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Original Article

Spectrophotometric Absorption Correction Methods for the Estimation of Fixed-Dose Combinations of Dapagliflozin and Metformin Hydrochloride

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ABSTRACT

Background: For the assessment of fixed-dose combinations of Dapagliflozin and Metformin Hydrochloride in bulk pharmaceuticals and pharmaceutical formulations utilizing distilled water as a solvent, a novel, straightforward, precise, and accurate UV-spectrophotometric absorbance correction approach was devised. On the additive absorbance property, an absorbance correction method was based. **Materials and Methods:** By keeping Dapagliflozin and Metformin hydrochloride apart, a distinct spectrum was generated, displaying absorption maxima peaks (max) at 275nm (DAPA), and 245nm (MET). Compared to metformin hydrochloride, dapagliflozin displayed some absorbance at 275nm. At 245nm, both drugs exhibit absorption. A method for solving equations is based on measuring the absorbance of two wavelengths, 275nm, and 245nm. Exactly validating the procedure was done. Dapagliflozin and metformin hydrochloride concentration ranges of 2–10 µg/mL and 20–100 µg/mL, respectively, were used in the assays utilizing the absorbance correction method at 275nm and 245nm, respectively. **Results:** The mean recovery for each compound was 100.25 ± 0.0459% and 99.82 ± 0.059. The accuracy (RSD 2%) was discovered to be within bounds. This approach had precise and accurate optical properties. The proposed method can be used with pharmaceutical formulations, and it was unaffected by the usual excipients included in the formulation. **Conclusion:** The findings demonstrate that the method is practical for routine estimation of the fixed-dose combination of dapagliflozin and metformin hydrochloride, in a pharmaceutical dosage form. It is straightforward, conscious, accurate, and applicable.

Keywords: Dapagliflozin, Metformin hydrochloride, Absorbance correction method, Method validation.

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INTRODUCTION

Anti-diabetic medications that contain dapagliflozin typically come in fixed-dose combinations with metformin hydrochloride. Chemically, "(DAPA) is (1s)- 1, 5-anhydro -1- C-[4- chloro -3- [(4 - ethoxy phenyl)methyl]phenyl].[1-3] D-glucitol, an oral anti-diabetic drug from a different family, inhibits sodium-glucose co-transporter 2. The kidney reabsorbs glucose due to sodium-glucose co-transporters. This is an important usage for dapagliflozin. It has "sodium-glucose transport protein sub-type 2 inhibitor" and is a "hypoglycemic agent." Hydrochloride of 1,1-Dimethylbiguanide is metformin HCl.[4,5] High blood sugar levels in people with type 2 diabetes can be decreased with metformin and diet. Metformin decreases the quantity of glucose absorbed from the intestines, decreases the quantity of glucose generated in the liver, and improves insulin sensitivity.[6] Dapagliflozin functions by helping your body eliminate extra glucose (sugar) through urination. Metformin reduces the amount of sugar the liver produces, delays the absorption of glucose from the intestines, and raises the body's sensitivity to insulin. Together, they offer improved blood sugar regulation.[7] The literature did not disclose a spectrophotometric absorbance adjustment method for fixed-dose combinations of Dapagliflozin and Metformin.[8,9] In the current investigation, an effort has been made to create a simple, reliable, and affordable approach for comparing the spectra of pharmaceutically formulated fixed-dose combinations of dapagliflozin and metformin hydrochloride.[10,11]

The study's objective was to develop spectrophotometric absorbance correction techniques for fixed-dose combinations that may be frequently used for drug analysis and the pharmaceutical dosage forms that contain them.[12]

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MATERIALS AND METHODS

Apparatus

Spectral and absorbance evaluations: UV-1800 SHIMADZU UV-spectrophotometer using 10 mm Quartz matched cells with pathlength 1 cm was used for all measurements. The absorption spectrum wavelength range is taken from 200-400 nm throughout the experimental Analysis work.

Analytical Balance: Sartorious BSA223S-CW.

Materials

Dapagliflozin (DAPA) and Metformin hydrochloride (MET) was gifted by MEDCHEMLABS (A Unit of Medchem Group) Hyderabad 502325, Telangana, India respectively. Double distilled water was prepared in the University laboratory.

Marketed formulation

Dapaglix M 10 mg/1000 mg are from Solitaire Healthcare Pharma India Ltd.

Reagents

Methnol, Distilled water obtain using Molecular Filter Membrane Assembly was used.

PROCEDURE

For bulk samples

Correctly weighed (100 mg) amounts of DAPA and MET were added to a 100 mL volumetric flask, where they were individually dissolved with 10 mL of Methanol before the volumes were made up (1000 µg/mL). Aliquots of DAPA and MAT with concentration ranges of 2–10 µg/mL for DAPA and 20–100 µg/mL for MET were appropriately prepared. 200-400 nm was chosen as the wavelength for scanning solutions. The wavelength for DAPA was chosen from the overlaid spectra in Figure 1 at 275 nm, where MET exhibits zero absorbance, and at 245 nm, where MET absorbance is compensated. In the region of 200-400 nm, various binary mixture solutions of DAPA and MET were run. When DAPA and MET are present in concentrations of 20–100 µg/mL and 2–10 µg/mL, respectively, the medicines adhere to beer's rule.

Following equations were used for the Quantitative estimation of these drugs

$$A = abc$$

$$DP = A1 / ab$$

$$DP = A1/ax1*b \text{---------- [1]}$$

$$A2 = A\ (DAPA) + A\ (MET)$$

$$A2 = [ay* MT* b] + [ax2* DP* b]$$

$$A2 = [ay2* MT] + [ax2* DP]$$

$$MT = [A2 - (ax2*DP]/ay2 \text{---------- [2]}$$

Where Ax1 and Ax2 stand for the absorbtivities of DAPA at λ1 and λ2, Ay1 and Ay2 for the absorbtivities of MET at λ1 and λ2, and DP and MT for the concentrations of DAPA and MET, respectively; λ1, λ2 stand for the mixture's absorbance at 275nm (for 1) and 245nm (for 2), respectively.

Standard stock solution preparation

Weighed precisely to create a standard solution with concentrations of DAPA (6 µg/mL) and MET (60 µg/mL), 100 mg for each one of DAPA and MET were transferred to separate 250 mL with Deionized water, the volumetric flasks were diluted to the mark.

Sample solution preparation

The exact measured 100mg labelled tablet powder of DAPA and MET was dissolved in 100 mL of methanol and stirred on a magnetic stirrer for 30 min. The mixture was then added to the 100 mL volumetric flask. The solution was filtered using Whatman membrane filter No. 41, and methanol was used to control the volume. The solution was properly diluted with Deionized water to provide the requisite DAPA (6 µg/mL) and MET (60 µg/mL) concentrations.

Validation of the proposed approach

The technique was validated under ICH (International Committee on Harmonization) requirements. [13,14]

Linearity (Calibration curve of DAPA and MET)

The calibration curves were plotted over a concentration range of 2-10 µg/mL for DAPA and 20-100 µg/mL (Figure 1) for MET (Figure 2).

Accurately measured standard stock solutions of each DAPA (2, 4, 6, 8, and 10 µg/mL) and MET (20, 40, 60, 80, and 100 µg/mL) were made in 10 mL volumetric flasks separately. The absorbance was measured at 275 nm and 245 nm. The calibration curves were

Table 1: Optical properties information.

Parameters	Observed value
Beer's Law Limit	Dapagliflozin Metformin HCl
	(2-10 µg/mL) (20-100 µg/mL)
Correlation Coefficient (R2)	0.9984 0.9994
Regression Equation (y = mx+ c)	Y = 0.0663x + 0.2599 y = 0.0095x + 0.0389
Slope	0.0663 0.0095
Intercept	0.2599 0.0389

Table 2: Precision Studies.

Drug Sample	Time selection	Conc. taken (µg/mL)	Conc. found (µg/mL)	% Gain	S D.	% RSD
Dapagliflozin	8:00AM	2	2.02	100.9	0.038	1.890
	1:00 PM	4	3.96	99	0.062	1.553
	5:00 PM	6	6.02	100.33	0.058	0.972
Metformin hydrochloride	8:00AM	40	39.99	99.975	0.356	0.891
	1:00 PM	60	60.12	100.2	0.979	1.648
	5:00 PM	80	79.90	99.875	0.391	0.781

Intraday evaluation of the formulation.

Drug sample	Time selection	Conc. taken (µg/mL)	Conc. found (µg/mL)	% Gain	S D.	% RSD
Dapagliflozin	8:00AM	2	2.01	100.5	0.040	1.969
	1:00 PM	4	3.99	99.75	0.059	1.463
	5:00 PM	6	6.03	100.5	0.058	0.973
Metformin hydrochloride	8:00AM	40	40.01	100.02	0.354	0.886
	1:00 PM	60	59.49	99.15	0.991	1.668
	5:00 PM	80	80.01	100.01	0.796	0.995

constructed by plotting absorbance versus concentration and equations were calculated (Figure 3).

The Precision of the method

Intraday - On the same day, three analyses of mixed standard solutions comprising 2, 4, 6 µg/mL DAPA and 40, 60, and 80 µg/mL MET were performed. the solution at 275nm and 245 nm (A1) (A2). The outcomes were provided as a relative standard deviation (Table 2).

Interday - On three different days, a combined standard solution that includes (2, 4, 6) µg/mL DAPA and (40, 60, 80) µg/mL MET was examined. Measure the solution at 275 nm and 245 nm (A1) (A2). The outcomes were provided as a relative standard deviation (Table 2).

Specificity

The quantitative detection of the analyte in the existence of a potential sample matrix component is made feasible by a method known as specificity. The dosage of the medication was calculated after measuring absorbance after a pre-weight quantity of the drug was introduced into frequently used excipients for making tablets.

Accuracy (Recovery explorations)

By calculating the recoveries of DAPA and MET using the conventional addition method, the procedure's accuracy was evaluated. The accuracy levels are 80, 100, and 120%. A pre-quantified test solution of DAPA (4 µg/mL) and MET (40 µg/mL) was combined with known portions of reference solutions of DAPA (2, 4, and 6 µg/mL) and MET (20, 40, and 60 µg/mL). With DAPA and MET, the solution's absorbance was calculated at a certain wavelength. Using the absorbance correction equation method, the amounts of DAPA and MET at each level were determined, and percentage recoveries were reported.

Accuracy (Recovery Studies of DAPA)

Accuracy (Recovery Studies of MET)

Limit of Detection and Limit of Quantitation

The lowest concentration of an analyte in a sample that can be quantitatively measured with sufficient accuracy and precision is known as the limit of quantitation. The limit of detection is the least amount of an analyte that can be detected but isn't necessarily quantitated as an exact value. The International Council on Harmonization (ICH) provided the following methods for computing the Signal-to-Noise ratio (S/N), from which the drug's Limit of Detection (LOD) and Limit of Quantification (LOQ) was obtained.

$$\{LOD = 3.3\ \sigma/S\}$$

$$\{LOQ = 10\ \sigma/S\}$$

where, σ = S.D. (standard deviation) of response and s = calibration curve slope.

Analysis DAPA and MET on Fixed Dosage Forms

The recoveries of the formulations were calculated at 275nm and 245nm for DAPA and MET, respectively, using the above-described absorbance correction method. Equations 1 and

Table 3: Recovery Studies. Accuracy (Recovery Studies of DAPA).

Drug (% recovery level)	Sl. No.	Amt Present, B	Amt Present, C	Amt Present, A	Amt Recovered (A-B)	% Recovered [(A-B/C)]*100	S D.	% RSD
DAPA (80%)	1.	4	2.6	6.62	2.66	100.8	0.036	0.459
	2.	4	2.6	6.59	2.59	99.57		
	3.	4	2.6	6.61	2.61	100.38		
					Mean	100.25		
DAPA (100%)	1.	4	4	8.13	4.15	100.55	0.451	0.343
	2.	4	4	7.96	4.96	99.85		
	3.	4	4	8.21	4.21	100.88		
					Mean	100.42		
DAPA (120%)	1.	4	6	9.95	5.95	99.16	0.030	0.168
	2.	4	6	9.92	5.92	98.67		
	3.	4	6	9.98	5.98	99.77		
					Mean	99.22		

Accuracy (Recovery Studies of MET).

Drug (% recovery level)	Sl. No.	Amt Present, B	Amt Present, C	Amt Present, A	Amt Recovered (A-B)	% Recovered [(A-B/C)]*100	S D.	% RSD
MET (80%)	1.	40	28.5	68.65	28.65	100.52	0.435	0.509
	2.	40	28.5	68.78	28.78	100.81		
	3.	40	28.5	67.97	27.97	98.14		
					Mean	99.82		
MET (100%)	1.	40	40	80.13	40.13	100.32	0.411	0.343
	2.	40	40	79.45	39.45	98.62		
	3.	40	40	80.19	40.19	100.47		
					Mean	99.80		
MET (120%)	1.	40	44	84.12	44.12	100.27	0.388	0.294
	2.	40	44	83.57	43.57	99.02		
	3.	40	44	84.32	44.32	100.72		
					Mean	100.03		

Table 4: Tablet formulation.

Marketed brand	Drugs Name	Amt Gain (mg)	% Purity	SD.	% RSD
Dapadax M (Daxai Ltd.)	DAPA	10.24	100.52	0.52	0.43
	MET	1000.87	100.08	1.69	0.17
Dapaglix M (Solitaire Ltd.)	DAPA	10.17	100.14	0.38	0.31
	MET	1000.88	100.09	1.02	0.11

2 were used to fit the sample solution's outputs into the regression equations for DAPA and MET. This enabled us to calculate the concentration of each analyte. Overlay spectra for both drug is shown in Figure 4.

RESULTS

Absorbance correction method

One of the most significant characteristics of dual-wavelength method is its ability to calculate the concentration of an unknown

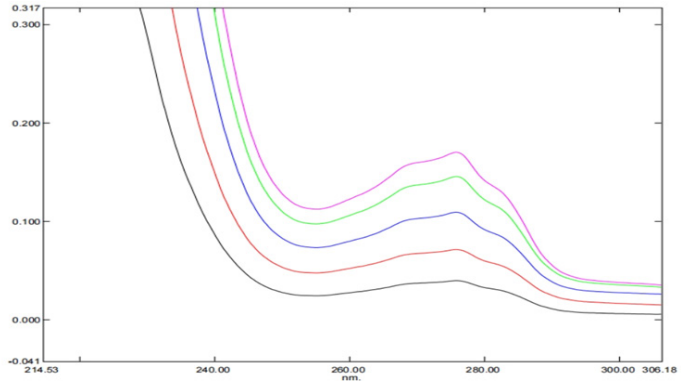


Figure 1: Overlay spectra of DAPAGLIFLOZIN (2-10µg/mL).

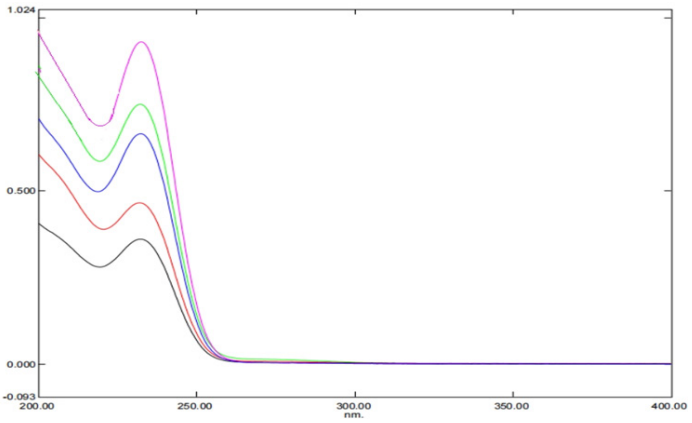
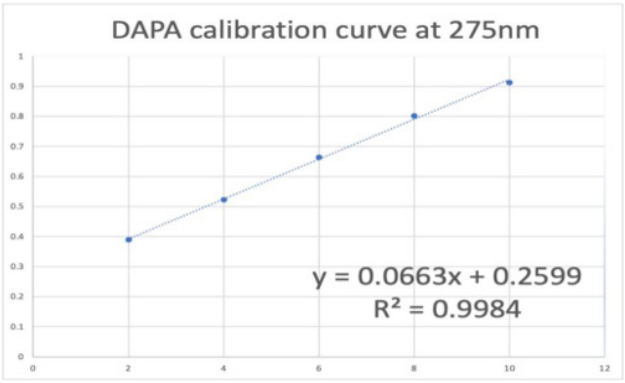


Figure 2: Overlay spectra of METFORMIN HYDROCHLORIDE (20-100µg/mL).



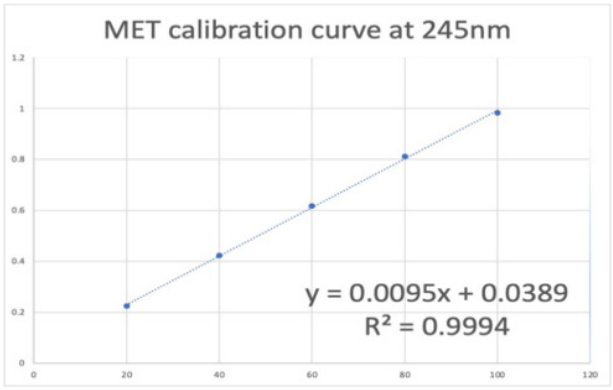


Figure 3: Linearity graph.

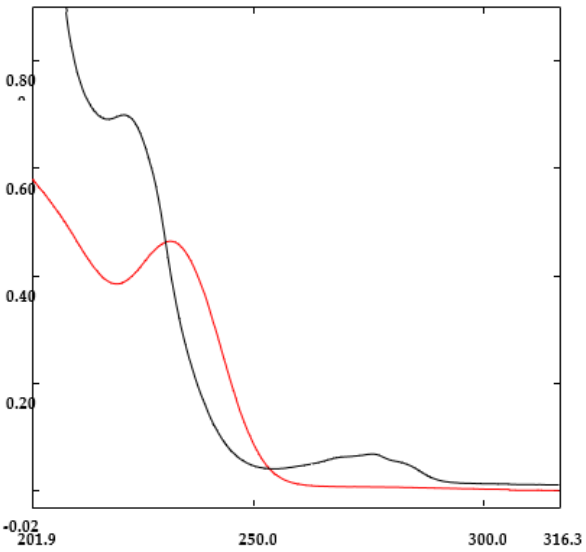


Figure 4: Overlay Spectra of DAPA (6µg/mL) and MET (60µg/mL).

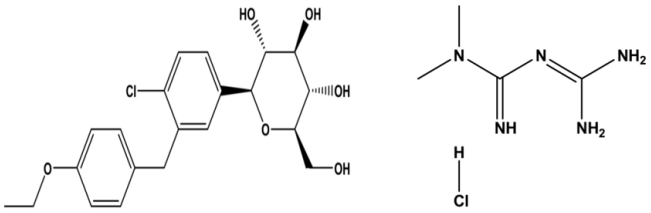


Figure 5: Chemical composition of [1] Dapagliflozin [2] Metformin Hydrochloride.

component in a combination that also contains an interfering component. To lessen the effects of an interfering component, two specific wavelengths were chosen.

The first wavelength where MET interference did not occur and where DAPA absorbance was lowest (275nm).

DAPA and MET both displayed their highest wavelength-dependent absorption at the second wavelength, or i.e., at (245nm). The influence of DAPA on the absorbance at 245nm (λ2) was abolished when it was found that the absorbance of MET was 0 at another wavelength, 275nm (λ1). Using these two selected wavelengths, the amount of DAPA in the DAPA and MET mixture was estimated (Figure 5). The difference in absorbance at these two wavelengths (A275-A245) wipes out the contribution of MET absorbance in the combination (Figure 4).

Validation parameters of the developed method

Linearity - Between the ranges of 2–10 µg/mL and 20–100 µg/mL, respectively, of absorbance and concentration of DAPA and MET, the linear association was found. The high value of the regression's correlation coefficients supported the calibration curves' linearity (Table 1).

Precision

The suggested method is accurate, as evidenced by the low RSD values of the intraday and interday variations for DAPA and MET, respectively (0.038 ±1.89% and 0.058 ± 0.97%) (Table 2).

Accuracy

The conventional addition approach was used to conduct the recovery studies. With DAPA and MET, respectively, the mean recovery was 100.25 ± 0.0459% and 99.82 ± 0.059 (Table 3). The approach is accurate, as evidenced by the high Numbers.

LOD and LOQ- The LODs for DAPA and MET were found to be 0.48 µg/mL and 2.99 µg/mL, respectively, whereas the LOQs were found to be 1.45 µg/mL and 9.07 µg/mL. The technique can correctly identify DAPA and MET within the required concentration range, according to the results.

DISCUSSION

Assay of the pharmaceutical formulation

To determine DAPA and MET in their combined dose forms, the suggested validated procedures were successfully used. The outcomes for DAPA and MET corresponded to the appropriate indicated quantities (Table 4).

CONCLUSION

The results of the suggested analytical method study of pharmaceutical formulations are highly repeatable, dependable, and in line with the drug's label claim. The tested samples pharmaceutical formulations' excipients did not affect on the results of the DAPA and MET determinations. The techniques can be applied frequently to analyze mixed-dose forms of DAPA and MET.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

DAPA: Dapagliflozin; **MET:** Metformin hydrochloride; **AMT:** Amount.

SUMMARY

Analytical Method Development and Validation for the simultaneous estimation of Empagliflozin, Dapagliflozin and Metformin hydrochloride using UV-spectrophotometer. A Simple, Precise, Accurate, cost effective HPLC and UV method is developed and validated as per ICH Guidelines for estimation of Empagliflozin and Metformin hydrochloride, Dapagliflozin and Metformin hydrochloride in bulk and fixed dose combination. Both the methods can be applied for quality control of fixed dosage forms of these drugs in marketed formulations.

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