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Analytical Spectrometric Study For Determining Dapagliflozin Propanediol Monohydrate Individually Or In Presence Of Metformin Hydrochloride In Tablets Formulation

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Abstract

First simple spectrophotometric method was developed and applied to determine Dapagliflozin Propanediol Monohydrate by Zero Spectrophotometry and First Derivative Spectrophotometric method for determining of Dapagliflozin Propanediol Monohydrate (DAPA) in the presence of Metformin Hydrochloride (MET). Zero spectrophotometric (ZS) was applied for the determination of (DAPA) at 223.5 nm. Linearity range was (2.61–31.23) µg/mL. Regression analysis showed a good correlation coefficients R^2 = 0.9989. The limit of detection (LOD) and limit of quantification (LOQ) were to be 0.569 µg/mL and 1.724 µg/mL, respectively. Derivative spectrophotometric (^{1}DS) was applied for the determination of (DAPA) in the presence (MET). (DAPA) was determined at 233 nm ($^1D_{233}$). Linearity ranges were (5.21 – 41.64) µg/mL for (DAPA). Regression analysis showed a good correlation coefficients R^2 = 0.9994. The limit of detection (LOD) and limit of quantification (LOQ) were to be 0.732 µg/mL and 2.218 µg/mL for (DAPA). The proposed Zero spectrophotometry method was applied to analysis individual (DAPA), and the derivative ($1D_{233}$) method was applied to analysis (DAPA) individually or with (MET) combination in Syrian trademark drugs.

Keywords: Dapagliflozin Propanediol Monohydrate (DAPA), Metformin Hydrochloride (MET), Zero spectrophotometry (ZS), Derivative Spectrophotometry (^{1}DS), Sodium-Glucose Cotransporter 2 (SGLT2).

Introduction

Dapagliflozin Propanediol Monohydrate is a crystalline solid, white to half white crystalline powder which is insoluble in water and soluble in ethanol, methanol, DMSO, dimethylformamide. It has a formula $C_{24}H_{35}ClO_9$. H_2O and molecular weight, 520.985 g/mol. It is inhibiting renal glucose reabsorption through the solid-glucose cotransporter (SGLT) offers an insulin-independent alternative to controlling blood glucose concentrations in patients with type 2 diabetes. It is a first-generation, selective SGLT inhibitor that blocks glucose transport with about 100-fold selective for SGLT2over SGLT1[1].

Dapagliflozin Propanediol Monohydrate is chemically known as (2S)-propane-1,2-diol(2S,3R,5S,6R)-2-{4-chloro-3-[(4-ethoxy phenyl)methyl]phenyl}-6-(hydroxy methyl)oxane-3-4-5-mono hydrate[2,3,4]. It is a new class of oral antidiabetic drugs; it is also called Sodium Glucose cotransporter 2 (SLGT2) inhibitors. These Sodium Glucose Cotransporter are responsible for glucose reabsorption in the kidney. Hence inhibiting the SLGT2 have been proposed as a new strategy in the treatment of diabetes. By suppressing the SLGT2. Dapagliflozin reduces plasma glucose concentration intern by elevating the renal glucose excretion. Dapagliflozin in Literature reveals few analytical methods which were reported like UV spectroscopy[5], liquid chromatography-mass spectrometry method in biologicalfluids[6], reversed-phase high-performance liquid chromatography (RP-HPLC) methods[7-12] and spectrophotometric methods[13] alone or combined with other pharmaceutical drugs.

Materials and Methods

Apparatus

All spectral measurements were carried out using a Spectro Scan 80 DV, UV/Vis spectrophotometer instrument Ltd (UK), connected to computer, quartz cells 1 cm. Ultrasonic bath Daihan (China), and stirrer Velp Scientifica (Europe).

Chemical regents

Methanol from LOBAL Chemie (India), hydrochloric acid from SURCHEM PRODUCTS LTD (ENGLAND), double distilled water. Dapagliflozin Propanediol Monohydrate material, purity 100.2 %, was obtained from Solan (Himachal Pradesh) India. Metformin hydrochloride material, purity 99.4 %, was obtained from Switzerland.

Standard preparation

Stock standard preparation: Stock solution 2.5 × 10^{-3} M of Dapagliflozin Propanediol Monohydrate (M_W = 520.985 g/mol) was prepared by dissolving 65.06 mg of Dapagliflozin Propanediol Monohydrate standard material in volumetric flask 50 mL of Methanol. The working standard solutions for first method (Zero spectrophotometry) were prepared by appropriate dilutions of stock solution 2.5 × 10^{-3} M with HCl (0.1 N) to give concentrations between (2.61 - 31.23) μg/mL of Dapagliflozin Propanediol Monohydrate.

The working standard solutions for the second method (Derivative spectrophotometry) were prepared by appropriate dilutions of stock solution 2.5 × 10^{-3} M with distilled water to give concentrations between (5.21 - 41.64) μg/mL of Dapagliflozin Propanediol Monohydrate.

Calibration Curve

To construct the calibration curve, five standard Dapagliflozin Propanediol Monohydrate solutions for each concentration were prepared and the absorbance was measured of each solution five times.

Sample preparation

Two products were studied

Zero spectrophotometry

✓ Ten DapaGold 10 (Golden Med Pharma) tablets were weighed and finely powdered and an accurate weight equivalent to one tablet (10 mg), dissolved in 10 mL methanol. The sample solution was filtered through a filter paper (Whatman 3, England). Then 0.3 mL was taken to 25 mL volumetric flask and adjusted to volume with HCl (0.1 N) to obtain theoretically equivalent to 12 μg/mL.

✓ Ten DapaGold 5 (Golden Med Pharma) tablets were weighed and finely powdered and an accurate weight equivalent to two tablets (5 mg), dissolved in 10 mL methanol. The sample solution was filtered through a filter paper (Whatman 3, England). Then 0.3 mL was taken to 25 mL volumetric flask and adjusted to volume with HCl (0.1 N), to obtain theoretically equivalent to 12 μg/mL.

Derivative spectrophotometric:

✓ Ten Dapa Gold 10 (Golden Med Pharma) tablets were weighed and finely powdered and an accurate weight equivalent to one tablet (10 mg), dissolved in 10 mL methanol. The sample solution was filtered through a filter paper (Whatman, England). Then 0.2 mL was taken to 25 mL volumetric flask and adjusted to volume with distilled water, to obtain theoretically equivalent to 8 μg/mL.

✓ Ten Dapa Gold 5 (Golden Med Pharma) tablets were weighed and finely powdered and an accurate weight equivalent to two tablets (5 mg), dissolved in 10 mL methanol. The sample solution was filtered through a filter paper (Whatman 3, England). Then 0.3 mL was taken to 25 mL volumetric flask and adjusted to volume with distilled water, to obtain theoretically equivalent to 12 μg/mL.

RESULTS AND DISCUSSION

Zero spectrophotometry: Absorption spectra of the standard raw material samples 10.41 μg/mL (DAPA) and 9.94 μg/mL (MET) solutions were recorded within a wavelength range of (210 – 300) nm against the blank (all the addition constituents without (DAPA) and (MET)). It was noticed, (DAPA) and (MET) cannot be determined by direct measurement of absorbance at 223.5 nm, because of the overlapped spectra.

On the other hand, derivative spectrophotometry showed more resolution, where the determination of (DAPA) and (MET) mixture was possible without pretreatment. The first derivative spectrum at zero-crossing point was used to determine (DAPA) in the presence of (MET) at 233 nm "Fig. 1".

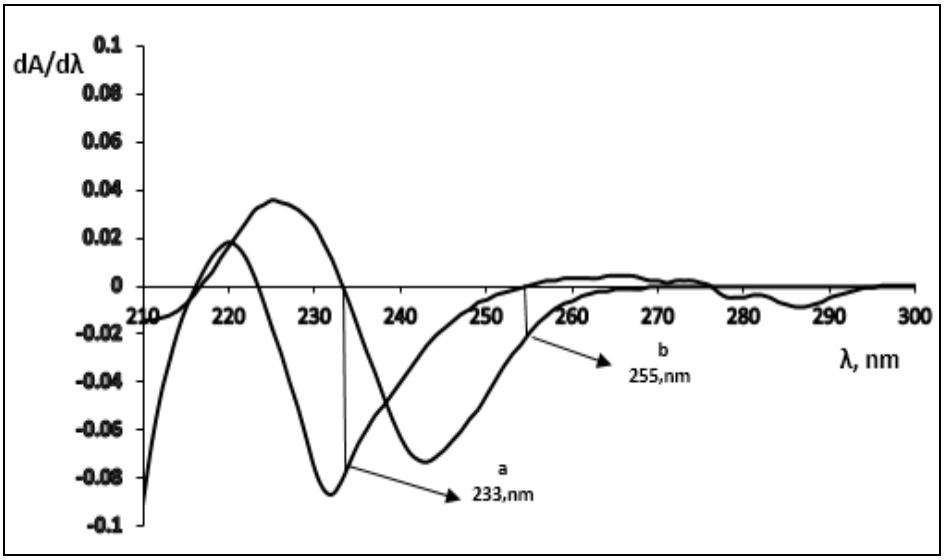


Fig. 1. First derivative spectra of: a- (DAPA), b- (MET).

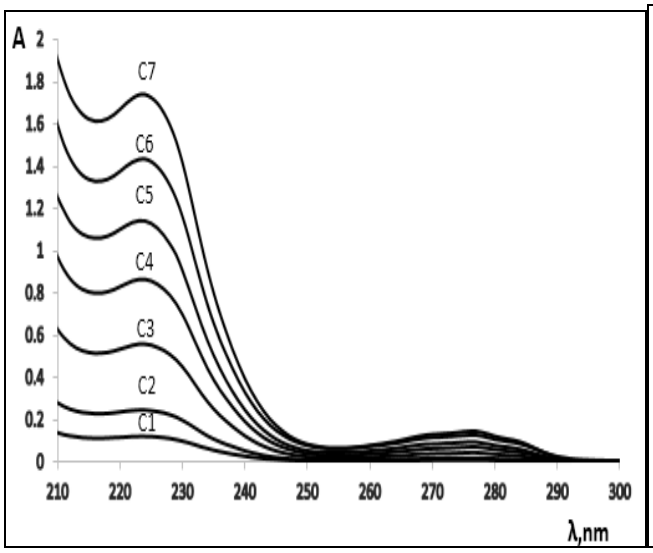
Stability of stock solution: Time effect on the stability of standard stock solution of Dapagliflozin Propanediol Monohydrate in Methanol was studied in three different concentrations 2 × 10^{-5} M, 3 × 10^{-5} M and 4 × 10^{-5} M. We did not notice any significant changes during the absorption measurement within two months.

Method's validation

The validity and suitability of the proposed method was assessed by linearity (evaluated by regression equation), limit of detection (LOD), limit of quantification (LOQ), accuracy (reported as percent %), precision (reported as RSD %), robustness, and Sandell's sensitivity.

Linearity

We studied the linearity of Dapagliflozin Propanediol Monohydrate standard concentrations at the optimal conditions where we made a series of 25 mL of separated volumetric flasks, each one contains variable concentrations of Dapagliflozin Propanediol Monohydrate stock solution 2.5 × 10^{-3} M, and completed to 25 mL with HCL (0.1 N). Finally, we measured the absorbance at 223.5 nm for each concentration. "Fig. 2" presents the Dapagliflozin Propanediol Monohydrate spectra. The range of linearity was obeyed to Beer's law in concentration (2.61 - 31.23) µg/mL and the linearity curve is presented in "Fig. 3".



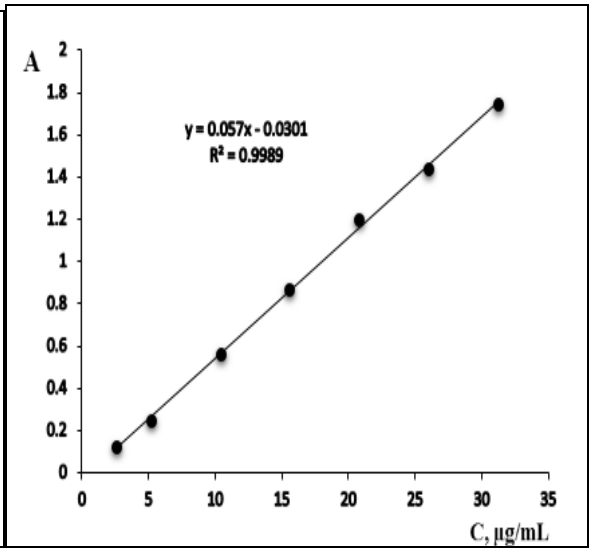


Fig. 2: spectra of (Dapagliflozin Propanediol .H_2O)

C1: 2.61µg/mL, C2: 5.21 µg/mL,

C3: 10.42 µg/mL, C4: 15.63 µg/mL,

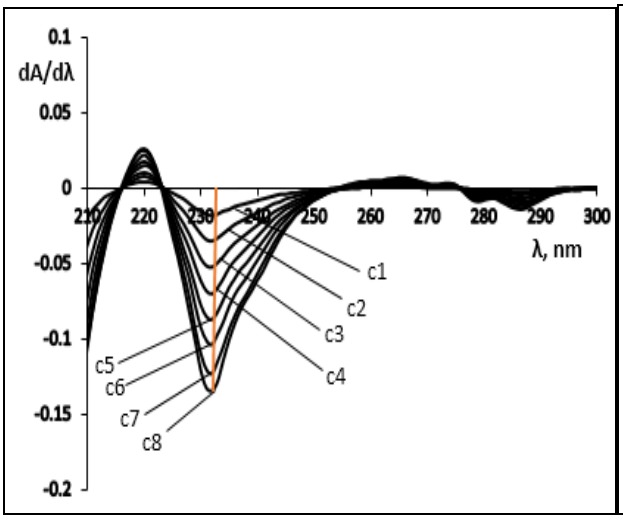
C5: 20.84 µg/mL, C6: 26.05 µg/mL,

C7: 31.26 µg/mL.

Fig. 3: (Dapagliflozin Propanediol .H_2O)

C1: 2.61µg/mL, C2: 5.21 µg/mL, C3: 10.42 µg/mL, C4: 15.63 µg/mL, C5: 20.84 µg/mL, C6: 26.05 µg/mL, C7: 31.26 µg/mL, n = 5.

The concentration linearity of (DAPA) in presence (MET) was in the range (5.21 - 41.64) μg/mL at 233 nm by $1D_{233}$ "Figs 4, 5".



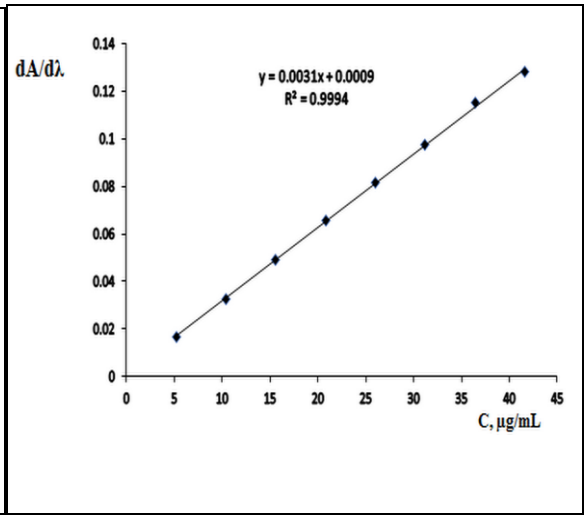


Fig. 4: First derivative spectra of (Dapagliflozin Propanediol .H_2O):

C1: 5.21 µg/mL, C2: 10.41 µg/mL,

C3: 15.62 µg/mL, C4: 20.82 µg/mL, C5: 26.03 µg/mL, C6: 31.23 µg/mL, C7: 36.44 µg/mL, C8: 41.64 µg/mL.

Fig. 5: Calibration curve for (Dapagliflozin Propanediol .H_2O)

C1: 5.21 µg/mL, C2: 10.41 µg/mL, C3: 15.62 µg/mL, C4: 20.82 µg/mL, C5: 26.03 µg/mL, C6: 31.23 µg/mL,

C7: 36.44 µg/mL, C8: 41.64 µg/mL. n = 5.

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

In spite of the measurement LOD and LOQ, five concentrations were analyzed in five replicates. LOD and LOQ for Dapagliflozin Propanediol Monohydrate and Metformin Hydrochloride were calculated by using the following equations:

$$LOD = \frac{3.3 \times SD}{m} \qquad LOQ = \frac{10 \times SD}{m}$$

Where SD is the standard deviation of y-intercepts (a) of regression lines and (b) is the slope of the equitation of calibration curve, y = a + b x and accuracy results are presented in "table 1".

Table 1: Statistical data for calibration curves.

Method	Analyte	Selected wavelength (nm)	Linearity rang µg/mL	Correlation coef. (R2)	LOD µg/mL	LOQ µg/mL	Temperature of solution
ZS	DAPA	223.5	2.61 - 31.23	0.9989	0.569	1.724	(25 ± 5) ºC
1DS	DAPA	$1D_{233}$	5.21 - 41.64	0.9994	0.732	2.218	

Accuracy

To determine the precision and accuracy of the proposed method, five replicate determinations were carried out on five different concentrations of standards (Dapagliflozin Propanediol Monohydrate). The precision and accuracy results are presented in "table 2".

Table 2: Accuracy for determination of Dapagliflozin Propanediol Monohydrate.

Method	Material	Theoretical concentration (µg/mL)	$\bar{x}$ observed concentration (µg/mL)	SD µg/mL	Precision (RSD %)	Accuracy (%)
ZS	Dapagliflozin Propanediol Monohydrate	5.21	5.17	0.06	1.16	99.23
		10.42	10.46	0.20	1.91	100.38
		15.62	15.45	0.04	0.26	98.91
		20.82	20.89	0.24	1.15	100.34
		26.03	25.93	0.13	0.50	99.62
1DS	Dapagliflozin Propanediol Monohydrate	10.41	10.39	0.13	1.25	99.81
		15.62	15.67	0.08	0.51	100.32
		20.82	20.61	0.34	1.65	98.99
		26.03	26.12	0.38	1.45	100.35
		31.23	31.21	0.60	1.92	99.94

$\bar{x}$: mean of five replicated determinations, Accuracy (%) = (observed concentration/theoretical concentration) × 100,

Precision (RSD %) = (standard deviation/mean concentration) *100.

Precision

In order to demonstrate the precision of the proposed methods, intra-day and inter-day variability studies were performed at three different concentrations for Dapagliflozin Propanediol Monohydrate at the same day and also at three different days. Method efficiency was tested in terms of RSD % for both intra-day and inter-day precision.

The precision was ascertained by carrying out five replicates of standard Dapagliflozin Propanediol Monohydrate under study and the mean was calculated. The RSD% results were not more than 2.47 and 1.94 for Dapagliflozin Propanediol Monohydrate (ZS) and Dapagliflozin Propanediol Monohydrate (1DS), respectively, where the method is considered very precise.

Robustness

The robustness of an analytical procedure is a measure of its capacity to maintain unaffected results by a very small variation of some parameters and provides an indication of its reliability during normal usage. The

studied variables parameters were slit, scan speed and the wavelength which performed at one concentration for Dapagliflozin Propanediol Monohydrate. the results showed no significant differences, "table 3".

Table 3: Robustness test study.

Method	Material	Work parameters	Measured deviation	$\bar{x}$* µg/mL	RSD %	Percent (%)
ZS	Dapagliflozin Propanediol Monohydrate (10.42 µg/mL)	Slit range	2 nm	10.34	0.62	99.23
		2 nm	1 nm	10.36	1.16	99.42
		scan speed	Fast	10.34	0.62	99.23
		medium	slow	10.31	0.58	98.94
		Wavelength	+2 nm	10.19	0.64	97.79
		223.5 nm	-2 nm	10.16	0.63	97.50
		pH	+0.05	9.99	0.74	95.87
		0.1 N	-0.05	10.46	1.86	100.38
^{1}DS	Dapagliflozin Propanediol Monohydrate (15.62 µg/mL)	Slit range	2 nm	15.67	0.52	100.32
		2 nm	1 nm	15.56	1.06	99.62
		scan speed	Fast	15.67	0.52	100.29
		medium	slow	15.61	0.96	99.94
		Wavelength $1D_{233}$	+1 nm	15.01	0.54	96.09
			-1 nm	15.31	0.90	98.02

*n = 5

Sandell's sensitivity

The mean molar absorptivity ε was (27734.82 L/mol.cm) , Sandell's sensitivity was (0.038 µg/cm^2), were calculated from the standard deviation of the absorbance measurements obtained from Beer's law.

RECOVERY

The recovery was studied by three addition standards for every product. "table 4" presents the recoveries of Dapagliflozin Propanediol Monohydrate results for the two products for DapaGold (5,10 mg/tab), Golden Med Pharma.

Table 4: Recoveries of Dapagliflozin Propanediol Monohydrate for (Dapa Gold 5, 10 mg/tab, Golden Med Pharma) Syrian products.

Method	Products	Pharmaceutical dosage	Sample µg/mL	Added µg/mL	Total Found $\bar{x}$ µg/mL	Recovery%	SD µg/mL	RSD%	Recovery Average %
	DapaGold 5	Dapagliflozin	4.89	3.91	8.77	99.23	2.01	2.03	

ZS		**Propanediol Monohydrate 5 mg/tab**		4.89	9.71	98.57	0.80	0.81	98.87
				5.87	10.69	98.81	1.66	1.68	
[1]DS	**DapaGold 5**	**Dapagliflozin Propanediol Monohydrate 5 mg/tab**	4.89	3.91	8.85	101.28	2.36	2.33	100.29
				4.89	9.71	98.57	2.88	2.92	
				5.87	10.82	101.02	1.85	1.83	
ZS	**DapaGold 10**	**Dapagliflozin Propanediol Monohydrate 10 mg/tab**	9.87	7.90	17.79	100.25	2.06	2.05	100.24
				9.87	19.82	100.81	0.07	0.07	
				11.85	21.68	99.66	0.16	0.16	
[1]DS	**DapaGold 10**	**Dapagliflozin Propanediol Monohydrate 10 mg/tab**	9.87	7.90	17.94	102.15	3.01	2.95	100.34
				9.87	19.67	99.29	2.69	2.71	
				11.85	21.67	99.58	2.21	2.22	

Application: Estimation of Dapagliflozin Propanediol Monohydrate in (DapaGold 5, 10 mg/tab, Golden Med Pharma) Syrian tablets products

The developed method was applied for quantitative determination and identification of Dapagliflozin Propanediol Monohydrate in (DapaGold 5, 10 mg/tab, Golden Med Pharma) Syrian pharmaceutical products. The samples were prepared as described in the section of samples preparation and analyzed. Quantitative analysis was done by using calibration curve. The obtained results are summarized in "table 5" for the two products.

The relative standard deviations RSD% (n = 5) were in the range of (0.61 - 1.62)%. Table 5 presents the determination results of Dapagliflozin Propanediol Monohydrate in (DapaGold 5, 10 mg/tab, Golden Med Pharma).

Table 5: Results of Dapagliflozin Propanediol Monohydrate (DapaGold 5, 10 mg/tab, Golden Med Pharma).

Method	**DapaGold 5, Golden Med Pharma (Dapagliflozin Propanediol Monohydrate 5mg/tab).**			
	Concentration $\bar{x}$mg/ tab	**SD mg/tab**	**RSD %**	**Per %**
ZS	5.00	0.08	1.60	100.00
DS	4.89	0.03	0.61	97.80
Method	**DapaGold10, Golden Med Pharma (Dapagliflozin Propanediol Monohydrate 10 mg/tab).**			

	Concentration $\bar{x}$ mg/ tab	SD mg/ tab	RSD %	Per %
ZS	10.09	0.13	1.29	100.90
DS	9.87	0.16	1.62	98.70

$\bar{x}$Mean for five replicates.

CONCLUSION

We developed a new method which is suitable for the identification and quantification of Dapagliflozin Propanediol Monohydrate in raw material. A good percentage shows that the method can be successfully used in routine analyses. The proposed method is simple, sensitive, rapid, specific, a little cost and could be applied for quality control of Dapagliflozin Propanediol Monohydrate. The method can be also proposed to determine Dapagliflozin Propanediol Monohydrate in pharmaceutical formulations.

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