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Original Article

DEVELOPMENT AND VALIDATION OF STABILITY-INDICATING RP-UPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF TEZACAFTOR AND IVACAFTOR IN FORMULATIONS

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ABSTRACT

Objective: Aim of the present research work was to develop a sensitive, rapid and accurate, stability-indicating RP-UPLC method for the simultaneous estimation of tezacaftor and ivacaftor in formulations.

Methods: The chromatographic separation of the mixture of tezacaftor and ivacaftor was attained in isocratic method utilizing a mobile phase of **0**.1 % orthophosphoric acid and acetonitrile in the proportion of 50:50%v/v utilizing a HSS C18 column which has dimensions of 100×2.1 mm, 1.7 µ particle size and the flow rate of 0.3 ml/min. The detection system was monitored at 292 nm wavelength maximum with 1.5 µl injection volume. The present method was validated as per the guidelines given by the ICH for specificity, accuracy, sensitivity, linearity and precision.

Results: The retaining time for tezacaftor and ivacaftor were achieved at 1.071 min and 0.530 min, respectively. Tezacaftor, ivacaftor and their combined drug formulation were exposed to thermal, acidic, oxidative, photolytic, and alkaline conditions. The developed method was highly sensitive, rapid, precise and accurate than the earlier reported methods. The total run time was decreased to 2.0 min; hence, the technique was more precise and economical. Stability studies directed for the suitability of the technique for degradation studies of tezacaftor and ivacaftor.

Conclusion: The projected method can be utilized for routine analysis in the quality control department in pharmaceutical trades.

Keywords: Tezacaftor, Ivacaftor, RP-UPLC, Stability studies, Validation

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INTRODUCTION

Tezacaftor (TZR) and ivacaftor (IVR) drugs were combined in a single dosage form (tablet) in the brand name of symdeko, used to treat cystic fibrosis (CF) in patients more than six years old having genetically specific mutations. A wide variety of cystic fibrosis transmembrane regulator (CFTR) mutations correlate to the CF-phenotype and are accompanied with different severity stages of the disease [1, 2]. The most common mutation, affecting approximately 70% of patients with CF worldwide, is known as F508del-CFTR or delta-F508 (ΔF508), in which a deletion in the amino acid phenylalanine at 508-position resulting in impaired production of protein CFTR, thereby producing a significant decrease in the quantity of ion transporter present on cell membranes. Ivacaftor as monotherapy has failed to show a benefit for patients with delta-F508 mutations, most likely due to an insufficient amount of protein available at the cell membrane for interaction and potentiation by the drug. CFTR correctors such as tezacaftor aim to repair F508del cellular misprocessing. This is done by modulating the position of the CFTR protein on the cell surface to the correct position, allowing for adequate ion channel formation and increased in water and salt movement through the cell membrane. The concomitant use of ivacaftor is intended to maintain an open channel, increasing the transport of chloride, reducing thick mucus production [3-5].

TZR chemically designated as 1-(2, 2-Difluoro-1, 3-benzodioxol-5–yl)–*N*-[1-[(2*R*)-2,3-dihydroxypropyl]-6–fluoro–2-(2-hydroxy-1, 1–dimethyl ethyl)-1*H*–indol–5-yl]–cyclopropanecarboxamide with molecular weight of 520.505 g/mol. IVR chemically designated as *N*-(2, 4-Di-*tert*–butyl-5-hydroxyphenyl)-4-oxo-1, 4-dihydroquinoline-3-carboxamide with molecular weight of 392.49g/mol (fig. 1) (Rowe and Verkman, 2012; Mohan, *et al.*, 2017). The literature review discloses that a very few UPLC [6-8] and high performance liquid chromatographic techniques [9-13] have been reported for the estimation of TZR and IVR. Based on the reported HPLC methods, there is a need to develop a rapid, sensitive reversed-phase UPLC method for simultaneous estimation of TZR and IVR in bulk and formulations.

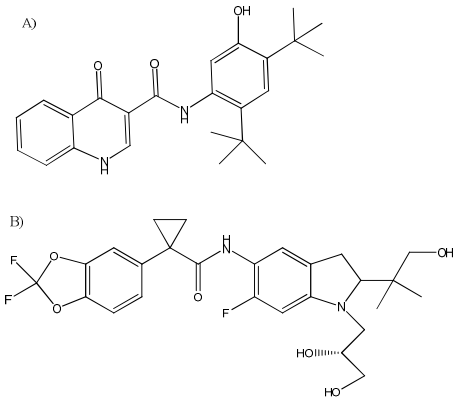

Fig. 1: Structures of A) ivacaftor and B) tezacaftor

MATERIALS AND METHODS

Chemicals and reagents

The standard components of TZR and IVR were provided as a gift sample from MSN Laboratories, Hyderabad, India. Symdeko tablets labeled to contain TZR 100 mg and IVR 150 mg were procured from the local market. HPLC grade acetonitrile was obtained from A. B enterprises, Mumbai, India. Orthophosphoric acid was bought from Ranchem, Mumbai, India. HPLC grade water was processed by utilizing Milli-Q Millipore water purification system used during the method development.

Liquid chromatography

Chromatographic system of Waters UPLC system furnished with photodiode array detector, auto-sampler, and HSS C18 column

which have dimensions of 100 × 2.1 mm, 1.7µ particle size. The output signal was monitored and integrated utilizing water Empower-2.0 software. The isocratic mobile consisting of 0.1% orthophosphoric acid and acetonitrile in the proportion of 50:50%v/v, pumped through the HSS C18 (100 × 2.1 mm, 1.7µ) column at a fixed flow of 0.3 ml/min. The injection volume of 1.5 µl was utilized to measure the chromatograms at 292 nm as the wavelength maximum in the detection system.

Preparation of buffer

To prepare 0.1% orthophosphoric acid buffer 1 ml of orthophosphoric acid was diluted to 1000 ml with HPLC grade water.

Preparation of stock and standard solutions

Accurately Weighed and transferred 25 mg of TZR and 37.5 mg of IVR working Standards into a 25 ml clean dry volumetric flask, add 3/4th volume of diluent (Water: ACN (50:50)), sonicated for 5 min and made up to the final volume with diluent to get 1000 µg/ml of TZR and 1500 µg/ml of IVR (stock solution). 1 ml of the resulting solution was transferred into a 10 ml volumetric flask and made up to 10 ml to get 100 µg/ml of TZR and 150 µg/ml of IVR.

Preparation of sample solution

20 tablets were weighed and calculated the average weight of tablets and then the weight equivalent to 1 tablet was transferred into a 100 ml volumetric flask containing 50 ml of diluent and sonicated for 25.0 min. Further, the volume made up with diluent and subjected for filtration by HPLC filters (1000 µg/ml of TZR and 1500 µg/ml of IVR). From the filtrate 1.0 ml solution was pipetted out into a 10.0 ml volumetric flask and made up to 10.0 ml with diluent to get 100 µg/ml of TZR and 150µg/ml.

Analytical method validation

The developed method for TZR and IVR was subjected for validation for the parameters like limit of detection (LOD), limit of quantification (LOQ), linearity, robustness, precision, system suitability and accuracy as per the guidelines of ICH [14-16].

RESULTS AND DISCUSSION

Optimized chromatographic conditions

After systematic trials with different mobile phase compositions and other parameters involved in the technique, HSS C18 100x 2.1 mm 1.7µ column, isocratic mobile consisting of 0.1% orthophosphoric acid and acetonitrile in the proportion of 50:50%v/v, column oven temperature of 30 °C with 1.5 ml injection volume, 0.3 ml/min flow rate and detection wavelength of 292 nm were optimized. Water and acetonitrile in the ratio of 50:50 %v/v was utilized as diluent.

Specificity

It is the ability of a method to unequivocally evaluate the analyte components in the presence of other components like impurities, degradants and excipients etc. expected to be present. This parameter was estimated by injecting and evaluating the blank, placebo, standard and sample solutions and chromatograms, respectively. Chromatograms of blank, placebo, and sample solution shown no peaks at the retaining time of TZR and IVR peaks. The chromatograms of TZR and IVR of standard, blank, formulation, and placebo were represented in fig. 2.

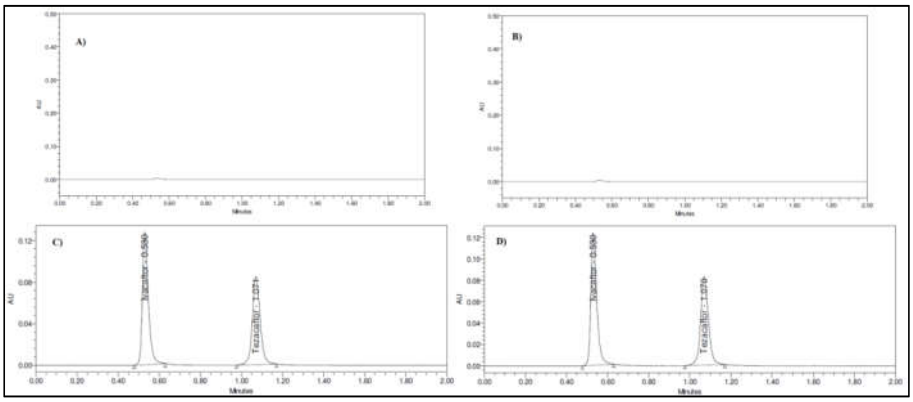

Fig. 2: Chromatograms of A) blank, B) placebo, C) standard and D) formulation

Linearity

Aliquots of 0.25, 0.50, 0.75, 1.0, 1.25, and 1.50 ml of standard stock solution were pipetted out from the standard stock solution of concentration 1000 µg/ml of TZR and 1500 µg/ml of IVR and made up to 10.0 ml mark with diluent. The resulting solutions were come into 25 to 150 µg/ml of TZR and 37.5 to 225 µg/ml of IVR concentration range. The resulting linearity solutions were infused into a chromatographic system and form the chromatograms linearity graph was plotted by taking the peak area on Y-axis and concentration on X-axis. The calibration graphs were shown in fig. 3, 4 and table 1, and all findings were within limits.

Table 1: Calibration curve data of TZR and IVR

TZR		**IVR**	
Concentration (µg/ml)	**Peak area**	**Concentration (µg/ml)**	**Peak area**
25	58564	37.5	81688
50	122099	75	164210
75	182333	112.5	245933
100	245412	150	324531
125	298585	187.5	403615
150	355250	225	484784
Regression equation			
y = 2384.4x+1488		y = 2151x+1552.3	
Correlation coefficient (R2)			
0.9994		0.9999	

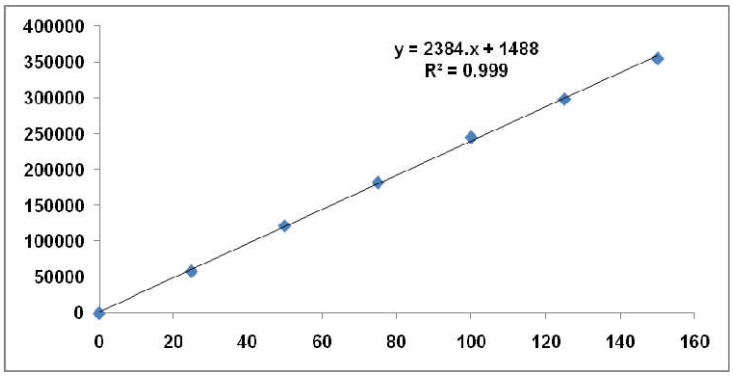

Fig. 3: Linearity of tezacaftor

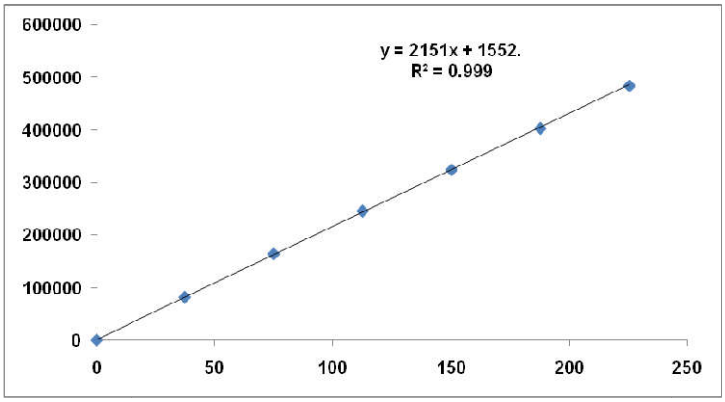

Fig. 4: Linearity of ivacaftor

System suitability

Six replicates of the standard reference solution were processed and infused to perform the system suitability parameter and the resulting chromatograms peak area, retention time, resolution, plate count, and tailing were measured. The findings of the system suitability parameter were shown in table 2 and related chromatograms were given in fig. 2(C).

Table 2: TZR and IVR system suitability results

S. No.	Peak name	Peak area	Retention time	Plate count	Resolution	Tailing
1	IVR	324491	0.531	2529	--	1.41
2	TZR	245595	1.072	4351	8.8	1.08

LOD and LOQ

LOD and LOQ parameters for TZR and IVR were calculated form the linear regression equation. Linearity values, graph and regression equation, were got from the linearity study and the LOD and LOQ values were represented in the table 3.

Precision

Analytical method precision is defined as the closeness of agreement between the replicate measurements of the analyte. It is expressed as the percentage coefficient of correlation or relative standard deviation (RSD) of the replicate measurements.

System precision

Working standard preparation of 1.5 µl solution was infused six times into the chromatographic system and chromatograms were obtained. %RSD of the peak area was calculated. The findings of system precision were shown in table 4.

Method precision

Working sample solutions of 1.5 µl were infused 6 times into the chromatographic system and chromatograms were obtained. The %RSD of the assay result of six preparations was determined. The findings achieved for assay were represented in table 5.

Table 3: Limit of detection and limit of quantification results

Parameter	Measured concentration (µg/ml)	
	TZR	IVR
LOD	0.41	0.47
LOQ	1.23	1.44

Table 4: System precision data

S. No.	Peak area response of drugs	
	TZR	IVR
1	245595	325617
2	246876	329213
3.	243399	325596
4	247294	324491
5	244267	324384
6	244682	326175
Average	245304	325913
STDV	1522.7	1761.1
% RSD	0.6	0.5

STDV: Standard deviation; RSD: Relative Standard deviation

Table 5: Method precision results

S. No.	Peak area response of drugs	
	TZR	IVR
1	249019	325135
2	243712	330729
3.	245330	328369
4	243060	323782
5	243574	323587
6	244391	323907
Average	244848	325918
STDV	2188.6	2959.9
% RSD	0.9	0.9

STDV: Standard deviation; RSD: Relative Standard deviation

Table 6: Intermediate precision results

S. No.	Peak area response of drugs	
	IVR	TZR
1	279082	230390
2	278896	232505
3.	286045	232781
4	282942	235868
5	284263	233126
6	286001	232951
Average	282872	232937
STDV	3224.0	1750.7
% RSD	1.1	0.8

STDV: Standard deviation; RSD: Relative Standard deviation

Table 7: Percentage recovery results

	IVR				TZR			
Spiked level	spiked (µg/ml)	recovery (µg/ml)	% recovery	Mean % recovery	spiked (µg/ml)	recovery (µg/ml)	% recovery	Mean % recovery
50%	50	50.53892	101.08	100.21	75	74.90	99.87	99.97
	50	50.04655	100.09		75	74.55	99.40	
	50	49.86412	99.73		75	74.89	99.86	
100%	100	100.4525	100.45		150	150.601	100.40	
	100	101.0942	101.09		150	150.1342	100.09	
	100	100.3124	100.31		150	148.3722	98.91	
150%	150	149.878	99.92		225	227.7316	101.21	
	150	149.6548	99.77		225	225.3397	100.15	
	150	149.2275	99.48		225	224.5833	99.81	

Intermediate precision

Working standard preparation of 1.5 µl was infused six times test preparations into the chromatographic system and chromatograms were obtained. The %RSD was evaluated for peak areas. The findings of intermediate precision study were represented in table 6.

Accuracy

A known amount of IVR and TZR at each three concentration levels of 50%, 100%, and 150% was added to a pre-analyzed sample solution and injected in triplicate at each level into the chromatographic system. The mean percentage recovery of IVR and TZR at each level was estimated. The findings were represented in tables 7.

Robustness

Working standard solution prepared as per test method was infused into the chromatographic system at variable conditions such as flow rate at±0.1 ml/min, mobile organic phase composition by±10%, and column temperature by±5 °C. The results of the robustness study parameter like peak area, retention time, plate count and tailing factor were within the limits.

Forced degradation studies

Acid degradation studies

To 1 ml of stock s solution IVR and TZR, 1 ml of 1N Hydrochloric acid were added and refluxed for 30 min at 60 °C. The resultant solution

was diluted to obtain 100 µg/ml of TZR and 150 µg/ml of IVR solution and 1.5 µl solution was injected into the chromatographic system and the chromatograms were recorded to assess the stability of sample (fig. 5 and table 8) [17, 18].

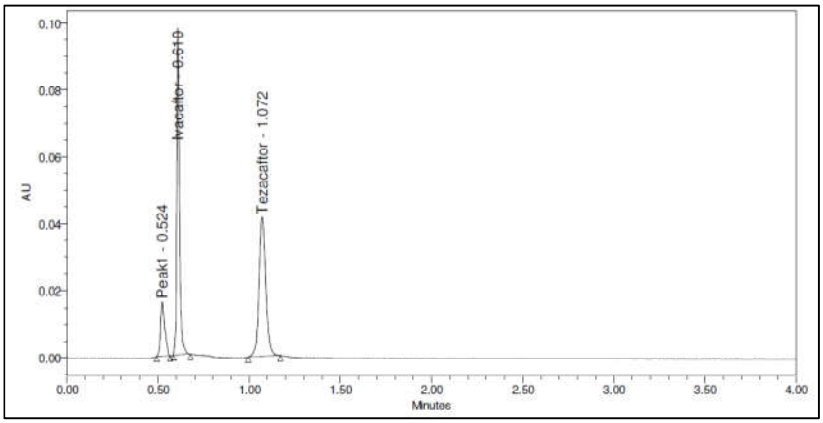

Fig. 5: Chromatogram for acid degradation study

Table 8: Results of stress degradation study

S. No.	Degradation condition	TZR		IVR	
		% recovery	% Degraded	% recovery	% Degraded
1	Acid hydrolysis	92.16	7.84	91.59	8.41
2	Base hydrolysis	93.56	6.44	92.82	7.18
3	Peroxide	93.19	6.81	94.82	5.18
4	Dry heat	97.98	2.02	96.60	3.40
5	Photostability	98.46	1.54	97.88	2.12
6	Water sample	99.51	0.49	99.14	0.86

Oxidation

To 1 ml of stock solution of VXR, SFR and VLR, 1 ml of 10% hydrogen peroxide (H_2O_2) was added separately. The solutions were kept for 30 min at 60 °C. For UPLC study, the resultant solution was diluted to obtain 100 µg/ml of TZR and 150 µg/ml of IVR solution and 1.5 µl solution was injected into the chromatographic system and the chromatograms were recorded to assess the stability of sample (fig. 6 and table 8).

Alkali degradation studies

To 1 ml of stock solution VXR, SFR and VLR, 1 ml of 1N sodium hydroxide were added and refluxed for 30 min at 60 °C. The resultant solution was diluted to obtain 100 µg/ml of TZR and 150µg/ml of IVR solution and 1.5 µl solution was injected into the chromatographic system and the chromatograms were recorded to assess the stability of sample (fig. 7 and table 8) [18, 19].

Dry heat degradation studies

The standard drug solution was placed in an oven at 105 °C for 6 h to study dry heat degradation [20]. For UPLC study, the resultant solution was diluted obtain 100 µg/ml of TZR and 150 µg/ml of IVR solution and 1.5 µl solution was injected into the chromatographic system and the chromatograms were recorded to assess the stability of sample (fig. 8 and table 8).

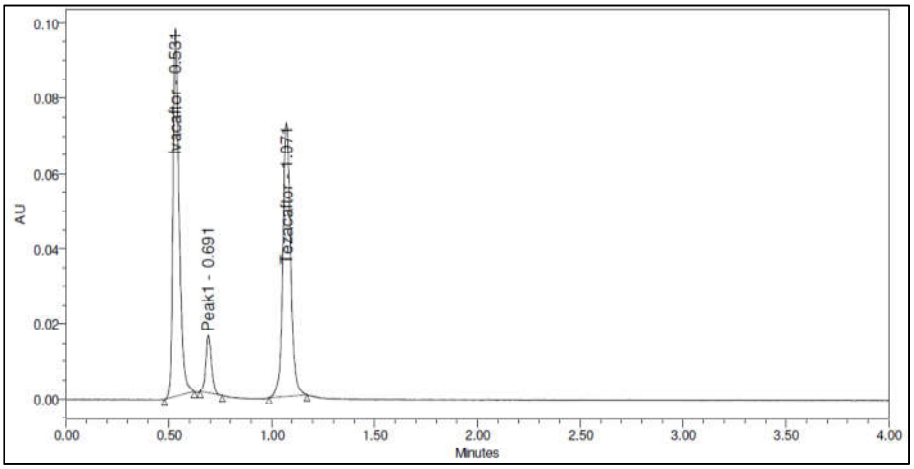

Fig. 6: Chromatogram for oxidation degradation study

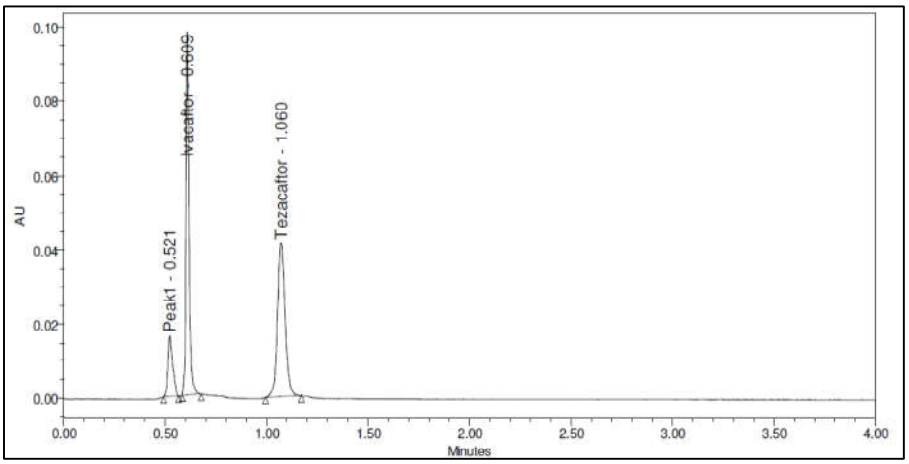

Fig. 7: Chromatogram for alkali degradation study

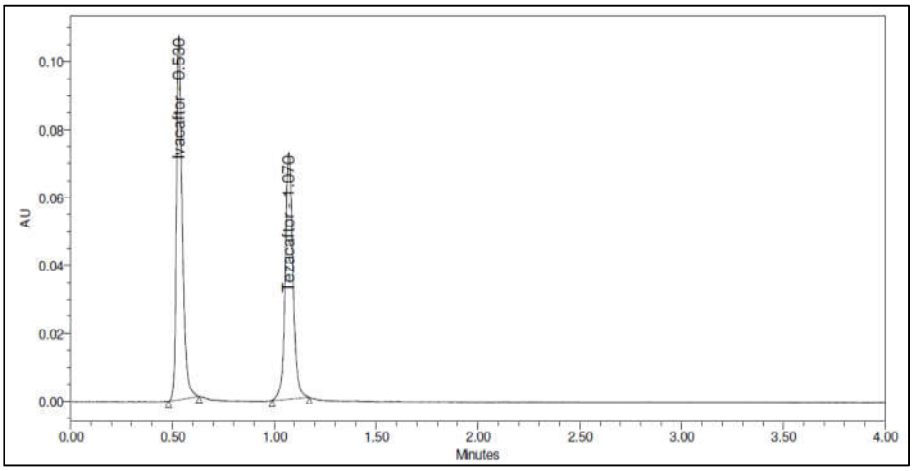

Fig. 8: Chromatogram for dry heat degradation study

Photostability studies

The photochemical stability of the drug was also studied by exposing the (100 µg/ml, 400 µg/ml and 100 µg/ml) solution to UV Light by keeping the beaker in UV Chamber for 7days or 200 Watt-hours/m2 in photostability chamber [21]. For UPLC study, the resultant solution was diluted to obtain 100 µg/ml of TZR and 150 µg/ml of IVR solution and 1.5 µl solution was injected into the chromatographic system and the chromatograms were recorded to assess the stability of sample (fig. 9 and table 8).

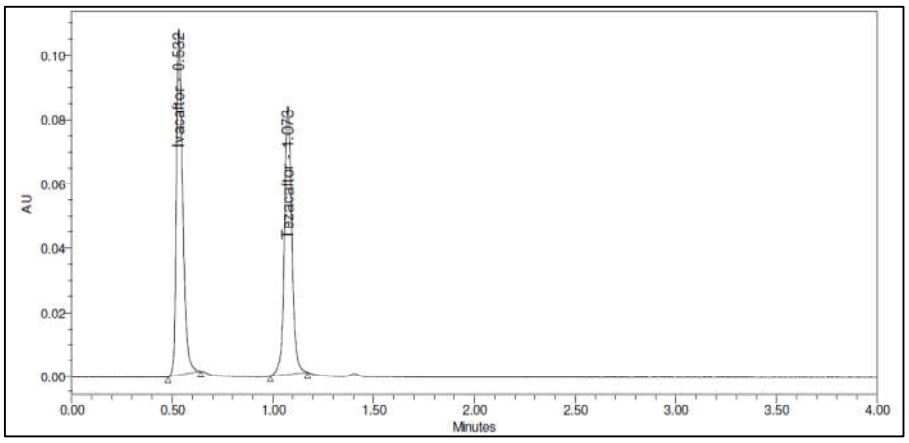

Fig. 9: Chromatogram for photostability study

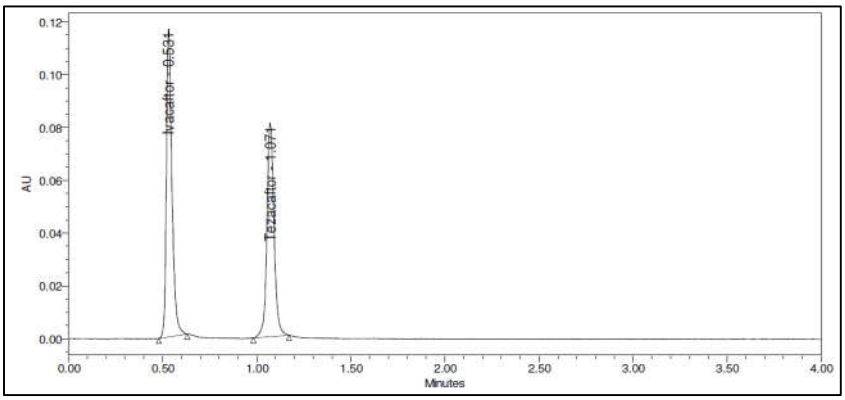

Fig. 10: Chromatogram for neutral degradation study

Neutral degradation studies

Stress testing under neutral conditions was studied by refluxing the drug in water for 6 h at a temperature of 60 °C. For UPLC study, the resultant solution was diluted to obtain 100 µg/ml of TZR and 150 µg/ml of IVR solution and 1.5 µl solution was injected into the chromatographic system and the chromatograms were recorded to assess the stability of sample (fig. 10 and table 8).

Assay of marketed formulation

The marketed formulation of Symdeko (tablet) was evaluated by infusing 1.5 µl of reference and analyte solutions six times into the chromatographic system and the resulting chromatograms of analytes were documented [22]. The quantity of anaytes existed in the marketed formulation was estimated by equating the peak area of reference and analyte. The % assay of TZR and IVR was found to be 99.0–101.0%.

CONCLUSION

A sensitive, rapid and accurate, stability-indicating RP-UPLC method for the simultaneous estimation of TZR and IVR in formulations was developed and validated as per the ICH guidelines. Retention times for TZR and IVR were achieved at 1.071 min and 0.530 min, respectively. Mean percentage recovery of TZR and IVR was found to be 100.21% and 99.97%, respectively. LOD and LOQ values obtained from regression equations of TZR and IVR and were found to be 0.41 µg/ml/1.23 µg/ml and 0.47 µg/ml/1.44 µg/ml. Regression equation of TZR and IVR were: y = 2384.4x+1488, y = 2151x+1552.3 respectively. Stability studies of these drugs proven that the percentage of degradation of analytes were found in between 0.49% to 8.41%. Retention time and total run times of analytes were decreased. Hence, the developed method was rapid and economical that can be applicable in routine analysis of these drugs in quality control department of pharmaceutical trades.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

Lakshmi Maneka performed experiments, analysed data and co-wrote the paper. Anjana performed experiments. Saravanakumar designed and drafted the article.

CONFLICTS OF INTERESTS

The authors declare that there are no conflicts of interest regarding the publication of the paper.

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