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Development And Validation Of Uv-Spectrophotometric Method For Simultaneous Quantification Of Dapagliflozin Propanediol Monohydrate And Eplerenone In Their Synthetic Mixture

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ABSTRACT

The present research attempts to develop a first order (D^1) UV-spectrophotometric method that is convenient to use, robust, cost-effective, quick, and precise for the simultaneous measurement of Dapagliflozin propanediol monohydrate and Eplerenone in their synthetic mixture. The method was validated as per ICHQ2(R2) guidelines. Methanol was utilized as solvent for preparation of sample solution and the detection wavelength to determine DAPA and EPLE in developed fist order UV spectrophotometric method was 240.41 nm and 254 nm respectively. The linearity range for DAPA is 4-12 µg/ml and EPLE is 10-30 µg/ml in methanol. The regression coefficient was found to be 0.9988 and 0.9969 for DAPA and EPLE respectively. The LOD and LOQ for DAPA was identified to be 0.4693 and 1.4222 and that for EPLE was 1.314 and 3.983 respectively. Thus, this method provides suitable advantages like improved resolution of overlapping peaks, better selectivity, minimizes background interference. The developed method was successfully applied for the quantification of DAPA and EPLE from their synthetic mixture.

KEYWORDS: Dapagliflozin Propanediol Monohydrate (DAPA), Eplerenone (EPLE), First order derivative (D^1) UV-Spectrophotometric method, ICH Q2(R2) Guideline

INTRODUCTION

Dapagliflozin Propanediol Monohydrate (DAPA) belongs to Sodium-glucose co-transport-2 (SGLT-2) inhibitor category. (1) Chemically, it is a 2-(4-Chloro-3-(4-ethoxybenzyl) phenyl)-6-(hydroxymethyl) tetrahydro-2H-pyran-3,4,5-triol.(2) Its molecular formula is $C_{24}H_{35}ClO_9$ and molecular weight is 503.0 g/mol. (3) Chemical structure of DAPA is shown in **Fig. 1**. The sodium-glucose cotransporter 2 (SGLT2), which is mostly found in the proximal tubule of the nephron, is inhibited by dapagliflozin. Because SGLT2 supports 90% of the kidneys' reabsorption of glucose, its blockage permits the excretion of glucose in the urine. Patients with type 2 diabetes can benefit from improved glucose control and possible weight loss because of this excretion. (4-6)

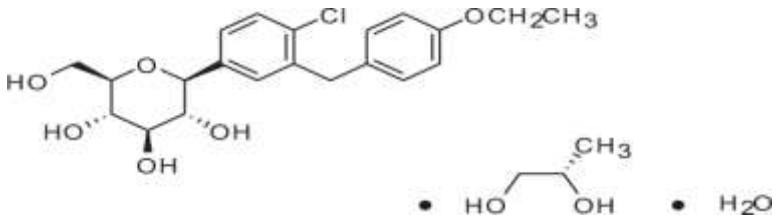

Figure 1 Chemical structure of Dapagliflozin Propanediol Monohydrate

Eplerenone (EPLE) is selective mineralocorticoid receptor antagonist (MRA). Chemically it is methyl (1*R*,2*S*,9*R*,10*R*,11*S*,14*R*,15*S*,17*R*)-2,15-dimethyl-5,5'-dioxospiro[18-oxapentacyclo[8.8.0.0^{1,17}.0^{2,7}.0^{11,15}]octadec-6-ene-14,2'-oxolane]-9-carboxylate.Molecular formula and molecular weight of eplerenone is $C_{24}H_{30}O_6$ and 414.5 g/mol respectively.(7) Chemical structure of EPLE is shown in **Fig. 2**. A highly specific mineralocorticoid receptor antagonist (MRA), eplerenone prevents aldosterone from binding to mineralocorticoid receptors in the kidney's collecting ducts and distal convoluted tubules. Aldosterone often facilitates the excretion of potassium and encourages the reabsorption of salt and water. Eplerenone increases urinary salt and water excretion (natriuresis) and decreases sodium reabsorption while preserving potassium by competitively blocking these receptors.(8-10)

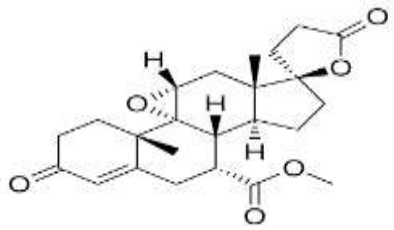

Figure 2 Chemical structure of Eplerenone

The Phase clinical III trial data represented that combination of both the drugs is effectively used to treat chronic kidney disease. The literature review shows that there is no established analytical method for DAPA and EPLE till date. So, the aim of present work is to developed and validate First order Derivative (D^1)UV visible spectroscopy method for both drugs in combination and validation of the same according to ICHQ(2)R2 guideline.

MATERIALS AND METHOD

Dapagliflozin Propanediol Monohydrate and Eplerenone were procured from the Exemed Pharmaceuticals, Vapi. The solvent used for both the drugs was methanol, which was acquired from Merck.

INSTRUMENTATION

A Double Beam UV–Visible Spectrophotometer (Shimadzu, Model UV-1800) equipped with two matched quartz cells of 1 cm path length was used for the analysis. The instrument, manufactured by Shimadzu Corporation, Analytical and Measuring Instruments Division, was operated using UV-Probe 2.35 software. To obtain the best resolution and an acceptable scanning time, the analysis was conducted using the Spectrum mode with a medium scan speed. The study's chosen wavelength range, which covered the UV region pertinent to the analytes, was 200–400 nm. Spectral characteristics were made more visible by using a scale factor of 10. To increase selectivity and reduce interference, the first-order derivative of the spectrum was recorded while the spectral bandwidth was kept at $\Delta\lambda = 2$ nm. An electronic analytical balance (Model AUW-220 d, Japan) was employed for accurate weighing of samples.

Preparation of Standard solutions

To reach a final concentration of 1000 µg/mL for both DAPA and EPLE, a carefully measured 10 mg of powdered drug was added to 10 ml of two separate volumetric flasks, dissolved, and diluted with a small amount of AR-grade methanol. After then, the volume was marked up to 10 ml.

Preparation of working standard solution of DAP and EPLE

One millilitre of the DAPA and EPLE stock solutions was transferred into a 10 mL volumetric flask and diluted to the mark with methanol to obtain 100 µg/mL working standards. From these working solutions,

appropriate aliquots of each analyte were taken and further diluted with methanol to prepare the linearity concentrations for DAPA (4–12 µg/mL) and EPLE (5–30 µg/mL).

Selection of Analytical wavelength

Zero-order spectra of DAPA and EPLE were recorded and converted to first-order derivative spectra (scale factor 10; $\Delta\lambda$ = 2 nm) to enhance selectivity and reduce interference. The wavelength showing linear absorbance of each analytes without interference of other analyte was selected for quantification of DAPA and EPLE.

Analysis of synthetic mixture

The recommended UV approach can be used to quantify DAPA and EPLE in a developed synthetic mixture. The assay of DAPA and EPLE from the produced synthetic combination was performed as follows: 10 mg of DAPA and 25 mg of EPLE were precisely weighed in a 100 mL volumetric flask to create the sample solution. A final concentration of 100 µg/mL DAPA and 250 µg/mL EPLE was achieved by adding 100 ml of methanol, then thoroughly mixing and sonicating for 15 minutes. The same was then used to further dilute the solution to a final volume of 100 mL. A final concentration of 100 µg/mL DAPA and 250 µg/mL EPLE was obtained by further diluting the solution prior to analysis. Three analyses of the sample were performed.

METHOD VALIDATION

The developed 1st Order UV Spectrophotometric Method was validated according to ICHQ2(R2) guideline in terms of Linearity and Range, Precision study (Repeatability, Intraday and Interday precision study), Limit of Detection (LOD) and Limit of Quantitation (LOQ) and accuracy study.

RESULTS AND DISCUSSIONS

The First order Derivative (D1) Spectrum data represented at the ZCP of EPLE (240.41 nm), DAPA showed linear absorbance, while at 254 nm DAPA showed zero absorbance and EPLE absorbed linearly. Hence, 240.41 nm was selected for DAPA and 254 nm for EPLE detection. **(Fig. 3)**

Method Validation

Linearity and Range

Linearity of the regression model was evaluated using the correlation coefficient. Calibration curves were prepared at five concentration levels in the ranges of 4–12 µg/mL for DAPA and 10–30 µg/mL for EPLE, and each level was analyzed five times to determine the linear response **(Figures 4 and 5, Table 1).** The correlation coefficients were obtained from calibration plots of amplitude versus concentration, and the corresponding linear regression parameters are summarized in **Table 2.**

LOD and LOQ

The LOD and LOQ of both drugs were determined using the signal-to-noise ratio in compliance with ICH recommendations using the following formulas: LOD = 3.3 × (σ/S) and LOQ = 10 × (σ/S), where σ is the response's standard deviation (Y-intercepts of the five calibration curves) and S is the average slope of the five calibration curves. LOD and LOQ of both the drugs is represented in **Table 2**.

Precision

The developed first-order UV spectrophotometric method's precision was assessed at two levels: repeatability and intraday and interday precision. The percentage RSD values for DAPA and EPLE repeatability, intraday precision, and interday precision were found to be less than 2 **(Table 3)** demonstrating the outstanding repeatability and reproducibility of the suggested method.

Accuracy

Standard addition method was used to study accuracy of developed 1st order derivative UV-Spectrophotometric method. The accuracy study of DAPA and EPLE were performed at 80%, 100% and 120% level and mean % recovery for DAPA and EPLE were shown **(Table 4)**. The data depicted satisfactory recovery of DAPA and EPLE which indicates that the method is accurate, and reliable.

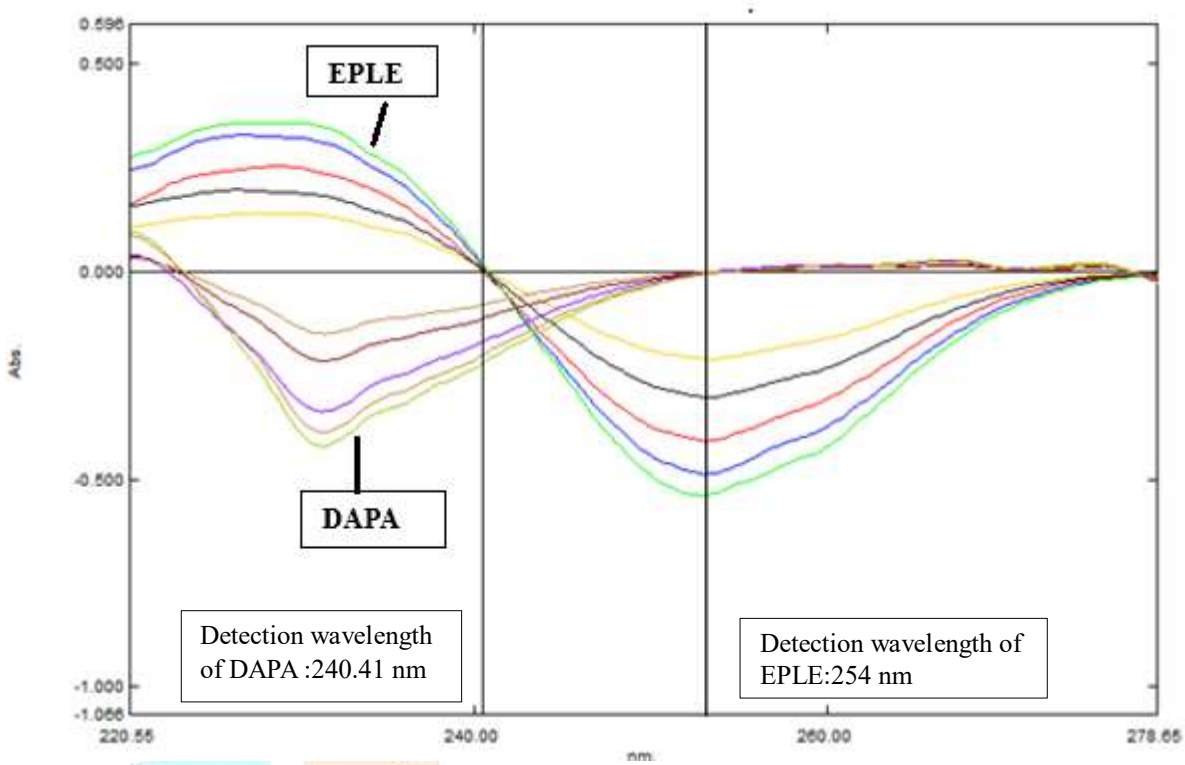

Figure 3: First order Derivative (D1) overlay Spectra of DAPA(4-12 µg/ml) and EPLE(5-30 µg/ml)

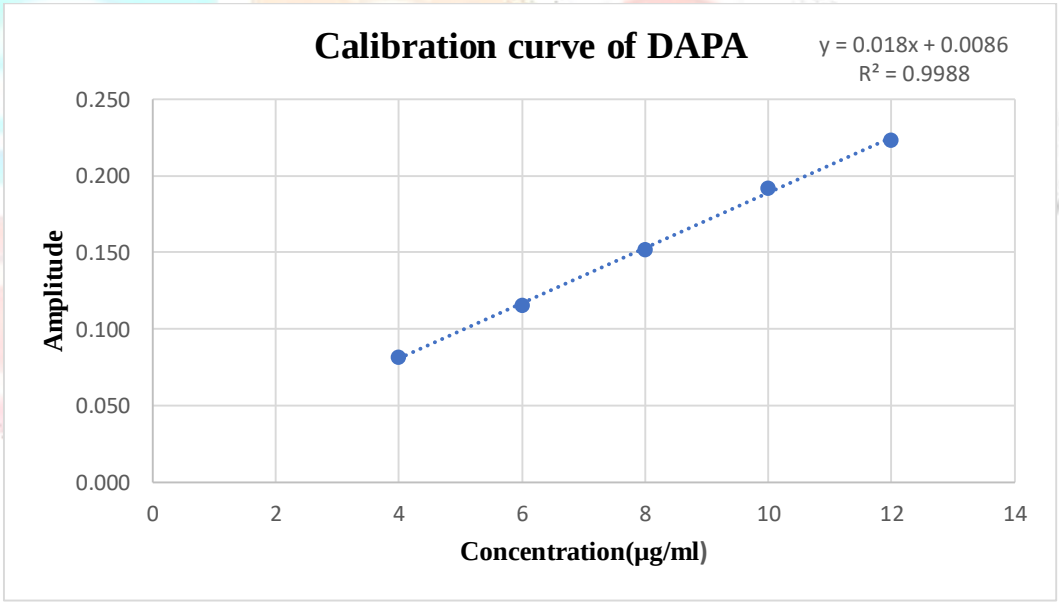

Figure 4 Calibration curve of DAPA (4-12 µg/ml) in Methanol

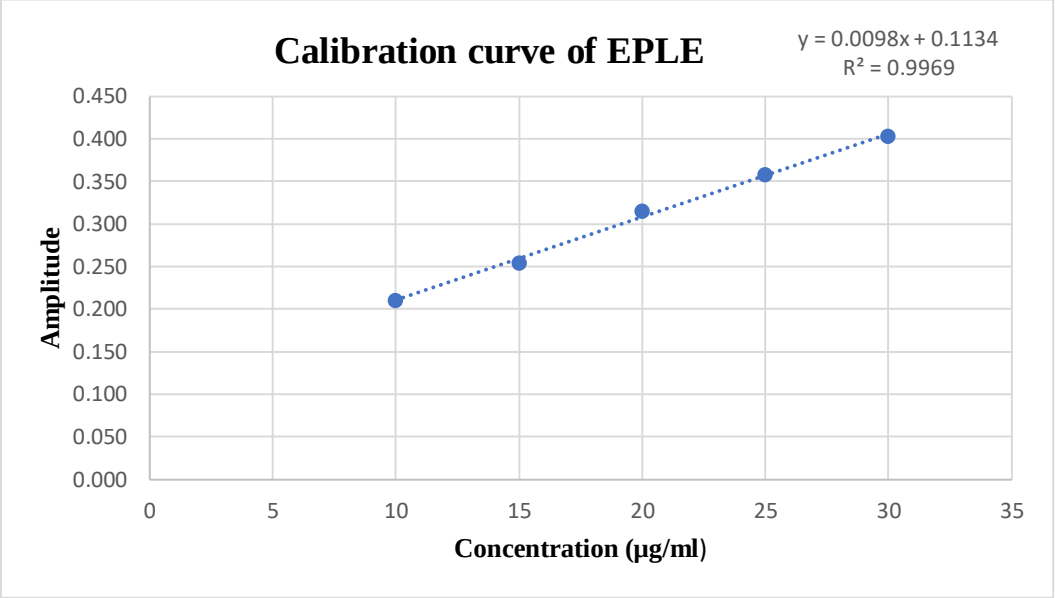

Figure 5 Calibration curve of EPLE (5-30 µg/ml) in Methanol

Table 1: Linearity data for DAPA and EPLE in Methanol

DAPA				EPLE			
Concentration	Avg. amplitude*	S. D	%RSD	Concentration	Avg. amplitude*	S. D	%RSD
4	0.081	0.0009	1.10	10	0.211	0.0013	0.62
6	0.115	0.0008	0.73	15	0.255	0.0034	1.33
8	0.152	0.0017	1.10	20	0.315	0.033	1.05
10	0.193	0.0013	0.68	25	0.358	0.0043	1.21
12	0.223	0.0026	1.16	30	0.403	0.0042	1.04

*n= 5, mean of five replicates, SD = standard deviation, %RSD = relative standard deviation

Table 2 : Linear regression parameter for DAPA and EPLE

Parameter	DAPA	EPLE
Calibration Range (µg/ml)	4-12 µg/ml	10-30 µg/ml
Regression equation	Y=0.018x+0.0086	Y=0.0098x+0.1134
Correlation coefficient	0.9988	0.9969
Slope[a]	0.018	0.0098
Intercept[a]	0.0086	0.1194
LOD (Limit of detection (µg/ml))	0.4693	1.3143
LOQ (Limit of quantitation (µg/ml))	1.4222	3.9830

n[a]=5, SD = standard deviation

Table 3: Precision study of DAPA and EPLE

Conc. (µg/ml)	Inter day Precision of DAPA and EPLE	Intraday Precision of DAPA and EPLE	Repeatability study of DAPA and EPLE
	%RSD*	%RSD*	%RSD#
DAPA			
6	0.50	0.87	-
8	0.66	1.32	0.804
10	0.52	0.80	-
EPLE			
15	1.37	1.63	-
20	0.80	1.45	0.528
25	1.38	1.21	-

*n = three replicates, #n = six replicates, SD = standard deviation, %RSD = relative standard deviation

Table 4: Accuracy study for DAPA and EPLE

DAPA			EPLE		
% Spike	Mean % Recovery* ±SD	% RSD	% Spike	Mean % Recovery* ±SD	% RSD
80	99.43± 0.803	0.808	80	101.54±0.327	0.322
100	100.72±1.060	1.053	100	100.57±0.779	0.775
120	101.03±0.657	0.650	120	100.09±0.878	0.877

*n =3 replicate, S.D = standard deviation, %RSD= relative standard deviation

Table 5:Analysis of Synthetic mixture

Drug	Dose(mg)	Conc.(µg/ml)	%Amount found			Mean% Amount	SD	%RSD
DAPA	10	8	99.88	99.86	100.02	99.9	0.0872	0.0872
EPLE	25	20	100.45	101.05	100.04	100.6	0.3617	0.3594

CONCLUSION

A simple, efficient, and affordable First order Derivative (D1) UV-spectrophotometric method for the simultaneous measurement of DAPA and EPLE in their synthetic combination was effectively developed and validated in this work. According to ICH Q2(R2) guidelines, the method showed linearity, accuracy, and precision, as per guideline mentioned. The overlapping spectra of both drugs were successfully resolved by using first-order derivative spectroscopy, which allowed for precise quantification free from interference. Both analytes can be successfully estimated in laboratory-prepared mixes and possibly in pharmaceutical formulations using the approved approach, which is appropriate for regular quality control analysis.

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