IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

DEVELOPMENT AND VALIDATION OF HPTLC BIOANALYTICAL METHOD FOR SIMULTANEOUS ESTIMATION OF DRUGS PRESCRIBED IN THE COMORBID CONDITION OF DIABETES AND HYPERTENSION

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Abstract

Type 2 Diabetes Mellitus and hypertension are the major contributors to the total burden of worldwide diseases. In around 32-87% of cases, patients are found to have both these diseases as a comorbid condition. Managing diabetes mellitus accompanied by hypertension is done by controlling blood glucose levels and blood pressure with antidiabetic and antihypertensive drugs respectively with their simultaneous consumption. Selection of the most accurate drugs and their effective concentration is a key for successful treatment in such patients with Type 2 Diabetes Mellitus due to its low dose and high selectivity. Telmisartan is one of the first-line drugs given in mild-to-moderate hypertension with a relative safety profile. The ability of Empagliflozin to cause a reduction in blood pressure necessitates monitoring of its concentration in patients consuming simultaneously an antihypertensive drug-like Telmisartan. The proposed method is an attempt to develop a high-performance thin-layer chromatographic bioanalytical method for simultaneous estimation of Empagliflozin and Telmisartan in human plasma. Chromatographic separation of drugs was performed over HPTLC plates pre-coated with silica gel $60F_{254}$ using Chloroform: Methanol: Toluene: Formic acid (8:2:4:0.1 v/v/v/v) as the mobile phase via a linear ascending technique. Detection and quantification were performed at a wavelength of 235 nm. The method was validated according to the European Medicines Agency guidelines for the bioanalytical method and gave satisfactory results. The developed method will be significant in the pharmacokinetic and toxicokinetic studies of the two drugs.

Keywords:

High-performance thin-layer chromatography, Comorbid condition, Bio-analytical method development, Empagliflozin, Telmisartan

1. Introduction

Diabetes mellitus is a chronic, metabolic disease identified by a high level of blood glucose which gives rise to severe complications and other comorbid conditions [1, 2]. Among the 10 largest countries within the world, India ranks second with 69.2 million people suffering from diabetes and another 36.5 million with pre-diabetes [3]. Among the two types of diabetes mellitus, Type 2 diabetes mellitus (T2DM) is more prevalent and contributes significantly to the upward trend [4, 5]. Diabetes is associated with several comorbidities and patients with diabetes are often treated with multiple medications. One of the comorbid conditions with T2DM is hypertension. A study conducted in 2017 on 1.3 million adults has described the prevalence of diabetes and hypertension as a comorbid condition and their concordance ranges from 32 to 82% [6, 7, 8]. The epidemiological studies have indicated that the coexistence of hypertension in patients with T2DM is an important contributing factor to the development and progression of macrovascular and microvascular complications with a four-times higher risk of cardiovascular disease (CVD) as compared to the non-diabetic patients [9-11]. Patients having T2DM along with complications of hypertension are prescribed an antidiabetic drug along with an antihypertensive drug for long-term use. This combination therapy necessitates the accurate selection of drugs from each class and their effective concentration to achieve the target blood sugar and blood pressure to avoid further complications [12].

Empagliflozin and Telmisartan are usually prescribed medicines for patients with this comorbid condition. As both these drugs belong to totally different drug categories, their combined formulation is not available in the market.

Empagliflozin (Figure 1A) is the most specific Sodium-glucose co-transporter 2 (SGLT2) inhibitor among all the currently tested SGLT2 inhibitors. SGLT2 inhibitors also known as gliflozins are the novel class of drugs available for the treatment of T2DM [13]. SGLT2 is the predominant transporter responsible for the reabsorption of glucose from the glomerular filtrate back into the circulation thereby increasing urinary glucose excretion [14]. They exert favorable effects beyond glucose control with a constant reduction in body weight, blood pressure, and serum uric acid reductions [15]. The reduction in the risk of hypoglycemia, absence of weight gain, and effective reduction in cardiovascular risk support the use of Empagliflozin as the first-line medication for patients with T2DM and CVD [16].

Telmisartan (Figure 1B) is considered a first-line drug in mild-to-moderate hypertension with a relative safety profile. Telmisartan is a potent, long-lasting, benzimidazole derivative that is a selective antagonist of the Angiotensin II receptor (AT-2 R) which works by blocking the hormone Angiotensin thereby relaxing blood vessels. AT-2 R blockers are a long-acting class of drugs that help in effective blood pressure control and reduce the risk of cardiovascular events. Telmisartan's dual mode of action also provides protective benefits against the vascular and renal damage caused by diabetes and CVD due to its multiple target action on AT-1 R and partial agonist action (25-30%) on PPARγ receptor which is involved in the glucose and lipid homeostasis [17].

The fact that Empagliflozin can cause a reduction in blood pressure, monitoring its concentration in patients consuming simultaneously an antihypertensive drug-like Telmisartan is of utmost importance. Bioanalytical methods are usually employed for the quantitative estimation of drugs and their metabolites in biological media. These methods play an important role in the estimation and interpretation of bioequivalence, pharmacokinetic and toxic kinetic studies.

Literature survey revealed that analytical and bioanalytical methods including UV spectroscopy [18-20], HPLC [21-30], HPTLC [31], and Mass spectrometry [32, 33] are reported for estimation of Empagliflozin alone or in its combination with other drugs. Similarly, various analytical and bioanalytical methods including UV spectroscopy [34, 35], HPLC [36-41], HPTLC [42-45], and Mass spectrometry [46-49], have been reported for the quantitative estimation of Telmisartan alone or with its combination with other drugs. However analytical and bioanalytical methods for the separation and quantitative estimation of Empagliflozin and Telmisartan are not available. In this research work, an attempt is made to develop an HPTLC bioanalytical method that can serve in finding the concentration of each one of them in plasma. Thus, the proposed research work was aimed at the development of a bio-analytical method for the separation and quantification of Empagliflozin and Telmisartan.

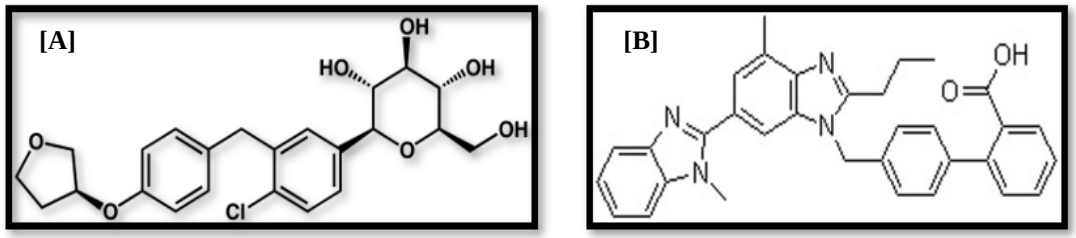

Figure 1. Chemical structure of [A] Empagliflozin, and [B] Telmisartan

2. Experimental

2.1 Materials

Empagliflozin (99.8%) and Telmisartan (99%) were obtained as a gift sample from an API manufacturing company (India). Drug-free Human Plasma was obtained from a blood bank, Kharghar (Navi Mumbai- India). All chemicals and reagents used in the study were of analytical grade and purchased from S.D Fine chemicals, Pvt Ltd. (Mumbai, India).

2.2 Instrumentation and software

A CAMAG HPTLC system (Muttenz, Switzerland) comprising of a CAMAG Linomat V semi-automatic sample applicator, CAMAG TLC Scanner IV, CAMAG TLC visualizer, flat bottom and twin-trough developing chamber (10×10 cm), UV cabinet with dual-wavelength UV lamp, Hamilton syringe (100 µL; Bonaduz, Switzerland), ultrasonic bath (Frontline FS-4, Mumbai, India) and CAMAG win CATS software were employed in the study. The samples were spotted in the form of bands having a bandwidth of 8 mm with a 100µL microsyringe (Linomat syringe 659.0014) on pre-coated silica gel HPTLC plate $60F_{254}$ (10×10 cm), 100 µm thickness, E. Merck, Darmstadt, Germany) employing a CAMAG Linomat V sample applicator. The densitometric scanning was performed at 235 nm in absorbance mode. The slit dimensions were set at 0.50 mm × 0.45 mm, the scanning speed at 20 mm/s, and the data resolution at 100µm/step.

2.3 Preparation of stock solution and working standard solutions

10 mg of Empagliflozin and Telmisartan were weighed accurately and transferred to a 10 mL volumetric flask. Methanol was used as a solvent and volume was made up to the mark of 10 mL to furnish a stock solution of 1000 µg/mL of each drug sample. Working standard solutions were prepared by appropriately diluting the stock solution (1000µg/mL) with methanol

in a 10 mL volumetric flask to get the concentration of 50, 100, 150, 200, 250, 300, and 350 µg/mL of each Empagliflozin and Telmisartan. All solutions were kept at 4-6°C and brought to room temperature before use.

2.4 Preparation of plasma samples and the quality control samples

Plasma samples were stored at -20°C and allowed to thaw at room temperature before processing. In brief, to 0.2 mL of centrifuged drug-free plasma, 0.02 mL working standard solution of Empagliflozin and Telmisartan was added to obtain calibration standards of 50, 100, 150, 200, 250, 300, 350 ng/spot. The quality control (QC) samples were prepared in plasma having a concentration of 50 ng/spot (LLOQ), 100 ng/spot (LQC), 250 ng/spot (MQC), and 350 ng/spot (HQC) and the tube was vortexed for 3 min. Protein precipitation technique was employed for the extraction of these spiked drugs from plasma by addition of 1.2 mL of a mixture of Tetrahydrofuran: Methanol in the ratio 1:1 v/v (1.2 mL) to the mixture of plasma and drug sample and the tube was centrifuged at 4000 rpm at 4°C for 10 min. The supernatant was collected and filtered through a 0.45 µm syringe filter and was used for HPTLC analysis using optimized chromatographic conditions

2.5 Chromatographic condition

The samples were spotted in the form of narrow bands having a bandwidth of 8 mm with a 100µL micro-syringe (Linomat syringe 659.0014) on pre-coated silica gel HPTLC plate $60F_{254}$ (10 × 10 cm), 100 µm thickness, E. Merck, Darmstadt, Germany) employing a CAMAG Linomat V sample applicator. The mobile phase was selected as a mixture of chloroform: methanol: toluene: formic acid in the ratio of 8:2:4:0.1 (v/v/v/v) for the development of plates. The time for chamber saturation was optimized to 20 min. The length of chromatographic development was 90 mm. The densitometric scanning was performed at 235 nm in absorbance/reflectance mode. The slit dimensions were set at 0.50 mm × 0.45 mm, the scanning speed at 20 mm/s, and the data resolution at 100µm/step. The results were evaluated to achieve an optimum separation between spots and migration of spots to ensure separation reproducibility

2.6 Method validation procedure

The developed method was validated based on EMA guidelines for bioanalytical method validation for sensitivity, selectivity, precision, accuracy, linearity, Limit of detection (LOD), limit of quantification (LOQ), robustness, recovery, carry-over, and stability. The selectivity of the method was investigated by analyzing six blank plasma samples. Each blank sample was tested for interference using the proposed extraction procedure. Three replicates of four QC samples LLOQ, LQC, MQC, and HQC were used for the determination of intra-day and inter-day precision and accuracy. Accuracy studies were performed at 80%, 100%, and 120% concentration levels. Linearity of the method was determined at seven concentration levels from 50-350 ng/band for both Empagliflozin and Telmisartan by employing the linear regression method. Co-efficient of regression, slope and y-intercept were estimated by plotting a graph of drug concentration against peak area for each standard to determine linearity. Limit of Detection (LOD) and Limit of Quantitation (LOQ) were calculated from the slope and standard deviation obtained from the graph of linearity of individual drugs. Robustness was performed in triplicate at LQC (100 ng/spot) and HQC (350 ng/spot) concentrations by deliberately making small changes in chromatographic conditions. Recovery of the drugs was obtained by comparing the results of extracted samples with the unextracted standard solution. Three types of stability studies, namely short-term stability studies (for 6 hr), freeze-thaw stability studies (three thaw cycles at -20°C), and long-term stability studies (20 days) were done at LQC and HQC concentrations as per EMA Guidelines for bioanalytical method development.

3. Result and discussion

3.1 Optimization of chromatographic condition

Based on the available literature, various combinations of mobile phases were tried for HPTLC separation of Empagliflozin and Telmisartan. The number of trails was performed to achieve proper and desired chromatographic separation. Mobile phase containing Chloroform: Methanol: Toluene: Formic acid (8:2:4:0.1 v/v/v/v) was found to give well-separated peaks after the photo densitometric HPTLC scanning at the isosbestic wavelength of 235 nm. The proposed method could successfully separate Empagliflozin and Telmisartan, in human plasma, with R_f values of 0.33±0.02 and 0.55±0.02, respectively (Figure 2).

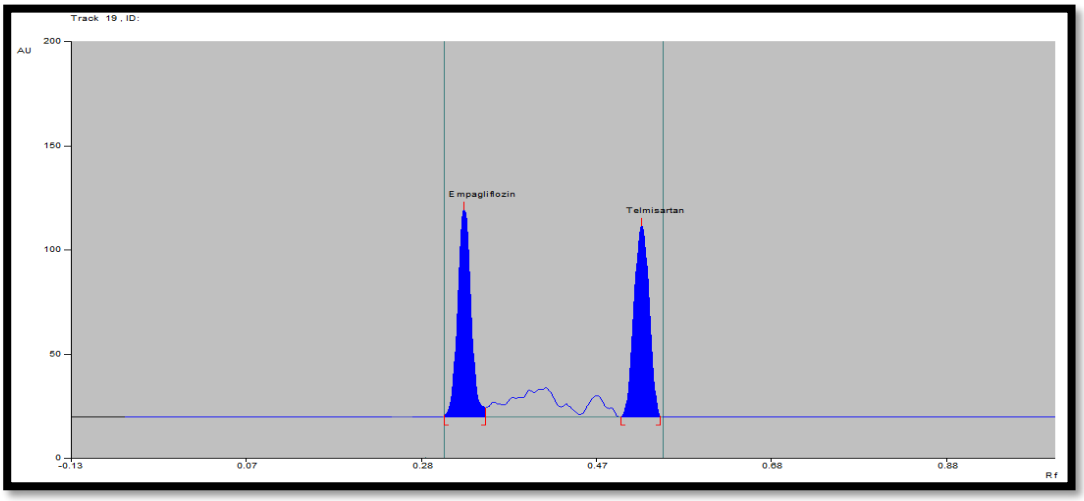

Figure 2: Densitogram of Empagliflozin and Telmisartan obtained using optimized chromatographic condition at the R_f of 0.33±0.02 and 0.55±0.02, respectively.

3.2 Optimization of the plasma extraction procedure

The bioanalytical method involves spiking of the plasma samples with the drug solutions and then it was further analyzed using various analytical techniques for quantitative and qualitative studies. However, the components in the plasma may cause interference in quantification and leads to poor analysis. To avoid this problem sample preparation is introduced in bioanalytical method development where the biological matrix is used. Sample preparation plays a key role in bioanalytical method development which ensures the recovery of the spiked drug from the biological fluid to avoid any interference of biological fluid during the quantification of the drug. The main objective of this process is to develop and optimize a simple, rapid, and economical extraction procedure giving the highest recovery of the spiked drug with minimal interference of plasma components. Plasma sample preparation was done by applying various extraction techniques depending upon the nature, and solubility characteristics of the drug of interest. By keeping this into consideration, a protein precipitation method was chosen for plasma sample preparation which involves a single-step procedure giving high recovery with minimal utilization of solvents, drug sample, and time. Development of plasma sample preparation method was carried out by employing a trial and error approach, where various protein precipitating agents have tried alone or in combinations to achieve the desired extraction of Empagliflozin and Telmisartan with possibly highest recovery. This was done by extracting thawed and centrifuged plasma samples spiked with Empagliflozin and Telmisartan solution, and blank plasma using a different set of protein precipitating agents that include methanol, acetone, acetonitrile, tetrahydrofuran, and this were then subjected to chromatographic analysis by HPTLC using the optimized chromatographic parameters. The selection of suitable extracting solvents was done by comparing the % recovery of drug extracted using different extracting solvents with that of unextracted drug samples. The extracting solvent giving the highest recovery with minimal plasma interference at the R_f of the drugs was selected. Blank plasma analysis was performed to detect the presence of any interfering components at R_f values of both the drugs. Extracting solvent giving the highest recovery with minimal plasma interference at R_f of both the drug was selected. Initially, acetonitrile was tried for the protein precipitation of plasma, however, the recovery was poor (30-40%). Extraction of the spiked plasma separately with methanol and tetrahydrofuran showed increased recovery ranging in 50-60% and 40-50%, respectively. Concerning this, a mixture of methanol and tetrahydrofuran was tried for extraction purposes which resulted in the highest recovery of 70-80%. After finalizing the extracting solvent, vortexing time and solvent volume were decided to be 3 min and 1.2 mL respectively. The optimized plasma sample preparation method for extraction of Empagliflozin and Telmisartan is summarized in table no 1.

Table 1: Optimized conditions for plasma sample extraction procedure

Sr. No.	Parameters	Conditions
1.	**Plasma sample Extracting technique**	Protein precipitation
2.	**Plasma sample extracting solvent**	Tetrahydrofuran: methanol (1:1)
3.	**The volume of extracting solvent**	1.2 mL
4.	**Vertexing time**	3 minutes
5.	**Centrifugation parameter**	4000 rpm at -4ºC for 10 minutes

3.3 Method validation

The developed method was then validated as per the validation protocol prepared for bioanalytical method validation employing EMA guidelines on Bioanalytical Method Validation guidelines. Performance characteristics of the bioanalytical HPTLC method were statistically validated for the selectivity, linearity, lower limit of quantification (LLOQ), LOD, accuracy, precision, robustness, carryover, and stability [50].

a) Linearity and Range

The standard stock solution was appropriately diluted and applied on an HPTLC plate to obtain linearity standard solutions containing Empagliflozin and Telmisartan in the concentration range of 50-350 ng/spot, respectively. Linearity was observed by plotting the graph of drug concentration (x) against peak area (y) for each standard. The standard deviation (SD), coefficient of determination (r^2), slope, and intercept of the calibration curves were estimated to determine the method's linearity. The regression equation showed that the method was linear over a wide range of concentrations. Interpretation of regression line validated the linearity and the data of the same are given in table 2. The linearity graph obtained for both the drugs is shown in Figures 3, 4. LOD and LOQ were calculated based on the standard deviation of the response and slope. The estimated values for LOD and LOQ for Empagliflozin is 16.21 ad 49.13 ng/ spot respectively. LOD and LOQ for Telmisartan are 10.54 and 31.96 ng/ spot respectively. This reflected the sensitivity of the method even at minimal concentrations.

Table 2: Linearity study data of Empagliflozin and Telmisartan

Concentration (ng/spot)	Empagliflozin							Telmisartan						
	50	**100**	**150**	**200**	**250**	**300**	**350**	**50**	**100**	**150**	**200**	**250**	**300**	**350**
Mean area	826	1037	1214	1362	1527	1699	1881	545.2	815	1149	1421	1748	2003	2304.2
% RSD	1.30	0.21	1.285	1.596	1.618	1.24	0.90	1.76	1.1	0.92	1.20	1.018	0.22	0.07
Linearity equation	y = 3.4292x + 678.43							y = 5.8949x + 247.66						
Correlation coefficient (r^2)	0.9983							0.9993						

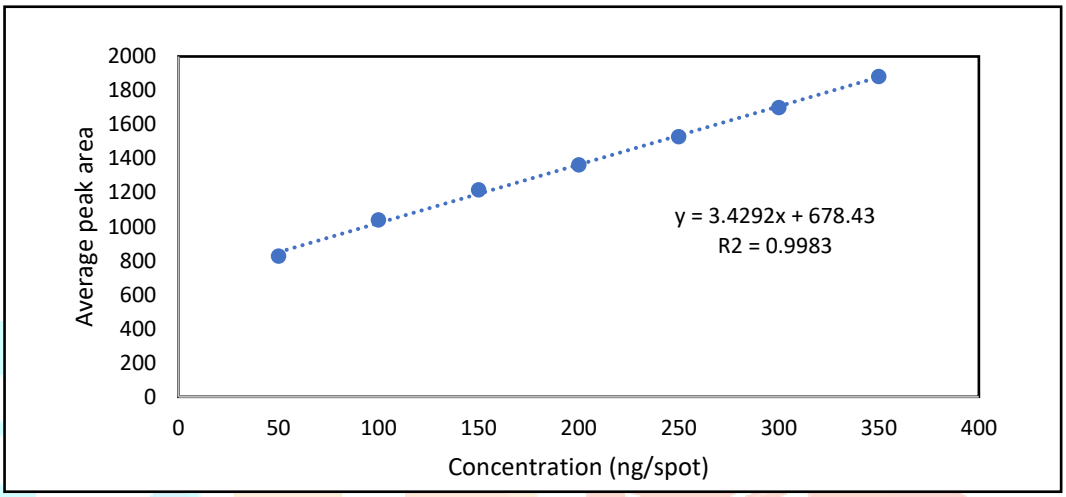

Figure 3: Representative calibration plot using linear regression method for Empagliflozin in the concentration range of 50-350 ng/spot

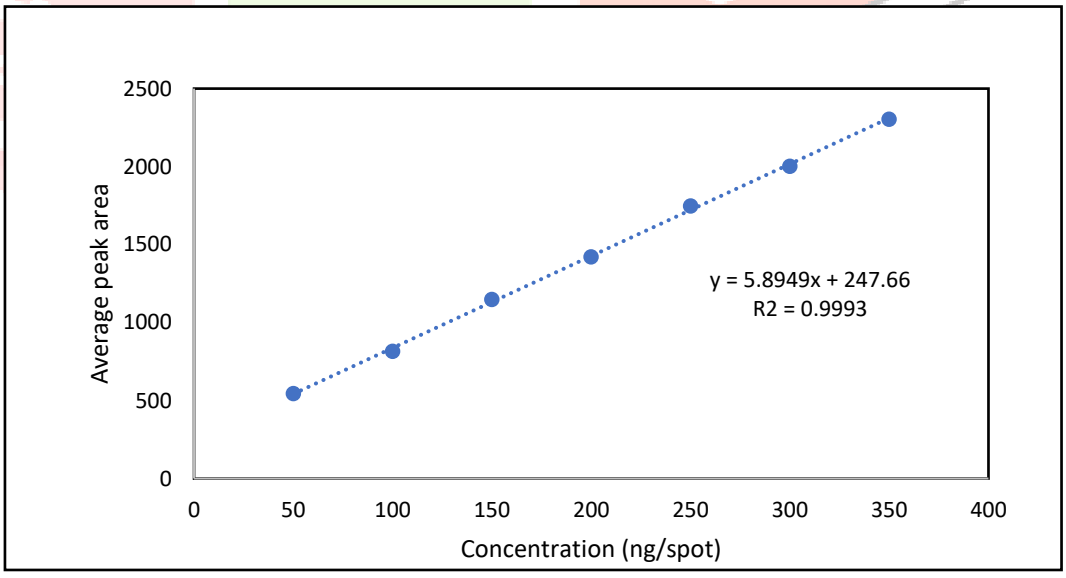

Figure 4: Representative calibration plot using linear regression method for Empagliflozin in the concentration range of 50-350 ng/spot

b) Selectivity

The selectivity of the method was investigated by analyzing six blank human plasma samples using the proposed protein precipitation procedure and HPTLC conditions. This was then compared with plasma spiked with Empagliflozin and Telmisartan at the concentration of their LLOQ in human plasma. As depicted in Figure 5, no extraneous peaks were observed in plasma at the R_f values of the drugs. Also, the peaks of Empagliflozin and Telmisartan were completely separated from each other. Thus, the method was found to be selective and specific for the analysis of Empagliflozin and Telmisartan in human plasma.

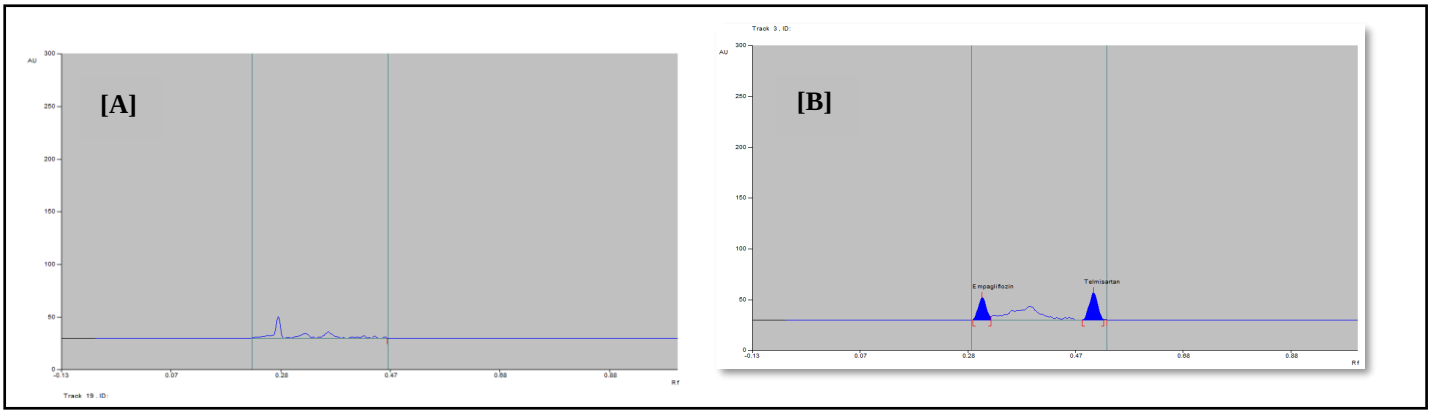

Figure 5: Densitogram of [A] blank plasma and [B] plasma spiked with Empagliflozin and Telmisartan at LLOQ level depicting the selectivity of the proposed method

c) Accuracy

The accuracy of the method was confirmed by conducting recovery studies at different concentrations of QC samples by triplicate analysis. Here the known amount of drug solution at different concentration levels of 80%, 100%, 120% was added to the QC samples, and recovery was calculated. The results summarized in table 3, indicated that the method enabled highly accurate simultaneous determination of Empagliflozin and Telmisartan from human plasma showing all the statistical results within the acceptable range associated with good recoveries at each added concentration.

Table 3: **Accuracy study data of Empagliflozin and Telmisartan**

	Empagliflozin			**Telmisartan**		
%Level	80	100	120	80	100	120
Initial amount (ng/spot)	100	100	100	100	100	100
Spiked amount (ng/spot)	80	100	120	80	100	120
Total amount (ng/spot)	180	200	220	180	200	220
Average area	1298.27	1363.36	1445.5	1311.7	1423	1549.96
Concentration found	180.75	199.73	223.68	180.5	199.38	220.92
Recovery (%)	100.41%	99.86%	101.67%	10020%	99.69%	100.41%

d) Precision

The precision of the method was determined by repeatability (intra-day) and intermediate precision (inter-day) for the set of QC samples including Lower Limit of Quantitation (LLOQ), LQC, MQC, HQC levels in triplicate. Repeatability or intra-day precision was investigated by analyzing three replicate QC samples of each drug at three different concentrations. Inter-day precision was assessed by analyzing the same QC samples over three days. The relative standard deviation (% RSD) of the obtained assay values at four different concentration levels was calculated. The resultant densitogram of quality control samples are given in Figure 6, 7, 8, 9 for LLOQ, LQC, MQC, HQC, respectively. The statistical data for the precision study are summarized in table 4. The results are summarized in Table 4 which were found in the range, thus confirming the method to be sufficiently precise.

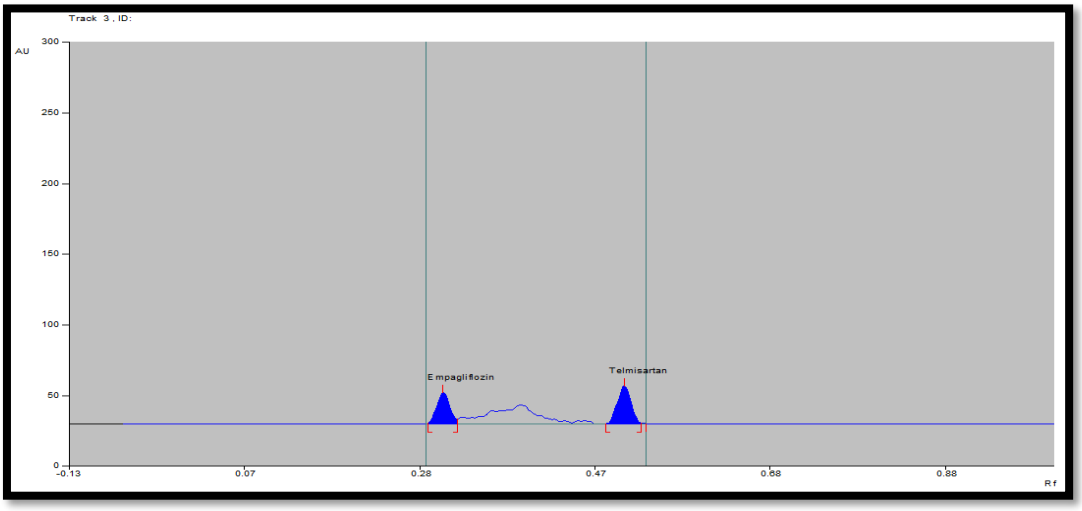

Figure 6: Densitogram of quality control sample at LLOQ (50 ng/spot) obtained using proposed bioanalytical method

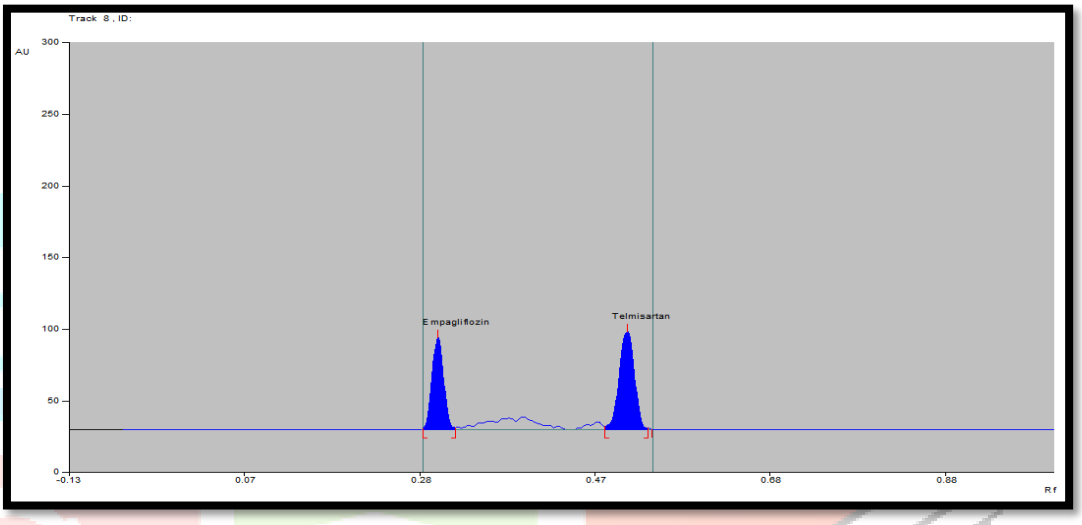

Figure 7: Densitogram of quality control sample at LQC (100 ng/spot) obtained using proposed bioanalytical method

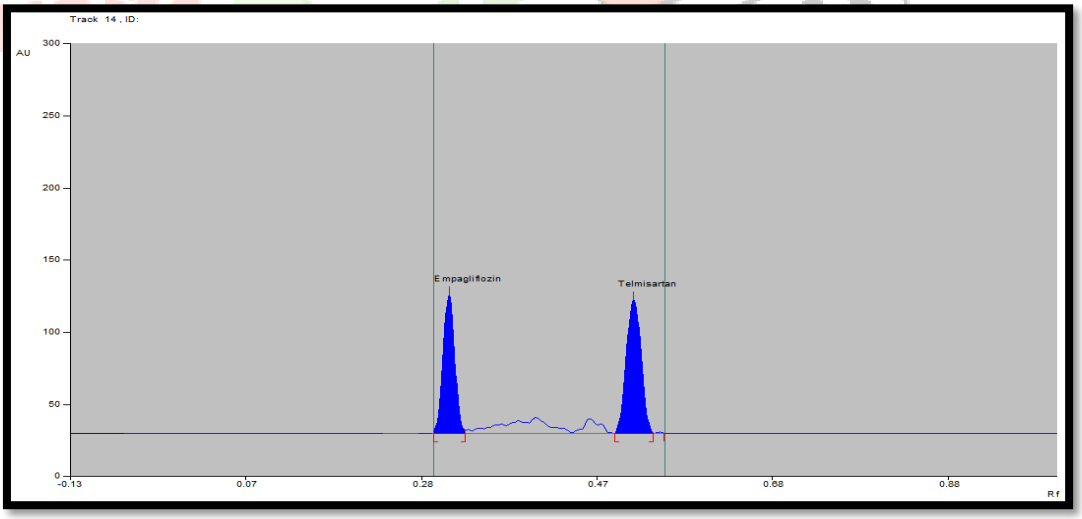

Figure 8: Densitogram of quality control sample at MQC (250 ng/spot) obtained using proposed bioanalytical

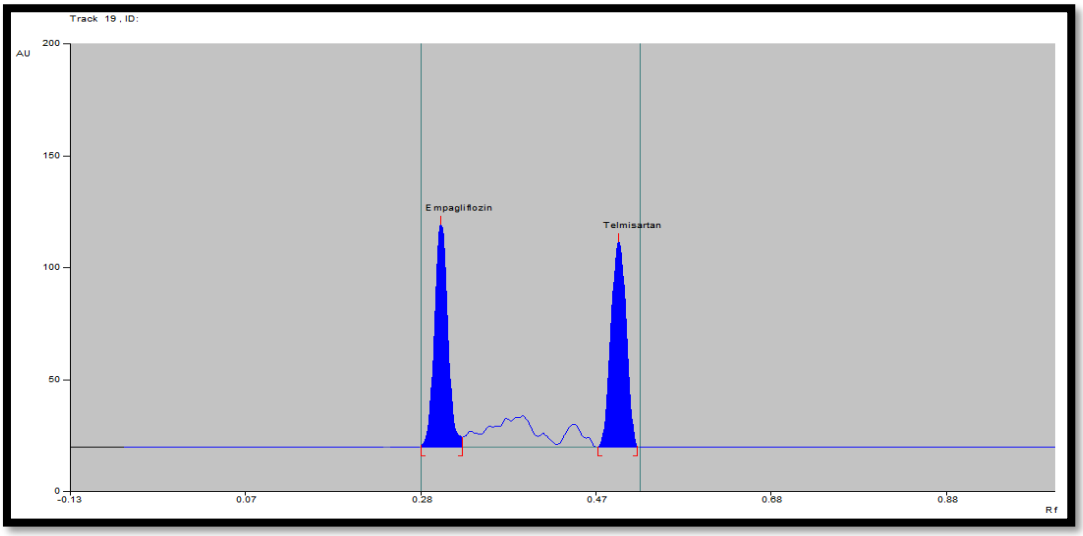

Figure 9: Densitogram of quality control sample at HQC (350 ng/spot) obtained using proposed bioanalytical method

Table 4: Precision study data of Empagliflozin and Telmisartan

		Concentration levels	Concentration (ng/spot)	Average peak area	Standard deviation	%RSD
Empagliflozin	**Intraday**	**LLOQ**	**50**	826.73	27.77	3.35
		LQC	**100**	1042.1	2.6620	0.2554
		MQC	**250**	1563.7	16.9038	1.0810
		HQC	**350**	1906.5667	7.23006	0.3792
	Inter-day	**LLOQ**	**50**	827.36	24.74	2.99
		LQC	**100**	1047.8667	11.0535	1.0548
		MQC	**250**	1561.5446	8.1190	0.5199
		HQC	**350**	1929.678	16.9550	0.8786
Telmisartan	**Intraday**	**LLOQ**	**50**	823.26	23.38	2.84
		LQC	**100**	834.6	1.8384	0.2202
		MQC	**250**	1781.9667	6.2983	0.3534
		HQC	**350**	2315.0334	5.6817	0.2454
	Inter-day	**LLOQ**	**50**	823.06	20.468	2.486
		LQC	**100**	835.112	4.5593	0.5459
		MQC	**250**	1771.82	12.2867	0.6934
		HQC	**350**	2314.90	6.2604	0.2704

e) Robustness

Robustness was studied by making small changes in chromatographic conditions such as changes in mobile phase composition (± 0.2), chamber saturation time (±2 min), detection wavelength (±2 nm), followed by its analysis in triplicate at LQC (100 ng/spot) and HQC (350 ng/spot) concentrations. The effects of these changes on peak area were evaluated by calculating % RSD. Table 5, depicts the data of robustness studies showing no significant deviation ensuring the robustness of the proposed method.

Table 5: Result of the robustness of Empagliflozin and Telmisartan

Method parameters	Level of variation	% RSD			
		Empagliflozin		Telmisartan	
		Low	High	Low	High
A] Mobile phase composition Chloroform: Methanol: Toluene: Formic acid (8: 2: 4: 0.1 v/v/v/v)	+0.2 to (volume of chloroform)	1.296	1.705	1.076	1.3042
	-0.2 of (volume of chloroform)	1.1004	2.3617	1.0062	1.4196
B] Saturation time (20 min)	+2	0.989	2.6042	1.4337	1.2531
	-2	1.8006	2.6696	1.1535	1.2741
C] Detection wavelength 235 nm	+2	0.7667	1.4514	1.6500	1.5361
	-2	1.294	0.1837	1.9284	1.7969

f) Recovery studies

Recovery was calculated by comparing the peak areas of freshly prepared and extracted LQC, MQC, and HQC samples of Empagliflozin and Telmisartan with the unextracted sample. The results of the same are shown in table 6, showing satisfactory recovery of both the drug ranging in 70-80%.

Table 6: Result of Recovery studies of Empagliflozin and Telmisartan

	Empagliflozin			Telmisartan		
QC sample	LQC	MQC	HQC	LQC	MQC	HQC
Mean peak area of standard solution	1294.3	1927.2	2419.9	1056.3	2148.9	2906.4
Mean peak area of Extracted solution	1037.7	1527.16	1881.56	815.06	1748.7	2304.2
Mean % recovery	80.1%	79.24	77.7%	77.16%	81%	79%

g) Stability studies

It is necessary to check the integrity of the drug solutions during storage and analysis. LQC and HQC were evaluated in triplicate for freeze-thaw, short-term (benchtop), and long-term stability studies. In the benchtop, stability samples were kept at room temperature for 6 hours. Freeze-thaw stability was done by analyzing the sample after three freeze-thaw cycles. For long-term stability, samples stored in the freezer for a period of 20 days were evaluated. The results represented in table 7 showed that the plasma samples containing Empagliflozin and Telmisartan can be handled under normal laboratory conditions without any significant stability issues.

Table 7: Result of Stability studies of Empagliflozin and Telmisartan

	Empagliflozin						Telmisartan					
	Short term stability		Freeze-thaw stability		Long term stability		Short term Stability		Freeze-thaw stability		Long-term stability	
	LQC	HQC	LQC	HQC	LQC	HQC	LQC	HQC	LQC	HQC	LQC	HQC
Mean	1015.2	1854.38	985.86	1768.85	974.4	1698.05	792.788	2296.48	727.35	2249.60	651.9	2033
SD	4.231	6.654	8.711	6.74	7.719	29.20	19.13	7.18	28.1	38.35	13.92	21.35
%RSD	0.41	0.35	0.88	0.381	0.79	1.719	2.41	0.31	3.865	1.7049	2.135	1.050

h) Carryover

Carry-over effects were assessed by injecting a blank plasma sample after a high concentration of a sample of calibration standard (HQC). This procedure was repeated three times to observe any carryover from the HQC level. Carryover in the blank plasma sample following the high concentration standard should not be greater than 20% of LLOQ. The representative densitogram of carry-over studies is shown in Figures 10, 11. The calculated value of carry-over for Empagliflozin and Telmisartan was found to be less than 20% of the area response of LLOQ, thus confirming the absence of any carryover from the previous sample.

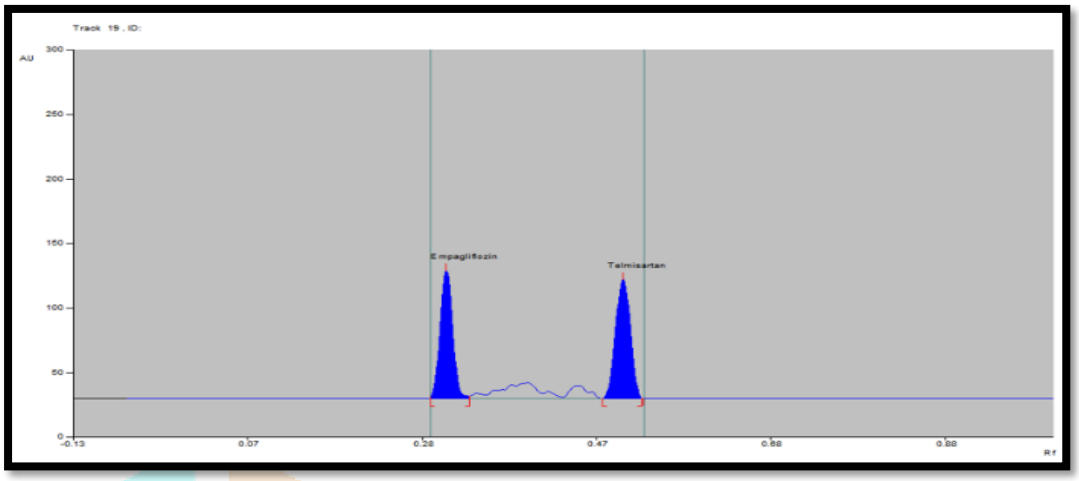

Figure 10: Densitogram of Empagliflozin and Telmisartan at concentration of HQC level

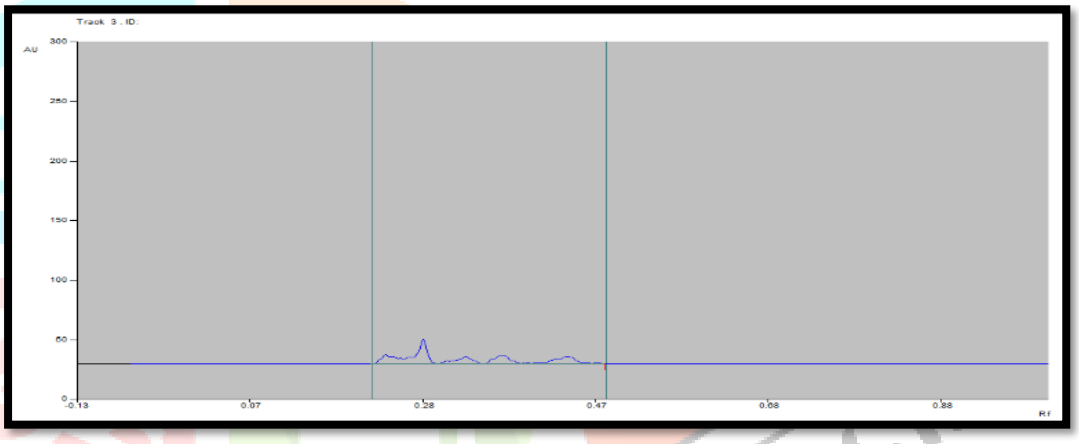

Figure 11: Densitogram of blank plasma sample applied after HQC depicting the carry over study

4. Conclusion

The proposed HPTLC method for estimation of Empagliflozin and Telmisartan in human plasma is economical, time-saving, and selective. The sensitivity of this method indicated its employability in the therapeutic drug monitoring of these two drugs in patients with the comorbid condition of hypertension and diabetes and thus can assist in pharmacokinetic and toxicokinetic studies.

5. Declaration

Funding and/or Conflicts of Interest/Competing interests

The authors did not receive support from any organization for the submitted work. All authors certify that they have no affiliations with or involvement in any organization for the submitted work. All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

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