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A New Validated Stability Indicating RP-HPLC Method for Simutaneous Estimation of Metformin Hydrochloride and Empagliflozin in Tablet Dosage Forms

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Abstract*—A combination of Metformin HCl and Empagliflozin is used to treat type 2 diabetes mellitus. A selective, accurate and precise RP-HPLC method was developed and validated for simultaneous estimation of these drugs in combined tablet dosage forms. The drugs were resolved on a Kromosil C18 column using Acetonitrile: 0.1% orthophosphoric acid (50:50 v/v) as the mobile phase. The detection wavelength was 260 nm. The retention times obtained for metformin HCl and empagliflozin were 2.192 & 3.200 min respectively. The linearity ranges were 125-750 & 1.25-7.5 µg/ml respectively with Regression coefficients of 0.999. The % R.S.D. of precision studies was found to be 0.57 & 0.44 respectively. The Accuracy of the proposed method was determined by recovery studies and the mean recovery was 100.55 & 100.52% respectively. The method was also applicable for quantitative analyses of the marketed tablet formulations and in studying stability of the drugs under acidic, alkaline, oxidation, thermal and UV conditions*.

***Keywords*—** *Empagliflozin, Metformin Hydrochloride, Tablets, Degradation studies, RP-HPLC.*

I. INTRODUCTION

Diabetes is a chronic, metabolic disease characterized by elevated levels of blood glucose. The most common is type2 diabetes, usually in adults, which occurs when the body became resistance to insulin or doesn't make enough insulin [1], [2].

Metformin HCl and empagliflozin is an oral anti diabetic that helps control blood sugar levels. Metformin HCl is a drug in biguanide class and chemically 1,1-dimethylimido dicarbonimidic diamide hydrochloride, works by decreasing glucose production by the liver and increasing the insulin sensitivity of the body tissues [3]. Empagliflozin is a drug of the gliflozin class with chemically 1- chloro-4-[b-D-glucopyranos-1-yl]-2-[4-([S]- tetrahydrofuran-3-yl-oxy) benzyl, it is an inhibitor of the sodium glucose cotransporter-2 (SGLT-2), it helps to stop sodium-glucose transport protein that have been filtered out of the blood by the kidney being reabsorbed back into the blood [4].

The literature survey shows that there are few methods for determination of metformin HCl and empagliflozin individually in tablet dosage form by various analytical equipment's like UV spectrophotometer [5-7], RP-HPLC [8-15], UPLC [16], [17] and LC-MS/MS [18]. So, the attempt has been made to develop a new validated stability indicating RP-HPLC method for simultaneous estimation of metformin hydrochloride and empagliflozin in tablet dosage form as per International Conference on Harmonization (ICH) guidelines.

II. MATERIALS AND METHODS

The API gift samples of MET & EMPA were provided by Spectrum Pharma Research solutions, Hyderabad. HPLC grade Acetonitrile, water and other chemicals obtained from the Rankem, Hyderabad. WATERS HPLC 2695 SYSTEM equipped with quaternary pumps, Photo Diode Array detector and Auto sampler integrated with Empower 2 Software. UV-VIS spectrophotometer T60 with special bandwidth of 2 mm and 10mm and matched quartz cells integrated with UV win 6 Software was used for measuring absorbances of Metformin and Empagliflozin solutions.

Preparation of 0.1% Orthophosphoric Acid:

Accurately pipette 1.0mL of OPA into clean & dried 1000mL volumetric flask, add 100mL of milli-Q water, stir well and finally makeup to the mark with milli-Q water.

Preparation of Mobile Phase:

It consisting mixture of 0.1%OPA and Acetonitrile at ratio 50:50 v/v.

Preparation of Diluent:

It is a mixture of Acetonitrile and milli-Q water at ratio 50:50 v/v.

Preparation of Standard Solution:

Accurately weighed 125mg of metformin HCl (API) and 12.5 mg of empagliflozin (API) and transferred into clean and dried 25 ml and 100 ml volumetric flask separately. Add 3/4th of diluents to both of these flasks, sonicate for 10minutes and finally made up to the mark with diluent. The resultant concentrations are 5000µg/ml of MET and 125µg/ml of EMPA.

Preparation of Standard Working Solutions (100% solution):

Pipette 1ml from each stock solution and transferred into clean and dried 10ml volumetric flask and finally make up to the mark with diluent. The resultant concentrations are 500µg/ml of MET and 12.5µg/ml of EMPA.

Preparation of Sample Stock Solutions:

10 tablets are randomly selected, weighed and the average weight of each tablet is calculated, all tablets were grounded into fine powder. The weight equivalent to 1 tablet was transferred into 100ml volumetric flask, add 50ml diluent, sonicated for 25 minutes and finally make up to the mark with

Vinay Kumar D and J. V. L. N. Seshagiri Rao, "A New Validated Stability Indicating RP-HPLC Method for Simutaneous Estimation of Metformin Hydrochloride and Empagliflozin in Tablet Dosage Forms," *International Research Journal of Pharmacy and Medical Sciences (IRJPMS)*, Volume 1, Issue 5, pp. 16-22, 2018.

diluent. All the content was passed through 0.45µ filter paper. The resultant concentration 5000µg/ml of MET and 125µg/ml of EMPA.

Preparation of Sample Working Solution (100% solution):

Pipette 1 ml of filtered sample stock solution, transfer it into 10 ml volumetric flask and make up to the mark with diluent. The resultant concentrations were 500µg/ml of MET and 12.5µ g/ml of EMPA.

Optimized Chromatographic Method:

The separation of Metformin Hydrochloride and Empagliflozin was achieved on a Kromosil C_{18} column (250x4.6 mm; 5.6 µ) and eluting with a mobile phase consisting of a 50:50 v/v mixture of Acetonitrile and Buffer [0.1% orthophosphoric Acid (pH 2.8)] at a flow rate of 1.0mL/min. The analytes were monitored at 260 nm. The injection volume was 10 µl. The total run time for elution of compound was 6 min.

Column: Kromasil C18; 50 x 4.6 mm; 5µ.
Column temperature: 30°C
Flow rate: 1 mL/min
Injection volume: 10 µL
Detector wave length: 260 nm
Run time: 6 min

III. METHOD VALIDATION [19]

The US Food and Drug Administration (FDA) and US Pharmacopeia (USP) both refer to ICH guidelines. The most widely applied validation characteristics are accuracy, precision, specificity, linearity, range, robustness, limit of detection, limit of quantification, limit of detection and limit of quantification.

System Suitability:

It is the checking of a system to ensure system performance before or during the analysis of unknown**.** It tests are an integral part of chromatographic method and are used to verify that the resolution & reproducibility of the system are adequate for the analysis tot be performed. In this, plate count (N), tailing factor (T), resolution (Rs) and reproducibility (%RSD) are determined from replicate injection of standard. The acceptable limit of %RSD is less than 2%. Table 1

TABLE 1. System suitability parameters.

Drug	Retention time (min)	Area	USP Plate Count	USP Tailing
Metformin HCl	2.192	9877896	4354	1.33
Empagliflozin	3.200	954988	7073	1.48

Specificity:

The ability of the method is to accurately measure the analyte response in the presence of all potential sample components. In this study, the method was evaluated by injecting 10µl of blank sample and placebo into HPLC. Fig No. 1, 2, 3 & 4.

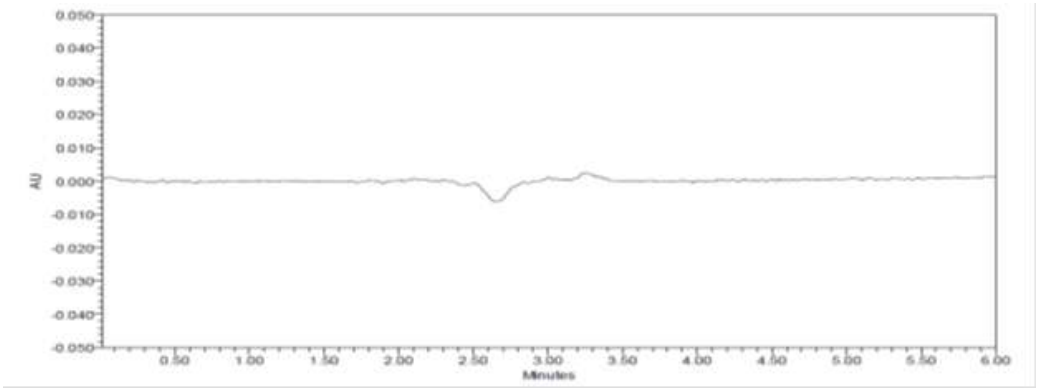

Fig. 1. Typical chromatogram of blank.

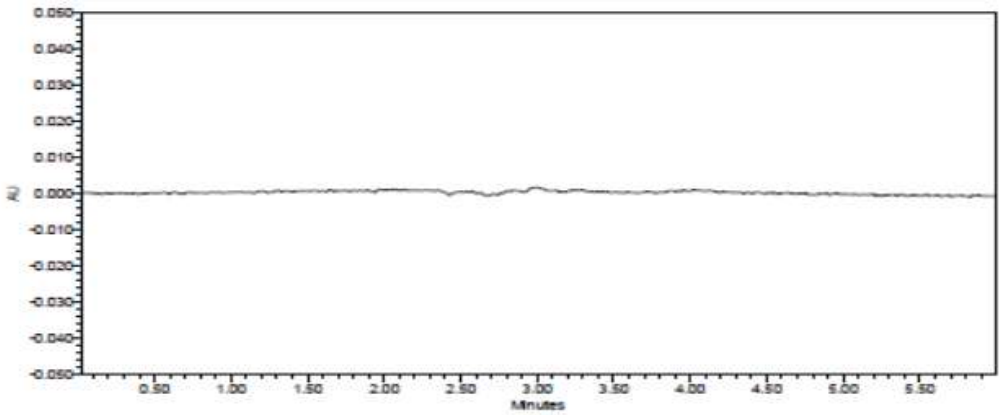

Fig. 2. Typical Chromatogram of Placebo.

Vinay Kumar D and J. V. L. N. Seshagiri Rao, "A New Validated Stability Indicating RP-HPLC Method for Simutaneous Estimation of Metformin Hydrochloride and Empagliflozin in Tablet Dosage Forms," *International Research Journal of Pharmacy and Medical Sciences (IRJPMS)*, Volume 1, Issue 5, pp. 16-22, 2018.

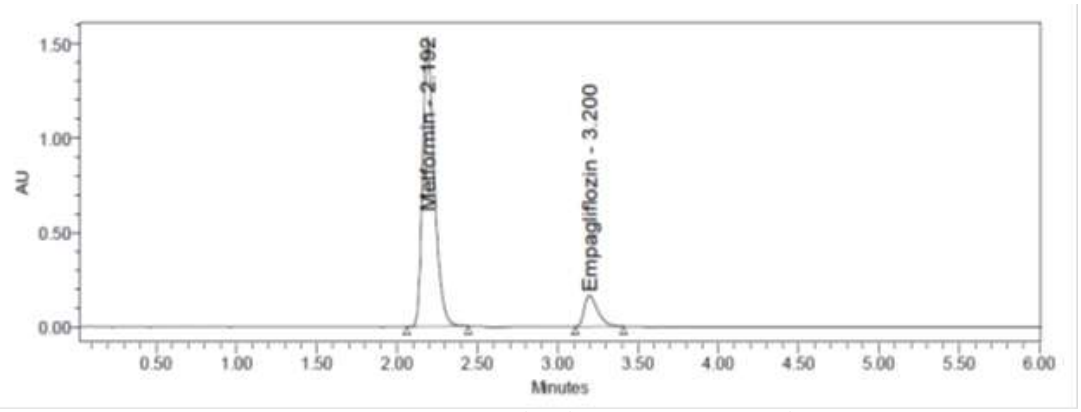

Fig. 3. Standard chromatogram of metformin HCL and Empagliflozin.

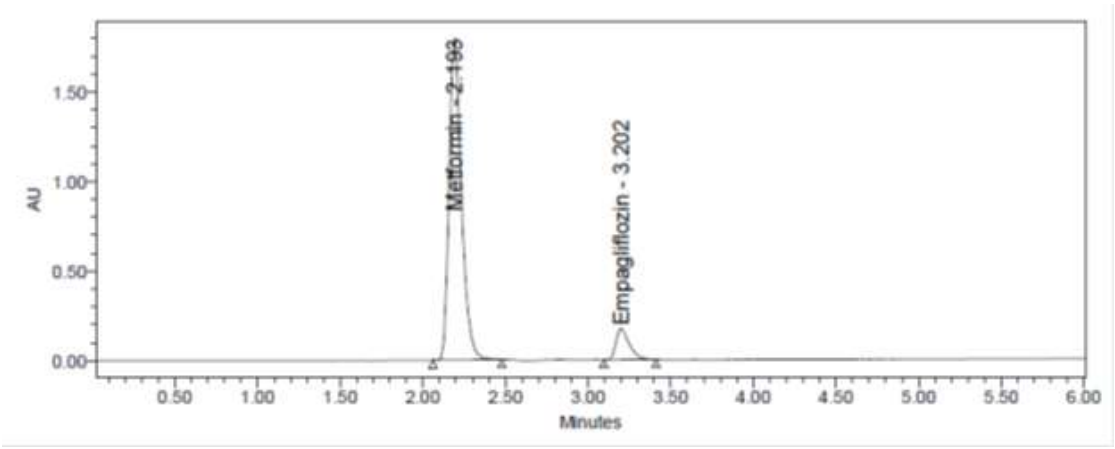

Fig. 4. Typical chromatogram of sample (Tablet Dosage Form).

TABLE 2. Recovery studies of metformin HCL and Empagliflozin.

Drug	Level of spike solution	Amount present (mg/mL)	Amount added	Amount recovered	% Recovery	% RSD
Metformin HCl	50%	500	250	249.82	99.93	0.73
	50%	500	250	254.81	101.93	
	50%	500	250	250.17	100.07	
	100%	500	500	500.01	100	
	100%	500	500	498.60	99.72	
	100%	500	500	502.45	100.49	
	150%	500	750	760.42	101.39	
	150%	500	750	754.75	100.63	
	150%	500	750	755.90	100.79	
Empagliflozin	50%	12.5	6.25	6.20	99.29	0.75
	50%	12.5	6.25	6.32	101.26	
	50%	12.5	6.25	6.30	100.92	
	100%	12.5	12.5	12.69	101.54	
	100%	12.5	12.5	12.60	100.87	
	100%	12.5	12.5	12.58	100.71	
	150%	12.5	18.75	18.66	99.55	
	150%	12.5	18.75	18.83	100.46	
	150%	12.5	18.75	18.76	100.67	

Accuracy:

The accuracy of the method was evaluated by standard addition method. The known amount of the reference standard was added to the known amount of standard solution at three different levels. The solutions were analyzed for mean recovery and %RSD. The studies were performed for both MET & EMPA at three different levels 50%, 100% and 150% solution. The 10 µL was injected into HPLC and % recovery and % RSD were noted as shown in table 2.

Precision:

Precision is the degree of agreement among individual test results when an analytical method is used repeatedly to multiple sampling of a homogenous sample. The precision was determined as reproducibility precision and studied for method precision and inter-day precision by injecting 10µL for six times and peak areas of replicated injections as shown in table 3.

Vinay Kumar D and J. V. L. N. Seshagiri Rao, "A New Validated Stability Indicating RP-HPLC Method for Simutaneous Estimation of Metformin Hydrochloride and Empagliflozin in Tablet Dosage Forms," *International Research Journal of Pharmacy and Medical Sciences (IRJPMS)*, Volume 1, Issue 5, pp. 16-22, 2018.

Linearity and Range:

Linearity is the ability of the method to elicit test results that are directly, or by a well-defined mathematical transformation to analyte concentration within a given range. Range is the interval between the upper and lower levels of analyte. The linearity of the samples concentration ranging 125-750 µg/ml for metformin Hcl and 3.125-18.75 µg/ml for empagliflozin. The linearity of the method was evaluated by linear regression analysis. The results were as shown in Fig. 5 & 6 and table 4.

TABLE 3. Precision studies of metformin HCL and Empagliflozin.

S.No.	Injection	Method Precision		Interday Precision	
		Metformin HCl	Empagliflozin	Metformin HCl	Empagliflozin
1	Injection -1	9827091	962995	9846584	928850
2	Injection-2	9813199	970369	9738723	941309
3	Injection-3	9951842	971088	9905008	940840
4	Injection-4	9920243	965830	9954662	955992
5	Injection-5	9896044	973026	9849647	944939
6	Injection-6	9925155	974147	9769625	938527
Average		**9888929**	**969576**	**9844042**	**941743**
SD		**56321**	**4314.0**	**80773**	**8841.1**
% RSD		**0.57**	**0.4**	**0.82**	**0.94**

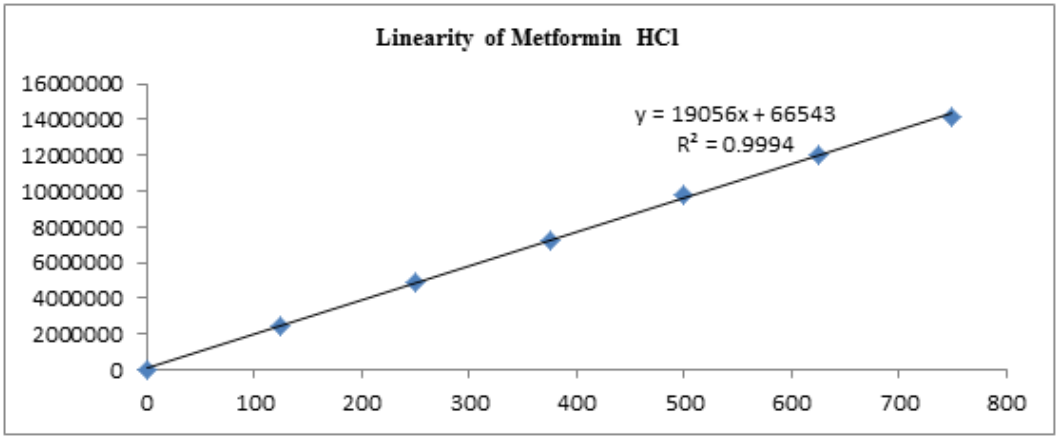

Fig. 5. Linearity curve of metformin HCL.

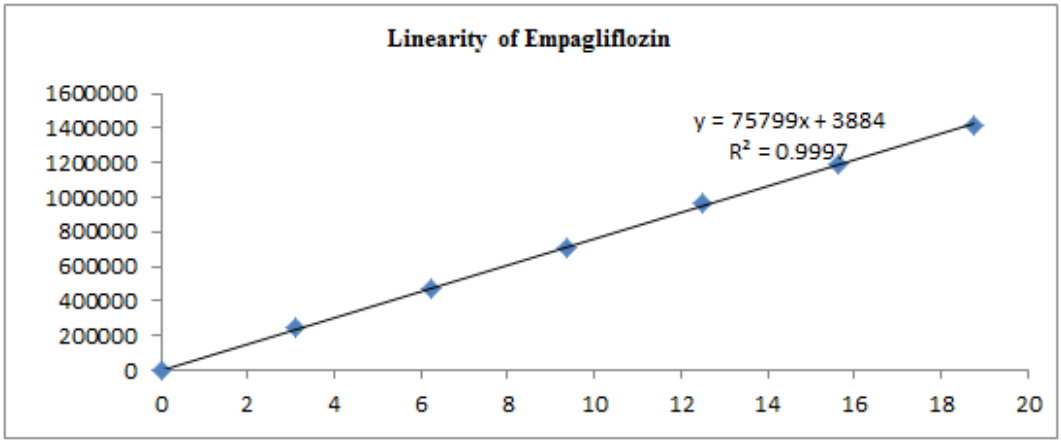

Fig. 6. Linearity curve of Empagliflozin.

TABLE 4. Linearity data of metformin HCL and Empagliflozin.

S.No.	Metformin HCl		Empagliflozin	
	Concentration (µg/mL)	Peak Area	Concentration (µg/mL)	Peak Area
01.	125	2405729	3.125	250343
02.	250	4893373	6.25	472422
03.	375	7146062	9.375	704841
04.	500	9818350	12.5	961172
05.	625	12030309	15.625	1193833
06.	750	14175281	18.75	1418900
Correlation coefficient (R^2)		**0.999**	**0.999**	

Vinay Kumar D and J. V. L. N. Seshagiri Rao, "A New Validated Stability Indicating RP-HPLC Method for Simutaneous Estimation of Metformin Hydrochloride and Empagliflozin in Tablet Dosage Forms," *International Research Journal of Pharmacy and Medical Sciences (IRJPMS)*, Volume 1, Issue 5, pp. 16-22, 2018.

Robustness:

It is the capacity of a method to remain unaffected by small, deliberate variations in method parameters. It was indicated by changing the flow rate, mobile phase composition and temperature. Table 5

TABLE 5. Robustness studies of metformin HCL and Empagliflozin.

Parameter	Change in parameter	Peak Area		SD		% RSD	
		Metformin HCl	Empagliflozin	Metformin HCl	Empagliflozin	Metformin HCl	Empagliflozin
Flow rate	0.8mL/min	9337797	99307	34443.9	3827.1	0.4	0.4
	1.2 mL/min	8477949	910414	47267.2	6918.6	0.6	0.8
Mobile phase composition	2.4	9350279	990058	43662.8	3530.9	0.5	0.4
	2.8	9945989	1.443365	54784	9037.1	0.6	0.6
Temperature	25^0C	10056055	1498926	52885.5	13640.7	0.5	0.9
	30^0C	8269993	879702	31506.0	3419.6	0.4	0.4

Limit of Detection (LOD) & Limit of Quantification (LOQ):

LOD is the lowest concentrations of an analyte in a sample that can be detected. LOQ is the lowest concentration of an analyte in a sample that can be quantized. The LOD and LOQ of MET & EMPA were determined from standard deviation of the response and the slope. Table 6

TABLE 6. LOD AND LOQ of metformin HCL and Empagliflozin.

Parameter	MET	EMPA
LOD	0.50	0.01
LOQ	1.52	0.03

Assay Procedure:

The assay performed by the marked product (Synjardy XR 12.5mg/500mg of EMPA & MET). The prepared sample and standard solution were injected into HPLC and peak areas were recorded. Finally percentage amount of drug was calculated. As shown in table 7.

TABLE 7. Assay of sample (Table Dosage Form).

Drug	Label Claim (mg)	Amount present (mg)	% Drug Content
Metformin HCl	500	499.42	99.88
Empagliflozin	12.5	12.53	100.24

IV. DEGRADATION STUDIES

Forced degradation is a degradation of new drug substances and drug products at conditions more than accelerated conditions. This studies show the chemical behavior of the molecules which in turn helps the development of formulation and packaging. Hence, in the present study forced degradation studies were established by subjecting the samples of MET and EMPA standard solution to degradation in Oxidation, Acid, Alkaline, Dry heat, Photo stability and Neutral degradation. As shown in table 8.

Oxidation:

Pipette 1ml of standard stock solution of MET and EMPA into volumetric flask separately, add 1ml of 20% hydrogen peroxide (H_2O_2), and these solutions were kept for 30minutes at 60^0C. The resultant solutions were diluted to obtain 500µg/ml and 12.5µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

TABLE 8. Forced degradation studies of metformin HCL and Empagliflozin.

Forced Degradation Studies				
Parameters		**% amount retained**	**Purity Angle**	**Purity threshold**
Metformin Hcl	Acid	96.61.	1.554	2.663
	Alkaline	97.30	1.462	2.283
	Oxidation	98.03	1.118	2.724
	Photo Stability	99.39	0.455	1.742
	Thermal	99.16	0.191	0.803
	Neutral	99.51	0.462	1.731
Empagliflozin	Acid	97.26	0.164	0.336
	Alkaline	97.95	0.152	0.316
	Oxidation	98.14	2.426	3.334
	Photo Stability	99.48	0.257	0.364
	Thermal	99.35	0.305	0.376
	Neutral	99.88	0.219	0.360

Acid Degradation Studies:

Pipette 1ml of stock solution of MET and EMPA into volumetric flask separately, add 1ml of 2N Hydrochloric Acid and reflex for 30 minutes at 60^0C.The resultant solutions were diluted to obtain 500µg/ml and 12.5µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Alkali Degradation Studies:

Pipette 1ml of stock solution of MET and EMPA into volumetric flask separately, add 1ml of 2N sodium hydroxide and reflex for 30 minutes at 60^0C.The resultant solutions were diluted to obtain 500µg/ml and 12.5µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry Heat Degradation Studies:

The standard Drug solutions were placed into oven at 105^0C for 6hours. The resultant solutions were diluted to obtain 500µg/ml and 12.5µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Photo Stability Studies:

The photo chemical stability of the drug was also studied by exposing the stock solutions to UV light by keeping the beaker in UV chamber for 7 days or 200 watt hours/m^2 in photo stability chamber. The resultant solutions were diluted

Vinay Kumar D and J. V. L. N. Seshagiri Rao, "A New Validated Stability Indicating RP-HPLC Method for Simutaneous Estimation of Metformin Hydrochloride and Empagliflozin in Tablet Dosage Forms," *International Research Journal of Pharmacy and Medical Sciences (IRJPMS)*, Volume 1, Issue 5, pp. 16-22, 2018.

to obtain 500µg/ml and 12.5µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Neutral Degradation Studies:

Stress testing under neutral conditions was studied by refluxing the drug in water for 6hours at 60^0C. The resultant solutions were diluted to obtain 500µg/ml and 12.5µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

V. Result and Discussion

The proposed method was simple, precise and accurate for the simultaneous determination of metformin Hcl and empagliflozin in combined tablet dosage form. The drugs were resolved on a Kromosil C18 column using Acetonitrile:0.1% orthophosphoric acid buffer 50:50v/v as mobile phase, flow rate of 1ml/min and detention wavelength was 260nm. The retention time for metformin Hcl and empagliflozin were found to be 2.192 and 3.200 min respectively.

The developed method was validated for accuracy, precision, linearity, robustness, LOD and LOQ. The linearity of the method was determined by Regression analysis. A linear relationship was evaluated in the concentration range of 125-750 µg/mL of metformin Hcl and 1.25-75 µg/mL of empagliflozin with correlation coefficient of 0.999 respectively. The system suitability studies and method precision were carried and %RSD were found to be less than 2%. The accuracy of the method was determined by recovery studies and mean recovery was observed to be 100.55% for metformin Hcl and 100.90% for empagliflozin. The LOD and LOQ were found to be 0.50µg/mL & 1.52µg/mL for metformin Hcl and 0.01µg/mL & 0.03µg/mL for empagliflozin. It indicates that the method was very sensitive. The robustness of the method was studies by deliberate changes in the flow rate, mobile phase composition and temperature. The %RSD were found to be not more than 2% and results indicates that the slight variations on the chromatographic conditions have negligible effect and conformed that the method was highly robust. The proposed method was successfully applied to the assay of commercial formulation and showed 99.88% and 100.24% of metformin Hcl and empagliflozin respectively.

The specificity of the developed method was evaluated by applying different stress conditions like acid, base, oxidation, thermal, photolytic and neutral to metformin Hcl and empagliflozin in combined dosage form. The result obtained indicates that the purity angle was always less than the purity threshold and it indicates the proposed method was stable.

VI. Conclusion

The developed method was simple, precise, accurate and reliable for the simultaneous estimation of metformin Hcl and empagliflozin in combined dosage form and envisages the stability behavior of both the drugs as per ICH guidelines. The %RSD of all results is less than 2% that shows high degree. Hence, the proposed method was simple, easy, cost-effective and can be used for routine analysis of metformin Hcl and empagliflozin combined dosage form.

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