International Journal of Pharmacy and Biological Sciences-IJPBS™ (2020) 10 (1): 65-72
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Pharmaceutical Sciences | Open Access | MCI Approved

Development and Validation of UV-Spectrophotometric Method for The Estimation of Canagliflozin and Metformin

S. J. Parmar[1], B. A. Patel*, A. B. Patel[1] and Abhishek. Pandey[1]
*,[1]**Department Of Pharmaceutical Sciences, Sardar Patel University, Vallabh Vidyanagar, Anand, Gujarat, India - 388120.**

Received: 10 Oct 2019 / Accepted: 08 Nov 2019 / Published online: 01 Jan 2020
***Corresponding Author Email:** bhavidg@gmail.com

Abstract
To develop and validate simple, sensitive, precise, rapid and cost effective method for determination of Canagliflozin and Metformin in synthetic mixture as per ICH Guidelines. For the First order derivative spectroscopy development, solvent used was in ratio of Methanol: Water (40:60 %) and wavelengths of MF at 240 nm (Zero absorbance of CF) and CF 319 nm (Zero absorbance of MF) were selected for analysis. Analysis of synthetic mixture was also done by same method. The percentage drug contents were found to be 99.48 ± 0.83 and 100.76 ± 1.29 for CF and MF respectively, the developed methods were validated as per ICH guidelines Q2 (R1) for linearity, range, accuracy and precision. Linearity of the methods was found to be in a range of 15 – 30 µg/ml and 4 – 12 µg/ml for CF and MF respectively. The accuracy of the methods was determined by recovery studies. The % of drugs recovered was found to be close 100, indicating accuracy of the method. Precision of the methods was estimated by repeatability and intermediate precision studies. The % RSD values were found to be less than 2, proving methods were precise. Therefore, the developed methods could be effectively used for routine quality control analysis in industry for simultaneous analysis of CF and MF in pharmaceutical formulation.

Keywords
Canagliflozin, Metformin, Derivative Spectroscopy, Synthetic mixture.

*****

INTRODUCTION
The Canagliflozin and Metformin is indicated as an adjunct to diet and exercise to improve glycemic control in adults with type-2 diabetes. Canagliflozin is chemically (1S)-1, 5-anhydro-1- [3- [[5-(4-fluorophenyl)-2-thienyl] methyl]-4-methylphenyl]-Glucitol and belongs to the class of SGLT2 inhibitors. It is used in the treatment of type-2 diabetes. Canagliflozin inhibits the reabsorption of glucose from kidneys and lowers the renal glucose threshold by inhibiting sodium-glucose transport protein (SGLT2). By blocking SGLT2, Canagliflozin decreases reabsorption of filtered glucose and reduces the renal threshold for glucose (RTG), thereby elevating the urinary glucose excretion (UGE) and reducing raised plasma glucose in patients with type-2 diabetes. Canagliflozin can be used as monotherapy or multi therapy in the treatment of type-2 diabetes. Metformin a biguanide antihyperglycemic agent used for treating type-2 diabetes. It acts by decreasing hepatic glucose production and glucose absorption, and it enhances insulin mediated glucose uptake. Metformin is recommended as first line therapy for patients with type-2 diabetes. Patients, from whom Metformin monotherapy is not sufficient to achieve glycemic goals, it is referred to use in

combination with other class of antidiabetic drugs.[1-12] The literature survey revealed that few analytical methods were reported for estimation of the drugs individually and in combination using UV, HPLC, HPTLC and LC-MS.[13-17] In the present study an attempt was made for simultaneous estimation of Canagliflozin and Metformin in pharmaceutical dosage form by UV spectrophotometry and validated as per ICH Guidlines[18]. The method can be applied for routine analysis in quality control laboratory.

MATERIALS AND METHODS

Drug: Canagliflozin and Metformin were obtained from ZYDUS Pharma., Ahmedabad

Year of Experimentation: 2019

Site: Department of Pharmaceutical Sciences, Sardar Patel University.

Chemicals and reagents:

AR Grade Methanol: Sisco Chem Pvt Ltd., Andheri Mumbai.

Water: Distilled water collected by distillation assembly.

Method: First order derivative spectroscopy

PREPRATION OF SOLVENT

Preparation of the CF standard stock solution

Accurately weighed 10 mg of CF was transferred into a 10 ml volumetric flask and dissolved in 4 ml of methanol and diluted up to the mark with Distilled water to get a stock solution containing 1000 µg/ml CF.

Preparation of CF working standard solution

10 ml of CF standard stock solution was transferred into the 100 ml VF and dilute with 40 ml methanol and 60 ml distilled water to get 100 µg/ml of CF.

Preparation of solution for calibration curve of CF

Aliquots (1ml, 1.5ml, 2ml, 2.5ml, 3ml) were withdrawn from a standard stock solution of CF and diluted up to 10 ml with solvent to get the calibration standards containing 10, 15, 20, 25, 30 µg/ml of CF. The calibration standards were analyzed by the proposed method.

Preparation of MF standard stock solution

Accurately weighed 10 mg of MF was transferred into a 10 ml volumetric flask and dissolved in water and diluted up to the mark with water to get a stock solution containing 1000 µg/ml MF.

Preparation of MF working standard solution

10 ml of MF standard stock solution was transferred into a 100 mlVF and dilute with the water to get 100 µg/ml of MF.

Preparation of solution for calibration curve of MF

Aliquots (0.4ml, 0.6ml, 0.8ml, 1.00ml, 1.2ml) were withdrawn from a standard stock solution of MF and diluted up to 10 ml with water to get the calibration standards containing 4, 6, 8, 10, 12 µg/ml of MF. The calibration standards were analyzed by the proposed method.

Preparation of standard solution containing a mixture of CF and MF

1 ml solution from CF and MF standard stock solution were mixed in a volumetric flask and diluted up to 10 ml with solvent to get 10 µg/ml of CF and 10 µg/ml of MF in the standard solution.

Preparation of sample solution of Synthetic Mixture for CF and MF

Powder mixture equivalent to 50 mg of CF and 70 mg of MF and 30 mg of starch, 140 mg lactose and 10 of magnesium stearate was transferred in 100 ml volumetric flask containing 50 mL Methanol, sonicated for 15 min and diluted to mark with same solvent to obtain 0.5 mg/ml of CF and 0.7 mg/ml of MF. The resulting solution was filtered using Whatman filter paper. From the above solution 2 mL was transferred into 10mL volumetric flask and diluted to mark with same solvent. Further take 1 ml of aliquot into 10 ml of volumetric flask and dilute up to mark with solvent. So, Resultant solution was found to contain 10 µg/ml of CF and 14 µg/ml of MF.

DEVELOPMENT OF FIRST DERIVATIVE UV SPECTROSCOPIC METHOD

Solubility

The solubility of CF and MF was practically determined. Solubility was determined by taking 10 mg of CF and MF in 10 ml volumetric flasks individually, adding the required quantity of solvent at room temperature and shaken for a few minutes. Solubility data for each study were observed and recorded.

Selection of wavelength for measurement

The standard solutions of 10 µg/ml of CF and 10 µg/ml MF were scanned over 200-400 nm. Then ZCP was selected from the First derivative spectrum of CF exhibits a maximum at 319 nm, 252 nm, 310 nm while MF reads zero and MF exhibits absorption at 240 nm while CF reads zero. Quantitative investigations using regression analysis have established that the concentration of CF and MF correlates very well with the measured first derivative peaks. Various ZCPs were obtained for CF and MF.

Spectrophotometric conditions

Mode: Spectrum

Scan speed: Medium

Wavelength range: 400-200 nm

Initial baseline correction:
Methanol: Water (40:60 v/v)
Absorbance scale: 0.00A – 2.00A

METHOD VALIDATION

Linearity and range

Aliquots were withdrawn from standard stock solution of CF and MF and diluted up to 10 ml with solvent to get the calibration standards containing 10, 15, 20, 25, 30 µg/ml of CF and 4, 6, 8, 10, 12 µg/ml of MF. The calibration standards were analyzed by the proposed method. The absorbance of CF and MF were measured. The calibration curves for CF and MF were constructed by plotting the absorbance against respective drug concentrations. A linear regression analysis was performed.

Precision

Interday and Intraday precision were evaluated by analyzing concentration CF (15-25 µg/ml) and MF (6-10 µg/ml) five times on the same day and on three different days and % RSD value obtained was calculated to determine any intraday and Interday variation.

Accuracy

Accuracy was determined by calculating the % recovery by the standard addition method. A known amount of standard solution of (0 ml, 18, 20 and 22 µg/ml) of CF and (25.2, 28 and 30.8 µg/ml) and MF were added in pre-analyzed sample solution of Synthetic mixture which gives a solution having the strength of 80%, 100% and 120% of middle concentration from the range. Each solution was scan in triplicates and recovery was calculated by measuring the Absorbance and fitting themselves into the regression equation.

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

The limit of Detection (LOD) and limit of quantitation (LOQ) of the drug were calculated using the following equations as per ICH guidelines.

$LOD=3.3(\sigma/S)$,

$LOQ=10(\sigma/S)$

Where σ is the Standard deviation of the response and S is the slope of the calibration curve.

RESULTS AND DISCUSSION

Selection of solvent

Solubility Data

Both the drugs were found to be freely soluble in Methanol, and partially soluble in Distilled Water. Hence the ration of methanol and water was selected as the final solvent for every measurement in the UV method.

Selection of Detection Wavelength

The standard solutions of 10 µg/ml of CF and MF were scanned over 200-400 nm. Overlay of zero order spectra of CF (10 µg/ml) and MF (10 µg/ml) was shown in Figure 1, Figure 2 shows

Method validation

Linearity

The linearity for MF and CF was found in the range of 15-35 µg/ml and 4-12 µg/ml respectively is shown in the overlain chromatogram of CF and MF, in figure 3. Calibration data and linear regression data are shown in Table no.1, 2 and 3 respectively for CF and MF, Calibration Curve were show in Figure 4, 5 for CF and MF respectively.

Precision

Intraday precision

Here, three different concentrations (15-25 µg/ml) of CF and Three different concentration (6-10 µg/ml) of MF were measured three times in a single day and % RSD value was calculated to determine Intraday variation which was within the limit (i.e. % RSD ≤2) as shown in Table no: 4 and 5 respectively to CF and MF.

Interday precision

Here, three different concentrations of CF and MF were measured at three different days and % RSD value was calculated to determine Intraday variation which is within the limit (i.e. % RSD ≤ 2) as shown in Table 6 and 7.

Repeatability

Instrument linearity was done by taking the absorbance of CF (10 µg/ml) and MF (14 µg/ml). The data show in Table no. 8.

Accuracy

The accuracy of the method was done by spiking pre-analyzed solution with a known amount of the drug and data obtained as shown in Table no.9 and 10, which suggests that the recovery of the present method ranges from 99.12-100.36 % for CF and 99.30 -100.46 % for MF.

LOD and LOQ

The LOD and LOQ were calculated by equation. The LOD and LOQ values were 0.3325 and 1.0076 µg/ml for CF and 0.10431 and 0.3161 µg/ml for MF, Show in Table no.11.

ANALYSIS OF SYNTHETIC MIXTURE

Content of CF AND MF found in the synthetic mixture from the proposed method are shown in Table 12. The % purity was found to be 99.87% for CF and 100.01% for MF.

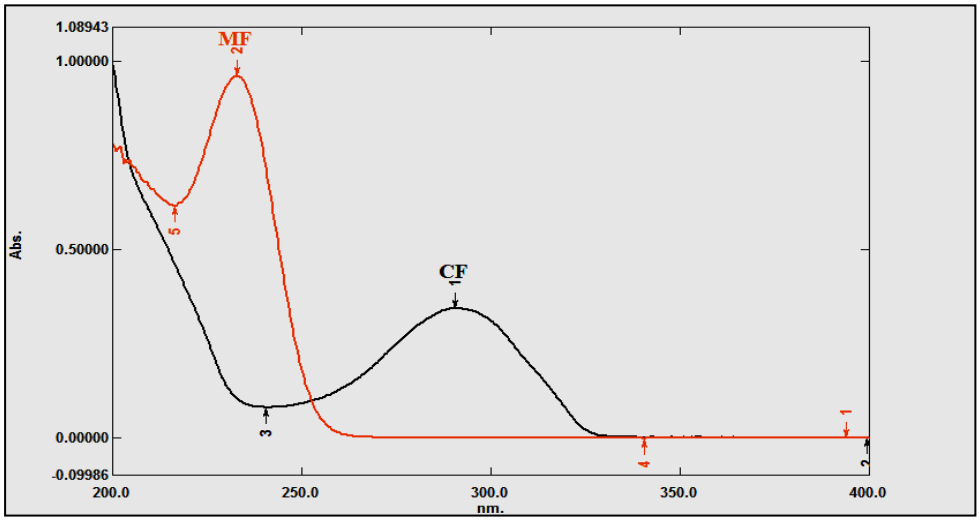

Figure 1: Overlay of zero order spectra of CF(10µg/ml) and MF CF(10µg/ml)

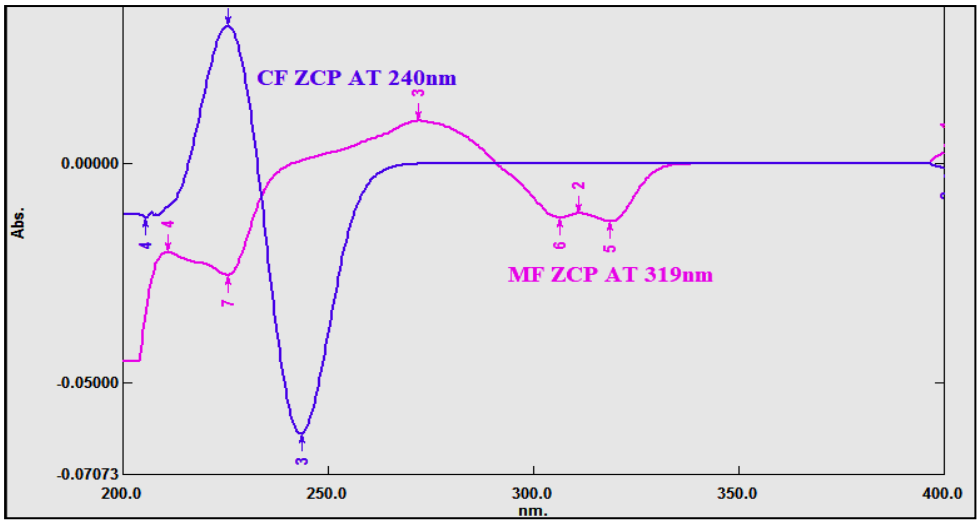

Figure 2: Overlay of First order derivative CF(10µg/ml) and MF(10µg/ml)

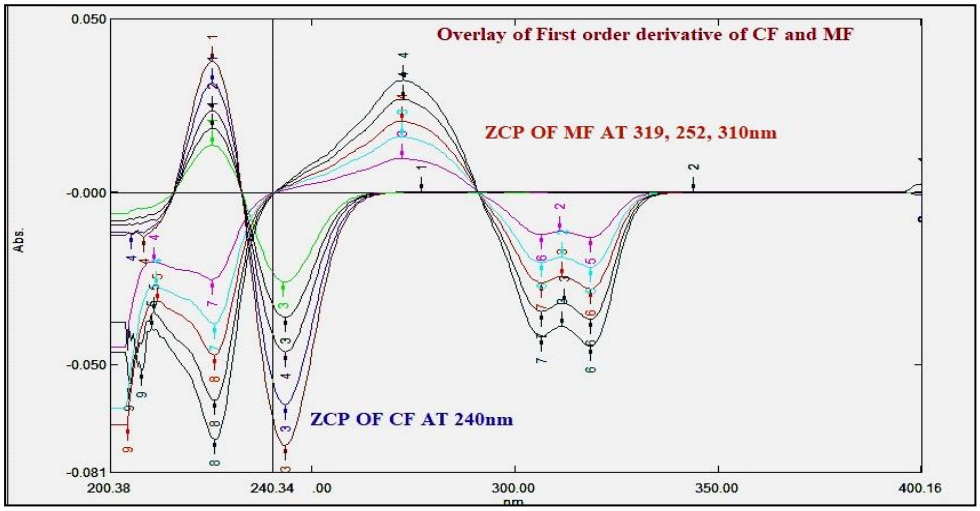

Figure 3: Overlay spectra of First order derivative of CF and MF with ZCP of both the Drugs.

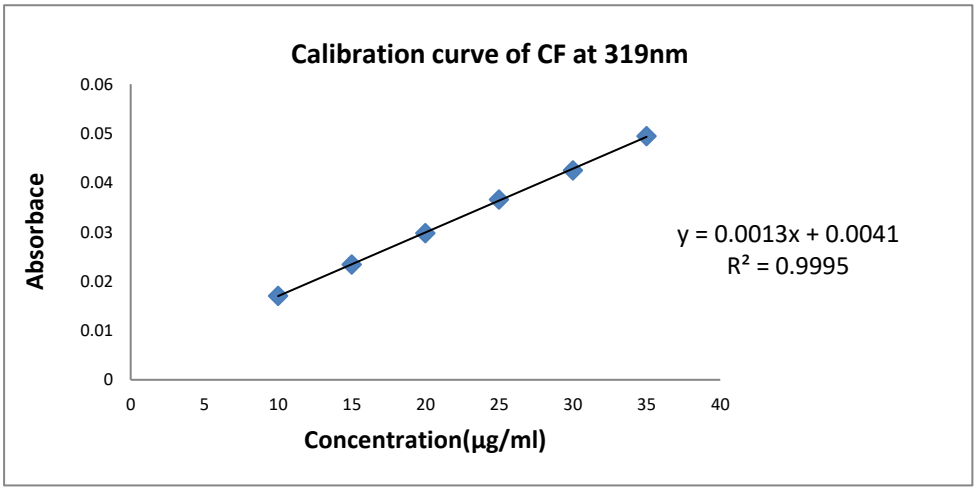

Figure 4: Calibration Curve of CF at 319nm

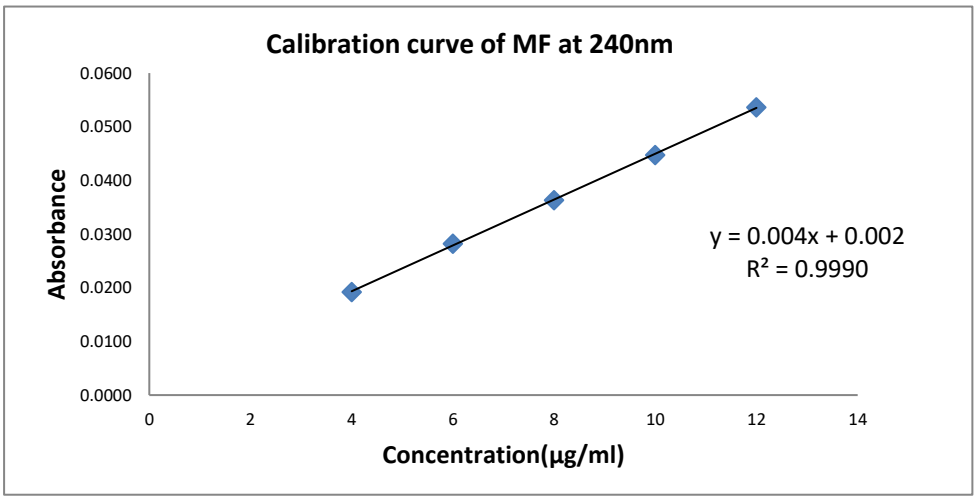

Figure 5: Calibration Curve of MF at 240nm

TABLE-1: LINEARITY DATA OF MF AT (240NM) (*n=5)

Sr No.	Concentration of MF(µg/ml)	*Mean absorbance± SD	%RSD
1	4	0.0192± 0.00010	0.54
2	6	0.0282± 0.00011	0.39
3	8	0.0365±0.00012	0.35
4	10	0.0447±0.00012	0.28
5	12	0.0536±0.00011	0.22

TABLE-2: LINEARITY DATA OF CF (319NM) (*n=5)

Sr No.	Concentration of CF(µg/ml)	*Mean absorbance± SD	%RSD
1	10	0.0172±0.00011	0.67
2	15	0.0234±0.00015	0.64
3	20	0.0295±0.00016	0.56
4	25	0.0364±0.00013	0.30
5	30	0.0425±0.00010	0.24

TABLE-3 REGRASSION DATA OF CF AND MF

Parameter	CF	MF
Linearity range	10-30µg/ml	4-12µg/ml
Regression equation	y = 0.013x + 0.0034	y = 0.0043x + 0.0023
Correlation co-efficient	0.9995	0.9997
Standard deviation of intercept	0.0015	0.002
Standard deviation of slope	0.00019	0.00013

TABLE-4 INTRADAY DATA OF CF (* n=3)

Concentration (µg/ml)	*Mean absorbance± SD	%RSD
15	0.0234±0.00016	0.75
20	0.0294±0.00019	0.71
25	0.0365±0.00020	0.57

TABLE-5 INTRADAY DATA OF MF (* n=3)

Concentration (µg/ml)	*Mean absorbance± SD	%RSD
6	0.0282±0.00011	0.40
8	0.3645±0.00013	0.36
10	0.0441±0.00026	0.59

TABLE-6 INTER DAY DATA OF CF (* n=3)

Concentration (µg/ml)	*Mean absorbance± SD	%RSD
15	0.0234±0.00016	0.69
20	0.0294±0.00019	0.65
25	0.0364±0.0019	0.52

TABLE-7 INTER DAY DATA OF MF (* n=3)

Concentration(µg/ml)	*Mean absorbance± SD	%RSD
6	0.0284±0.00018	0.63
8	0.0364±0.00016	0.45
10	0.0447±0.00026	0.61

TABLE-8 REPEATABILITY

Concentration	CF 10 (µg/ml)	MF 14 (µg/ml)
Absorbance	0.01631	0.06285
	0.01635	0.06267
	0.01621	0.06254
	0.01641	0.06285
	0.01651	0.06271
	0.01639	0.06273
Mean	0.01636	0.06272
± SD	0.0001	0.0001
%RSD	0.59	0.18

TABLE- 9: RECOVERY DATA OF CF (* n=3)

Concentration of Sample Taken (µg/ml)	Concentration of Pure API spiked) (µg/ml)	Total Concentration (µg/ml)	*Mean Total Concentration Found (µg/ml)	%Recovery Mean
10	8	18	18.63	100.36
10	10	20	19.88	99.12
10	12	22	23.40	101.60
Average				100.15

TABLE-10: RECOVERY DATA OF MF (* n=3)

Concentration of Sample Taken (µg/ml)	Concentration of Pure API spiked (µg/ml)	Total Concentration (µg/ml)	*Mean Total Concentration Found (µg/ml)	%Recovery Mean
14	11.2	25.2	25.5	100.10
14	14	28	28.38	100.46
14	16.8	30.8	29.79	99.30
Average				100.28

TABLE-11: DATA OF LOD AND LOQ

	CANAGLIFLOZIN	METFORMIN
LOD	0.3325	0.10431
LOQ	1.0076	0.3161

TABLE 12: ASSAY RESULT OF SYNTHETIC MIXTURE

Parameters	CF	MF
Actual Concentration (µg/ml)	10	14
Concentration Obtained (µg/ml)	9.25	14.31
%Purity	99.87	100.60
%RSD	0.739	0.394

CONCLUSION

In this proposed method the linearity was observed in the concentration range of 10-30 µg/ml and 2-12 µg/ml with co-efficient of correlation, r^2 = 0.9994 and r^2 = 0.9997 for CF and MF at 319 nm and at 240 nm, respectively. The method was validated as per International Conference on Harmonization (ICH) guidelines.

The result of the analysis of combined mixture by the proposed method was found to be highly reproducible and reliable. The additive present in the combined mixture of the assayed samples did not interfere with determination of CF and MF.

So, the developed first derivative UV spectroscopy method is simple, cost effective, precise, accurate and can be used for simultaneous determination of CF and MF in pharmaceutical dosage forms in routine quality control laboratory.

ACKNOWLEDGMENT

The author is thankful for the Department of Pharmaceutical Science, Vallabh Vidyanagar for providing all necessary facilities to carry out this work. And also thankful to Zydus Pharma, for gift an API of Canagliflozin and Metformin.

REFERENCES

1. Rang H.P, Dale M.M, Ritter J.M, Pharmacology, Churchill Livingstone Publication, New York, 6th edition, 2007, pp. 234-35,389-90.
2. Parker K.L., Brunton LL., Goodman & Gilman's the pharmacological basis of therapeutics; 10th Edn; Mac Graw-Hill Companies, Inc. USA, 2001, pp. 555-559.
3. Harvey R.A., Champe PC., Lippincott's illustrated reviews: Pharmacology; 4th Edn; 2009, pp 216-228.
4. Anjeneyulu Y., Chandrasekhar K., and Manikam V. A textbook of Analytical Chemistry; 2nd Edn; Parma Book Syndicate, 2005, pp 3.
5. Willard H., Meritt L., Dean J., and Settle F. Instrumental methods of Analysis; 6th Edn; CBS Publishers and Distributors, New Delhi, pp 19-90.
6. Sharma.B.K, Instrumental method of chemical analysis, Goel Publication House, Meerut, 24th Edition, 2005, pp:46-68, 54-83
7. Bechett, A.H., Stenlake, J.B, Practical Pharmaceutical Chemistry, Part 1, CBS Publishers and Distributors, New Dehli, 4th Edition, 2007, pp: 1-4.
8. Jeffery G.H., Bassett J., Vogels textbook of Quantitative Chemical Analysis, 5th edition, 1991, pp:217-235.
9. Drug Bank: METFORMIN, https://www.drugbank.ca/drugs/DB08907 metformin (24/01/2019).
10. Drug Bank: Canagliflozin, https://www.drugbank.ca/drugs/DB08907 canagliflozin (20/01/2019).
11. Canagliflozin:http://www.ebi.ac.uk/chebi/searchId.do? chebiId=CHEBI:73274 (15/09/2018).
12. The European agency for the evaluation of medical products, ICH Topic Q2B Note for guidance on validation of analytical procedures: Methodology. GPMP/ICH/281/95, 1996
13. USP 27, NF 22. The United States Pharmacopeia and the National Formulary, Rockville, MD: The United States Pharmacopoeial Convention: 20852, 2004.
14. Analytical Method Development and Validation of Canagliflozin: Review Shuchithra S JSS College of pharmacy, Mysore, India US FDA. Technical Review Guide: Validation of Chromatographic Methods, 1993.
15. Dr. Swamy Gandla., Kumara A, *Validated RP-HPLC Method for Simultanious Determination of Metformin*

and Canagliflozin in Pharmaceutical Formulation, Asia journal of Pharmaceutical Analysis, 2018; 8(2): 73-77.
16. Vijaya Laxami Marella, *Development and Validation of a Stability-Indicating Reverse Phase HPLC-PDA Method for Determination of Canagliflozin in Bulk and Pharmaceutical Dosage Form,* International Journal of Advances in Pharmaceutical Analysis, 2017; 07(03): 24-27.
17. Patel B.A., Parmar S.J. et.al., *Development and Validation of Chromatographic Determination of Carvedilol Phosphate in Bulk and Pharmaceutical Dosage Form Using Fluorescence detector*, International Journal of Pharmaceutical Sciences and Research, 2012 3(11): 4404-4407.
18. ICH, Q2B (2005) Validation of Analytical Procedure: Methodology, International Conference on Harmonization, IFPMA, Geneva, Switzerland.