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METHOD DEVELOPMENT AND VALIDATION OF ERTUGLIFLOZIN IN BULK AND PHARMACEUTICAL DOSAGE FORM BY UV-VISIBLE SPECTROPHOTOMETRIC METHOD

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ABSTRACT

The objective of the study was to develop and validate easy, accurate and precise UV-Visible spectrophotometric method for the determination of Ertugliflozin in bulk and pharmaceutical dosage form. Spectroscopic method development for the estimation of Ertugliflozin was carried out using Shimadzu 1800 UV Visible Spectrophotometer with a pair of 10mm path length matched quartz cells. All the solutions were scanned in the range of 200-400nm. Zero order and first order derivative spectrum for both Method A and Method B Spectrophotometric methods have been developed for the determination of Ertugliflozin in borate buffer pH 9 and phosphate buffer pH 6.8. The result of the study, absorbance of Ertugliflozin was measured at 220nm for the zero order. Zero order spectra were derivatized into first order. Beer's law was obeyed in the concentration range of 10-60 and 10-70µg/ml. The methods were validated with respect to linearity, accuracy, precision and LOD and LOQ. The linearity concentration range was 10-60 and 10-70µg/ml and correlation coefficients (r^2) were found to be > 0.999. The mean percentage recoveries were found to be in the range of 98-102%. The percentage relative standard deviation were found to be <2.0%. It can be concluded that the proposed method is recommended for routine analysis since it is rapid, simple, accurate and also sensitive and specific.

KEYWORDS

Ertugliflozin, UV Visible spectrophotometer, Zero order and First-order derivative.

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INTRODUCTON

Type 2 Diabetes Mellitus (T2DM) is a global pandemic, were evident from the global cartographic picture of diabetes by the International Diabetes Federation[1]. Diabetes mellitus is the chronic, progressive, incompletely understood metabolic condition chiefly characterized by hyperglycemia. Impaired insulin secretion, resistance to the tissue actions of insulin, or a combination of both are thought to be the most commonest reasons contributing to the

pathophysiology of T2DM, a spectrum of disease that were originally arising from the tissue insulin resistance and gradually progressing to a state characterized by complete loss of secretory activity of the beta cells of the pancreas. T2DM is the a major contributor to the very large rise in the rate of non-communicable diseases[2,3].

Ertugliflozin is chemically known as ertugliflozin l-pyroglutamic acid is (1s, 2s, 3s, 4r, 5s)-5-(4-chloro-3-(4ethoxybenzyl) phenyl)-1-(hydroxymethyl)-6, 8-dioxabicyclo [3.2.1] octane-2, 3, 4-triol, compound with (2s)-5oxopyrrolidine-2-carboxylicacid. The molecular formula is $C_{27}H_{32}CINO_{10}$ and the molecular weight is 566.00. Ertugliflozin which belongs to the class of various potent and that were the selective inhibitors of the sodium dependent glucose co transporters (SGLT), more specifically the type 2 which is responsible for about 90% of the glucose reabsorption from glomerulus[4]. Administration of ertugliflozin increases urinary glucose excretion which leads to a negative balance and osmotic diuresis. Thus, the anti diabetic agent has been reported to significantly reduce the body weight and blood pressure of diabetic patients[5].

Literature survey revealed that no analytical methods were reported so far for single drug namely ertugliflozin by UV Visible Spectrophotometry. Thus, the objective of present study was to develop and validate precise, accurate and economical UV spectrophotometric method for estimation of ertugliflozin for both methods (Method A and Method B) namely Zero order and first order derivative.

MATERIAL AND METHODS

Chemicals

Ertugliflozin was marketed by Merck and Pfizer under the brand name "STEGLATRO". Analytically pure ertugliflozin sample was obtained from Spectrum Pharma Research Solutions (Hyderabad). All the chemicals used were of analytical grade.

Instrumentation

UV-1800 double beam UV Visible Spectrophotometer (Shimadzu) with a pair of 10mm path length matched quartz cells were used for the study. All the solutions were scanned in the range of 200-400nm. The analysis was carried out by using UV solutions 2.42 software. An electronic balance (Model Shimadzu) was used for weighing purpose. Volumetric flasks and pipettes of borosilicate glass were used in the study.

Preparation of standard drug solution

Accurately weighed 10mg of ertugliflozin was dissolved in 50ml methanol in a 100ml volumetric flask. The solution was sonicated for 10mins. The final volume was adjusted to 100ml with methanol (standard stock solution 100µg/ml). The prepared standard solution was scanned in the range of 200-400nm for determination of wavelength of maximum absorption.

Preparation of borate buffer (p^H 9)

Dissolve 6.20g of boric acid in 500ml of water, adjust to p^H 9.0 with 1M sodium hydroxide (about 41.5 ml) and dilute with water to 1000ml.

Preparation of phosphate buffer (p^H 6.8)

Dissolve 28.80g of disodium hydrogen phosphate and 11.45g of potassium di hydrogen phosphate in sufficient water to produce 1000ml.

Validation procedure

The method was validated according to ICH guidelines, in terms of linearity, accuracy, precision and LOD and LOQ. Methods are evaluated to determine its effectiveness for future use[6].

Linearity

The linearity was determined by plotting the concentration against corresponding absorbance. A standard stock solution (100µg/ml) was further diluted with buffer to obtain 10µg/ml - 60µg/ml and 10µg/ml - 70µg/ml solutions for ertugliflozin. The calibration curves were constructed by plotting absorbance versus concentration and the regression equations were calculated[7].

Accuracy

The accuracy of the proposed method was assessed by recovery studies which were carried out at three different levels therefore 50%, 100%, and 150%. Known amount of stock standard solutions was added to pre-analyzed samples at three different concentration levels and they were subjected to analysis by the proposed method. The percentage recovery was then calculated[8].

Precision

Intra-day precision

Standard stock solutions (2 ml, 4 ml, and 8 ml) were taken in 10 ml volumetric flasks and final volume was made up to the mark with buffer. The absorbances of these solutions were individually measured thrice within a day and recorded[9].

Inter-day precision

Standard stock solutions (2 ml, 4 ml, and 8 ml) were taken in 10 ml volumetric flasks and final volume were made up to the mark with buffer. The absorbances of these solutions were measured thrice in three days and recorded[10].

Limit of detection

LOD was calculated based on the standard deviation of response and the slope of the corresponding curve using following equation LOD = 3.3 σ/ S[11].

Limit of quantification

LOQ was calculated based on the standard deviation of response and the slope of the corresponding curve using following equation LOQ = 10 σ/ S[12].

RESULTS AND DISCUSSION

The standard solution of ertugliflozin in acetonitrile (10µg/ml) was subjected to a scan individually at a series of wavelengths of 200 nm to 400 nm at zero order derivative mode. The first order derivative spectra were taken at a smoothening factor of the instrument using Shimadzu 1800 spectronic UV Visible spectrophotometer. The absorption maximum of ertugliflozin was found to be at 220nm. An overlain spectrum is depicted in Figure No.1-4 and summary of validation parameters is represented in Table No.1.

Linearity

Beer Lambert's law was obeyed in the concentration range of 10-60µg/ml and 10-70µg/ml. The linearity of an analytical procedure is its ability to elicit test results that are directly proportional to the concentration of analyte in the sample[13]. The linear regression data for the calibration plot were indicative of a good relationship between the peak area and concentration over wide range. The calibration curves are shown in Figure No.5-8.

Accuracy

Accuracy of the methods were ascertained by standard solution method at three levels. Standard quantity equivalent to 50%, 100%, and 150% is to be added in sample. Known amount of stock standard solutions was added to tablet samples at three different concentrations level and they were subjected to analysis by the proposed method. The results shown that best recoveries in between 98-102%. The percentage RSD values were found to be <2 indicating the method was accurate and the results were shown in Table No.2,3.

Precision

Precision of the method was considered by repetitive measurements of drug solution and the result showed lower %RSD values. The %RSD for intra-day precision and inter-day precision for ertugliflozin were given in Table No.4-7.

Lod and Loq

The limit of detection and limit of quantification were determined to be 0.068 and 0.182µg/ml for method A and 0.081 and 0.197µg/ml for method B.

The methods discussed in the present work provide a convenient and accurate way for analysis of ertugliflozin in its bulk and pharmaceutical dosage form. Absorbance maxima of ertugliflozin at 220nm were selected for the analysis. Linearity for detector response was observed in the concentration range of 10-60µg/ml and 10-70µg/ml. The slope, intercept, correlation coefficient and optical characteristics were summarized. Accuracy of proposed methods was ascertained by recovery studies and the results are expressed as percentage recovery was in the acceptance limit of 98 to 102%. Regression analysis of Beer's law plot revealed a good correlation. The RSD was not more than 2%. The proposed method were validated as per ICH guidelines. The precision was measured in terms of inter-day and intra- day, which was determined by a sufficient number of aliquots of a homogeneous sample. The percentage RSD was found within the range of 2%. The limit of detection LOD is defined as the lowest concentration of an analyte. The limit of quantification LOQ is defined as an individual analytical procedure is the lowest amount of analyte in a sample that can be determined with suitable

precision and accuracy. The LOD and LOQ of ertugliflozin were determined by using the standard deviation of response and slope approach defined by ICH guidelines.

Table No.1: Summary of validation parameters

Methods	Parameters						
	Beer's Law Limit	**Correlation coefficient**	**%Recovery ± SD**	**LOD µg/ml**	**LOQ µg/ml**	**Sandell's Sensitivity**	**Molar absorptivity**
Method A Zero order	10-60 µg/ml	0.9997	99.5±0.3	0.068	0.182	0.05406	6105.308
First-order derivative		0.9996	99.7±0.8	0.066	0.184		
Method B Zero order	10-70 µg/ml	0.9995	99.7±0.3	0.076	0.192	0.05263	5783.976
First-order derivative		0.9992	98.52±1.1	0.081	0.197		

Table No.2: Accuracy data of method A

S.No	Method A	Initial amount (µg/ml)	Amount added (µg/ml)	Amount recovered (µg/ml, n=3)	Mean ± SD
1	Zero order	10	30	29.9	99.7±0.3
		10	40	40.47	101.2±0.3
		10	50	49.63	99.3±0.3
2	First order	10	30	29.68	99.0±0.6
		10	40	39.82	99.0±1.0
		10	50	50.07	100.2±0.5

*Each value is average of three determinations

Table No.3: Accuracy data of method B

S.No	Method B	Initial amount (µg/ml)	Amount added (µg/ml)	Amount recovered (µg/ml, n=3)	Mean ± SD
1	Zero order	10	30	30.28	100.93±1.1
		10	40	40.26	100.66±0.69
		10	50	50.3	100.7±0.063
2	First order	10	30	29.08	99.35±0.8
		10	40	40.54	101.36±0.6
		10	50	50.54	101.09±0.6

*Each value average of three determinations

Table No.4: Intraday precision data of method A

S.No	Method A	Concentration (µg/ml)	Amount found (µg/ml)	Mean ± SD (µg/ml, n=3)	%RSD
1	Zero order	20	19.75	98.8±0.8	0.8
		40	39.73	99.3±0.2	0.2
		80	79.14	98.9±0.2	0.2
2	First order	20	19.83	99.2±0.8	0.8
		40	39.71	99.3±0.3	0.3
		80	79.08	98.8±0.1	0.1

*Each value average of three determinations

Table No.5: Inter day precision data of method A

S.No	Method A	Concentration (µg/ml)	Amount found (µg/ml)	Mean ± SD (µg/ml, n=3)	%RSD
1	Zero order	20	19.79	99.0±0.5	0.5
		40	39.87	99.7±0.6	0.6
		80	79.23	99.0±0.9	0.9
2	First order	20	19.75	98.8±0.5	0.5
		40	39.66	99.1±0.4	0.4
		80	78.9	98.6±0.2	0.2

*Each value average of three determinations

Table No.6: Intraday precision data of method B

S.No	Method B	Concentration (µg/ml)	Amount found (µg/ml)	Mean ± SD (µg/ml, n=3)	%RSD
1	Zero order	20	19.61	98.1±0.6	0.6
		40	40.40	101.0±0.2	0.2
		80	80.79	101.0±0.1	0.1
2	First order	20	19.8	99.5±1.6	1.6
		40	40.38	101.0±0.4	0.4
		80	79.78	99.74±0.15	0.15

*Each value average of three determinations

Table No.7: Inter day precision data of method B

S.No	Method B	Concentration (µg/ml)	Amount found (µg/ml)	Mean ± SD (µg/ml, n=3)	%RSD
1	Zero order	20	19.7	98.3±0.55	0.6
		40	40.0	100.0±1.44	1.4
		80	80.8	101.03±0.1	0.1
2	First order	20	19.65	98.3±1.8	1.8
		40	40.33	100.9±0.5	0.5
		80	79.83	99.8±0.23	0.23

*Each value average of three determinations

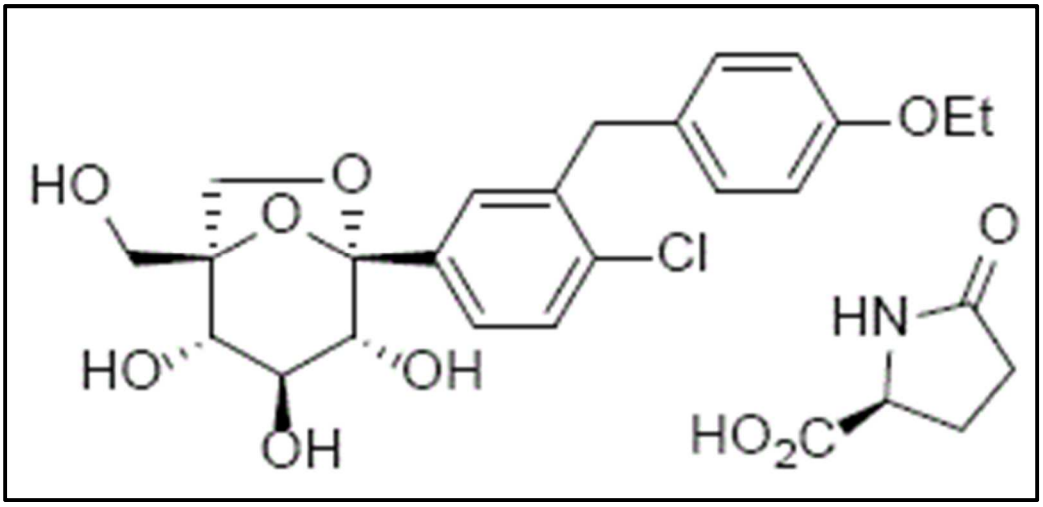

Figure No.1: Chemical structure of ertugliflozin

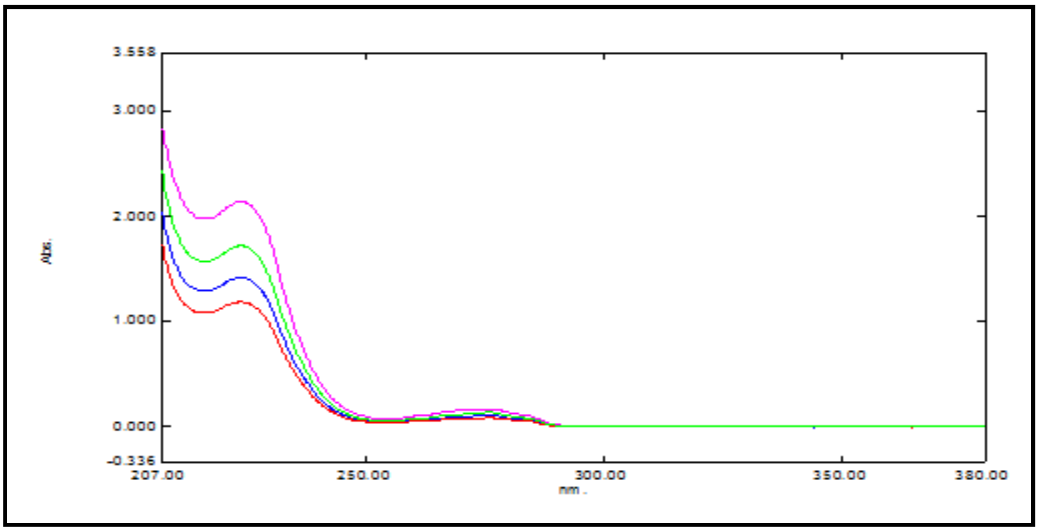

Figure No.1: Overlain spectrum of method A (Zero order)

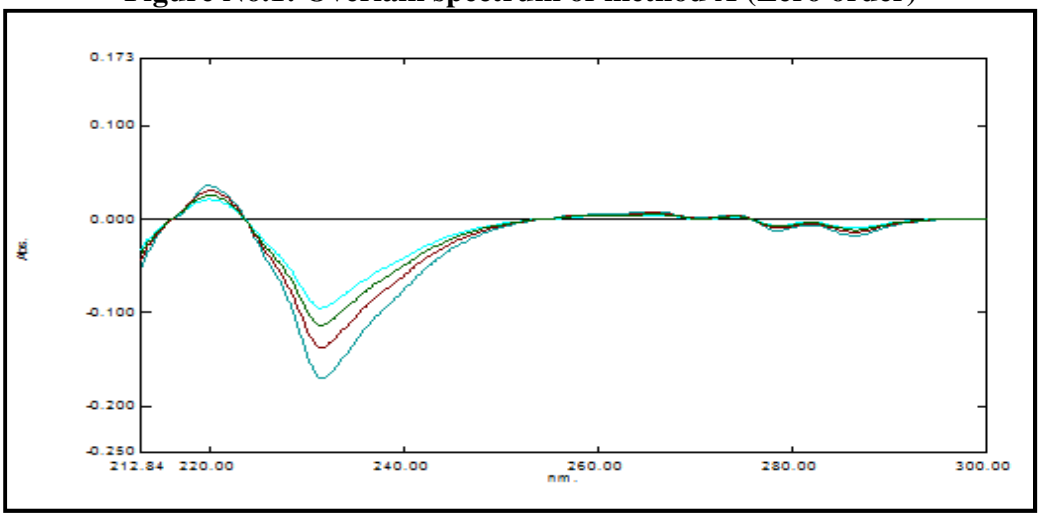

Figure No.2: Overlain spectrum of method A (First order derivative)

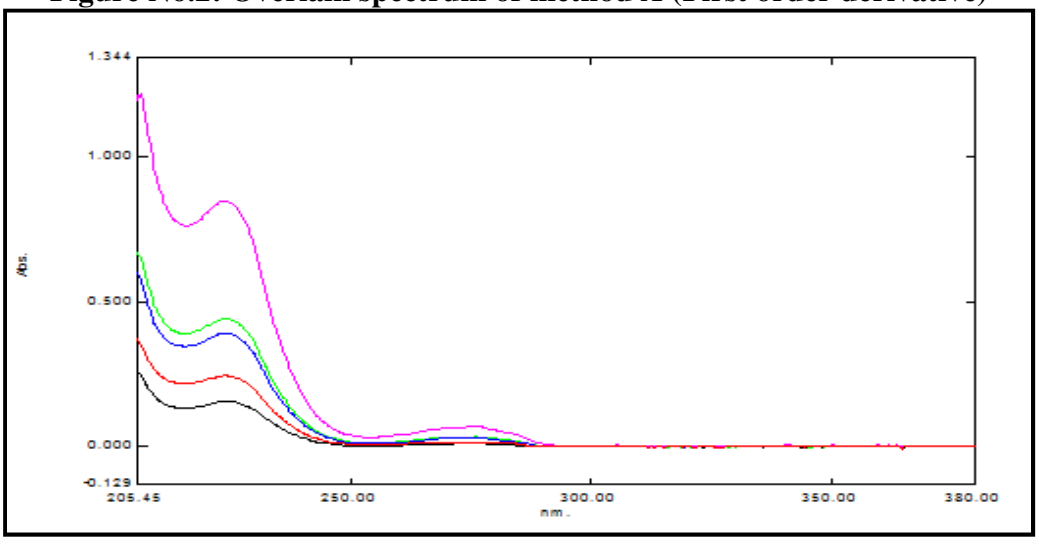

Figure No.3: Overlain spectrum of method B (Zero order)

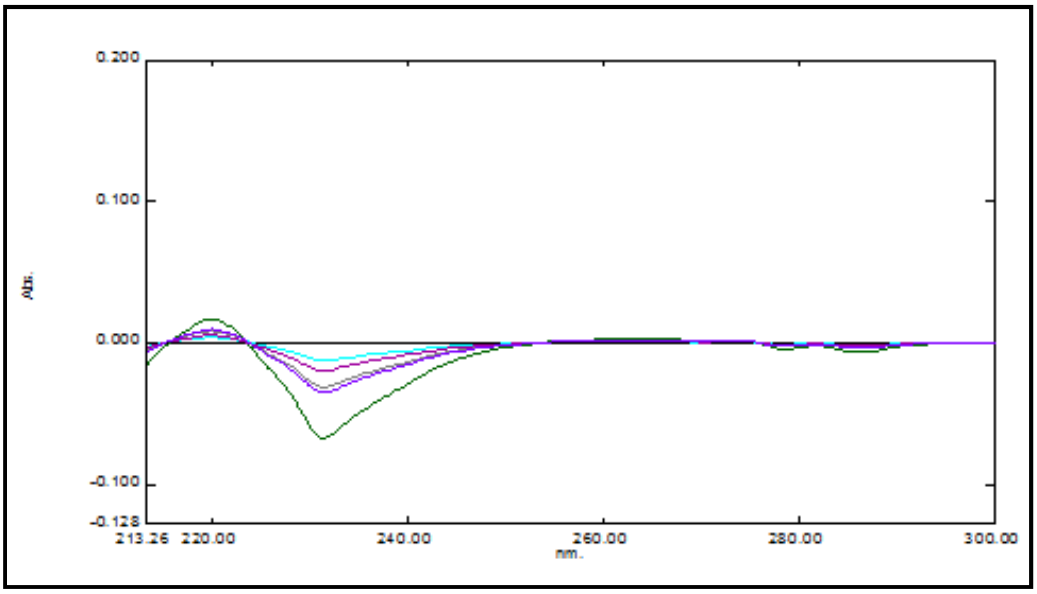

Figure No.4: Overlain spectrum of method B (First order derivative)

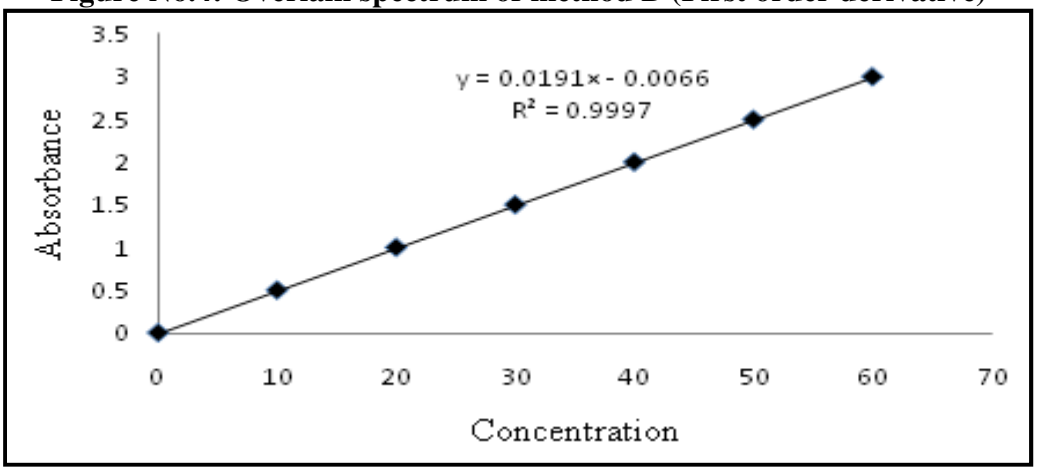

Figure No.5: Calibration curve of method A (Zero order)

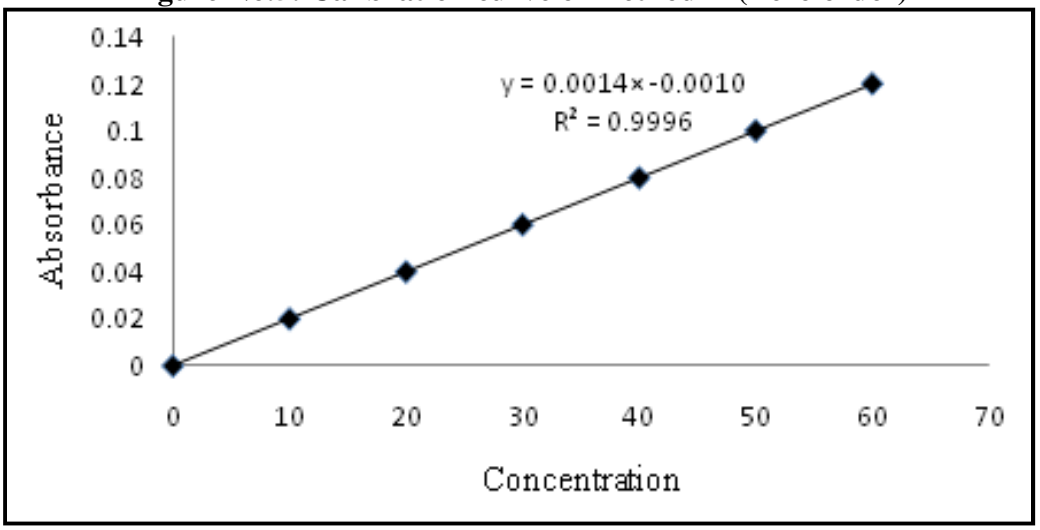

Figure No.6: Calibration curve of method A (First order derivative)

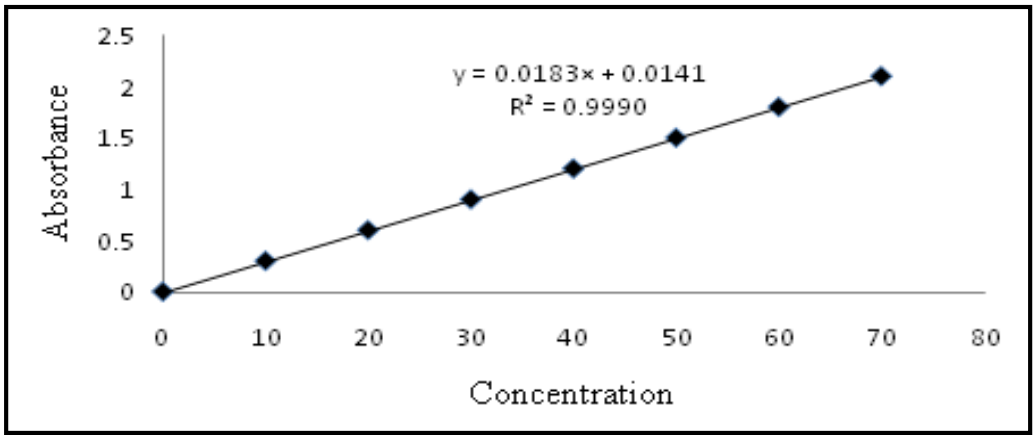

Figure No.7: Calibration curve of method B (Zero order)

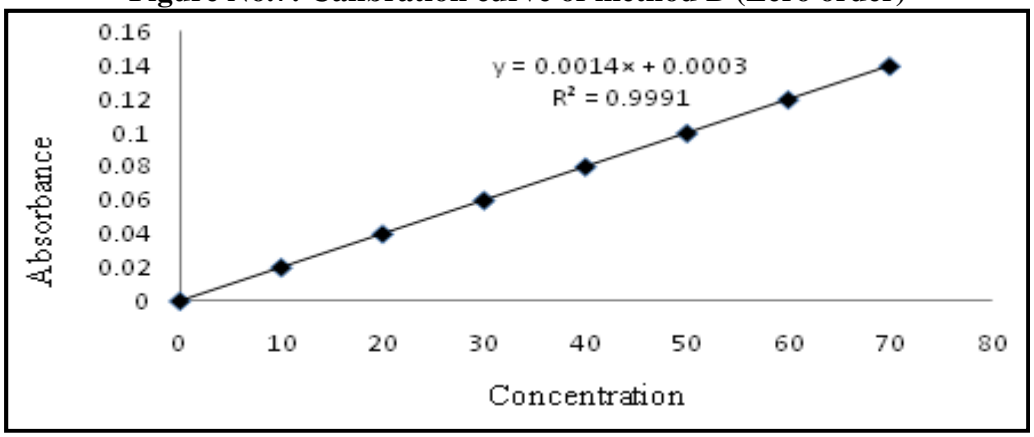

Figure No.8: Calibration curve of method B (First order derivative)

CONCLUSION

Finally, it is concluded that Zero order and First order derivative methods were developed for estimation of ertugliflozin in bulk and pharmaceutical dosage form. The developed methods were validated and found to be simple, sensitive, accurate and precise hence, the proposed methods can be used for routine quality control analysis of ertugliflozin in bulk and pharmaceutical formulation.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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