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Development and Validation of Stability-Indicating HPTLC Method for Simultaneous Determination of Telmisartan and Cilnidipine in Combined Tablet Dosage Form

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ABSTRACT

A new simple, accurate, precise and selective stability-indicating high performance thin layer chromatographic (HPTLC) method has been developed and validated for simultaneous estimation of Telmisartan and Cilnidipine in combined tablet dosage form. The mobile phase selected was Toluene: Methanol: Glacial acetic acid (8: 2: 1, v/v/v) with UV detection at 260 nm. The retention factor for Telmisartan and Cilnidipine were found to be 0.38 ± 0.004 and 0.62 ± 0.007. The method was validated with respect to linearity, accuracy, precision and robustness. The drugs were subjected to stress condition of hydrolysis (acid, base), oxidation, photolysis and thermal degradation. Results found to be linear in the concentration range of 200-1400ng band^{-1} and 50-600ng band^{-1} for Telmisartan and Cilnidipine, respectively. The method has been successfully applied for the analysis of drugs in pharmaceutical formulation. The % assay (Mean ± S.D.) was found to be 100.79 ± 1.38 for Telmisartan and 99.55 ± 1.13 for Cilnidipine. The developed and validated stability indicating method can be used for assessing the stability of Telmisartan and Cilnidipine in bulk drug and pharmaceutical dosage form.

Keywords: Telmisartan, Cilnidipine, Forced degradation, Validation, Tablet Dosage Form.

INTRODUCTION

Telmisartan (TELMI), chemically, 2-(4-{[4-Methyl-6-(1-methyl-1*H*-1, 3-benzodiazol-2-yl)-2-propyl-1*H*-1, 3-benzodiazol-1-yl] methyl} phenyl) benzoic acid is an angiotensin II receptor antagonist used in the management of hypertension. [1] Cilnidipine (CILNI), *O*3-(2-methoxyethyl) *O*5-[(*E*)-3-phenylprop-2-enyl] 2, 6-dimethyl-4-(3-nitrophenyl)-1, 4-dihydropyridine-3, 5-dicarboxylate is a calcium channel blocker which act on the N-type calcium-channel that existing sympathetic nerve end besides acting on L-type calcium-channel that similar to most of the calcium antagonists. [2]

Literature survey reveals reverse phase high performance liquid chromatographic (HPLC) [3-12], LC MS [13], HPTLC [14] and UV [15] methods for determination of TELMI either as single or in combination with other drugs in pharmaceutical preparations and in human plasma. Analytical methods reported for CILNI includes HPLC [16-19], HPTLC [20-21] and spectrophotometric [22] either as single drug or in combination with other drugs. Also HPLC [23], HPTLC [24] and spectrophotometric [25] methods have been reported for simultaneous determination of TELMI and CILNI as bulk and in pharmaceutical dosage form.

To best of our knowledge, no reports were found for stability-indicating high performance thin layer

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chromatographic (HPTLC) method for simultaneous determination of TELMI and CILNI in tablet dosage form. This paper describes simple, precise, accurate and selective HPTLC method development and validation as well as stability study (hydrolysis, oxidation, photo-degradation and thermal degradation) as per International Conference on Harmonisation Guidelines. [26-27]

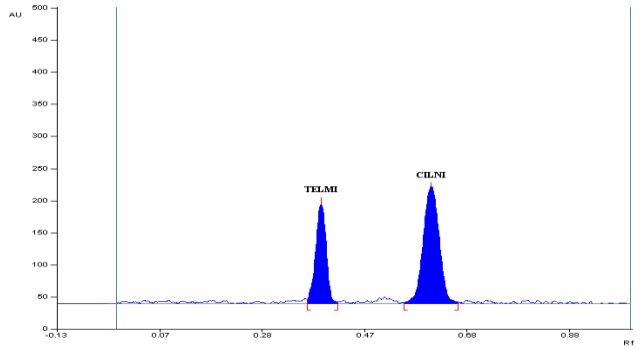


Fig. 1: Representative densitogram of mixed standard solution of TELMI (600 ng band^{-1}, R_f = 0.38 ± 0.03) and CILNI (300 ng band^{-1}, R_f = 0.62 ± 0.05)

MATERIALS AND METHODS

Chemicals and Reagents

Pharmaceutical grade working standards TELMI and CILNI were obtained as gift samples from J. B. Chemicals and Pharmaceuticals Ltd. (Mumbai, India) and FDC Ltd. (Goa, India), respectively. The pharmaceutical dosage form used in this study was Cilacar T tablets J. B. Chemicals and Pharmaceuticals Ltd., Mumbai, India) labeled to contain 40 mg of TELMI and 10 mg of CILNI were procured from the local market. Toluene, methanol, Glacial acetic acid (all AR grade) was purchased from Merck specialties Pvt. Ltd. (Mumbai, India).

Instrumentation and Chromatographic conditions

Chromatographic separation of drug was performed on precoated silica gel aluminium plate 60 F_{254} (10 × 10) with 250µm thickness (E. MERCK, Darmstadt, Germany) using a CAMAG Linomat 5 sample applicator (Switzerland). Samples were applied on the plate as a band with 6 mm width using Camag 100µL sample syringe (Hamilton, Switzerland).

Linear ascending development was carried out in 10 × 10 cm twin trough glass chamber (CAMAG, Muttenz, Switzerland) using Toluene: Methanol: Glacial acetic acid (8: 2: 1, v/v/v) as mobile phase. The optimized chamber saturation time for mobile phase was 20 min. The length of chromatogram run was 9 cm and development time was approximately 20 min. TLC plates were dried in a current of air with the help of a hair drier. Densitometric scanning was performed on CAMAG thin layer chromatography scanner at 260 nm for all developments operated by WINCATS software version 1.4.2. The source of radiation utilized was deuterium lamp emitting a continuous UV spectrum between 200 to 400 nm.

Preparation of Standard Stock Solution

Standard stock solution of TELMI and CILNI were prepared by dissolving 10 mg of each drug in 10 mL of methanol to get concentration of 1 mg mL^{-1} from which 1 mL was further diluted to 10 mL to get stock solution of 100ng µL^{-1} for both the drugs.

Selection of Detection Wavelength

After chromatographic development bands were scanned over the range of 200-400 nm and the spectra were overlain. It was observed that both drugs showed considerable absorbance at 260 nm. So, 260 nm was selected as the wavelength for detection.

Analysis of Tablet Formulation

Twenty tablets were weighed accurately and finely powdered. A quantity of powder equivalent to 5 mg of CILNI (20 mg of TELMI) was weighed and transferred to a 10 mL volumetric flask containing 5 mL of methanol and the content was sonicated for 15 min. The solution was filtered using Whatman paper No. 41 and the volume was made up to the mark with methanol to obtain the final concentration of 500ng band^{-1} for CILNI and 2000ng band^{-1} for TELMI. One mililitre volume of above solution was diluted with methanol to obtain final concentration of 50 ng band^{-1} for CILNI and 200ng band^{-1} for TELMI. Two µL volume of this solution was applied on TLC plate to obtain final sample concentration of 100ng band^{-1} for CILNI and 400ng band^{-1} for TELMI. After chromatographic development peak areas of the bands were measured at 260 nm and the amount of each drug present in each sample was estimated from the respective calibration curves. Procedure was repeated six times for the analysis of homogenous sample.

Stress Degradation Studies

Stress degradation studies were carried out on bulk drug substance under condition of acid, base, neutral hydrolysis, oxidation, and dry heat in order to evaluate the ability of the proposed method to separate both the drugs from their degradation products. Dry heat degradation was carried out in solid state.

Acid treatment

1 mL working standard solution of TELMI and CILNI (1000ng µL^{-1} each) was mixed separately with 1 mL of 0.1 N methanolic HCl and 8 mL of methanol. Solutions were kept at room temperature for one hour. 6µL and 3µL of resulting solution was applied separately on TLC plate and developed under optimized chromatographic condition. 12.06 % of degradation was observed for TELMI with degradation peaks at Rf 0.13, 0.71 and 13.30 % of CILNI degraded with degradation peaks at Rf 0.06, 0.26 and 0.47. The representative densitogram obtained after acid treatment is shown in Figure 2.

Alkali treatment

1 mL working standard solution of TELMI and CILNI (1000ng µL^{-1} each) was mixed separately with 1 mL of 0.1 N methanolic NaOH and 8 mL of methanol. Solutions were kept at room temperature for six hours. 6µL and 3µL of resulting solution was applied separately on TLC plate and developed under

optimized chromatographic condition. 13.97% of degradation of TELMI was observed in alkaline condition while 10.93% degradation of CILNI was observed with degradation products. The representative densitogram obtained after alkali treatment is shown in Figure 3.

Neutral Hydrolysis

1 mL working standard solution of TELMI and CILNI (1000ng μL^{-1} each) was mixed separately with 1 mL of water and 8 mL methanol. Solutions were kept at room temperature for four hours. 6µL and 3µL of resulting solution was applied separately on TLC plate and developed under optimized chromatographic condition. 26.44% degradation was observed for TELMI in neutral condition when refluxed for 4 h with degradation peaks at Rf 0.50, 0.77 while 20.43% of degradation was observed for CILNI. The representative densitogram after neutral degradation is shown in Figure 4.

Oxidative degradation

1 mL working standard solution of TELMI and CILNI (1000ng μL^{-1} each) was mixed separately with 1 mL of 3 % solution of H_2O_2 and 8 mL of methanol. Solutions were kept at room temperature for two hours. 6µL and 3µL of resulting solution was applied separately on TLC plate and developed under optimized chromatographic condition. 18.04 % degradation was observed for TELMI when treated with 3 % H_2O_2 while CILNI exhibited 14.27% degradation. The representative densitogram after oxidative degradation is shown in Figure 5.

Degradation under dry heat

Dry heat study was performed by keeping both drugs individually in oven at 60°C for period of 24 hours. A sample was withdrawn at appropriate times, weighed and dissolved in methanol to get solution of 100ng μL^{-1} for both the drugs. 6µL and 3µL of resulting solution was applied separately on TLC plate and developed under optimized chromatographic condition. 10.41% of degradation was observed for TELMI with degradation peak at Rf 0.27 and and 10.70% of degradation was observed for CILNI with degradation peak at Rf 0.16. The representative densitogram obtained from sample subjected to dry heat is shown in Figure 6.

RESULTS AND DISCUSSION

Optimization of Chromatographic conditions

The primary objective in developing this stability indicating HPTLC method is to obtain the resolution between TELMI, CILNI and its degradation products. The separation was carried out by linear ascending development in 10 cm × 10 cm twin trough glass chamber using Toluene: Methanol: Glacial acetic acid (8: 2: 1, v/v/v) as mobile phase and detection was carried out at 260 nm. The retention factors for TELMI and CILNI were found to be 0.38 ± 0.03 and 0.62 ± 0.05 respectively. Representative densitogram of mixed standard solution of TELMI and CILNI is shown in Figure 1.

Stress degradation studies

The results of stress degradation studies showed that the method is highly specific and the degraded products were well separated from both the drugs. The amount of drug recovered after degradation studies and the Rf values of degradation products are given in Table 1.

Table 1: Data of forced degradation studies of TELMI and CILNI

Stress conditions	TELMI		CILNI	
	% Assay of active substance	Rf values of degraded products	% Assay of active substance	Rf values of degraded products
Acidic/ 0.1 N HCl/ Kept for 1 h at RT	87.94	0.13, 0.71	86.70	0.06, 0.26, 0.47
Alkaline /0.1N NaOH/ Kept for 6 h at RT	86.03	0.08, 0.18	89.06	0.18 0.74
Oxidative /3 % H_2O_2 / Kept for 2 h at RT	81.95	0.70, 0.74	85.72	0.20, 0.45, 0.50
Neutral/H_2O/ Kept for 4 hrs at RT	73.55	0.50, 0.77	79.56	0.46, 0.52
Dry heat/ 60°C/ 24 h	89.58	0.27	89.29	0.16

Table 2: Recovery Studies of TELMI and CILNI

Drug	Amount taken (ng band^{-1})	Amount added (ng band^{-1})	Total amount found (ng band^{-1})	% Recovery	% RSD*
TELMI	400	320	717.80	99.95	1.54
	400	400	805.81	100.72	1.25
	400	480	880.78	100.08	0.75
CILNI	100	80	179.67	99.81	1.84
	100	100	199.91	99.95	1.29
	100	120	221.06	100.47	1.92

*Average of three determinations

Table 3: Robustness Data in Terms of Peak Area (% RSD)

S. No.	Parameter	(% RSD)*	
		DVR	RTV
1	Mobile phase saturation (± 10 %)	0.60	1.36
2	Mobile phase variation (± 2 % methanol)	0.67	1.14
3	Time from application to development (0, 10, 20, and 30 min)	0.75	0.80
4	Development to scanning (0, 30, 60, and 90 min)	0.91	0.77

*Average of three determinations

Method Validation

The method was validated for linearity, accuracy, intra-day and inter-day precision and robustness, in accordance with ICH guidelines. [26-27]

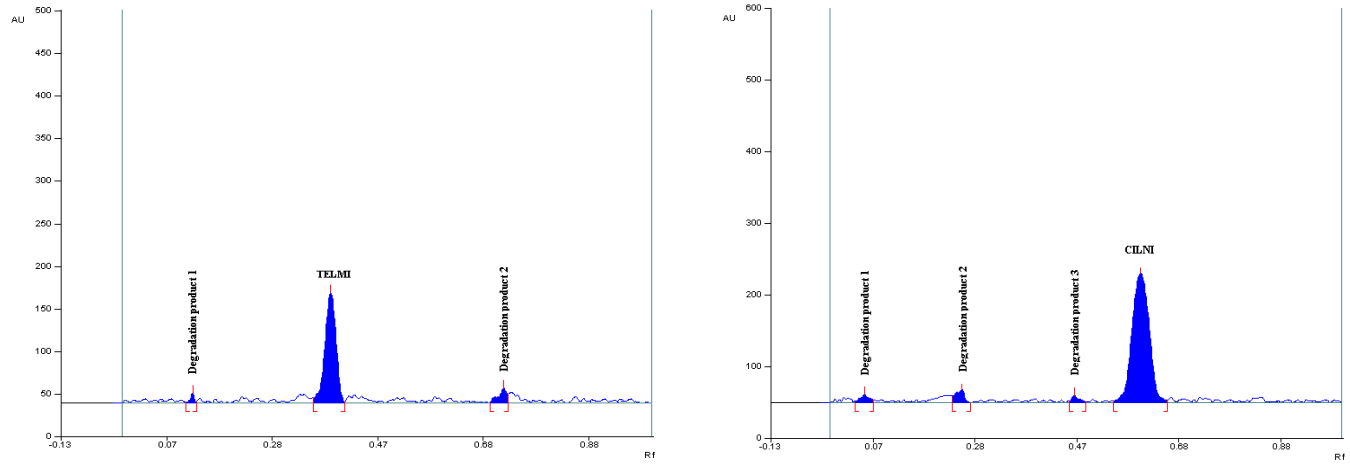
Linearity

The standard stock solutions of TELMI and CILNI (100ng μL^{-1} each) were applied separately on TLC plate in range of 2, 4, 6, 8, 10, 12µL and 0.5, 1, 2, 3, 4, 5, 6µL respectively. Straight-line calibration graphs were obtained in the concentration range 200-1200ng band^{-1} for TELMI and 50-600 ng band^{-1} for CILNI with high correlation coefficient > 0.99.

Precision

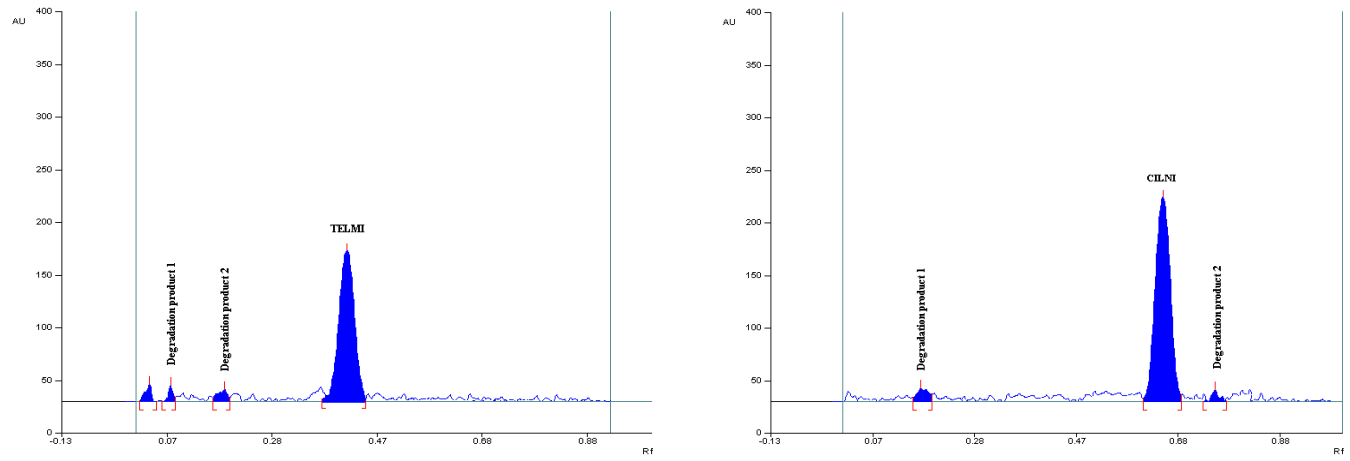
Set of three different concentrations in three replicates of mixed standard solutions of DVR and RTV were prepared. All the solutions were analyzed on the same

day in order to record any intraday variations in the results. Intra-day variation, as RSD (%), was found to be in the range of 0.69 to 1.61 for TELMI and 1.03 to 1.70 for CILNI. For Inter day variation study, three different concentrations of the mixed standard solutions in linearity range were analyzed on three consecutive days. Interday variation, as RSD (%) was found to be in the range of 0.99 to 1.40 for TELMI and 0.72 to 1.38 for CILNI. The lower values of % RSD obtained has proved that the developed method is precise.



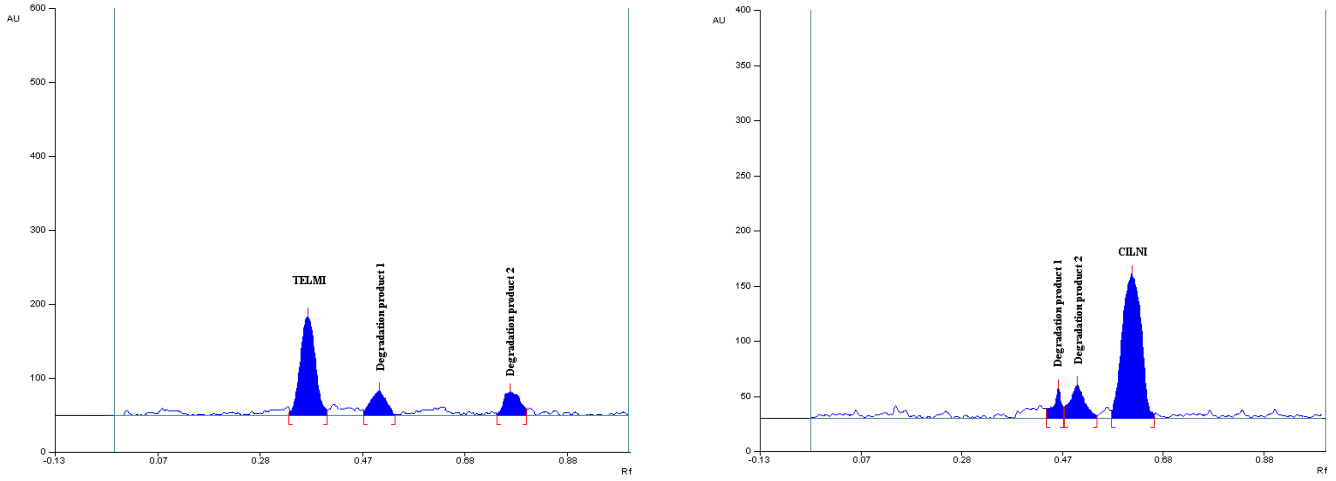
(a) (b)

Fig. 2: Representative densitogram after acid treatment (a) TELMI with degradation products at Rf 0.13, 0.71 and (b) CILNI with degradation products at Rf 0.06, 0.26 and 0.47



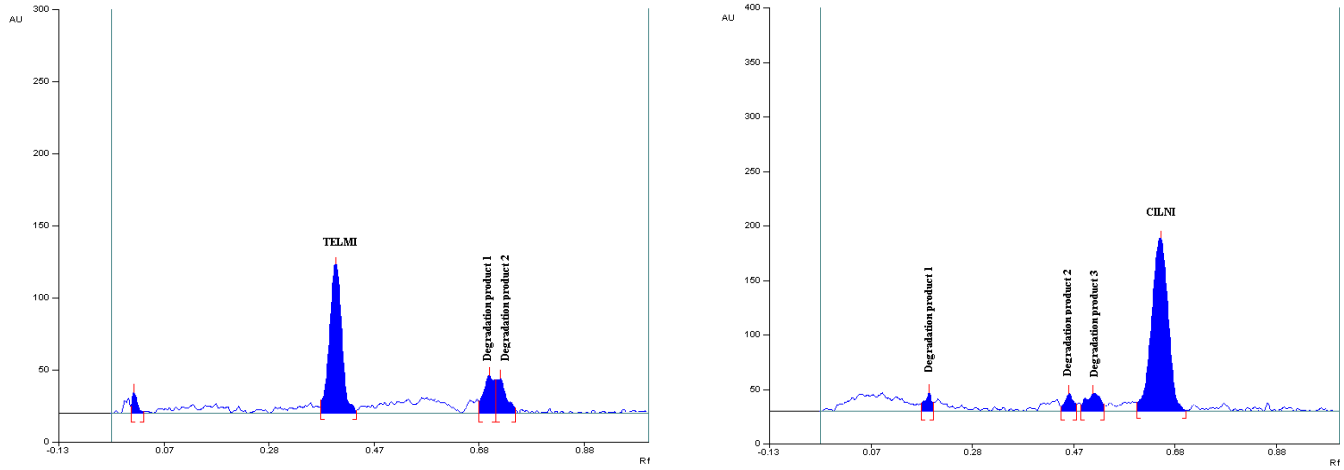
(a) (b)

Fig. 3: Representative densitogram after alkali treatment (a) TELMI with degradation products at Rf 0.08, 0.18 and (b) CILNI with degradation products at Rf 0.18 and 0.74



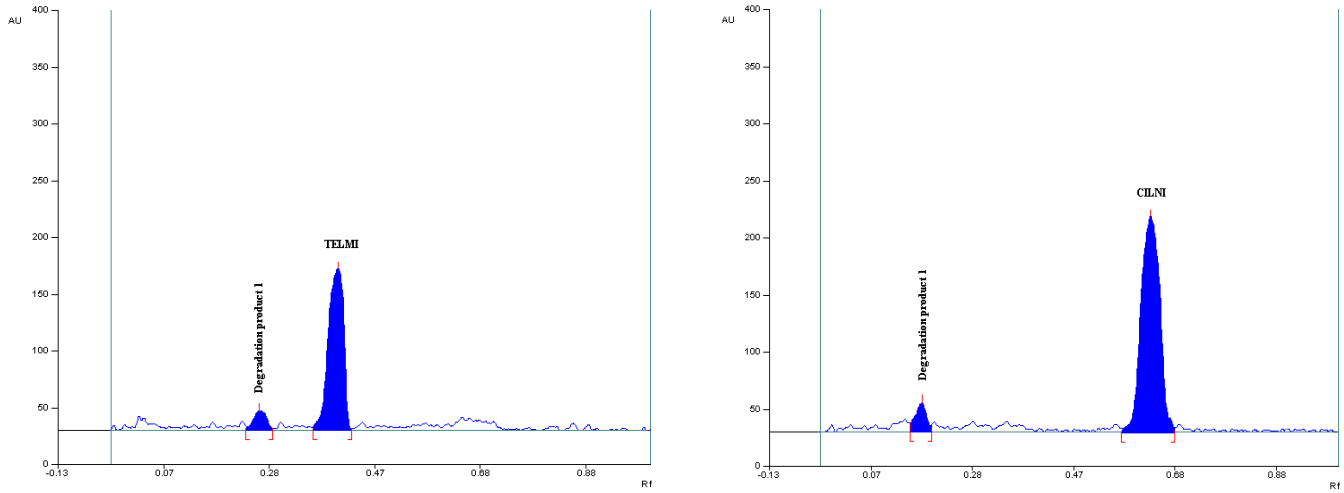
(a) (b)

Fig. 4: Representative densitogram obtained after neutral degradation with (a) TELMI with degradation products at Rf 0.50, 0.77 (b) CILNI with degradation products at Rf 0.46, 0.52



(a) (b)

Fig. 5: Representative densitogram after oxidative degradation (a) TELMI with degradation products at Rf 0.70, 0.74 (b) CILNI with degradation products at Rf 0.20, 0.45, 0.50



(a) (b)

Fig. 6: Densitogram obtained from sample (a) TELMI (b) CILNI subjected to dry heat

Limit of detection (LOD) and Limit of quantitation (LOQ)

LOD and LOQ were calculated as 3.3 σ/S and 10 σ/S, respectively; where σ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot. The LOD of TELMI and CILNI were found 97ng band^{-1} and 15ng band^{-1}, respectively. The LOQ of TELMI and CILNI were 115ng band^{-1} and 47ng band^{-1}, respectively.

Recovery Studies

To check accuracy of the method, recovery studies were carried out by adding standard drug to sample at three different levels 80, 100 and 120%. Basic concentration of sample chosen was 400ng band^{-1} of TELMI and 100ng band^{-1} of CILNI. The drug concentrations were calculated from respective linearity equation. The results of recovery studies indicated that the method is accurate for estimation of drugs in tablet dosage form. The results obtained are shown in Table 2.

Specificity

The specificity of the method was ascertained by peak purity profiling studies. The peak purity values were found to be more than 991, indicating the no interference of any other peak of degradation product, impurity or matrix.

Robustness Studies

Robustness of the method was determined by carrying out the analysis under conditions during which mobile phase saturation time, wavelength, time from application to development and from development to scanning was altered and the effect on the area of drug was noted. The deliberate variations made to the method conditions showed no marked changes in chromatographic behaviour, indicating that the method is robust. The results are given in Table 3.

The standard deviation, % RSD calculated for the method are low, indicating a high degree of precision of the method. The results of the recovery studies performed indicated that the method is accurate for estimation of drugs in tablet dosage form. The results of the stress studies indicated the specificity of the method. The method gives well-resolved peaks of TELMI and CILNI without interference from the excipients or from degradation products even after

exposure to different stress conditions when analyzed individually. The method can be used to determine the purity of the drugs available from various sources by detecting the related impurities. Hence, it can be concluded that the developed TLC-densitometry method is accurate, precise, and selective and can be employed successfully in the estimation of TELMI and CILNI in bulk and in pharmaceutical formulation.

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