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Development Of Stability Indicating HPTLC Method For Simultaneous Estimation Of Telmisartan And Indapamide In Bulk Drug And Pharmaceutical Dosage Form

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Abstract: A new stability indicating HPTLC method has been developed for the simultaneous estimation of TEL and IND in their formulation in the presence of their degradation products generated by forced degradation, as per the ICH [Q1A (R2)] prescribed stress conditions. The method employed TLC aluminium plates precoated with Silica Gel $60F_{254}$ as the stationary phase. The solvent system comprised of Toluene: Ethyl Acetate: Acetone: Methanol (7: 4: 3: 1, v/v/v/v). Densitometric analysis of both the drugs was carried out in the absorbance mode at 259 nm. This system yielded compact spots for Indapamide and Telmisartan, (R_f value of 0.61 ± 0.03 and 0.34 ± 0.03, respectively for IND and TEL). As the method could effectively detect the drugs in the presence of their degradation products, it was employed to establish degradation kinetics of IND and TEL in various conditions.
Key Words: HPTLC, Indapamide, Stability- Indicating, Telmisartan.

Introduction:

Telmisartan (TEL) is indicated in the treatment of hypertension. IUPAC name of the drug is 4'-[[4-Methyl-6-(1-methyl-1*H*-benzimidazol-2-yl)-2-propyl-1*H*-benzimidazol-1-yl] methyl] biphenyl-2-carboxylic acid (Figure 1). It is an angiotensin II receptor antagonist, blocks vasoconstricting and aldosterone secreting effects of angiotensin II by selectively blocking the binding of angiotensin II to the AT_1 receptor in vascular smooth muscle and the adrenal gland. The drug is freely soluble in methanol, sparingly soluble in methylene chloride and practically insoluble in water. The drug is official in European pharmacopoeia [1] and British Pharmacopoeia [2].

Indapamide (IND) is a thiazide-like diuretic and it is indicated as antihypertensive due to diuretic property. IUPAC name of the drug is 4-Chloro-N-[(2*RS*)-2-methyl-2, 3-dihydro-1*H*-indol-1-yl]-3-sulfamoyl-benzamide (Figure 2). The drug is freely soluble in methanol, ethanol, ethyl acetate, and acetic acid, sparingly soluble in chloroform and ether and practically insoluble in water. The drug is official in British Pharmacopoeia, European Pharmacopoeia and United States Pharmacopoeia[3]. Combination of IND and TEL results in synergistic effect and thus poses superior blood pressure lowering activity and is approved by regulatory authorities to treat hypertension.

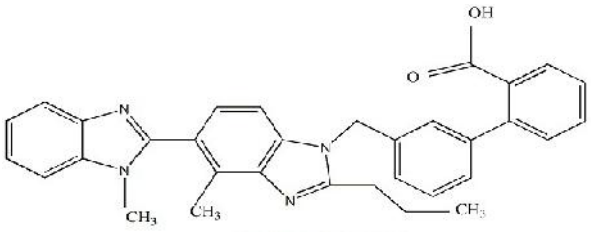

Figure 1: Chemical structure of Telmisartan

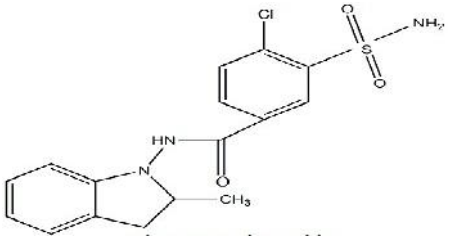

Figure 2: Chemical structure of Indapamide

Literature review revealed that various analytical methods have been developed for TEL, based on Spectrophotometry, Polarography, HPLC and HPTLC either individually or in combination [4,5,6,7]. Similarly, various methods have also been reported for estimation of IND, using Colorimetry, Spectrophotometry, Fluorometry, HPLC, UPLC and HPTLC, either individually or combination with other drugs [8,9,10,11,12,13]. Stability indicating analytical method is reported for TEL individually and the combined dosage form of IND and TEL using HPLC [14,15.]

The aim of the study was to develop a stability indicating analytical method for the simultaneous estimation of IND and TEL in the presence of their probable degradation products using HPTLC. The method was further extended to assess the stability of drugs, to study the effect of various degradation conditions on them and lastly to evolve their degradation kinetics.

Materials and Methods:

The solvents and regents utilized in the set of experiments were of analytical grade, purchased from M/s. Qualigens LTD (Mumbai, India). TLC plates were from Merck India LTD (Precoated Silica Gel G $60F_{254}$ TLC plates with aluminum support, Catalogue No. 1.0554.0007).

Linomat V sample applicator, twin trough developing chamber and TLC scanner IV were used for the set of the experiments. The chromatographic data was collected and integrated using WINCATS software Ver. 1.4.7 supplied by CAMAG.

Preparation of standard solutions:

The stock solution was prepared by dissolving IND and TEL in methanol (5 mg IND and 10 mg TEL in 10 ml methanol), separately. The aliquot from the stock solutions were mixed in a volumetric flask and diluted with methanol to produce the working standard solution of concentration 50 µg/ml and 100 µg/ml for IND and TEL respectively.

Chromatographic Conditions:

The samples were spotted in the form of bands of 6.0 × 0.45 mm size on precoated TLC plate using Camag 100 µl syringe and Camag Linomat V (applicator). The plates were washed with methanol and activated at 60^{o}C for 10 min prior to application. The application rate was set at 150 nl/sec. The plate was developed with 30 ml mobile phase with 15 min chamber saturation and the developing distance was set at 90 mm. The plates were allowed to dry at room temperature (25 ± 2.0^{o}C) at relative humidity of 60% ± 5. The dried plates were scanned and quantified in reflectance- absorbance mode at 259 nm using the Camag TLC Scanner-IV. The slit dimension was kept at 4.0 × 0.45 mm with scanning speed of 10 mm/sec. The monochromator band width was set 20 nm and each track was scanned thrice.

Calibration curve of IND and TEL:

Various dilutions of combined working standard solution were spotted on the TLC plates which covered the range of 300-1000 ng/spot for IND and 600-2000 ng/spot for TEL. Each concentration was spotted six times on TLC plate.

Method Validation:

The method was validated in terms of specificity, interday and intraday precision, accuracy, limit of detection (LOD) and limit of quantitation (LOQ) as described in ICH guidelines [16].

Specificity of the method was ascertained by analyzing standard drug and sample. The purity of the spot for IND and TEL was ascertained by comparing the R_f value and UV spectra of the spot of drugs in track of sample with that of standard.

Intraday precision was determined by analyzing standard solutions of three different concentrations for 6 times in same day. The interday precision was measured similarly at the interval of 6 days. The results of intraday and interday precision are tabulated as in Table 2.

Accuracy of the analysis was evaluated by performing recovery studies. The results are shown in Table 3.

In order to estimate the Limit of Detection (LOD) and Limit of Quantitation (LOQ), blank solution and various concentration of the standard solution were spotted on the plate. The plate was developed and scanned.

Robustness:

Mobile phase having different composition like Toluene: Ethyl Acetate: Acetone: Methanol A) (7.0: 4.0: 1.0: 1.0, v/v/v/v), B) (2.5: 4.0: 3.0: 1.0, v/v/v/v), C) (7.0: 1.5: 3.0: 1.0, v/v/v/v) were selected and chromatograms were developed. The effects obtained by altering the chamber saturation time to 0, 10, 20 min were studied. Time duration between spotting and developing the plate were altered and the alteration in area of the spots were studied and compared with the optimized chromatographic conditions.

Analysis of Marketed Formulation

A total of 20 capsules were accurately weighed and powdered in a mortar. The powder equivalent to about 20.0 mg of TEL was weighed accurately. The weighted amount was quantitatively transferred to volumetric flask and dissolved in 30 ml methanol by sonication for 45 min. The content was then diluted up to 50 ml using methanol. The solution was filterd using whatman filter paper Grade 41. The aliquot of test solution was further diluted using methanol to produce the solution containing approximately 15 µg/ml IND and 400 µg/ml TEL. Test solution thus prepered was spotted and analyzed (4 µL). The experiment was repeated thrice and results are tabulated in Table 4.

Table: 1 Linear Regression Data for Calibration Curves

	IND	TEL
Linearity Range (ng/spot)	30-1000	600-2000
r ± SD	0.9993 ± 0.000136	0.9971 ± 0.000048
Slope ± SD	7.755 ± 0.053649	4.192 ± 0.00506
Intercept ± SD	1316 ± 56.70979	2127.667 ± 5.46504

n= 6, SD: Standard Deviation

Table: 2 Intra-day and Inter-day precision of HPTLC method

Name of the Drug	Amount (ng/spot)	Intra-day Precision			Inter-day Precision		
		SD	RSD	SE	SD	RSD	SE
IND	300	21.632	0.589	8.831	29.875	0.572	12.197
	600	9.143	0.143	3.733	31.936	0.439	13.038
	1000	50.853	0.614	20.761	159.381	1.457	65.067
TEL	600	25.400	0.556	10.369	10.628	0.286	4.339
	1200	39.464	0.545	16.111	29.657	0.499	12.108
	2000	34.498	0.354	14.084	167.248	1.955	68.279

n=6, RSD: Relative Standard Deviation, SE: Standard Error

Table: 3 Recovery Studies

Name of the Compound	Amount of Standard Spiked (ng)	Average of Amount Recovered (ng)	Recovery (%) ± SD	RSD	SE
IND (n=3)	0	328.99	---	---	---
	600	594.18	99.05± 1.19	1.21	0.39
	650	655.2	100.8± 1.79	1.79	0.59
	700	701.89	100.27± 1.30	1.30	0.43
TEL (n=3)	0	751.07	---	---	---
	1200	1196.9	99.74± 1.20	1.20	0.40
	1300	1312.4	100.96±1.69	1.66	0.56
	1400	1402.8	100.2± 1.97	1.96	0.65

Table: 4 Assay of Marketed Formulation

Name of Drug	Amount Spotted (ng/spot)	Amount Detected (ng/spot) ± SD	% Assay ± RSD
IND	60	60.84 ± 0.24	101.4 ± 0.39
TEL	1600	1580.33 ± 9.32	98.77 ± 0.59

Degradation Kinetic Study:

Preparation of standard stock solution:

The stock solution was prepared by dissolving 50 mg IND and 50 mg TEL in 50 ml methanol separately.

Acidic/ Basic degradation: In 10 ml of stock solutions of IND and TEL, 10 ml of 0.1 N HCl/ 0.1 N NaOH was added separately and refluxed at 40^{o}C, 60^{o}C, and 80^{o}C for 8 hrs. 1 ml Sample was withdrawn at different time interval for 8 hrs.

Oxidative degradation: In 10 ml of stock solutions of IND and TEL, 10 ml of 3% H_2O_2 was added separately and refluxed at 40^{o}C, 60^{o}C, and 80^{o}C for 3 hrs. 1 ml Sample was withdrawn at different time interval i.e. for 3 hrs.

In case of oxidative degradation, samples were dissolved in methanol and diluted to get 100 µg/ml as final conc. 10 µl of the solution was applied on TLC plate. In case of basic and acidic degradation, samples were neutralized with 0.1 N HCl and 0.1 N NaOH respectively then diluted with methanol to get 100 µg/ml as final conc. 10 µl solution was applied on TLC plate.

Thermal degradation studies:

Thermal degradation studies were performed by keeping TEL in oven at 40^{o}C, 60^{o}C, 80^{o}C and IND at 40^{o}C, 50^{o}C, 60^{o}C for 8 hrs. Samples were withdrawn after appropriate time intervals, dissolved in methanol and diluted to get 300 µg/ml concentration. 2 µl of the solution was spotted on TLC plate.

Photolytic Degradation studies:

Photolytic studies were carried out by exposure of drug to UV light (254 nm) up to 8 hrs. Sample was weighed, dissolved and diluted to get 300µg/ml as final conc. 2 µl of the solution was spotted on TLC plate.

Rate constant k was calculated using graphical method and substitution method. The rate constants were determined and energy of activation was calculated.

Results and Discussion:

Development and validation of HPTLC method:

The solvent system was developed and optimized using trial and error method. The optimized mobile phase was Toluene: Ethyl Acetate: Acetone: Methanol (7.0: 4.0: 3.0: 1.0, v/v/v/v), in which spots of the degradation products were well resolved from the spot of the drugs. R_f values obtained for IND and TEL were 0.61 ± 0.03 and 0.34 ± 0.03, respectively (Figure 3).

The purity of the spot obtained by each drug was confirmed by overlapping the recorded spectra of the drug with that of the standard (Figure 4). The peak purity of IND and TEL was assessed by comparing their respective spectra at the peak start, apex and peak end positions of the spot *i.e.*, r (s, m): 0.999999, r (m, e): 0.999999 in standard and r (s, m):0.999990, r (m, e): 0.999968 in sample for IND and r (s, m): 0.999965, r (m, e): 0.999981 in standard and r (s, m): 0.999993, r (m, e): 0.999999 in sample for TEL. When overlain spectra of both the drugs was considered, both the drugs showed significant absorbance at 259 nm (Figure 5) hence, this wavelength was selected for detection and estimation of both the drugs.

Calibration range selected for the present experiment was 300-1000 ng/spot for IND and 600-2000 ng/spot for TEL. Calibration data, results of intraday and interday precision and accuracy are shown in Table 1, 2 and 3, respectively. Drug spiked in the degradant sample solution at 3 different levels showed recovery in the range of 98-100 % confirming the accuracy of the method.

Limit of Detection was found to be 100 ng/spot and 200 ng/spot for IND and TEL, respectively. Limit of Quantitation was 300 ng/spot and 600 ng/spot for IND and TEL, respectively.

The results of robustness studies suggested that the amount of Toluene in the proposed system was critical as alterations in the relative proportion of Toluene affected the sharpness of TEL spot. Alteration in Ethyl Acetate proportion in the system did not affect the resolution much, except the alteration in R_f values of the spots. Alteration in Acetone proportion resulted in change in R_f values but R_f value of TEL showed more than 10% alteration. The results were not affected by altering the other parameters except the saturation timing for the tank. As saturation time increased, R_f value for TEL decreased. Saturation time less than 15 min could not prevent the fronting effect. The area under the peak remains unaltered up to 15 min when plates were exposed to the room temperature after application and also after development. The temperature of the area was maintained at 25^{o}C throughout the experiments.

Assay values of marketed formulation are shown in Table 4.

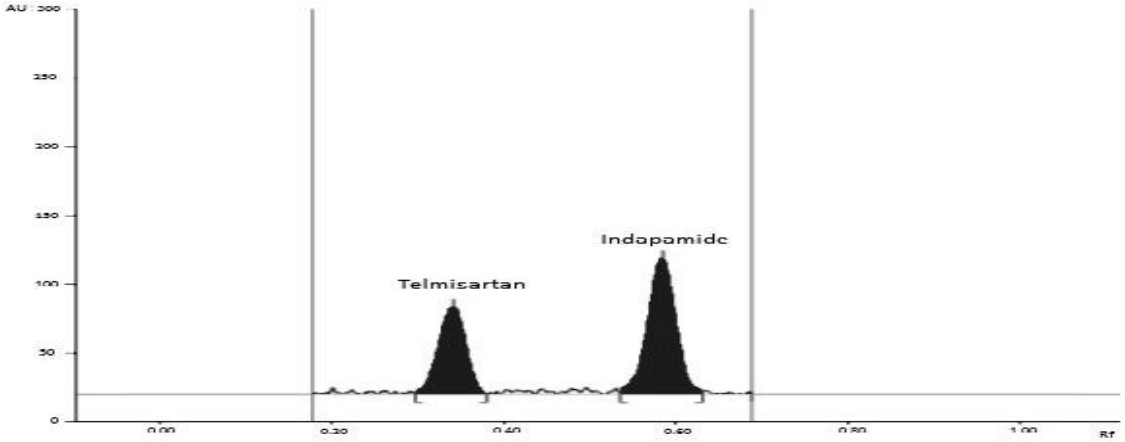

Figure 3: Chromatogram of IND and TEL

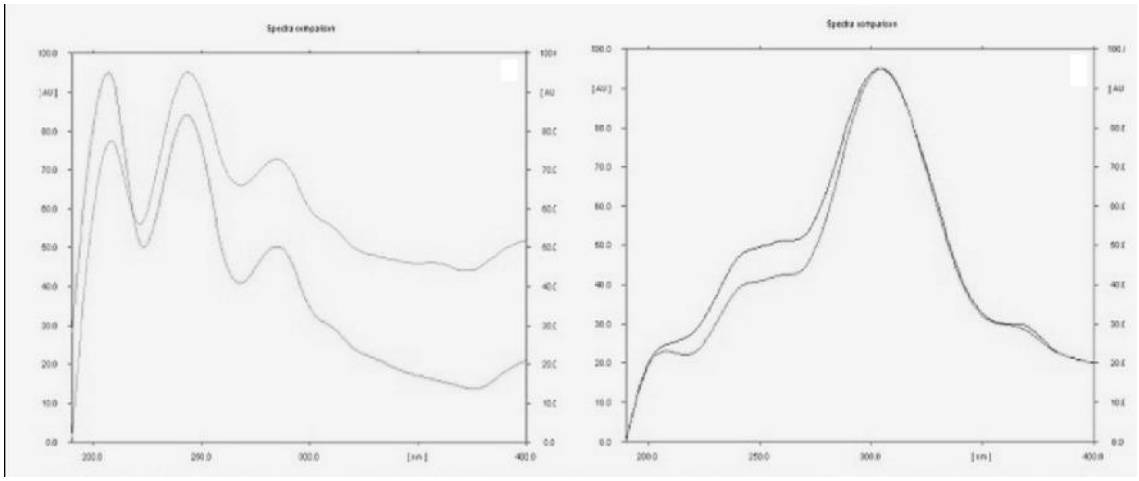

Figure 4: Superimposed Spectra of IND and TEL Spot in Standard Track and in Corresponding Spot of Sample Track respectively

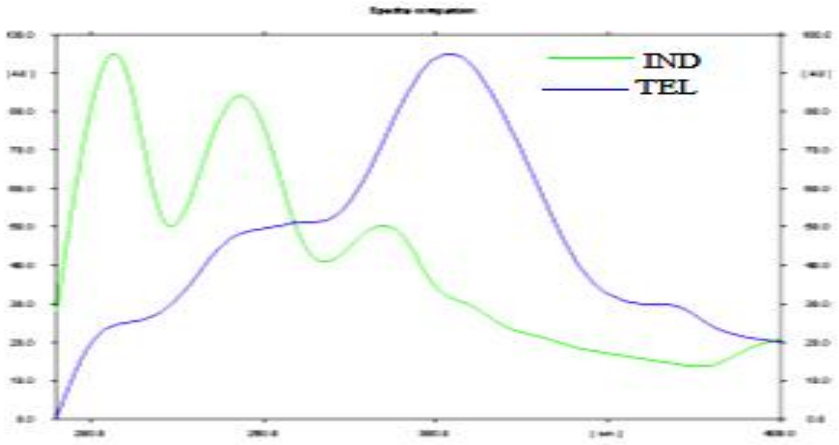

Figure 5: Overlay Spectra of IND and TEL

Degradation Kinetic Study:

Results of percentage drug degradation are shown in Table 5. The data obtained at various time interval fits into equation of first order kinetics i.e.

$$k = \frac{2.303}{t} \log \frac{C_o}{C}$$

Here, t = Time
C_o = Initial concentration at time 0
C= Concentration remaining after time t
Units of K= time $^{-1}$ [17]

When the graph of logarithm of percent drug remaining versus time (min) was plotted, it showed a straight line that depicts that the degradation reaction followed first order kinetics. The results showed that as temperature increased, degradation rate also increased (Table 6). Arrhenius plot of log k+3 versus 1/T*1000 gave a straight line. Energy of activation (Ea) was calculated from the line equation obtained from Arrhenius plot (Table 7).

Table: 5 Results of Percentage Degradation

Stress condition	% degraded (IND)						% degraded (TEL)					
	Standard			Formulation			Standard			Formulation		
	40°C	60°C	80°C	40°C	60°C	80°C	40°C	60°C	80°C	40°C	60°C	80°C
Acid	25.9	48.6	57.2	29.2	55.9	58.8	31.2	44.7	59.8	35.5	51.1	64.1
Alkali	18.4	43.1	52.8	22.5	46.1	55.1	34.5	42.8	47.1	41.8	47.7	50.4
Oxidative	80.7	89.9	97.2	83.1	96.8	99.7	43.1	80.9	95.8	50.4	80.4	95.4
Thermal	30.6	46.0	43.5*	---	---	---	26.7	50.5	63.7	---	---	---
Photolytic	35.4	---	---	---	---	---	47.8	---	---	---	---	---

*: 50 °C

Table: 6 Degradation Rate Constant (k) for IND and TEL

Stress condition	Degradation Rate Constant (k)											
	IND						TEL					
	40°C		60°C		80°C		40°C		60°C		80°C	
	k_1	k_2	k_1	k_2	k_1	k_2	k_1	k_2	k_1	k_2	k_1	k_2
Acid	0.00069	0.00091	0.00138	0.00107	0.00207	0.00189	0.00046	0.00069	0.00138	0.00173	0.00138	0.00263
Alkali	0.00069	0.00109	0.00092	0.00139	0.00115	0.00152	0.00046	0.00051	0.00092	0.00176	0.00138	0.00231
Oxidative	0.00115	0.00176	0.0023	0.00496	0.00484	0.00624	0.00299	0.00589	0.0129	0.0153	0.02073	0.0184
Thermal	0.00069	0.00067	0.00161	0.00153	0.00207	0.00208	0.00046	0.00089	0.00115	0.0011	0.00138	0.00118
Photolytic	0.00138	0.00108	---	---	---	---	0.00112	0.00069	---	---	---	---

k_1: k determined using graphical method
k_2: k determined using substitution method

Table: 7 Energy of Activation

Stress condition	Ea* (TEL)				Ea (IND)			
	Standard		Formulation		Standard		Formulation	
	Equation	Graph	Equation	Graph	Equation	Graph	Equation	Graph
Acid	30.52	25.30	24.65	22.34	16.43	24.96	14.71	18.34
Alkali	34.34	24.94	30.38	25.13	7.61	11.58	6.44	5.15
Oxidative	31.81	83.85	32.26	54.27	28.94	32.42	31.84	28.17
Thermal	6.29	25.13			25.99	25.09		

*: Energy of Activation (Joule/ mole)

Conclusion:

The stability indicating HPTLC method for simultaneous estimation of IND and TEL from pharmaceutical dosage form was developed and validated as per ICH guidelines. The marketed pharmaceutical formulation containing IND and TEL when subjected to quantitative analysis using the developed method yielded nearly 100% assay result for IND and TEL. The force degradation studies performed by selecting various degradation conditions at different temperatures confirmed that IND and TEL followed first order kinetic in the selected degradation conditions. The rate constant k was determined for both the drugs in each set of condition. The activation energy was also estimated for each degradation condition at different temperature. The applicability of the study lies in controlling the critical process parameters during manufacturing and storage conditions based on the observed degradation behavior of IND and TEL in their combined dosage form.

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