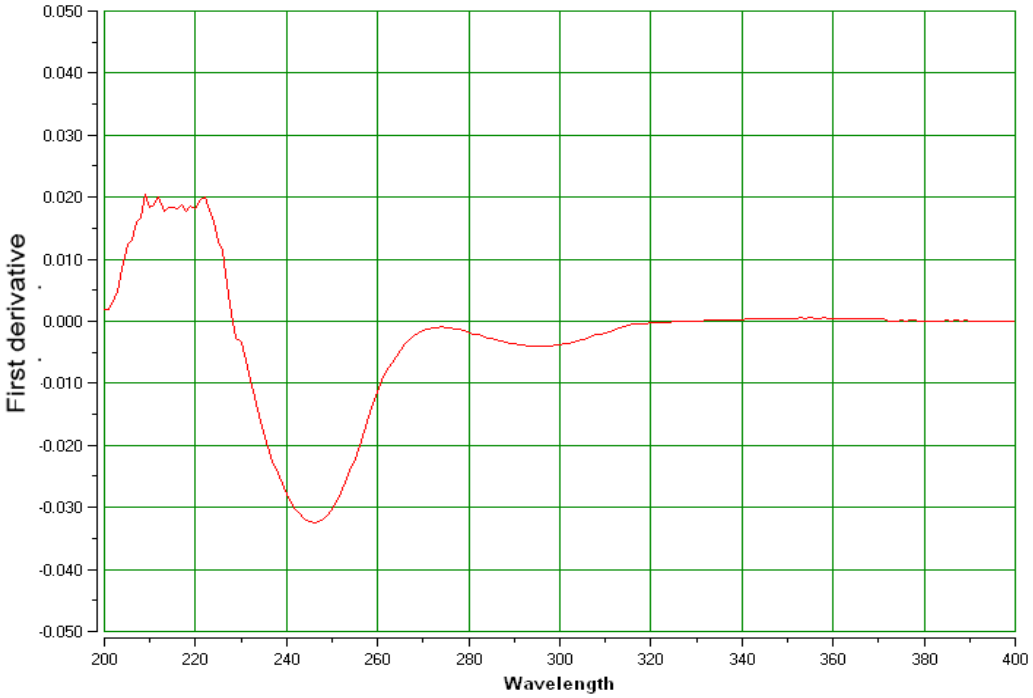

Figure 1: First derivative UV Spectrum of Dapagliflozin

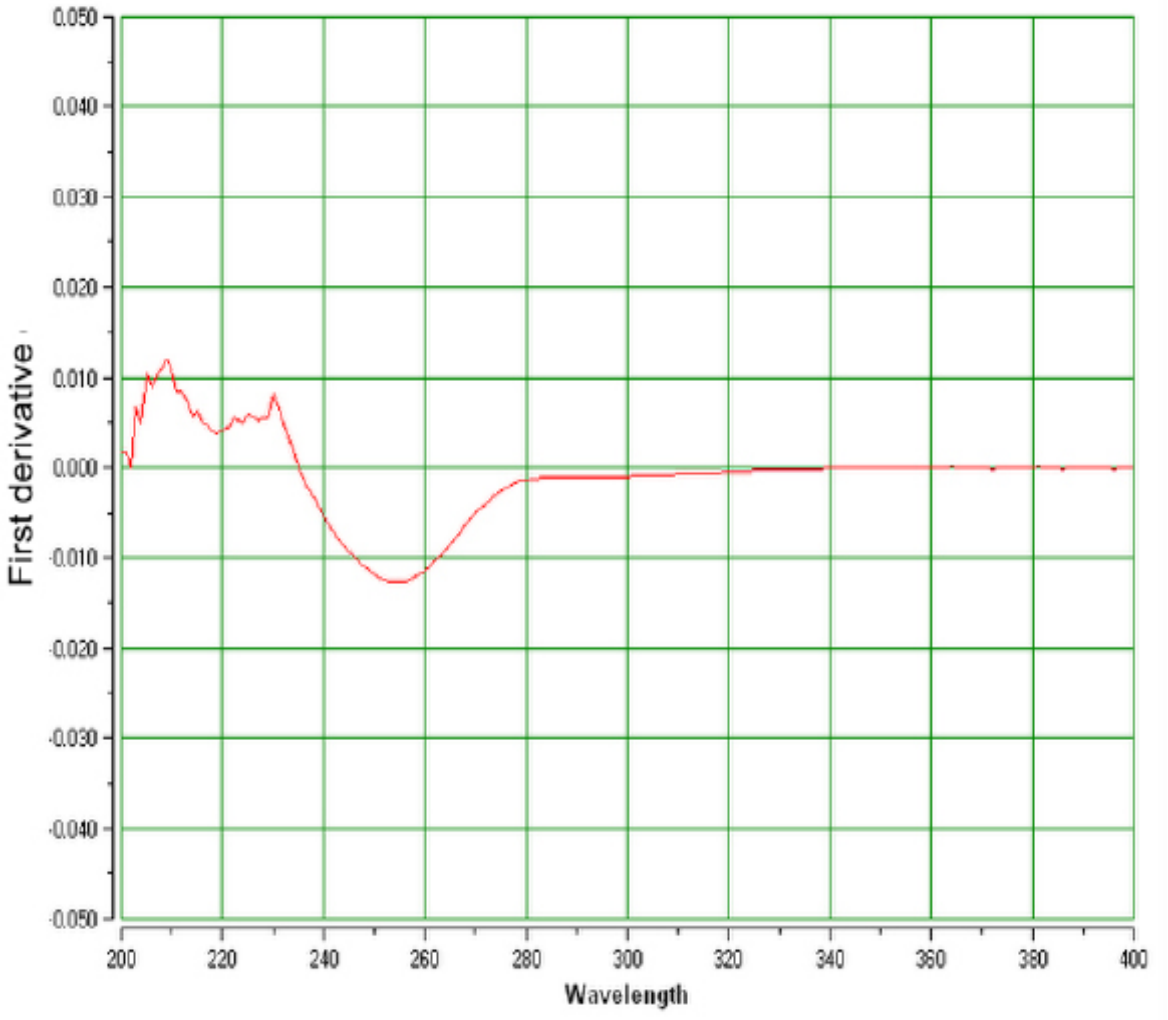

Figure 2: First dervivative UV Spectrum of Metformin HCl

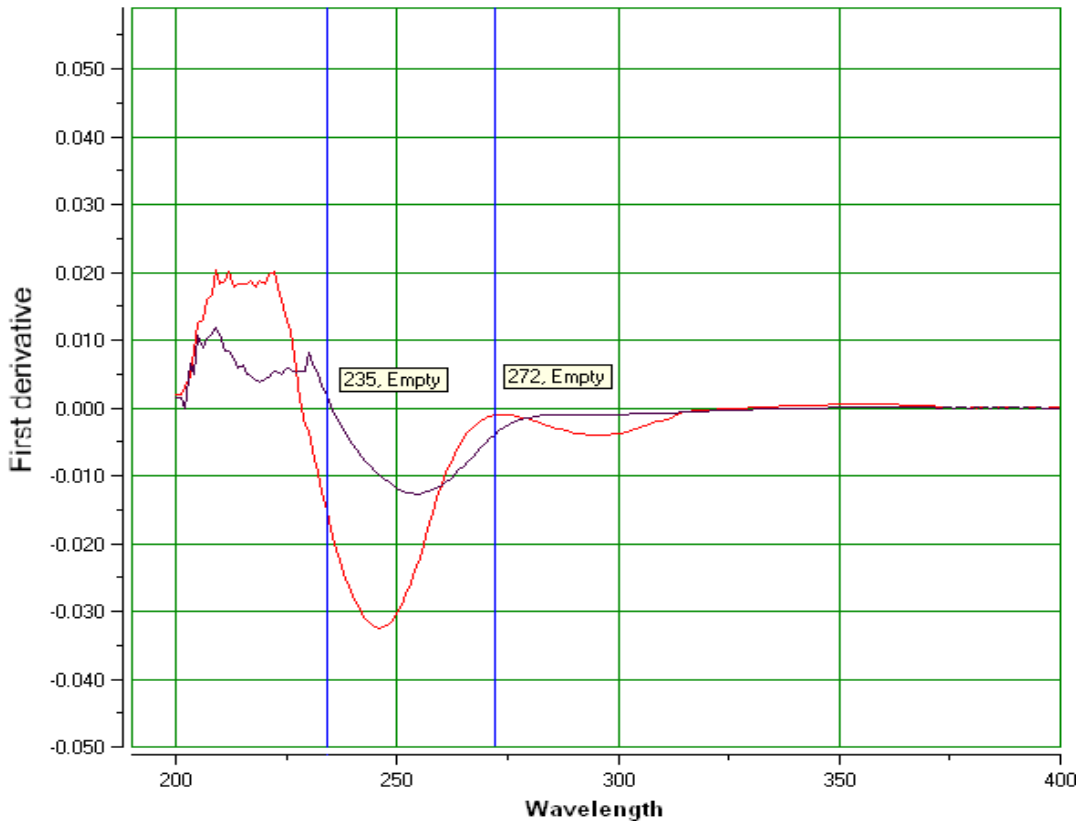

Figure 3: First derivative UV spectrum of Overlay of Dapagliflozin and Metformin HCl

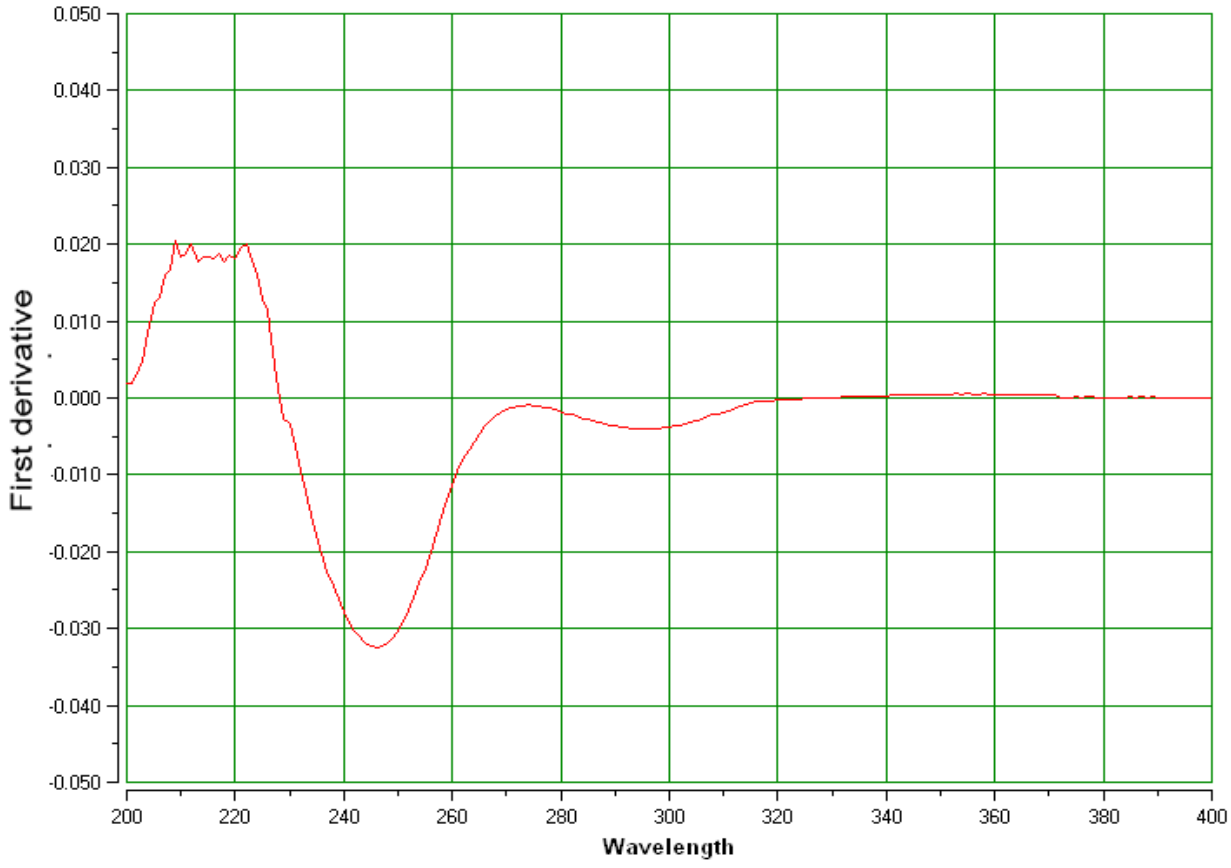

Figure 4: First derivative UV spectrum of powder mixture of Dapagliflozin and Metformin HCl

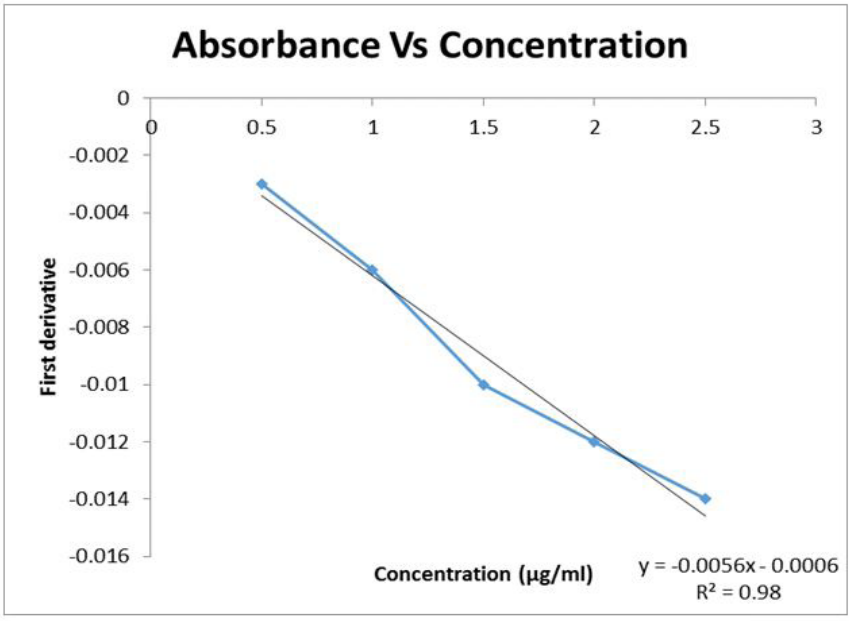

Figure 5: Calibration Curve of Dapagliflozin at 235 nm in Methanol Solution within the range of 0.5 to 2.5 µg/ml the drug obeyed Beer's law

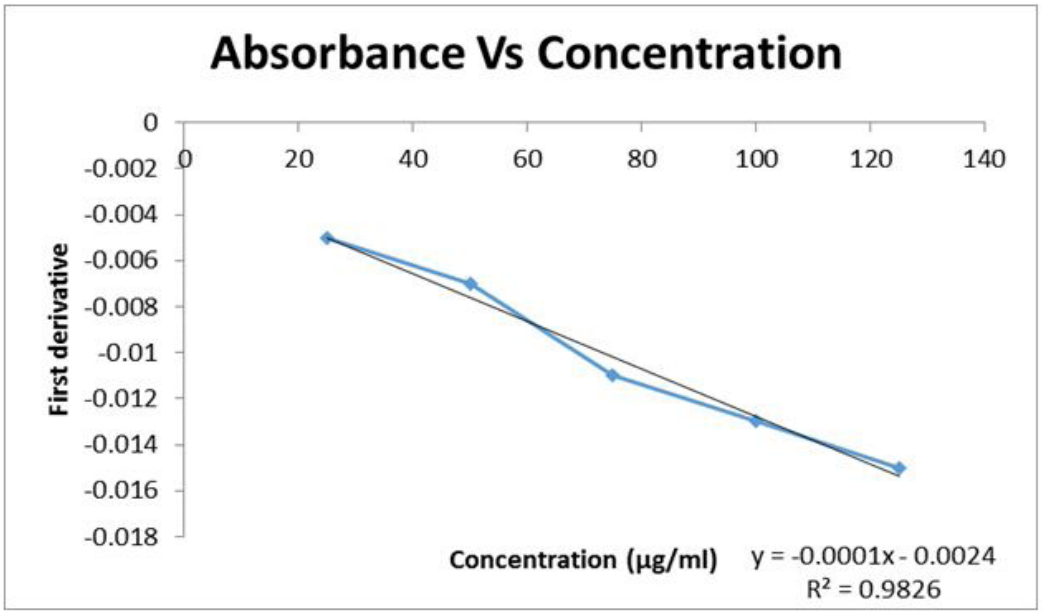

Figure 6: Calibration Curve of Metformin HCl at 272 nm in Methanol Solution Within the range of 25 to 125 µg/ml the drug obeyed Beer's law

Validation study

The following method parameters were evaluated in order to validate the quality of the proposed method: linearity, recovery, precision. Linear range of the proposed method was established by analysis of five standard calibration solutions. Recoveries were calculated using the slope of the linear regression. The intra and inter-day precision were evaluated by repeating the assay method three times (five replicates each time) on the same day and on three consecutive days (five replicates each day), respectively.

Accuracy and Precision

The accuracy study was performed by addition of known amounts of DAPA and MET to known concentration (addition method). Precision of the method were assessed by intra and inter-day validation. The intra and inter-day precision were determined by determining the concentrations of DAPA and MET in synthetic mixture in five replicates for three different concentration levels. The intra and inter-day precision were obtained by repeating the assay method three times on the same day and on three consecutive days, respectively. The repeatability of the method was expressed as the %RSD. Accuracy was expressed as the percent deviation of the mean determined concentration against the spiked concentration. (Table 3), summarizes the mean values of accuracy and precision for both intra and inter-day assays. Both precision and accuracy results indicated satisfactory precision of the proposed methods according to the FDA guidelines.

Parameter	Dapagliflozin	Metformin HCl
Working λ	235	272
Beer's Law range	0.5-2.5 µg/ml	25-125 µg/ml
Regression Values:		
i. Slope	-0.0056	-0.0001
ii. Intercept	-0.0006	-0.0024
iii. Regression coefficient (r^2)	0.980	0.982

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

Calibration curve

The calibration curves showed a good linearity in the concentration range of 0.5-2.5 µg/ml and 25-125 µg/ml with correlation coefficient (r^2 > 0.984 and 0.982) respectively for Dapagliflozin and Metformin hydrochloride (Table 4). The Limit of Detection (LOD) and Limit of Quantification (LOQ) values for Dapagliflozin and Metformin hydrochloride were determined according to ICH recommendations [15] considering the SD of the response and the slope.

Drug	LOD (µg/ml)	LOQ (µg/ml)
DAPA (235 nm)	0.009	0.039
MET (272 nm)	0.013	0.041

Table 4: Data for LOD and LOQ of Dapagliflozin and Metformin hydrochloride

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 235 nm and 272 nm for both drugs were measured. From the calibration curve concentration of Dapagliflozin and Metformin HCl respectively in Methanol solution could be measured Table 5, 6 and 7.

Sr. No.	Amount present in µg/ml		Amount found in µg/ml		Amount found in %	
	DAPA	MET	DAPA	MET	DAPA	MET
1	1	50	0.99	50.57	99	101.14
2	1	50	1.01	49.28	101	98.56
3	1	50	0.98	48.85	98	103.7
4	1	50	1.02	50.71	102	101.42
5	1	50	0.98	50.01	98	100

* n=5

Table 5: Data for Powder Mixture Analysis

Name of Component	Amount Present (µg/ml)	Mean*	Standard Deviation	% Co-efficient of Variation	Standard Error of Mean
DAPA	1	99.6	1.817	1.824	0.812
MET	50	100.16	1.906	1.904	0.849

* n=5

Table 6: Statistical validation of Powder mixture

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

Level of % Recovery	% Mean Recovery*		Standard Deviation*		Co-efficient of Variation* (% R.S.D.)		Standard Error*	
	DAPA	MET	DAPA	MET	DAPA	MET	DAPA	MET
80	98.15	99.95	0.851	0.061	0.867	0.061	0.491	0.035
100	99.66	99.34	0.763	0.373	0.766	0.375	0.441	0.215
120	99.08	99.72	0.457	0.163	0.461	0.164	0.264	0.094

Table 7: Statistical Validation for Recovery Studies

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 8 and 9.

Drug	% Mean*	S.D*	% R.S.D.*	S.E.*
DAPA	99.02	0.759	0.760	0.339
MET	99.54	0.397	0.399	0.171

* n=6

Table 8: Intra-day Precision

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.

Drug	% Mean*	S.D*	% R.S.D.*	S.E.*
DAPA	98.98	0.920	0.929	0.411
MET	99.87	0.235	0.235	0.105

* n=6

Table 9: Inter-day Precision

Results

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 first derivative 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 7, 8 and 9. 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 Dapagliflozin 1 mg 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.

Acknowledgement

The authors thank Manus akkteva, Ahmedabad and Aarti Drugs, Mumbai for supplying gift samples of Dapagliflozin and Pioglitazone hydrochloride respectively to carry out this work.

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