

## DEVELOPMENT OF HPLC STABILITY DEMONSTRATING METHODOLOGY FOR QUANTIFYING AZELNIDIPINE AND TELMISARTAN IN TABLETS AND BULK TYPES: VALIDATION FOLLOWING ICH DIRECTIVES

**KRISHNAPHANISRI PONNEKANTI<sup>1</sup>, K. SUNITHA<sup>1</sup>**

<sup>1</sup>Department of Pharmacy, GITAM Institute of Pharmacy, GITAM Deemed to be University, Visakhapatnam, India 530045  
 Email: krishnaphanisri@gmail.com

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### ABSTRACT

**Objective:** Azelnidipine (AZEL) and Telmisartan (TELM) combination is referred to the sufferers of hypertension. No analytical process has yet been mentioned for the TELM and AZEL combination analysis. We, therefore, have designed for its first time stability demonstrating methodology based on HPLC for analysing TELM and AZEL in the tablets and bulk.

**Methods:** The assay of TELM and AZEL was get done on a 250 mm length C18 column (Supelco, 4.6 mm inner diameter, 5.0  $\mu$ m particle size), and utilized 0.1M Na<sub>2</sub>SO<sub>4</sub> (pH 3.6) and acetonitrile (55% volume: 45% volume) as the mobile solvents phase, at a stream rate 1.0 ml/min. HPLC recognition of TELM and AZEL was taken by a photodiode array sensor set at 258 nm. For validation of the stability demonstrating methodology proposed in terms of sensitivity, precision, specificity, linearity, device adequacy, robustness and accuracy, ICH directives were followed.

**Results:** Calibration curves of TELM and AZEL were generated in the array of 20-60  $\mu$ g/ml and 4-12  $\mu$ g/ml with recovery percentage ranges of 99.62%-101.05% and 97.76%-100.17%, and detection limits of 0.020  $\mu$ g/ml and 0.009  $\mu$ g/ml, respectively. TELM and AZEL stability was inspected in the existence of acid, base, light, heat, and oxidation and it was realised to be more stable under oxidation degradation testing conditions employed when compared to acid, alkaline, photo, and heat degradation testing conditions applied.

**Conclusion:** The observations demonstrated that the described HPLC stability demonstrating methodology was suitable for quantitating TELM and AZEL combination in tablets and bulk.

**Keywords:** Azelnidipine, Telmisartan, Hypertension, Stability demonstrating, Analysis

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### INTRODUCTION

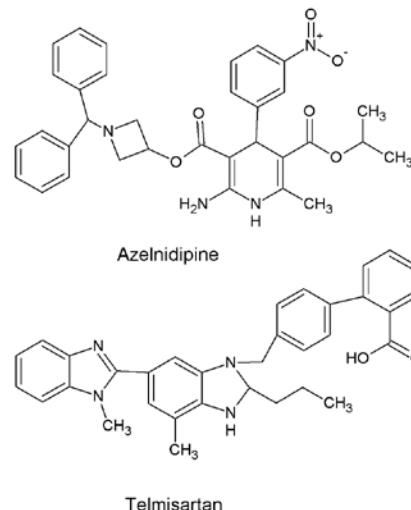
Hypertension is the extremely avoidable contributing factor for cardiovascular impairments namely coronary cardiac disease, heart attack, heart stroke, atrial fibrillation, pulmonary artery disease and myocardial infarction, progressive renal disease and cognitive decline [1]. Hypertension is the foremost single contributor to the world's leading basis of demise and disability. In 2010 it is valued that 1.39 billion (31.1%) of adults globally be ill with hypertension. In middle and small revenue nations (1.04 billion people and 31.5%), the occurrence of hypertension among adults was elevated than in the high revenue nations (349 million individuals and 28.5%) [2].

Azelnidipine (AZEL), structure in fig. 1, is a modern dihydropyridine calcium passage antagonist that is particular for the L-kind calcium passages and has received FDA authorization for therapy of the hypertension clients [3]. Antihypertensive outcomes of AZEL are equivalent to that of amlodipine [4]. AZEL is indeed increasingly lipid-soluble and has greater selectivity for the vascular surface than older generational calcium passage antagonists, and in animal experiments treated with AZEL, blood flowing to the brain was markedly enhanced [5].

Telmisartan (TELM), structure in fig. 1, is an angiotensin (II) receptor blockade being used to alleviate minimal to severe hypertension [6-8]. TELM is extremely selective for the type 1 angiotensin (II) receptors. TELM is an extra lipophilic angiotensin (II) receptor blocker than utmost other angiotensin (II) receptor inhibitors, which aids its oral uptake and tissue as well as cell permeation [9]. TELM too has an impact on the peroxisome proliferator triggered receptors, which are nuclear hormone receptor superfamily ligand triggered transcription elements. TELM, a drug that treats both diabetes and hypertension, may well be an alternative therapy choice [10, 11].

The combination of TELM and AZEL is recommended for hypertension sufferers. In hypertension sufferers, TELM and AZEL

combination drops blood pressure, enables blood to flow more effectively to numerous tissues, allows the heart to operate more effectively, and increases oxygen flow across the body, and thereby reducing heart-associated chest pain [12].



**Fig. 1: Azelnidipine (AZEL) and Telmisartan (TELM) structures**

Stress tests must be implemented on a medication compound to ascertain its basic stability properties, bestowing to the "ICH Q1A (R2)" parent drug permanency evaluation recommendations [13]. Understanding how a drug substance's consistency varies with times and the nature of deterioration products generated under different

storage circumstances affects the potency and wellbeing of pharmaceuticals is critical [14-16]. Stability demonstrating methods are a group of analytical methodologies that demonstrate the sample stability and must be completely validated. No Stability demonstrating method has yet been recommended for the TELM and AZEL combination. We developed, for the initial time, stability demonstrating methodology for TELM and AZEL combination in tablets and bulk in this report. The approach developed is indeed dependent on reverse-phase liquid chromatography with a photodiode sensor, that has been configured for the investigation of the TELM and AZEL combination in a fast, precise, and sensitive manner.

## MATERIALS AND METHODS

### Instrumentation and analysing conditions of TELM and AZEL combination

High-performance liquid chromatography (Waters 2695 model HPLC system, Autosampler) accompanying with photodiode detector (2998 model) was used in the procedure development and combination analysis of TELM and AZEL. The very fine particles loaded 250mm length C18 column (Supelco, 4.6mm inner diameter, 5.0  $\mu$ m particle size) was utilised for TELM and AZEL chromatographic separation. Flow of 1 ml/min, utilised for the TELM and AZEL chromatographic separation. The mobile phase components of 0.1M Na<sub>2</sub>SO<sub>4</sub> (pH 3.6) and acetonitrile (55% volume: 45% volume) was used. The complete process was completed at room temperature. To every sample, the specimen (10  $\mu$ l) was chosen as the injection quantity. The quantitation of TELM and AZEL was done at 258 nm.

### Chemicals

"SD Fine chem Ltd (India)" supplied analytical mark Na<sub>2</sub>SO<sub>4</sub>, peroxide and NaOH; "Merck (India)" supplied analytical mark HCl, H<sub>3</sub>PO<sub>4</sub> and HPLC mark acetonitrile; Milli Q System base prepared Milli Q water was employed in the combined analysis of TELM and AZEL.

### Reference drugs and tablets

"Rainbow pharma training laboratories (India)" provided TELM and AZEL reference bulk samples. TELMA-AZ tablet type (Glenmark Pharmaceutical Ltd, India) was bought from a local shop drug retailer and claimed to comprise 8 mg of AZEL and 40 mg of TELM.

### TEL M and AZEL combination solutions

A stock TELM and AZEL combination solution (concentration: 400  $\mu$ g/ml TELM and 80  $\mu$ g/ml AZEL) was made by solubilizing 40 mg of TELM and 8 mg of AZEL in 25 ml of mobile phase and then diluting to 100 ml volume with the very similar solvent. A working TELM and AZEL combination solution (concentration: 40  $\mu$ g/ml TELM and 8  $\mu$ g/ml AZEL) was made by solubilizing 1 ml of stock TELM and AZEL combination solution in 3 ml of mobile phase and then diluting to 10 ml volume with the very similar solvent.

### Procedure for evaluating the TELM and AZEL combination in bulk

Different aliquots covering 20  $\mu$ g/ml–60  $\mu$ g/ml of TELM and 4.0  $\mu$ g/ml–12.0  $\mu$ g/ml of AZEL were correctly shifted from stock TELM and AZEL combination solution (concentration: 400  $\mu$ g/ml TELM and 80  $\mu$ g/ml AZEL) into separate sets of 10 ml volumetric flasks, and then diluting to 10 ml volume with the very similar solvent. 10  $\mu$ l volume of each solution were infused into 250mm length C18 column (Supelco, 4.6mm inner diameter, 5.0  $\mu$ m particle size) and evaluated with "stability-indicating HPLC methodology" conditions given (see section: Instrumentation and analysing conditions of TELM and AZEL combination). The peak response values of TELM and AZEL at 258 nm were recorded and afterward calibration curves of TELM and AZEL were drawn followed by calculating regression equations of TELM and AZEL. The concentration of TELM and AZEL in nameless specimen solution can be assessed by exploiting the calibration curves of TELM and AZEL or regression equations of TELM and AZEL, respectively.

### Procedure for evaluating the TELM and AZEL combination in tablets

A total of 20 tablets (TELMA-AZ tablet, claimed to comprise 8 mg of AZEL and 40 mg of TELM) were collected, with the median weight

estimated and mashed to a fine powder. Dose comparable to 40 mg of TELM and 8 mg of AZEL was shifted to a volumetric flask (100 ml), blended with 10 ml of mobile and stirred with ultra sonicator at 27 °C for 20 min, diluted with similar solvent up to 100 ml mark and filtered via a filter membrane (0.45 m). This tablet stock solution has 400  $\mu$ g/ml TELM and 80  $\mu$ g/ml AZEL. A sample tablet TELM and AZEL combination solution (theoretical concentration: 40  $\mu$ g/ml TELM and 8  $\mu$ g/ml AZEL) for the analysis was made by solubilizing 1 ml of tablet stock TELM and AZEL combination solution in 3 ml of mobile phase and then diluting to 10 ml volume with the very similar solvent. 10  $\mu$ l volume of sample tablet TELM and AZEL combination solution were infused into 250mm length C18 column (Supelco, 4.6 mm inner diameter, 5.0  $\mu$ m particle size) and evaluated with "stability-indicating HPLC methodology" conditions specified (see section: Instrumentation and analysing conditions of TELM and AZEL combination). At 258 nm, the peak response values of TELM and AZEL were measured. The amount of TELM and AZEL in the tablet specimen solution can be established by employing TELM and AZEL calibration curves or regression equations, respectively.

### Stability testing of TELM and AZEL

The stability of TELM and AZEL should be assessed by a stress testing stability analysis. This research illustrates the stability of TELM and AZEL in the presence of acid, base, light, heat, and oxidation [13].

### Acid degradation testing

The investigation was done out using 0.1N hydrochloric acid (10 ml). The TELM and AZEL stock tablet solution (400  $\mu$ g/ml TELM and 80  $\mu$ g/ml AZEL) was combined in an equivalent volume proportion with acid and blended into the volumetric flask (100 ml). The blend was stirred for 30 min at 27 °C using a ultra sonicator. The sample was mixed with mobile phase up to 100 ml mark and filtered via a filter membrane (0.45 m) before being inserted (10  $\mu$ l) into the HPLC instrument for TELM and AZEL analysis with "stability-indicating HPLC methodology" conditions specified (see section: Instrumentation and analysing conditions of TELM and AZEL combination).

### Oxidation degradation testing

The oxidation degradation testing of TELM and AZEL was worked out with peroxide (30%, 10 ml) as the oxidising agent. In a similar quantity volume, the stock TELM and AZEL tablet solution (400  $\mu$ g/ml TELM and 80  $\mu$ g/ml AZEL) is blended with peroxide. The sample was stirred with ultra sonicator at 27 °C for 30 min, diluted with mobile phase up to 100 ml mark and filtered via a filter membrane (0.45 m). The sample (10  $\mu$ l) was inserted into HPLC machine and the TELM and AZEL content were established with "stability-indicating HPLC methodology" conditions specified (see section: Instrumentation and analysing conditions of TELM and AZEL combination).

### Alkaline degradation testing

The investigation was done out using 0.1N sodium hydroxide (10 ml). The tablet TELM and AZEL stock solution (400  $\mu$ g/ml TELM and 80  $\mu$ g/ml AZEL) was combined in an equivalent volume proportion with alkaline and blended into the volumetric flask (100 ml). The blend was stirred for 30 min at 27 °C using a ultra sonicator. The sample was mixed with mobile phase up to 100 ml mark and filtered via a filter membrane (0.45 m) before being inserted into the HPLC instrument for TELM and AZEL analysis with "stability-indicating HPLC methodology" conditions specified (see section: Instrumentation and analysing conditions of TELM and AZEL combination).

### Photodegradation testing

For this investigation, the stock solution of TELM (400  $\mu$ g/ml) and AZEL (80  $\mu$ g/ml) was placed into the volumetric flask (100 ml) and exposed with sunlight for 6 h. After that, the sample was mixed with mobile phase up to 100 ml mark, filtered via a filter membrane (0.45 m) and analysed for TELM and AZEL content using "stability-indicating HPLC methodology" conditions specified (see section: Instrumentation and analysing conditions of TELM and AZEL combination).

### Heat degradation testing

For this report, the stock solution of TELM (400 µg/ml) and AZEL (80 µg/ml) was placed into the volumetric flask (100 ml) and exposed with 60 °C for 30 h using an oven. After that, the sample was mixed with mobile phase up to 100 ml mark, filtered via a filter membrane (0.45 m) and analysed TELM and AZEL content using "stability-indicating HPLC methodology" conditions specified (see section: Instrumentation and analysing conditions of TELM and AZEL combination).

### RESULTS

#### Optimizing the analysing conditions of TELM and AZEL combination

With a "Supelco C18 column (5 µm; 4.6 × 250 mm)" with a column slit temperature having 25 °C and 0.1M Na<sub>2</sub>SO<sub>4</sub>-acetonitrile (pH 3.6 and ratio 55:45, v/v) as mobile solvents phase and an isocratic stream form run of 1.0 ml/min, optimal response, good system fittingness values (table 1) and proportioned peak nature for TELM and AZEL were

obtained. The best match for the finest peak response and to quantify TELM and AZEL was recognized to be UV identification with a 258 nm configuration. Chromatographic analysis run phase for the TELM and AZEL evaluation was 7.0 min with retaining times of 2.757 and 3.664 min perceived for TELM and AZEL, respectively (fig. 2).

### Validation

The HPLC-based stability demonstrating approach for TELM and AZEL was validated bestowing to the "ICH Q2 (R1)" recommendations [17, 18].

### Selectivity

The specificity of TELM and AZEL analysing method was witnessed by analysing diluent (0.1M Na<sub>2</sub>SO<sub>4</sub>-acetonitrile, pH 3.6 and ratio 55:45, v/v), standard TELM and AZEL solution (40 µg/ml TELM and 8 µg/ml AZEL) and formulation solution (40 µg/ml TELM and 8 µg/ml AZEL). The specificity chromatograms of TELM and AZEL are presented in fig. 3.

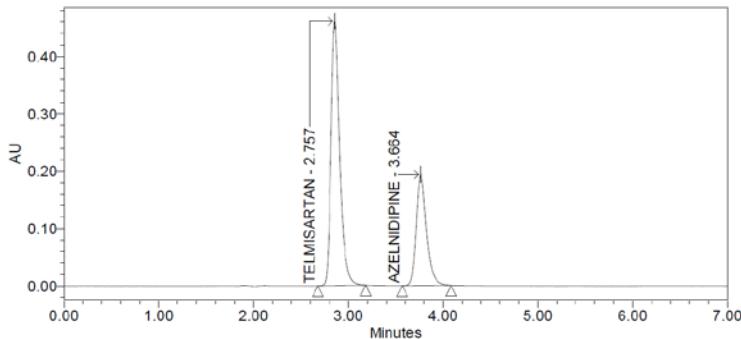


Fig. 2: TELM and AZEL chromatogram

Table 1: TELM and AZEL system appropriateness measures

Parameter	TEL M			AZEL		
	Mean value*	SD value	RSD value	Mean value*	SD value	RSD value
Retention time	2.754	0.002	0.077	3.666	0.004	0.116
Resolution	-	-	-	4.974	0.011	0.229
Tailing	1.372	0.004	0.326	1.308	0.004	0.342
Plate count	5074	58.034	1.144	6109	40.054	0.656
Peak response	2838577	9979.936	0.352	1570555	5471.114	0.348

\*mean of five measures; SD value—standard deviation value for five measures; RSD values—percentile standard deviation value for five measures

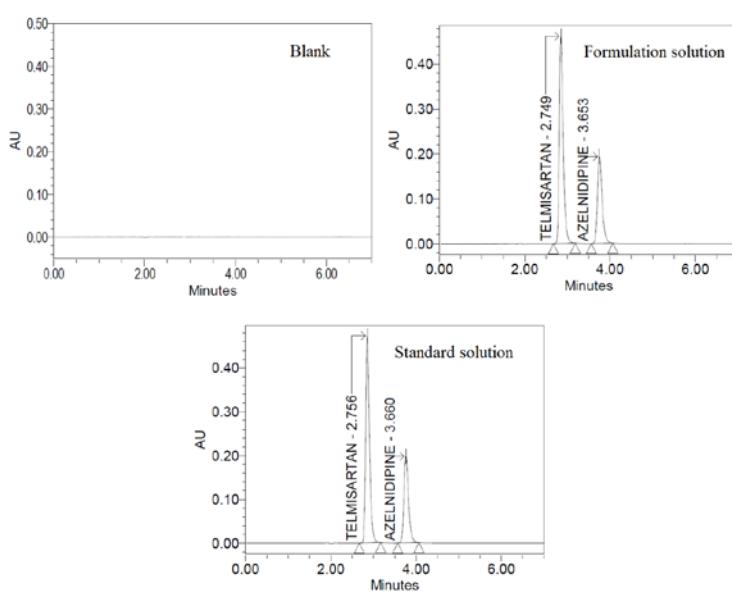


Fig. 3: TELM and AZEL specificity chromatograms

**Linearity**

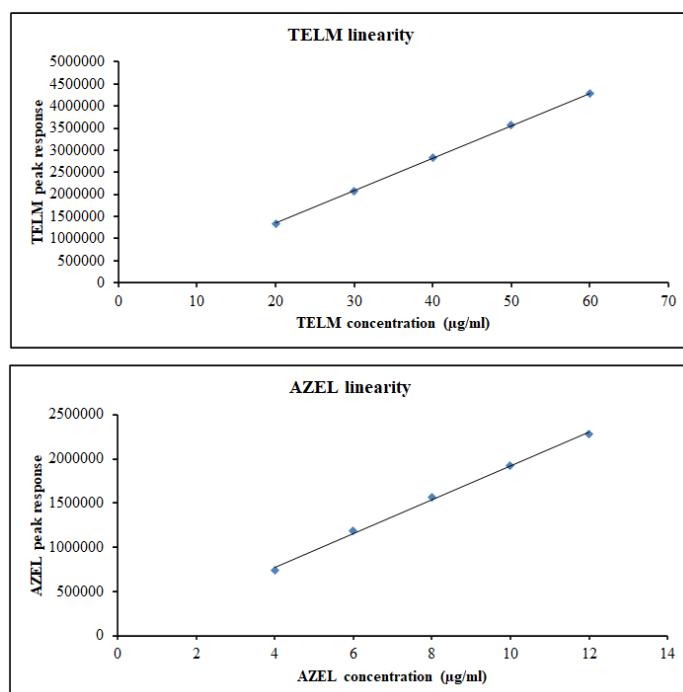
By analysing six solutions in the 20-60 µg/ml (TELM) and 4-12 µg/ml (AZEL) concentration limits, we generated a calibration chart to illustrate linearity (fig. 4). The correlation coefficient, intercept and slope for TELM and AZEL calibration charts were recorded utilizing linear regression evidence analysis.

TELM regression equation:  $y = 73577.83 x - 124711.8$

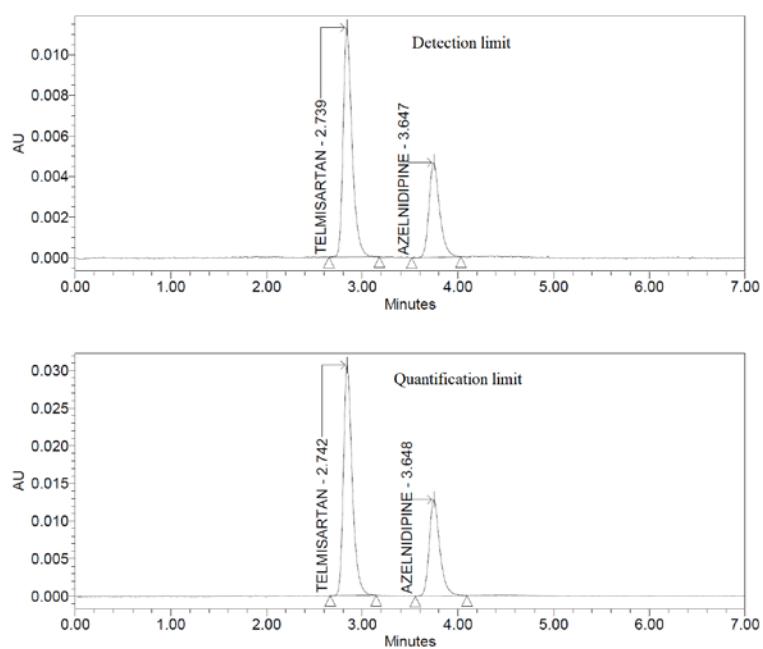
TELM correlation coefficient: 0.9996

AZEL regression equation:  $y = 190619.35 x + 14475.2$

AZEL correlation coefficient: 0.9980



**Fig. 4: Calibration charts of TELM and AZEL**



**Fig. 5: TELM and AZEL chromatograms at their quantification and detection limits concentration**

**Quantification and detection limits for TELM and AZEL**

The detection limits were characterized as the amount (µg/ml) of TELM and AZEL that should have been sensed and will yield

signal/noise proportion of 3:1. The calculated detection limits for TELM and AZEL were 0.020 µg/ml and 0.009 µg/ml, respectively. The quantitation limits were characterized as the amount (µg/ml) of TELM and AZEL that should have been sensed and will yield

signal/noise proportion of 10:1. The calculated quantification limits for TELM and AZEL were 0.065 µg/ml and 0.031 µg/ml, respectively. The TELM and AZEL chromatograms at their quantification and detection limit concentrations were represented in fig. 5.

#### Precision and accuracy

These two criteria for HPLC based stability demonstrating approach was weighed up by the analysis of standard TELM and AZEL solution (40 µg/ml TELM and 8 µg/ml AZEL) by six repeated evaluation of two pure samples of TELM and AZEL on one day, the calculations resulted were exemplified in table 2.

#### Recovery

This criterion for the HPLC-based stability demonstrating approach was weighed up employing the standard adding methodology. The formulation solutions (40 µg/ml TELM and 8 µg/ml AZEL) were prepared and spiked at quantities comprising 19.8 µg/ml TELM and 3.96 µg/ml AZEL (50% standard adding level), 39.6 µg/ml TELM and 7.92 µg/ml AZEL (100% standard adding level) and 59.4 µg/ml TELM and 11.88 µg/ml AZEL (150% standard adding level). These formulation solutions were assessed by three repeated evaluations, the calculations resulted were exemplified in table 3.

**Table 2: TELM and AZEL measures for precision and accuracy**

Sample Inj* No.	Precision		Accuracy	
	TEL M peak response	AZEL peak response	TEL M % assay	AZEL % assay
1	2821726	1561001	98.71	99.09
2	2824535	1558797	98.81	98.95
3	2825550	1557448	98.84	98.87
4	2818158	1565516	98.59	99.38
5	2819465	1560065	98.63	99.03
6	2825565	1562141	98.84	99.17
Mean value	2822500	1560828	98.74	99.08
SD value	3209.399	2823.571	0.112	0.179
RSD value	0.114	0.181	0.114	0.181

\*mean of six measures; SD value—standard deviation value for six measures; RSD values—percentile standard deviation value for six measures; Sample Inj\* No.—Sample injection number

**Table 3: TELM and AZEL recovery measures**

Spiked percent	Value spiked (µg/ml)	Value recovered (%)	Mean value (%)	SD value	RSD value
<b>TEL M recovery</b>					
50	19.800	100.23	100.37	0.428	0.426
	19.800	100.85			
	19.800	100.03			
100	39.600	99.44	99.62	0.159	0.160
	39.600	99.75			
	39.600	99.66			
150	59.400	100.92	101.05	0.168	0.166
	59.400	101.24			
	59.400	100.99			
<b>AZEL recovery</b>					
50	3.960	99.42	99.41	0.036	0.036
	3.960	99.37			
	3.960	99.44			
100	7.920	100.00	100.17	0.150	0.150
	7.920	100.25			
	7.920	100.27			
150	11.880	97.51	97.76	0.240	0.246
	11.880	97.77			
	11.880	97.99			

\*mean of three measures; SD value—standard deviation value for three measures; RSD values—percentile standard deviation value for three measures

#### Robustness

HPLC based stability demonstrating approach robustness was weighed up by the analysis of standard TELM and AZEL solution (40 µg/ml TELM and 8 µg/ml AZEL) by rendering some modest modifications in acetonitrile ratio (optimized 45% volume; altered 40% volume % and 50% volume), column rate of flow (optimized 1 ml/min; altered 0.9 ml/min and 1.1 ml/min) pH (optimized 3.6 unit; altered 3.4 unit and 3.8 unit), column temperature (optimized 25 °C; altered 23 °C and 27 °C) and wavelength (optimized 258 nm; altered 256 nm and 260 nm). Findings of robustness for TELM and AZEL are tabularized in table 4.

#### TEL M and AZEL stability testing

The stability of TELM and AZEL in the presence of acid, base, light, heat, and oxidation was illustrated with the formulation solution.

The TELM and AZEL chromatogram after acid degradation testing showed four additional peaks at 1.908 min, 5.569 min, 5.835 min and 6.422 min except than TELM peak (2.751 min) and AZEL peak (3.656 min). After acid degradation testing, 10.74% of TELM was degraded and 8.8% of AZEL was degraded. But for the TELM peak (2.752 min) and AZEL peak (6.079 min), the TELM and AZEL chromatograms after alkaline degradation testing revealed four additional peaks at 1.484 min, 1.892 min, 4.982 min, and 6.079 min. After acid degradation testing, 8.51% of TELM and 6.8% of AZEL were noticed to be degraded. The TELM and AZEL chromatogram after oxidation degradation testing showed three additional peaks at 1.192 min, 2.058 min and 5.061 min except for than TELM peak (2.755 min) and AZEL peak (3.661 min). After oxidation degradation testing, 5.34% of TELM was degraded and 4.29% of AZEL was degraded. After a photodegradation testing the chromatogram of TELM and AZEL had three extra peaks with a range of 1.261 min,

2.303 min, and 6.610 min other than TELM and AZEL peaks at 2.756 min and 3.660 min, respectively. 7.59% of TELM was found degraded and 5.12% of AZEL was found degraded after photodegradation testing. The TELM and AZEL chromatogram after heat degradation testing showed four additional peaks at 1.183 min, 1.789 min, 4.747 min and 6.100 min except for than TELM peak

(2.752 min) and AZEL peak (3.657 min). After heat degradation testing, 11.42% of TELM was degraded and 9.82% of AZEL was degraded. The TELM and AZEL chromatograms after acid degradation testing, oxidation degradation testing, alkaline degradation testing, photodegradation testing, and heat degradation testing were represented in fig. 6.

Table 4: TELM and AZEL robustness measures

Condition	TEL M Peak response	Mean value	SD value	RSD value	AZEL Peak response	Mean value	SD value	RSD value
<b>Acetonitrile ratio</b>								
Optimized 45% vol	2852318	2813478	37163	1.3	1589088	1561666	26804	1.8
Altered 40% vol.	2809861				1560384			
Altered 50% vol.	2778256				1535526			
<b>Column rate of flow</b>								
Optimized 1 ml/min	2768902	2817027	52079	1.8	1539116	1562862	25078	1.7
Altered 0.9 ml/min	2872318				1589088			
1.1 ml/min	2809861				1560384			
<b>pH</b>								
Optimized 3.6 unit	2831726	2825374	13507	0.5	1571001	1570060	9242	0.6
Altered 3.4 unit	2834535				1578797			
Altered 3.8 unit	2809861				1560384			
<b>Column temperature</b>								
Optimized 25 °C	2809861	2815219	49858	1.7	1560384	1562652	28329	1.9
Altered 23 °C	2868256				1535526			
Altered 27 °C	2867541				1592048			
<b>Wavelength</b>								
Optimized 258 nm	2811832	2788037	39518	1.4	1608996	1588918	25385	1.7
Altered 256 nm	2742420				1597376			
Altered 260 nm	2809861				1560384			

\*mean of three measures; SD value—standard deviation value for three measures; RSD values—percentile standard deviation value for three measures

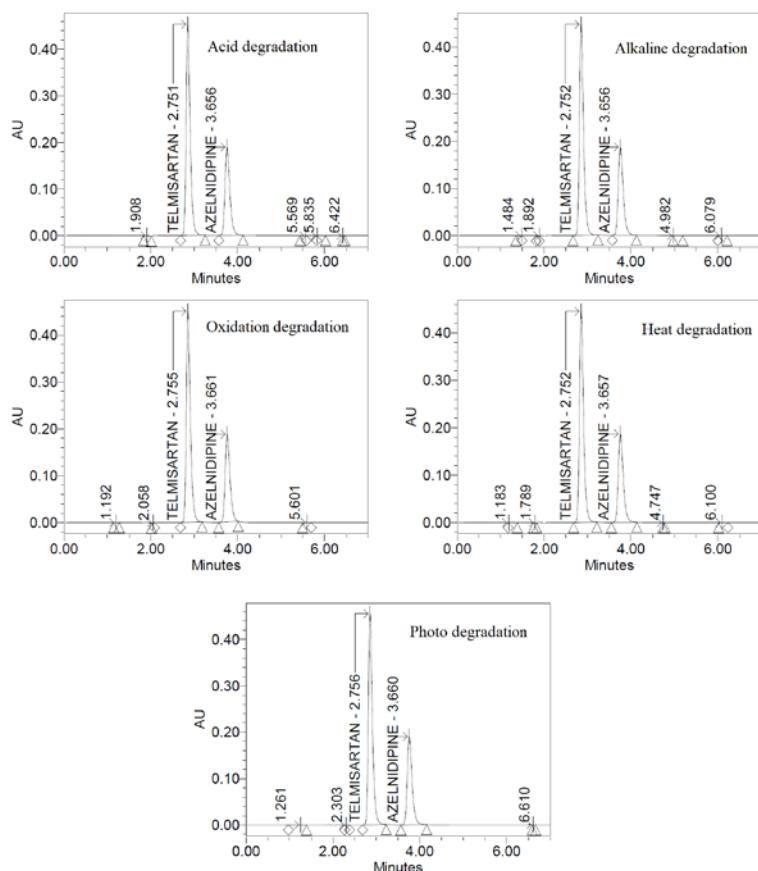


Fig. 6: Chromatogram of TELM and AZEL formulation solution after degradation

## DISCUSSION

The intention of the present work was to create HPLC-based stability demonstrating approach proficient in separating and determining TELM and AZEL well in the petite feasible analysis time with sensible precision, robust, selective and reliability. The column and solvents of the mobile phase were standardized till a best possible responses and peak structures for TELM and AZEL were achieved [19]. The columns that were examined at were: Kromasol C18, Develosil C18, Sunsil C18, Aligent C18 and Supelco C18. All examined stationary columns have identical dimensions (5 µm; 4.6 × 250 mm). The solvent combinations that were examined at were: 0.1% H<sub>3</sub>PO<sub>4</sub>-methanol; 0.1M NaH<sub>2</sub>PO<sub>4</sub>-methanol; and 0.1M Na<sub>2</sub>SO<sub>4</sub>-acetonitrile. The temperature (25 °C) at the stationary column, column flow stream (1 ml/min), and injection analysis volume (10 µl) were all held steady throughout the trial experimentations. Finally, the settings explained in section "Instrumentation and analysing conditions of TELM and AZEL combination" were opted to analyse the TELM and AZEL combination.

No peaks were spotted nearby to the RT of TELM and AZEL in diluent (55% volume 0.1M Na<sub>2</sub>SO<sub>4</sub> with pH 3.6 and 45% volume acetonitrile), approximately the same RT of (55% volume 0.1M Na<sub>2</sub>SO<sub>4</sub> with pH 3.6 and 45% volume acetonitrile) in standard TELM and AZEL solution and formulation solution. Hence evidenced high selectivity of TELM and AZEL analysing method [20].

The TELM and AZEL's concentration was evidently linear for the proposed HPLC-based stability demonstrating approach in the possibility of 20-60 µg/ml and 4-12 µg/ml, with strong linearity as correlation coefficient was 0.9996 and 0.9980, respectively [21].

The low quantification and detection limits concentration values were considered reasonable, ample and sensitive for the TELM and AZEL analysing method [22].

The RSD assessments for the TELM and AZEL peak response were within 2%, indicating that the HPLC-based stability demonstrating process was precise and repeatable for determining TELM and AZEL [23]. The recovery assessments for the TELM and AZEL assay within 100±2% recommended that HPLC based stability demonstrating approach was accurate and reliable for determining the TELM and AZEL [23].

Good recoveries for TELM (99.62%-101.05%) and AZEL (97.76%-100.17%) were achieved in standard addition methodology applied and proved no interfering from pharmaceutical excipients of TELMA-AZ tablets while analysing the TELM and AZEL [24].

The misplays in expressions of RSD for TELM and AZEL peak response after making some modest modifications in acetonitrile ratio (1.3% for TELM and 1.8% for AZEL), column rate of flow (1.8% for TELM and 1.7% for AZEL) pH (0.5% for TELM and 0.6% for AZEL), column temperature (1.7% for TELM and 1.9% for AZEL) and wavelength (1.4% for TELM and 1.7% for AZEL) within 2%, indicating that the HPLC based stability demonstrating process was robust for determining the TELM and AZEL [25].

The choice of formulation production, storage, shipping, shelf life, packaging, and chemical stabilization of TELM and AZEL is directly influenced by stability testing [13-16, 26]. The TELM and AZEL were realised more stable under oxidation degradation testing conditions employed. The TELM and AZEL was realised more sensitive under heat degradation testing situations employed. The retention times of TELM, AZEL and degradation products got during acid degradation testing, oxidation degradation testing, alkaline degradation testing, photodegradation testing, and heat degradation testing conditions are completely different. Hence evidenced high stability demonstrating feature and specificity of TELM and AZEL analysing method.

## CONCLUSION

A stability demonstrating methodology for analysing the TELM and AZEL combination in the tablets and bulk was elucidated in this report. This method found fit, based on the values obtained during validation experiments, for the quality regulatory analysis of TELM and AZEL combination in the quality declaration laboratories deprived of interference from pharmaceutical excipients of TELMA-AZ tablets and degradation products got in acid degradation testing, oxidation

degradation testing, alkaline degradation testing, photodegradation testing, and heat degradation testing conditions employed.

## FUNDING

Nil

## AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

## CONFLICTS OF INTERESTS

Declared none

## REFERENCES

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