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ORIGINAL ARTICLE

Development and Validation for the Quantification of Ivacaftor and Tezacaftor by RP-UPLC

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ABSTRACT
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 1.251 min and 0.543 min respectively. Mean percentage recovery of TZR and IVR were 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 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.
Keywords: Ivacaftor, Tezacaftor, RP-UPLC, Validation, Stability.

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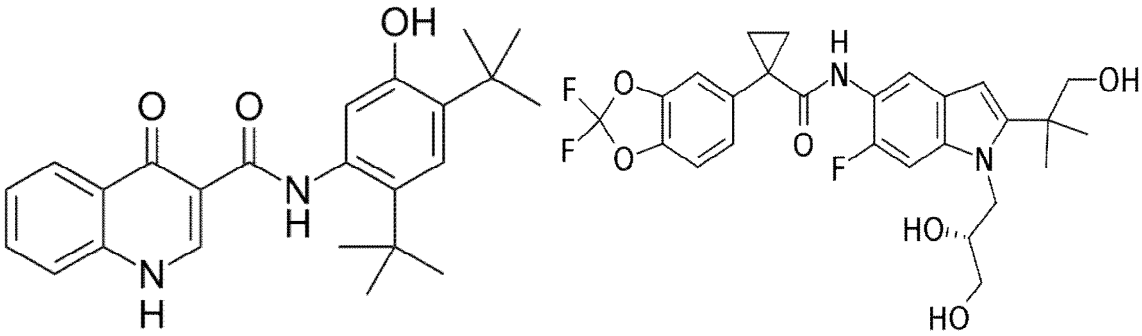
INTRODUCTION

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 CFphenotype 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). 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.



A **B**

Fig. 1: Structure of A) Tezacaftor B) Ivacaftor.

MATERIAL 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 100mg and IVR 150mg 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.

Method development

During the method development various mobile phase compositions consisting of methanol, acetonitrile, water, phosphate buffers and different stationary phases were executed to get fine chromatographic conditions like theoretical plates, resolution, tailing and peak shape.

Optimized conditions

Chromatographic system of Waters UPLC system furnished with photodiode array detector, auto-sampler, and zorbax C18 column which have dimensions of 100 × 2.1mm, 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 40:60%v/v, pumped through the zorbax C18 (100 × 2.1mm, 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 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 25mg of TZR and 37.5mg 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 minutes 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[14-19].

RESULTS AND DISCUSSION

Method development

With different mobile phase compositions and stationary phases two different trials were executed and third trail was optimized. In all the two trials there merged peaks were observed in trail-1 and peak shape was poor and tailing in the trail-2. Optimized chromatographic peaks were shown in Fig. 2.

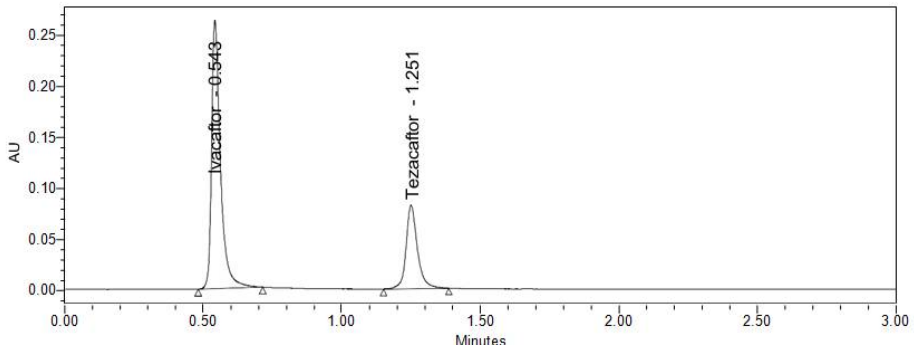


Fig. 2: Optimized Chromatogram.

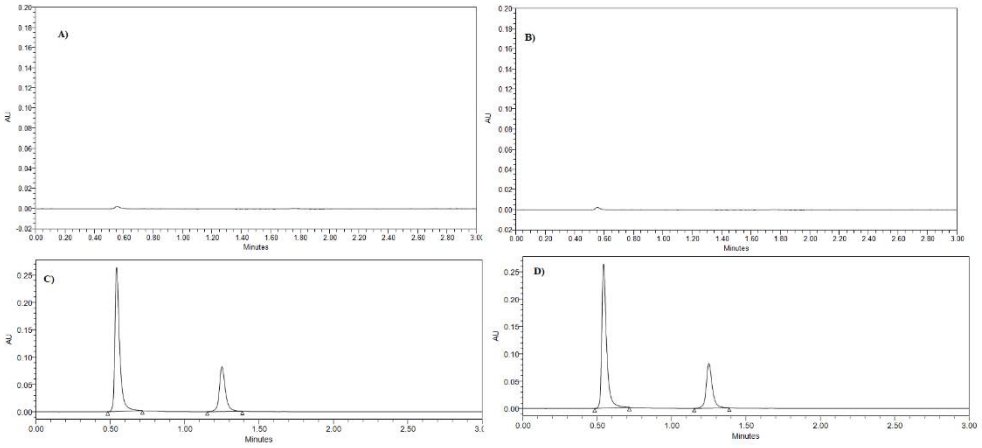


Fig. 3: Chromatograms of A) blank, B) placebo, C) standard and D) formulation

Specificity

It is the ability of a method to unequivocally evaluate the analyte components in 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 [16] 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. 3.

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 [17]. 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. 4, 5. and Table. 1, and all findings were within the 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	

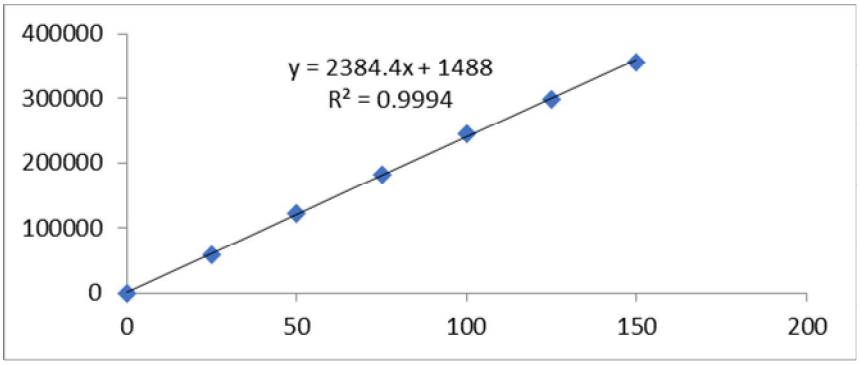


Fig. 4: Linearity of tezacaftor

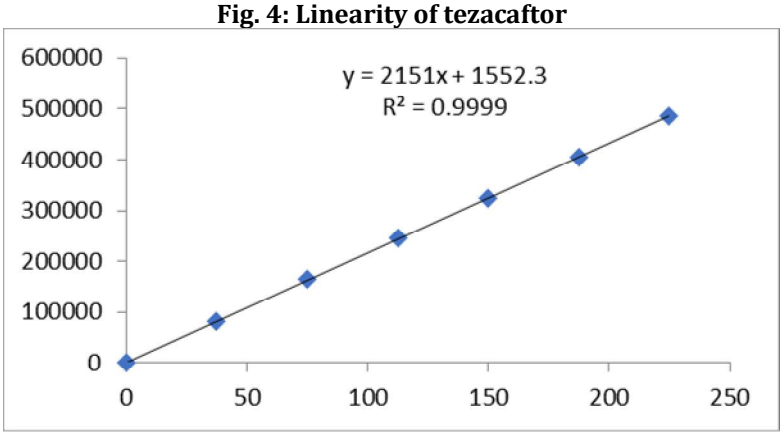


Fig. 5: Linearity of ivacaftor

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 system suitability parameter were shown in the Table. 2.

Table. 2: TZR and IVR system suitability results

S No	Peak name	Peak area	Retention time	Plate count	Resolution	Tailing
1	IVR	324491	0.543	2529	--	1.41
2	TZR	245595	1.251	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.

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

Precision

Analytical method precision is defined as 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[11-14].

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.

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

Method precision

Working sample solutions of 1.5 µL was 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. 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

Intermediate precision

Working standard preparation of 1.5 µL was infused six times test preparations into the chromatographic system and chromatograms were obtained[11]. The %RSD was evaluated for peak areas. The findings of intermediate precision study were represented in Table. 6.

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

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[12-15]. The mean percentage recovery of IVR and TZR at each level was estimated. The findings were represented in Tables.7.

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	

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 [15-18] composition by ±10%, and column temperature by ±5°C. The results of 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 was added and refluxed for 30mins at 60°C[17-19]. 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).

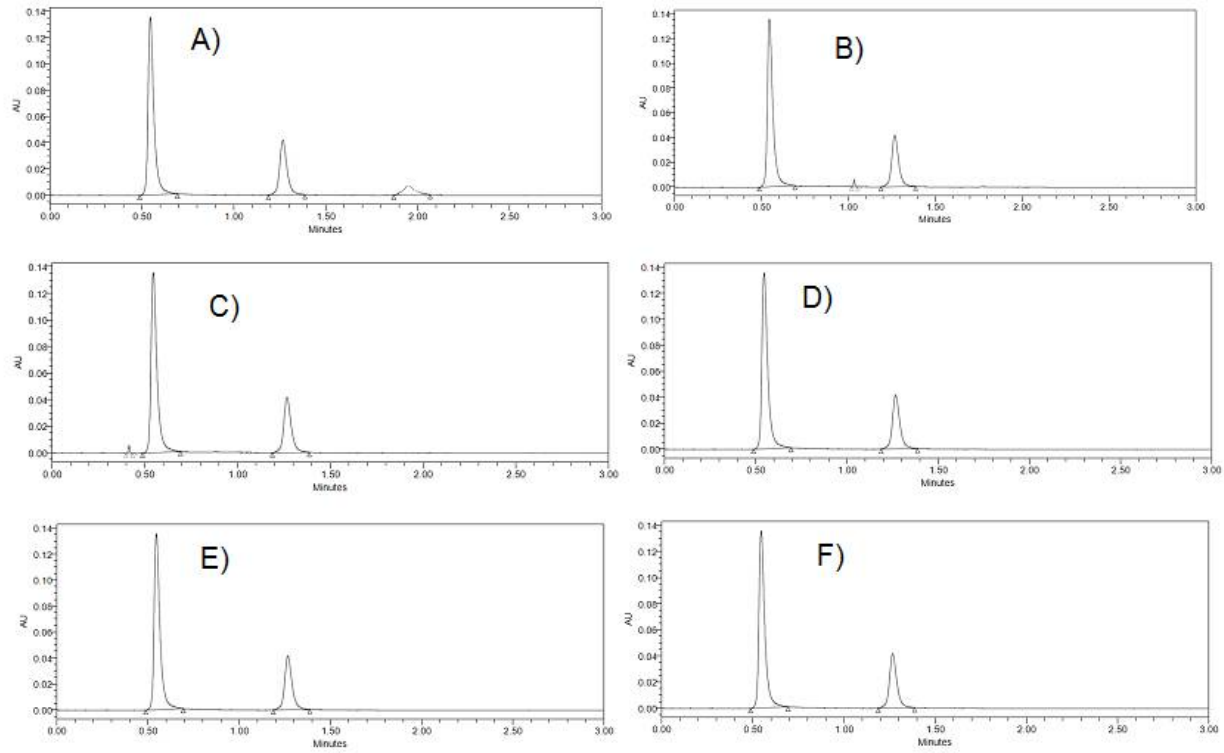


Fig. 6: Chromatogram for acid degradation study

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	Photo stability	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 was added and refluxed for 30mins at 60°C. The resultant solution was diluted to obtain 100 µg/ml of TZR and 150µg/ml of IVR solution and 1.5µL solution was injected into the chromatographic system and the chromatograms were recorded to assess the stability of sample (Fig. 6 and Table. 8).

Dry heat degradation studies

The standard drug solution was placed in oven at 105°C for 6 h to study dry heat degradation. 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. 6 and Table. 8).

Photo stability 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 photo stability chamber. 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).

Neutral degradation studies

Stress testing under neutral conditions was studied by refluxing the drug in water for 6hrs 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. 6 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. The quantity of anaytes existed in marketed formulation was estimated by equating the peak area of reference and analyte. The % assay of TZR and IVR were 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 1.251 min and 0.543 min respectively. Mean percentage recovery of TZR and IVR were 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 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.

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