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SIMULTANEOUS ESTIMATION OF ANTI-DIABETIC DRUGS CANAGLIFLOZIN AND METFORMIN IN BULK AND DOSAGE FROM BY RP-HPLC

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ABSTRACT: The development and validation of an RP-HPLC technique for the simultaneous Estimation of metformin and canagliflozin in a combined dosage formulation is described in this paper. The mobile phase in this new method is (water 50: Methanol 50 at pH 3) and the column is Cosmosil C18 (250mm x 4.6ID, particle size: 5 micron). Canagliflozin was detected using UV detection at 254 nm, and the drug was eluted after an 8-minute retention time. The method was validated according to the International Conference on Harmonization (ICH) guidelines, with precision, accuracy, linearity, limit of detection, limit of quantitation, and robustness as the parameters. The proposed RP-HPLC approach was found to be useful, practical, and reliable in the routine analysis of metformin and canagliflozin in bulk and dose form.

KEYWORDS: RP-HPLC, Canagliflozin, Metformin, Anti-diabetic Drug, Linearity Accuracy, Precision, LOD, LOQ.

INTRODUCTION

Canagliflozin, {(2S,3R,4R,5S,6R)-2-(3- [5-(4-fluorophenyl) thiophen-2-yl] methyl-4-methyl phenyl) -2- (3-[5-(4-fluorophenyl) thiophen-2-yl] methyl-4-methyl phenyl) Invokana, also known as -6- (hydroxymethyl)oxane-3,4,5-triol, is a sodium-glucose co-transporter 2- (SGLT2) inhibitor used in the treatment of type 2 diabetes mellitus in combination with lifestyle changes such as diet and exercise. mIt was approved by the FDA for the first time in 2013 for diabetes management, and then again in 2018 for a second indication of decreasing the risk of cardiovascular events in persons with type 2 diabetes. Canagliflozin was the first oral diabetes medication to be approved for the prevention of cardiovascular events in people with type 2 diabetes. The most common cause of death in these patients is cardiovascular disease. Metformin, also known as 1-carbamimidamido-N, N- dimethylmethanimidamide, is an antihyperglycemic medicine that decreases blood glucose levels without producing hypoglycemia in people with type 2 diabetes. Metformin is an insulin sensitizer that reduces insulin resistance and fasting insulin levels in the blood by a clinically significant amount. Another well-known benefit of this medication is that it aids with weight loss. For obese type 2 diabetic patients, metformin is the treatment of choice. A review of the literature revealed that there are only a few HPLC-based analytical techniques for determining Canagliflozin and Metformin. In addition, the reported approach has a number of drawbacks. Furthermore, none of the methodologies are listed in the Quality by Design methodology. As a result, the goal of this study is to develop and validate a simple, precise, accurate, and cost-effective HPLC method for determining Canagliflozin and Metformin in bulk as API, as well as to assess the method's applicability in completed product formulation.

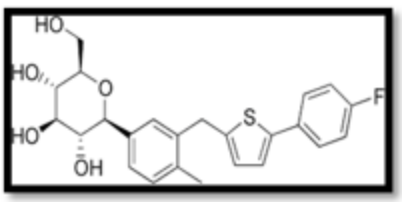


Fig. 1: Structure of Canagliflozin.

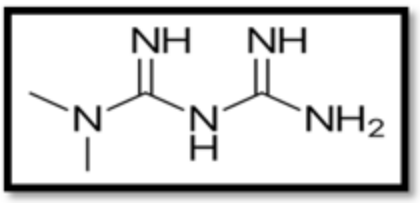


Fig. 2: Structure of Metformin.

MATERIALS AND METHODS

HPLC: HPLC Binary Isocratic System

Column: Cosmosil C18 (250mm x 4.6ID, Particle size: 5 micron)

Detector: UV Visible

Sonicator: Wensar Ultra Sonicator

Membrane Filter: Nylon 0.45µm

Chemicals and solvents: Chromatographic condition

Table 1: Chromatographic condition.

Sr. No.	Parameter	Observation
1	Column	Cosmosil C18 (250mm x 4.6ID, Particle size: 5 micron)
2	Flow rate	1.0 ml/min
3	Wavelength	254nm
4	Injection volume	20µl
5	Mobile phase ratio	80:20 (Methanol: Water at pH 3)
6	Run time	8 min
7	Temperature	Ambient

Detection Wavelength by UV Spectroscopy

Between 400nm and 200nm, the standard solution was scanned. The wavelength of maximal absorption from the spectrophotometric study was used as the analytical wavelength for the examination, which was 254nm. As a result, 254nm is used as an analytical wavelength for further analysis.

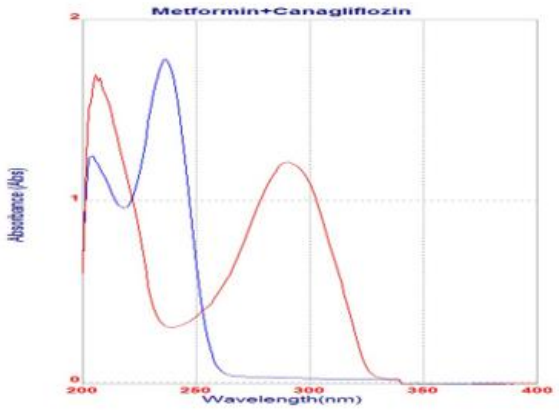


Figure 3: Overlay UV spectrum of CGF and MFM.

Preparation of Standard Solutions

Preparation of mobile phase

Mobile phase was prepared by mixing HPLC grade Methanol and water. This was prepared in different ratio as per the requirement of an experiment from time to time. The pH of resulting solution was maintained at pH 3 by using ortho phosphoric acid.

Preparation of standard stock solution of CGF and MFM

10mg of CGF and MFM was weighed individually and transferred in two separate 100ml volumetric flasks containing few ml of mobile phase (water 50: Methanol 50 at pH 3). The volume of both flasks was made up to the mark using mobile phase to make up the consequential solutions of 100µg/ml respectively. These solutions were ultra-sonicated for 30min in three cycles each of 10 min.

Furthermore, these solutions were filtered separately through 0.45µ membrane filter in order to remove small traces if any.

Preparation of working solution of CGF and MFM

Aliquot of 0.3ml and 3.0ml of standard stock solutions of CGF and MFM respectively were pipette out using micropipette and transferred separately in to two 10.0ml volumetric flask and diluted with mobile phase (Water: Methanol in the ratio of 50:50 at pH 3) to make solutions of 3µg/ml and 30µg/m respectively. These solutions were ultra-sonicated for 20 min in two cycles each of 10 min. Also, they were filtered through 0.45µ membrane syringe filter and filled in HPLC vials for injection. These solutions were then used for auxiliary investigation.

Preparation of working solution of CGF and MFM in combination

Aliquot of 0.3ml and 3.0ml of standard stock solutions of CGF and MFM respectively were pipette out using micropipette and transferred in single 10ml volumetric flask and diluted with mobile phase (Water: Methanol in the ratio of 50:50 at pH 3) to make solutions of 3µg/ml and 30µg/m of CGF and MFM respectively. This solution was ultra-sonicated for 20 min in two cycles each of 10 min. Also, it was filtered through 0.45µ membrane syringe filter and filled in HPLC vials for injection. These solutions were then used for auxiliary investigation.

RP-HPLC method Validation

Linearity and Range

Aliquots from standard stock solution (100µg/ml) of CGF and MFM equivalent to 0.1, 0.2, 0.3, 0.4, 0.5ml and
1.0, 2.0, 3.0, 4.0, 5.0ml respectively were pipette out using micro-pipette and moved to five diverse 10ml volumetric flasks. The aliquot of each volumetric flask was diluted up to 10ml using the mobile phase (Methanol 80: Water 20 at pH 3) to achieve the resulting solutions of 1, 2, 3, 4, 5 of CGF and 10, 20, 30, 40, 50µg/ml of MFM respectively. Each of this solution was injected to the set optimized chromatographic conditions and chromatograms were recorded.

Precision

Precision testing was carried out to study repeatability. From range of linearity quality control (QC) standards was defined 3µg/ml for CGF and 30µg/ml for MFM respectively. The standard solutions of CGF and MFM were prepared by diluting standard stock solutions (100µg/ml) of CGF and MFM equivalent to 0.3ml and 3.0ml respectively up to 10ml using mobile phase to obtain consequential solutions of 3µg/ml of CGF and 30µg/ml of MFM. This solution was injected to given chromatographic conditions (Table 10) in triplicate and peak area was determined. Outcomes were recorded and supplementary used to calculate mean, SD, %RSD.

% Accuracy

Accuracy of the method can be determined by different method. Only the requirement as per ICH Q2R1 guideline is that, it should be evaluated at three levels with minimum nine determinations of standard or test concentration of analyte across the range. Here in this projected research work, accuracy was determined by two different methods. Firstly, from results obtained for three QC standards in the precision experiment. Secondly, by percent recovery experiment as explained in the later section. % Accuracy was determined from the information obtained for precision testing. Here in this case, it was determined from the observations of mean peak area obtained in the case of three QC standards of CGF and MFM defined for precision study. The % accuracy was determined by using following formula.

Percent recovery

Preparation of standard stock solution of MFM and CGF from API

Precisely weighed 10mg of CGF and 100mg MFM (API) and transferred in to a 100ml volumetric flasks containing few ml of mobile phase and volume was made up to the mark (100ml) using mobile phase (Methanol 80: Water 20 at pH 3) to get ending concentration standard stock solution of 100µg/ml of CGF and 1000µg/ml of MFM. The subsequent solution was filtered through 0.45µ membrane filter and ultra- sonicated for 30 min in three cycles each of 10 min.

Preparation of standard working solution MFM and CGF

From the above standard stock solution of MFM and CGF further dilutions were performed as mentioned in Table 5. These standard working solutions of CGF and MFM were injected for set chromatographic system in triplicate and mean peak area was determined.

Table 2: Dilutions of standard stock sol. of MFM and CGF for % recovery.

Sr. No.	Vol. of stand. Stock sol. (ml)	Final vol. (ml)	Final Conc. of CGF	Final Conc. of MFM
1.	0.3	10	3	30
2.	0.4	10	4	40
3.	0.5	10	5	50

Preparation of standard sample solution from dosage form

Twenty tablets (Label claim INVOKAMET, 50mg of Canagliflozin and 500mg of Metformin Hydrochloride, Janssen Pharmaceuticals) were weighed and average weight was determined. Further, the tablets triturated to obtain powder. Powder equivalent to 10mg of CGF along with it contain 100mg MFM were transferred to 100ml volumetric flask containing some amount of mobile phase in ml and diluted with mobile phase slowly with wobbling to achieve the main stock solution of CGF 100µg/ml and MFM 1000µg/ml. The substantial solution was filtered through 0.45µ membrane filter and ultra- sonicated for 30min in three cycles each of 10 min.

Preparation of sample solution for % recovery by spike method

Aliquots of 0.2ml of standard stock solution (100µg/ml of CGF and 1000µg/ml of MFM) was pipette out using micro pipette in three different 10ml volumetric flasks. In the respective 10ml flasks add 0.1, 0.2 and 0.3ml of sample stock solutions serially and finally make up the volume up to 10ml with same solvent to obtain final concentrations as 3, 4 and 5µg/ml of CGF and 30, 40 and 50µg/ml of MFM to attain test solutions at 50%,

100% and 150% likewise. Each of these three level test solutions of CGF and MFM was injected to the given set of chromatographic conditions and peak area for each level was determined by integration of chromatograms. The peak area obtained for standard solution of CGF and MFM injected (in the past estimated) was compared with sample to get % recovery. The % recovery was determined from the sample and standard.

Table 3: Dilutions of sample sol. of MFM and CGF for % recovery.

Sr. No.	Vol. of stand. stock sol. (ml)	Vol. of sample stock sol. (ml)	Final vol. (ml)	Final Conc. of CGF	Final Conc. of MFM
1.	0.2	0.1	10	3	30
2.	0.2	0.2	10	4	40
3.	0.2	0.3	10	5	50

Experimental work System suitability testing

Table 1: Observations for system suitability testing of CGF & MFM.

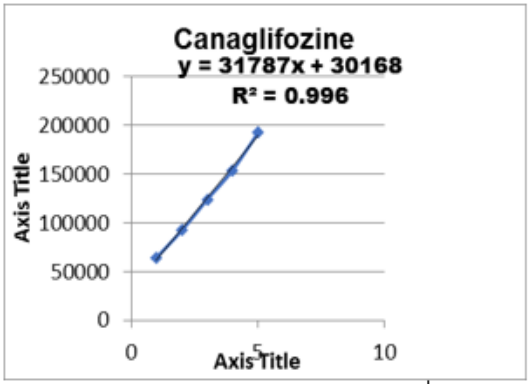
Sr. No.	Parameter	Observations of CGF			Observations of MFM			Acceptance criteria	Inference
		Mean	SD	%RSD	Mean	SD	%RSD		
1.	Peak Area	125176	649.85	0.519	2644804	14318.38	0.54	< 2	Pass
2.	Retention time	5.819	0.0542	0.93	3.75	0.018	0.49	< 0.5%	Pass
3.	Number of Theoretical plates	7589.3	65.87	0.87	8540.5	80.66	0.94	> 2000	Pass
4.	Resolution	6.06	0.04	0.68	6.06	0.04	0.68	> 1.75	Pass

Linearity and Range

Table 5: Observations obtained for linearity experiment of CGF and MFM.

Sr. No.	Conc. of CGF std. solution (µg/ml)	peak Area	Sr. No.	Conc. of MFM std. solution (µg/ml)	peak Area
1	1	64571	1	10	436070
2	2	92297	2	20	1523861
3	3	124088	3	30	2656039
4	4	154056	4	40	3628938
5	5	192624	5	50	4862786

Figure 4: Calibration curve of CGF.



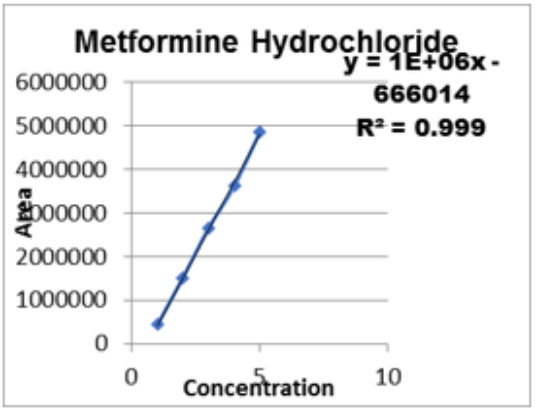


Figure 5: Calibration curve of MFM.

Table 6: Observations of CGF obtained for intra and inter-day precision.

Conc. (µg/ml)	Peak Area		Intra-day precision (Repeatability)			Peak Area		Inter-day precision (Intermediate precision)		
	Morning	Evening	Mean area	% RSD	Inference	Day 1	Day 2	Mean area	% RSD	Inference
3	124088	125310	124623.2	0.55	Pass	124088	123863	125869	0.57	Pass
3	124551	123572				124551	124569			
3	125003	125215				125003	125869			

Table 7: Observations of MFM obtained for intra and inter-day precision.

Conc. (µg/ml)	Peak Area		Intra-day precision (Repeatability)			Peak Area		Inter-day precision (Intermediate precision)		
	Morning	Evening	Mean area	% RSD	Inference	Day 1	Day 2	Mean area	% RSD	Inference
30	2656039	2663461	2658578	0.19	Pass	2656039	2656396	2660754	0.14	Pass
30	2651421	2663465				2651421	2658888			
30	2662048	2655031				2662048	2660754			

% Accuracy

Table 8: Observations obtained for accuracy of CGF.

Sr. No.	Conc. (µg/ml)	Peak Area	Mean	SD	%SD
1	1	64571	64705.34	208.06	0.32
		64600			
		64945			
2	3	124088	124547.34	457.51	0.36
		124551			
		125003			
103	5	192624	193136.34	468.52	0.24
		193242			
		193543			

Table 9: Observations obtained for accuracy of MFM.

Sr. No.	Conc. (µg/ml)	Peak Area	Mean	SD	%SD
1	10	436070	435168.66	874.37	0.20
		435112			
		434324			
2	30	2656039	2656502.66	5328.65	0.20
		2651421			
		2662048			
3	50	4862786	4871545.66	7599.16	0.16
		4876371			
		4875480			

Percent recovery

Table 10: Observations obtained for percent recovery experiment of CGF at three levels viz. 50, 100 and 150%.

% Recovery Level	Conc. of standard spiked (µg/ml)	Conc. of sample (µg/ml)	Total conc. of sample (µg/ml)	Peak Area of Std.	Total peak Area of sample	% Recovery	Inference (Standards 95-105% w/w)
50	2	1	3	124088	125205	100.90	Pass
100	2	2	4	154056	155374	100.85	Pass
150	2	3	5	192624	191044	99.17	Pass

Table 11: Observations obtained for percent recovery experiment of MFM at three levels viz. 50, 100 and 150%.

% Recovery Level	Conc. of standard spiked (µg/ml)	Conc. of sample (µg/ml)	Total conc. of sample (µg/ml)	Peak Area of Std.	Total peak Area of sample	% Recovery	Inference (Standards 95-105% w/w)
50	20	10	30	2656039	2693771	101.42	Pass
100	20	20	40	3628938	3674364	101.25	Pass
150	20	30	50	4862786	4878342	100.31	Pass

LOD and LOQ

LOD and LOQ indicate the minimum concentration of CGF and MFM that can be determined and quantified using proposed method. The LOQ was determined using following formulae and were to be 0.32 of CGF and 0.33 µg/ml of MFM. The LOD was determined using following formulae and were to be 0.97 of CGF and 0.99 µg/ml of MFM.

Table 12: Observations for LOD and LOQ.

Standard Drug Solution	LOD (µg/ml)	LOQ (µg/ml)
CGF	0.57	1.70
MFM	0.16	0.49

SUMMARY AND CONCLUSION

The proposed work was intended to expand an easy, vulnerable, precise, accurate and economic RP-HPLC method for the estimation of Canaglifozin (CGF) and Metformin Hydrochloride (MFM) as API. Also, method projected to explore an applicability of the method to test for assay of CGF & MFM in marketed formulations (Tablet dosage form). At the beginning of the experiment of method development with RP-HPLC, trial runs were recorded and preliminary chromatographic conditions were defined with mobile phase composition of Methanol and Water pH 3 in the ratio of 80:20. Above chromatographic conditions were used for further RP-HPLC method development as the chromatographic peak was better defined and roughly complimentary from tailing. The retention time obtained for CGF & MFM was 5.819 min and 3.75min respectively (Mean RT of SST) with C18 stationary phase (Column 250mm x 4.6mm, 5µm particle size).

System suitability experiment was carried out to learn an efficiency of the system and to make it appropriate for additional learn with six repeated measurements of standard solution of the CGF & MFM. The calculated statistical parameters were contained by the receiving standard as per ICH Q2R1 guidelines for CGF & MFM. The equivalent peak area and retention time of both CGF & MFM were reproducible as indicated by % RSD within limit (<2 and <0.5 for Peak area and RT respectively). Linearity of the method was assessed by practical regression coefficient of about r2 = 0.996 between the standard concentration of CGF and the respective peak areas. The regression curve was plotted by linear regression fitting and its regression equation was y = 31787x – 30168 (Where, Y gives peak area and X is the concentration of the CGF). Linearity of the method was assessed by practical regression coefficient of about r2 = 0.999 between the standard concentration of MFM and the respective peak areas. The regression curve was plotted by linear regression fitting and its regression equation was y = 109585x – 66601 (Where, Y gives peak area and X is the concentration of the MFM).

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