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STABILITY INDICATING RP-HPLC METHOD FOR ESTIMATION OF ANTIDIABETIC DRUG IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT:

To develop precise, accurate, reproducible and validate stability indicating rp-hplc method for determination of canagliflozin in bulk and pharmaceutical dosage form as per ICH Q2R1 guidelines. The adequate separation was carried using mixture of acetonitrile :water :glacial acetic acid (58:42:0.2% v/v) as a mobile phase, hypersil BDS c18 column as stationary phase with the flow rate of 1.0 ml/min and the effluent was monitored at 290nm using uv detector. A stability- indicating hplc method has been developed for analysis of the drug in the presence of the degradation products and is validated with different parameters such as linearity, precision, accuracy, robustness etc. Beer's law was obeyed in the concentration range of 25-75 µg/ml. A retention time of canagliflozin api and tablet were 4.08 min and 4.10 min respectively. A mean recovery of canagliflozin in tablet formulation was observed in the range of 100.17-101.25%. Degradation of canagliflozin was found to occur in acid, alkaline, hydrogen peroxide and but not in thermal and photolytic condition. The proposed method was found to be specific, accurate, precise and robust can be used for estimation of canagliflozin in api and pharmaceutical formulation.

KEYWORDS: rp-hplc, canagliflozin, api, pharmaceutical formulation, ICH.

INTRODUCTION:

Stability testing and stress degradation studies play a very crucial role in drug development. Stability is fundamental to all product characteristics, and the term "Stability indicating assay" has been used to describe a procedure which affords specific determination of drug substance in the presence of its degradation products. The prime goal of studying the stability of a drug is to determine the shelf life of the drug. The various conditions specified for stress degradation studies include acidic, alkaline, oxidation, photolytic and thermal.[1-3]

Canagliflozin belongs to a new class of oral antidiabetic drugs, called Sodium Glucose Co- Transporter 2 (SGLT2) inhibitors. These sodium glucose co-transporters are responsible for glucose reabsorption in the kidney. Hence inhibiting the SGLT2 have been proposed as a new strategy in the treatment of diabetes.[1,2,3] suppressing the SGLT2, Canagliflozin plasma glucose concentration intern by elevating the renal glucose excretion by the kidney,used to improve glycemic control in patients with type 2 diabetes.

It is chemically known as (1s)-1,5-Anhydro-1-C-[3-[[5-(4-fluorophenyl)-2-thienyl] methyl]-4-methylphenyl]-D-glucitol hemihydrate (fig.1).It has a molecular formula C24H25FO5S with molecular weight 444.5g/mol. Canagliflozin is a light yellow powder which is soluble in ethanol, methanol and acetonitrile,partially soluble in water.[1-7]

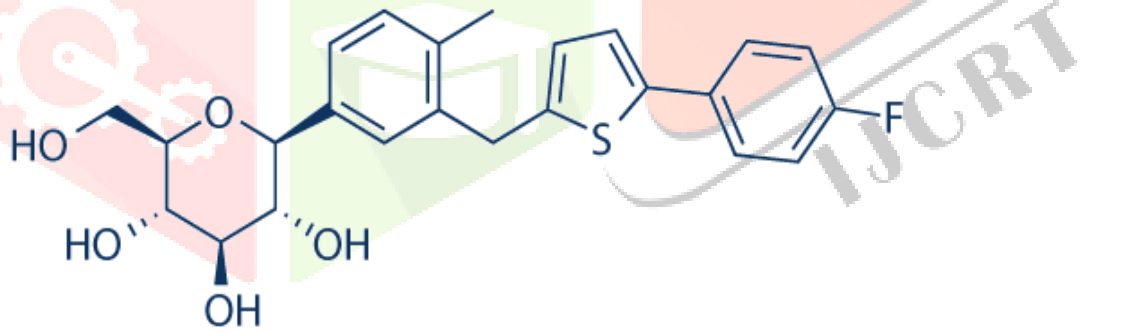

Figure No: 1 Chemical structure of Canagliflozin

AIM AND OBJECTIVES:

A thorough literature survey has revealed that there are few analytical methods reported for quantitative estimation of Canagliflozin and its stability study. In the present work a successful attempt has been made to develop accurate, precise, economical and rapid analytical method for estimation and its stability study is done by HPLC.

LITERATURE REVIEW:

1. **P. Sahare et al., 2021[8]** have reported a Simple, accurate, rapid & precise has been developed and validated for estimation of Canagliflozin hemihydrate in pharmaceutical tablet Formulation by Uv-spectrophotometer and RP-HPLC method.
2. **A. Murugesan et al., 2021[9]** have reported reversed phase high-performance liquid chromatogrophy method for the determination of oral antidiabetic drug Canagliflozin in bulk and pharmaceutical dosage form. The Chromatography was reported on ZORBAX C18 Column with acetonitrile, water (53:47% v/v) mobile phase.
3. **S. Singh et al., 2021[10]** have reported precise RP-HPLC method for estimation of Canagliflozin by using INERTSIL Column C18 with mobile phase ratio 70:30 of water and acetonitrile as the detection Wavelength was 264nm.
4. **H. Sadasivuni et al., 2020[11]** have reported new selective and sensitive high performance liquid chromatography method with UV detection at 260nm for the quantification of Canagliflozin hemihydrate in pharmaceutical dosage form.
5. **G. Reddy et al., 2020[12]** have reported simple and rapid reverse phase high performance liquid chromatography method for estimation of Canagliflozin in bulk and pharmaceutical formulation.
6. **D. Singh et al., 2019[13]** have reported spectrophotometric and high performance liquid chromatography method for estimating Canagliflozin in bulk and tablet dosage form having lambada max 280 nm.
7. **S. Patil et al., 2019[14]** have reported determination of Canagliflozin with grace C18 column consisting mobile phase methanol:water (90:10% v/v) by using RP-HPLC method.
8. **G. Mounika et al., 2019[15]** have reported accurate,precise, specific high performance liquid chromatography method for quantification of Canagliflozin in bulk and dosage form with uv detector at 291 nm.
9. **M.D. Game et al., 2018[16]** have reported precise, accurate, reproducible and validate stability indicating HPLC method for the determination of antidiabetic drug in API and pharmaceutical formulation as per ICH Q2R1 guidelines.
10. **V.L. Marella et al., 2017[17]** have reported simple, specific and accurate reverse phased high performance liquid chromatography method for determination of Canagliflozin in bulk and pharmaceutical dosage form where the eluents were monitored at 230nm using mobile phase 0.02% formic acid:acetonitrile (40:60).

MATERIALS AND METHODS

Materials:

Standard Canagliflozin (99.15% purity) was obtained as gift sample from Morepen Laboratories, Baddi, India. The available tablet formulation of Canagliflozin [Invokana© – 100 mg] was purchased from local medical shop. Hydrochloric acid,hydrogen peroxide, sodium hydroxide and other LR (Laboratory reagent)

grade and methanol, acetonitrile, water,GAA were HPLC grade and were purchased from Merck chemicals, Mumbai.

Preparation of standard stock solution and selection of detecting wavelength:

1] Standard stock solution:

An accurately weighed quantity of about 10 mg of Canagliflozin was taken in 10.0 ml volumetric flask, dissolved in HPLC grade methanol and volume was made up to mark with same solvent (conc. 1 mg/ml). The aliquot portion of the standard stock solution was diluted appropriately with the same solvent to obtain the concentration of 10µg/ml. This solution was scanned in 1 cm cell using double beam UV- Visible Spectrophotometer over the range of 400-200 nm and the UV absorbance spectrum was recorded. From the spectrum, the detecting wavelength selected for estimation of the drug was 290 nm as shown in Figure No: 2

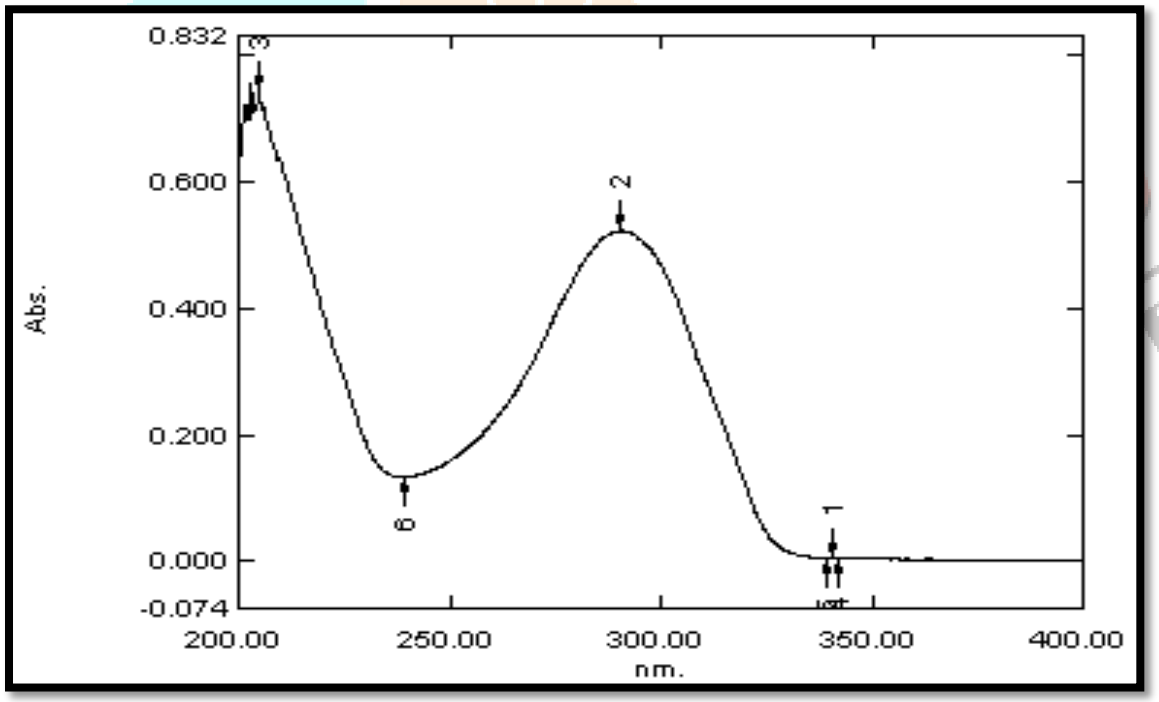

Figure No: 2 UV absorption spectrum of Canagliflozin

2] Selection of mobile phase

The pure drug of Canagliflozin was injected into the HPLC system and run in different solvent systems. Each mobile phase was allowed to equilibrate with stationary phase until steady baseline was obtained. Different mobile phases like methanol and water, acetonitrile, and water in various proportions were tried.

Different individual solvent, as well as combinations of solvents, were tried to get a stable peak. Each mobile phase was filtered through 0.45µm nylon membrane filter and sonicated on the ultrasonic bath. After several trials acetonitrile: water: glacial acetic acid (58:42:0.2% v/v) was found to be the most satisfactory since it gave sharp, peak with symmetry within limits and significant reproducible retention time.

Table No: 1 Selection of mobile phase

Sr. No.	Mobile phase strength (v/v)	Rt of Canagliflozin [min]	Conclusion
1	Methanol:acetonitrile:water (80:10:10)	3.47	Peak not satisfactory
2	Acetonitrile:Water (60:40)	2.97	Peak not satisfactory
3	Acetonitrile:0.01M ammonium formate (60:40)	4.92	Peak not satisfactory
4	Methanol: water:glacial acetic acid (80:20:0.1)	7.30	Peak not satisfactory
5	Acetonitrile:water:glacial acetic acid (58:42:0.2)	4.08	Peak satisfactory

3] Preparation of mobile phase and construction of calibration curves

Mobile phase was prepared by homologous mixture of 580ml of acetonitrile ,420ml of water and 2ml of glacial acetic acid,shake well , sonicated for about 5minute ,filter the mobile phase through 0.45 µm membrane filter paper. Accurately weighed quantity of 50 mg Canagliflozin dissolved in diluent and volume was made up to 100ml mark by same to obtain 500 µg/ml. stock solution. The appropriate aliquot of Canagliflozin stock solution was transferred in series of 20.0 mL volumetric flask and volume was made up to the mark with mobile phase to obtain the various concentration of 25- 75 µg/ mL.These solutions were injected using a 20µL fixed loop system separately in 5 times and chromatographed under conditions described above and peak areas were recorded. The graph was plotted as concentration of drug Vs peak area and depicted in **Figure No: 3**

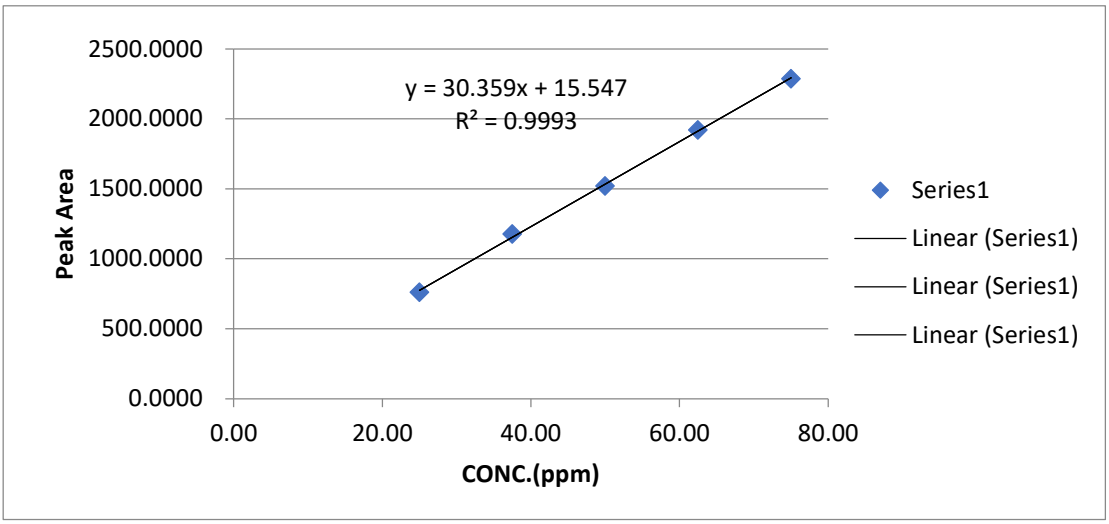

Figure No.3 : Calibration curve for Canagliflozin

Table No: 2 Final chromatographic conditions

Chromatographic mode	**Chromatographic conditions**
Standard solution	50µg/mL of Canagliflozin
Stationary phase	Hypersil C18 (5µm, 250mm X 4.6mm. ID.)
Mobile phase	Acetonitrile:water:Glacial acetic acid (58:42:0.2)
Detection wavelength	290 nm for Canagliflozin
Flow rate	1.0 mL/min
temperature	Ambient
Elution mode	isocratic
Software	Autochrome 3000
Injection volume	20µL

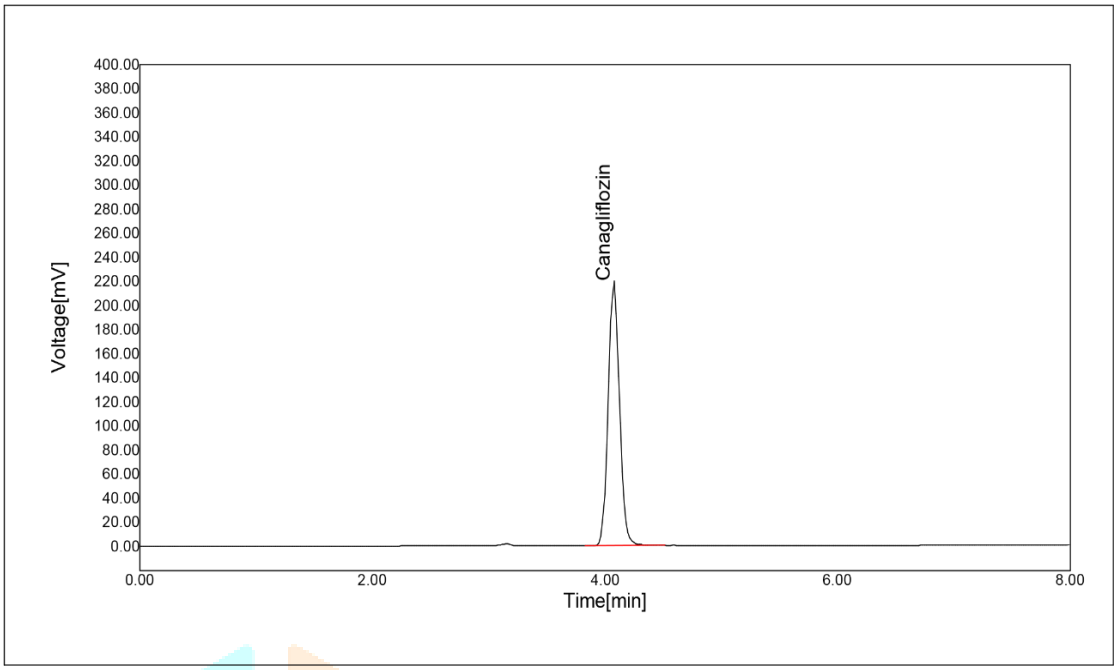

Fig no 4: Method development of Canagliflozin by RP-HPLC

SYSTEM SUITABILITY :

System suitability test is a pharmacopoeial requirement and is used to verify, whether the resolution and reproducibility of the chromatographic system is adequate for analysis to be done. The tests were performed by collecting data from five replicate injections of standard drug solution. Previously filtered mobile phase was allowed to equilibrate with stationary phase until baseline was achieved. 20µL standard drug solution was injected separately and their system suitability parameters were recorded.[12,14]

SPECIFICITY:

The specificity of method was performed by comparing the chromatogram of blank,standard and sample. The retention time found are stated below.

Table no 3 : Specificity

Sr no.	Solution	Retention time
1.	Blank	0
2.	Canagliflozin Standard	4.05
3.	Canagliflozin Sample	4.03

PRECISION:

The precision study was performed using an interday and intraday precision method. The proposed method was determined by analyzing the canagliflozin solution at different time intervals and on different days.

The precision of the method was determined by intraday studies. Prepare 50µg/mL solutions from a standard solution and inject five times in a day on to analytical column. The percentage relative standard deviation (%RSD) was calculated and lower % RSD indicates that there are less variation and there is high precision in the valves. %RSD= (S.DX100)/mean

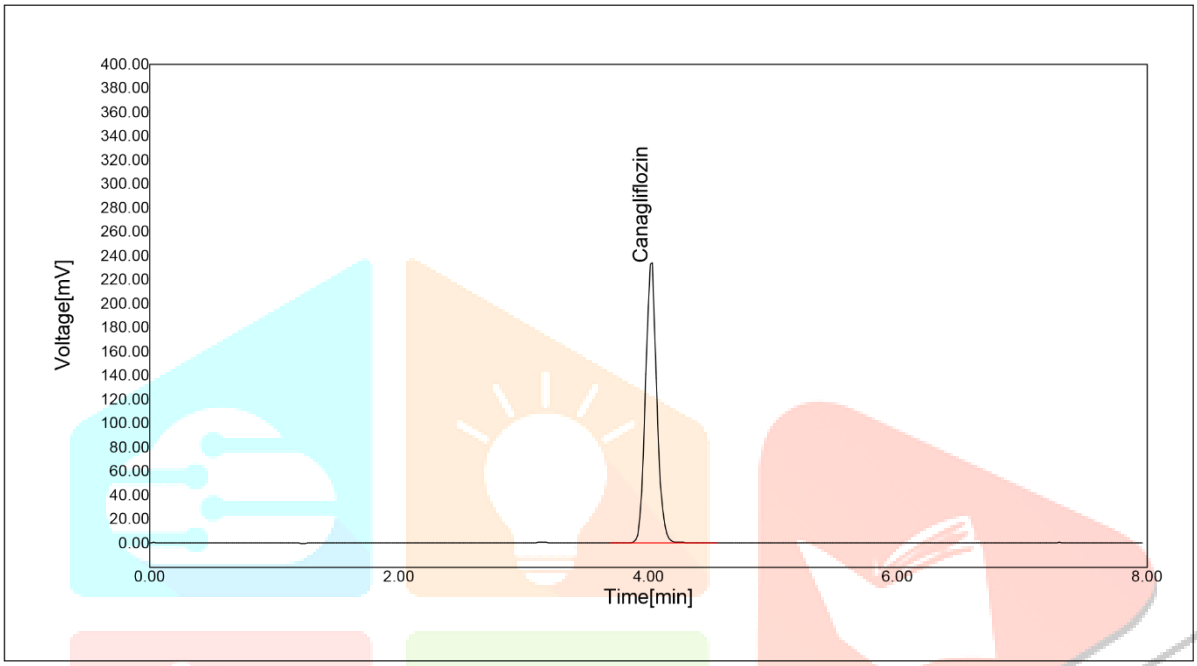

Figure 5: precision studies of canagliflozin

LINEARITY:

Preparation of stock solution

Accurately weighed quantity of 50 mg Canagliflozin dissolved in diluent and volume was made up to 100ml mark by same to obtain 500 µg/ml. stock solution ,level 1-5 perform to obtain 25-75µg/ml of Canagliflozin.

Table no. 4 : Linearity data of Canagliflozin

Drug name: Canagliflozin		
Sr no.	**Concentration (µg/ml)**	**Area**
1	25.00	761.6894
2	37.50	1176.8043
3	50.00	1520.7268
4	62.50	1921.5770
5	75.00	2286.7588
Regression Equation		y = 30.359x + 15.547
Correlation coefficient (R^2)		0.9993
Steyx		17.8309
Slope		30.359

LOD(µg/ml)	1.94
LOQ(µg/ml)	5.87

ROBUSTNESS:

The Robustness of a method is its ability to remain unaffected by small deliberate changes in parameters. To evaluate the robustness of the proposed method. Small but deliberate variations in the optimized method parameters were done. The effect of change in mobile phase composition and flow rate, wavelength on retention time and tailing factor of drug peak was studied.The mobile phase composition was changed in (±3%) proportion and the flow rate was varied by (±0.1ml/min) of optimized chromatographic condition. Robustness parameters were also found satisfactory; hence the analytical method be concluded.

Table no 5 : Robustness data of Canagliflozin

Variables	**Drug name: Canagliflozin**			
	t_R (min)	**Area**	**T_f**	**N**
Flow rate (+0.1 ml/min)	3.63	1363.8793	1.13	10711
Flow rate (-0.1 ml/min)	4.52	1715.0775	1.12	10032
ACN-H2O-GAA (55:45:0.2%)	4.30	1589.5349	1.00	21339
ACN-H2O-GAA (61:39: 0.2%)	3.82	1393.3657	1.05	22900

ACCURACY:

Percentage drug accuracy of three different concentrations; 80%, 100% and 120% (injected thrice) to estimate the Canagliflozin from nano formulation and results obtained have been reported in Table. The accuracy of the method was determined by calculating the recovery of Canagliflozin by the spiked method.For the both the drug, the values of standard deviation were satisfactory and the %recovery were found close to 100%. The %RSD value was found less than 2% which indicates the accuracy of the method.

Table no. 6 : Drug recovery data of Canagliflozin

		MEAN % reovery	**SD**	**%RSD (NMT 2)**
Accuracy at 80 %		100.17	0.7499	**0.75**
Accuracy at 100 %		99.57	0.8412	**0.84**
Accuracy at 120 %		101.25	0.8939	**0.88**

The %RSD was found less than 2% and in range of 0.75% to 0.88%

OBSERVATION TABLE:

Table no.7 : Overall results of system suitability and validation parameters.

System suitability parameters	Canagliflozin	Acceptable Values
Theoretical plates (*N*)	10239	≥ 2000
Tailing factor (T_f)	1.02	< 2
Retention time (t_R)	4.08	> k’
Wavelength of Detection (nm)	290 nm	> 200 nm
Repeatability (% RSD)	1.54	< 2%
Intra-Day Precision (% RSD)	0.32	< 2%
Inter-Day Precision (% RSD)	0.29	< 2%
Accuracy (%)	99.57-101.25	98%-102%
Linearity range	25–75 µg/ml	NA
Regression equation	y =30.359x +15.547	NA
Correlation Coefficient (r^2)	0.9993	NA
LOQ (µg/ml)	1.94 µg/ml	NA
LOD (µg/ml)	5.87 µg/ml	NA

The above table shows the result of system suitability and validation parameters. All result were found within the acceptance criteria

APPLICATION OF PROPOSED METHOD TO MARKETED FORMULATION (INVOKANA):

Preparation of standard drug solution

Accurately weighed quantity of 50mg Canagliflozin dissolved in diluent and volume was made up to 100ml mark by same to obtain 500µg/ml. stock solution. Pipette out 2ml from standard stock solution and diluted it with 20ml diluent to obtain 50µg/ml of Canagliflozin.

Weigh all 10 tablets for getting their average weight. single tablet weight is 200mg i.e. it contains 100 mg API & 100 mg excipients.

Average wt. of tablet = 200.2 mg

Triturate all the tablets and select weight of tablet powder equivalent to preparation of standard drug solution so we select 100 mg of tablet powder, dissolved in diluent and volume was made uo to 100ml mark.pipette out 2ml from above solution and another make up volume in 20ml volumetric flask with diluent.and inject it in HPLC.

Stability studies of Canagliflozin

Acid degradation:

Take 50mg of canagliflozin API ,transfer in 100ml vol. flask ,add 25ml diluent + 50ml of 1N HCL Solution and refluxed on a heating mantle at 40^0C for 6 hrs. After cooling add drop by drop 1 N NAOH solution for frequently shaking to check to neutralize the above solution and finally make up the volume upto 100ml.pipette out 2ml from above solution and transfer into 20ml vol. flask and make up volume upto mark with diluent and inject it.[30-34]

Base degradation:

Take 50mg of canagliflozin API ,transfer in 100ml vol. flask ,add 50ml diluent + 25ml of 1N NAOH Solution and refluxed on a heating mantle at 40^0C for 6 hrs. After cooling add drop by drop 1 N HCL solution for frequently shaking to check to neutralize the above solution and finally make up the volume upto 100ml.pipette out 2ml from above solution and transfer into 20ml vol. flask and make up volume upto mark with diluent and inject it

Peroxide degradation:

Take 50mg of canagliflozin API ,transfer in 100ml vol. flask ,add 25ml diluent + 50ml of 3% H2O2 Solution and refluxed on a heating mantle at 50^0C for 6 hrs. After cooling finally make up the volume upto 100ml.pipette out 2ml from above solution and transfer into 20ml vol. flask and make up volume upto mark with diluent and inject it.

Thermal degradation:

The pure drug was heated at the oven which is maintained at 60^0C for 6 hrs and cool at room temperature. Make up the volume upto 100ml.pipette out 2ml from above solution and transfer into 20ml vol. flask and make up volume upto mark with diluent and inject it.

Photolytic degradation:

The pure drug was placed in petri plate and exposed to UV light for 254 nm in a UV chamber for 12 hrs. Later weigh accurately 50mg of canagliflozin , dissolved in 100 ml vol.flask with diluent (mobile phase). pipette out 2ml from above solution and transfer into 20ml vol. flask and make up volume upto mark with diluent and inject it.

Acceptance criteria to all condition

Result shows in terms of % degradation.

Table no. 8 : Overall Result of stability study

Stress condition	% Area Canagliflozin observed after degradation	% of degradation
Acidic	93.84	6.16
Basic	94.76	5.24
Peroxide	95.76	4.24
Thermal	97.78	2.22
Photolytic	99.78	No degradation

RESULTS AND DISCUSSION:

A literature survey of Canagliflozin revealed that some analytical method has been reported for its determination in bulk drugs and its pharmaceutical dosage form. Also, there is no more stability indicating assay method reported for estimation of Canagliflozin. In present investigation a new RP-HPLC method was to develop and validated for Canagliflozin as per ICH guideline and used as a stability-indicating method. For the Canagliflozin, isocratic method was developed by taking trials for the ratio of methanol, ACN and water, ACN and Water and then the ratio of ACN, water and GAA. From various mobile phases trial, ACN:Water:GAA (58:42:0.2% v/v) was selected, since it gave sharp, well resolved peaks with

symmetry within limits and significant reproducible retention time for Canagliflozin. The detection was carried out at 290 nm. The retention time obtained for Canagliflozin was 4.08 min with C18 stationary phase.[20]

The Developed Method was validated by various parameter like Linearity and Range, Specificity, Accuracy, Precision, Robustness. The system suitability parameter is obtaining within the limit for Canagliflozin that is % RSD while, Retention time 4.08min. The linearity range for CFZ was found to be 25-75µg/ml. The mean Regression equation was found to be y = 30.359x+15.547. Hence, the method is linear within the range. The LOD and LOQ value was observed as 1.94 and 5.87 (µg/ml) respectively.

Specificity of the method was evaluated by injecting the blank, standard and sample solution prepared as per proposed method and injected into the HPLC system to check Interference if any at the retention time of Canagliflozin. Chromatogram of blank solution showed no peaks at the retention time of Canagliflozin standard and test peaks. Hence, the method can be termed as specific.

The mean % recovery was found in the range of 100.17-101.25 and that of the %assay of CFZ was 98.82. It gives the response within the limit.

Average canagliflozin observed (in mg) in marketed tablet – (INVOKANA 100mg) was 97.09 against lable claim.

The precision study was performed using an interday and intraday precision method.The Proposed method was determined by analyzing the Canagliflozin solution at a different time interval and on different days. The %RSD for both interday and intraday precision study was found to be 0.29% and 0.32% respectively. It is under the limit that is not more than 2%. Hence, the method is found to be precise.

The robustness method was determined by varying the method parameter, such as a change in flow and a change in mobile phase composition. The robustness was calculated as the % assay and RSD. The result of the assay of two test preparation was not affected by varying the condition.

The stability study of Canagliflozin indicate that the drug significantly degrade under acidic,basic and peroxide conditions, but not in thermal and photolytic condition.

CONCLUSION:

A novel simple, precise, accurate, RP-HPLC method has been developed for the estimation of Canagliflozin in the pure and marketed formulation by using an optimized mobile phase containing ACN:Water:GAA (58:42:0.2% v/v) and detection wavelength at 290nm. In the present assay, the mobile phase preparation was easy and the solvent used was low cost making the method more economical. The validated method is Specific, Linear, Precise, Acurate, Robust for Assay of Canagliflozin. The % RSD for all parameters was found to be less than two, which indicated the validity of the method and assay result obtained by this method is fare agreement with ICH norms, therefore, it is concluded that this developed RP-HPLC method can be conveniently used in future.[25]

Degradation study indicates the stability of the drug. All the peak of the degradation products formed during stress degradation studies were well separated from the analyte peak. Hence, this method regarded as more specific, stability-indicating, and can be successfully used for routine analysis for the determination of Canagliflozin in the tablet dosage form.[44-47]

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REFERENCES:

1) "Importance of medicines in daily life" by Jennifer Alinio - ezinearticles.com, March 07, (2017).
2) Medicines in the Society by Malcom S. M. Watts, Calif Med, 102(2), 134, (1965).
3) Nair S, Wilding JP. Sodium-glucose co-transporter 2 inhibitors as a new treatment for diabetes mellitus. J Clin Endocrinol Metab 2010;95:34-42.
4) Misra M. SGLT2 inhibitors: a promising new therapeutic option for treatment of type 2 diabetes mellitus. J Pharm Pharmacol 2013;65:317-27.
5) Kim Y, Babu AR. Clinical potential of sodium-glucose cotransporter 2 inhibitors in the management of type 2 diabetes. Diabetes Metabolism Syndrome Obesity 2012;5:313-27.
6) Introduction to pharmaceutical analysis, http://www.pharmatutor.org/pharma-analysis
7) David Harvey., A text book of Modern Analytical Chemistry, McGraw-Hill Higher Education (ISBN: 0-07-237547-7).
8) Praful Sahare, Amit Nayak, Alok Pal Jain, Harikishor Barange Asian Journal of Pharmaceutical Analysis 11 (1), 29-37, 2021
9) ARULSELVAN MURUGESAN, MUKTHINUTHALAPATI MATHRUSRI ANNAPURNA Int J App Pharm 13 (5), 358-363, 2021
10) Swapna Singh, Prem Kumar Bichala, Abhishek Agrawal A & V Publications 13 (3), 209-212, 2021
11) Hemambika Sadasivuni, Narayana Rao Gundoju Mukt Shabd J 9 (VI), 1690-8, 2020
12) Gouru Santhosh Reddy, Sonam Patel, Krishna Veni Nagappan, Khagga Bhavyasri, Chejati Mounika, M Sumakanth, Gullapalli Kowmudi Journal of Young Pharmacists 12 (4), 321, 2020
13) D Singh, NEENA Bedi, AK Tiwary Indian J Pharm Sci 81 (1), 39-44, 2019
14) Sushil D Patil, Shaikh Abdul Muqeet, Sanjay J Kshirsagar Asian Journal of Research in Chemistry 12 (1), 11-15, 2019
15) Gudipally Mounika, K Bhavya Sri, R Swethasri, M Sumakanth SAMRIDDHI: A Journal of Physical Sciences, Engineering and Technology 11 (02), 85-92, 2019

16) M.D.Game, Naglaxmi Bopudi et al. Ijppr.Human, 2018; Vol. 12 (3): 123-144.

17) Vijaya Lakshmi Marella, A Syed, L Prasanna, BN Nalluri Int J Adv Pharm Anal 7 (3), 24-27, 2017

18) http://www.adorablescience.com/why-is-medicine-important/

19) Douglas A. Skoog., James Holler, F., and Timothy A Nieman., Principals of Instrumental analysis, Thomson Asia Pte. Ltd., Singapore, 5 th Ed., 342 – 344, (2004).

20) Introduction to Pharmaceutical Analysis, www.safaribooksonline.com

21) Kaila Harshad, O., "Analytical Study of Pharmaceutical Substances, Method Development and Validation Study, Few Case Studies", Ph.D. thesis, Saurashtra University, (2011).

22) Siddiqui, Masoom Raza; AlOthman, Zeid A.; Rahman, Nafisur. "Analytical techniques in pharmaceutical analysis: A review". Arabian Journal of Chemistry. 10: S1409–S1421. 2013.04.016.

23) Jennifer Knaack. "The Importance of Analytical Chemistry in Quantitative Pharmaceutical Sciences". Pharmaceuticanalytica Acta, 2012.

24) Kasture. A.V., Wadodkar, S.G., Mahadik, K. R., "Textbook of pharmaceutical analysis Il". Published by NiraliPrakashan, 2005. 13th Edn:

25) AlallFrakHussain. Uv-Visible Spectrometry. Researchgate. 2019.

26) Fundamental of Analytical chemistry 8th ed. By DouglasA. Skoog, Donald M. West, F. James Holler, Stanley R. Crouch. 2003.

27) Davidson A.G. "Ultra - Visible absorption Spectroscopy. "Practical Pharmaceutical Chemistry," 4 th edition, part-II. CBS Publisher and Distributors, 1997,

28) Malviya R. Bansal V, Pal O.P. and Sharma P.K, "High Performance Liquid Chromatography: A Short Review", Journal of Global Pharma Technology,2010. Volume 2, Issue 5,

29) Skoog, Douglas A.; Holler, F. James; Crouch, Stanley R. (2007). Principles of Instrumental Analysis (6th ed.). Belmont, CA: Thomson Brooks/Cole.

30) Beckett A.H. and Stellate JB "Practical Pharmaceutical Chemistry". 4th edition, CBS Publication and Distributors, New Delhi. 1977.

31) Panchumarthy Ravisankar. Ch. Naga Navya, D. Pravallika, D. NavyaSri,"A Review on StepBy-Step Analytical Method Validation", Journal of Pharmacy, Volume 5. Issue 10, 2015.

32) Br. Jay, J. Kelvin, and B. Pierre, Understanding and Implementing Efficient Analytical Methods Development and Validation, 2003

33) R.M. Christopher, and W.R. Thomas, Quality Systems approach to Pharmaceutical cGMP Development and validation of Analytical Methods, (1st Ed., 2005)

34) International Conference on Harmonization, "Q2A: Text on Validation of Analytical Procedures," Federal Register 60 (40), 11260–11262 (1995).

35) International Conference on Harmonization, "Q2B: Validation of Analytical Procedures: Methodology; Availability," Federal Register62(96), 27463–27467 (1997).

36) Polumbryk M, Ivanov S, Polumbryk O. Antioxidants in food systems. Mechanism of action. Ukrainian Journal of Food Science. 2013;1(1)

37) https://www.intechopen.com/chapters/65225

38) https://www.medicalnewstoday.com/articles/301506#_noHeaderPrefixedContent

39) Shebis Y et al. Natural antioxidants: Function and sources. Food and Nutrition Sciences. 2013; 4:643-649

40) Vibha Gupta, V., Jain, A. D. K. J., N.S, G., &Guptan, K. Development and validation of HPLC method - a review. International Research Journal of Pharmaceutical and Applied Sciences, 2(4), (2012)

41) Pratap B, P., Akalanka Dey, G H, S. rao, P, J., & P, A. A Review - Importance of RP-HPLC in Analytical Method Development. International Journal of Novel Trends in Pharmaceutical Sciences, 3(1), (2013),

42) Khan hamid,'Analytical method development in pharmaceutical research: Step involved in HPLC method Development' Asian Journal of Pharmaceutical Research, Vol (7),2017

43) Santosh kumar Bhardwaj, k.Dwivediaand D. D. Agarwal, "A Review : HPLC Method Development and Validation" international journal of Analytical and Bioanalytical chemistry 2015, Volume 5.

44) G. Geetha Karanam NageGanika Raju, B. Vignesh Kumar and M. Gnana raja," Analytical method validation: An Updated Review" International Journal of Advance in Pharmacy, Biology and Chemistry, Vol. 1(1), 2012.

45) S. B.Kanthale.S. S. Thonteand B. B. Supekar, "Development of Stability Indicating Assay Method: A Review". Ijpsr, 2019, Vol. 10

46) N.Bhavana. Pr.Likhitha. Hanumanth and Pv. Suresh, "Design and Development of Stability Indicating Assay Methods as Per ICH Guidelines A Review". International Journal of Pharma and Chemical Research, Volume 3. Issue 2, 2017.

47) ICH guidelines, Q1A(R2): Stability Testing of New Drug Substances and Products (revision2), International Conference on Harmonization with Q1B: Photostability Testing of New Drug Substances and Products. US Department of Health and Human Services; Rockville, MD: 1997;62(95):27115–27122

48) Reynolds D., et al. "Available Guidance and Best Practices for Conducting Forced Degradation Studies." Pharmaceutical Technology, Feb. 1, 2002.