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Validated RP-HPLC method for Determination of Erlotinib HCl in Tablet Dosage forms and its Application to Stress Degradation Studies.

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ABSTRACT

An accurate and precise stability-indicating RP-HPLC method has been developed and validated for the analysis of Erlotinib HCl in tablet dosage forms. The method was developed on a Qualisil Gold C18 (250×4.6mmi.d, 5µm particle size) analytical column using methanol: water in the ratio of 58:42(v/v) as the mobile phase. The instrumental settings were flow rate 1mL/min, column temperature 23^{o}C and the detection wavelength of 246nm using a Photo Diode Array (PDA) detector. The method is linear over a range of 5-50µg/ml. The LOD and LOQ values were found to be 0.81µg/mL and 2.466µg/mL respectively. Erlotinib was exposed to acidic, alkaline, oxidative, photolytic, thermal stress conditions and these stressed samples were analyzed by the proposed method. The drug product was completely separated from the degradants which indicates that the method is specific.

Key-words: ErlotinibHCl, RP-HPLC, Stress degradation studies.

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INTRODUCTION

Erlotinib is a drug used to treat non-small cell lung cancer, pancreatic cancer and several other types of cancer. It is a reversible tyrosine kinase inhibitor, which acts on the epidermal growth factor receptor (EGFR).It is a quinazolinamine with the chemical name N-(3-ethynylphenyl)-6,7-bis(2-methoxyethoxy)-4-quinazolinamine available as hydrochloride salt. Erlotinib hydrochloride has the molecular formula $C_{22}H_{23}N_3O_4$.HCl and a molecular weight of 429.90.The structure of ErlotinibHCl is shown in figure 1.Literature survey reveals that several RP-HPLC methods [4, 5] and very few stability indicating [7] assay methods were reported. Our main aim was to develop a simple, cost effective method, validate and apply the developed method to stress degradation studies.

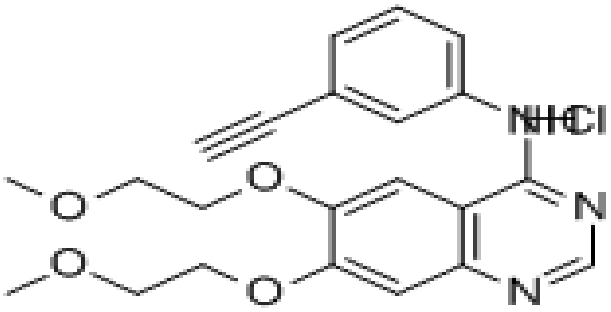

Figure: 1 Structure of Erlotinib HCl

MATERIALS AND METHODS:

Chemicals and Reagents:

Pure form of drug was provided by Hetero labs Hyderabad, India. Methanol of HPLC grade was purchased from Merck, Mumbai (India). HPLC grade water was prepared in- house. Erlocip (Erlotinib) 25mg label claim tablet was purchased from local market.

Instrumentation:

Chromatographic Analysis was performed on HPLC Agilent 1200 with UV-PDA detection and the output signal was monitored and integrated using EZ Chrome Elite software.

Chromatographic Conditions:

Stationary phase: Qualisil Gold C_{18}column (250 x 4.6 mm id, 5µm)

Mobile phase: Methanol: Water in the ratio of 58:42 (P^H of water adjusted to around 3 with OPA)

Flow Rate: 1mL/min, Column Temperature: 23^{o}C, Detection Wave length: 246nm

Injector : Manual Rheodyne Injector (20 µL).

Preparation of Standard solutions:

Weigh accurately 10mg of the API and transfer it in to a 10ml dry volumetric flask. Dissolve drug with methanol and make up to the volume with methanol to obtain a concentration of 1000µg/mL. From the primary stock, 5mL is withdrawn in to a 50ml dry volumetric flask and made up to the mark with mobile phase to obtain a concentration of 100µg/mL.

Preparation of sample solution:

Twenty tablets were weighed accurately and powdered. An amount of the powder equivalent to 10 mg of Erlotinib was dissolved in 50 mL of mobile phase. The solution was sonicated for 5 minutes filtered into a 100 mL volumetric flask through 0.45 µm filter paper and then the volume was made up to 100 mL with mobile phase to give 100 µg/mL**.**

RESULT&DISCUSSION

Method Development:

The method was developed on Qualisil Gold C_{18} (250 x 4.6 mm id, 5µm) column. Several trails were conducted with various mobile phase compositions. The optimized mobile phase was methanol:water in the ratio of 58:42 (pH of water was adjusted to around 3 with OPA).The detection wavelength was 246nm.The Retention time was obtained at 7.587min.Tailing factor was less than 2.

Method Validation:

The method was validated as per ICH Guidelines which include System suitability, Specificity, Linearity, Precision, Repeatability, Accuracy, Assay, LOD, LOQ and Robustness.

System suitability:

System suitability of the method was determined by injecting a concentration of 20µg/mL. System suitability parameters that are to be monitored are Plate count, Asymmetry, Peak tailing, Resolution, and Capacity factor and Height equivalent to theoretical plates (HETP).The results were tabulated in Table 1.

Table 1 System Suitability Parameters

S. No	Parameter	Values obtained	Acceptance criteria
1	Plate count	6340	>2000
2	Tailing factor	1.121	≤ 2.0
3	Asymmetry (10%)	0.99	0.9-1.2
4	Resolution	---	>1.5
5	Capacity factor	2.083	1-10

Specificity:

Specificity is the ability of a method to discriminate between the intended analyte(s) and other components in the sample. Volume of 20 µL of working placebo sample solution was injected.

As no peaks were found at retention time of 7.587min, the proposed method was specific for the detection of Erlotinib HCl. The chromatogram was shown in the Figure 2.

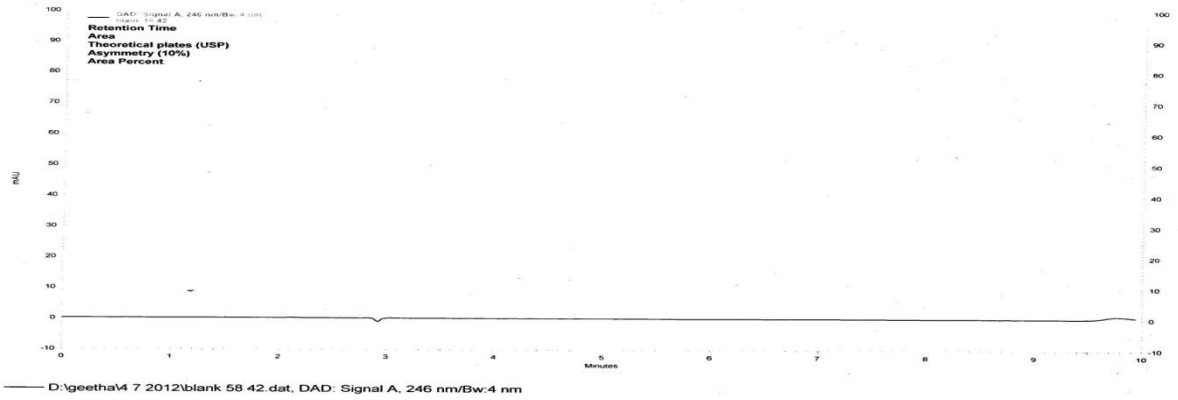

Figure 2 Mobile phase blank

Linearity:

Linearity for Erlotinib was determined by triplicate injections of drug solutions in concentration range of 5-50µg/mL. The peak areas versus concentration data were evaluated by linear regression analysis. The correlation coefficient was found to be 0.9991.The linearity data was reported in Table 2.The linearity plot was shown in the Figure 3.

Table 2 Linearity data of Erlotinib hydrochloride

S. No	Parameter	ErlotinibHCl
1	Linearity range	5- 50 µg/ml
2	Regression equation	y=28696x+60355
3	Correlation coefficient	0.9991
4	Intercept	60355
5	Slope	28696

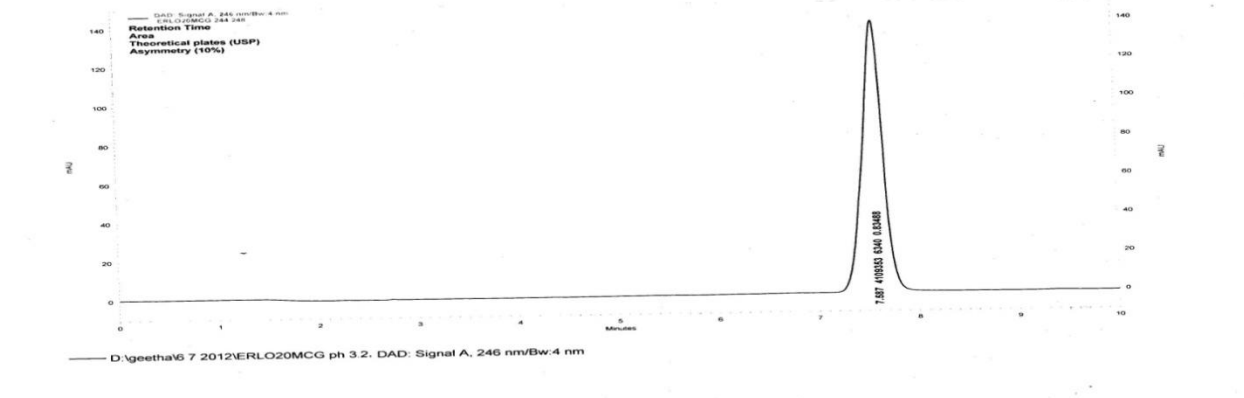

Figure 3 Chromatogram of ErlotinibHCl (20µg/ml)

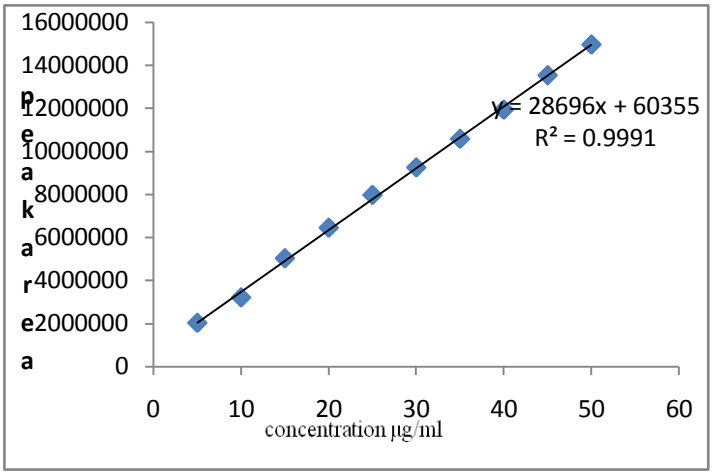

Figure 3.Linearity plot of Erlotinib

Precision:

To assess the precision of the method, intra-day and inter-day measurements of the drug was completed with computation of %RSD for replicate samples (n=3) using concentrations of 15µg/mL, 25 µg/mL, 45 µg/mL, both intra-day and inter-day samples were calibrated with standard curve concurrently prepared on the day of analysis. The %RSD values found to be less than 2, which indicate that the proposed method is precise for analysis. The results were tabulated in Table 3.

Table 3 Precision data of Erlotinib hydrochloride

S.No	Concentration (µg/mL)	Peak area ±S.D(n=3)		%RSD	
		Intraday	Inter day	Intraday	Inter day
1	15	5081763±46371.69	5118096±85070.56	0.9	1.6
2	25	7965810±70237.69	7982477±135277.5	0.88	1.6
3	45	13656363±149072	13733030±194728.3	1.09	1.41

Repeatability:

Repeatability was determined by analyzing 20µg/mL of drug solution six times and the %RSD was found to be 1.08, which is less than 2.The results were shown in Table 4.

Table 4 Results of Repeatability

Drug	Conc. (µg/ml) (n=6)	Peak Area mean ± SD	%RSD
Erlotinib HCl	20	6527013 ±70945.99	1.08

Accuracy:

Accuracy of the method was determined by Recovery studies. To the pre-analyzed sample (20µg/mL), known amount of the standard drug was added at the level of 80%, 100%, 120%.The individual recovery ranges from 99.33% to100.5%.The mean recovery was 99.92%.The results were shown in the Table 5.The recovery plot was shown in Figure 4.

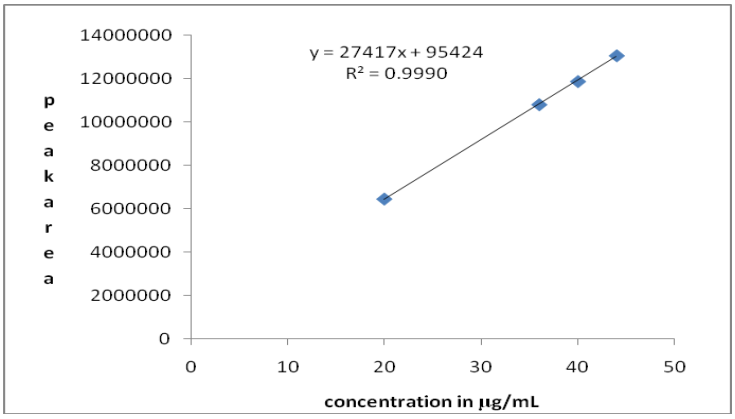

Figure 4.Recovery Plot of Erlotinib

Table 5 Recovery data of Erlotinib

QC conc. (µg/mL) (A)	Recovery level	Added drug (µg/mL) (B)	Peak Area mean ± SD (n=3)	Amount Found ± SD (µg/mL)	%Recovery	%RSD
20	80%	16	10968231±148921.5	35.76±0.306	99.33	1.3
	100%	20	11863639±71344.43	39.81±0.343	99.94	0.6
	120%	24	12778858±96105.9	44.22±0.366	100.5	0.7

Assay:

20 tablets of Erlocip (25mg) were weighed accurately weigh tablet powder equivalent to 10mg of API. Extract with few ml of methanol and make up the volume to 10ml.The solution was sonicated for 15min and filtered using 0.45mm filter. From this 1ml was taken into a 10ml flask and made up to the mark by mobile phase to obtain 100mcg/ml. Pipette out 2ml and make up to 10ml with mobile phase and inject the sample(n=3) and measure the peak area. The amount found and % recovery were calculated and shown in the Table 6.

Table 6 Assay of Formulation

Formulation	Peak Area mean ± SD(n=3)	Amount found ±SD	%Recovery	%RSD
Erlocip(25mg)	6565105± 54895.54	25.312±0.358	101.25	0.83

LOD and LOQ:

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations designated by International Conference on Harmonization (ICH) guidelines[18].

$$LOD = 3.3 \times \sigma/S$$

$$LOQ = 10 \times \sigma/S$$

Where,

σ = the standard deviation of the response and S = slope of the calibration curve

Robustness:

The robustness of the method was evaluated by analyzing the system suitability standards and evaluating system suitability parameter data after varying the HPLC pump flow rate(±0.2%), p^H(±0.2%), and mobile phase 60: 40(±2%). Results were tabulated in the Table 7.

Table 7 Robustness data of Erlotinib hydrochloride

S.No	Parameter	Optimized conditions	Modification	Retention time (R_t)	Asymmetry
1	Flow rate	1mL/min	1.2mL/min	6.533	0.992
			0.8mL/min	8.620	1.12
2	Mobile phase composition	58:42	60:40	7.467	0.993
			56:44	7.902	0.997
3	p^H	3.2	3.00	7.587	0.94
			3.40	7.652	0.99

STRESS DEGRADATION STUDIES:

The drug was subjected to acidic, alkaline, oxidative, thermal, and photolytic degradations. The drug was found to be stable to thermal and photolytic conditions. The drug was found to be degraded in acidic, alkaline, oxidative conditions. The results of these studies indicates that no interference from excipients, impurities and degraded products due to various stress conditions and assured that the peak response was due to a single component only.

Acid Degradation

Accurately weigh tablet powder equivalent to 10mg and transfer in to a 10mL dry volumetric flask. Pipette out 5mL from the above solution in to a 50mL dry volumetric flask and make up to the mark with 5N HCl sonicate for 5min.Keep aside for 24hrs. After 24hrs 5mL is withdrawn and the sample is neutralized by using NaOH and made up to the mark by mobile phase. Filter the solution through 0.45µm filter and inject. The chromatogram was shown in the Figure 5.

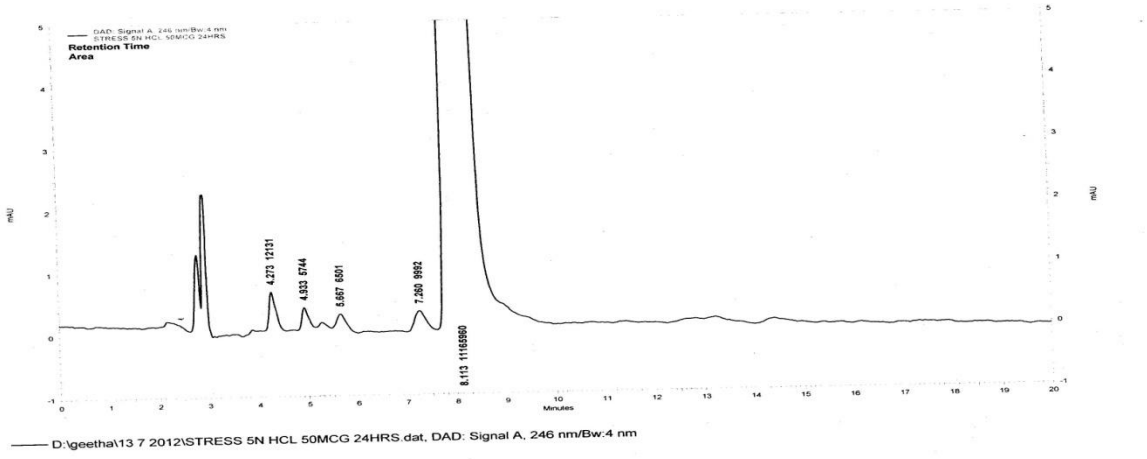

Figure 5 Chromatogram of 5N HCl degraded sample

Alkaline Degradation:

Accurately weigh tablet powder equivalent to 10mg and transfer in to a 10mL dry volumetric flask. Pipette out 5mL from the above solution in to a 50mL dry volumetric flask and make up to the mark with 10N NaOH, sonicate for 5min.Keep aside for 24hrs. After 24hrs 5mL is withdrawn and the sample is neutralized by using HCl and made up to the mark by mobile phase. Filter the solution through 0.45µm filter and inject in to the HPLC system. The chromatogram was shown in the Figure 6.

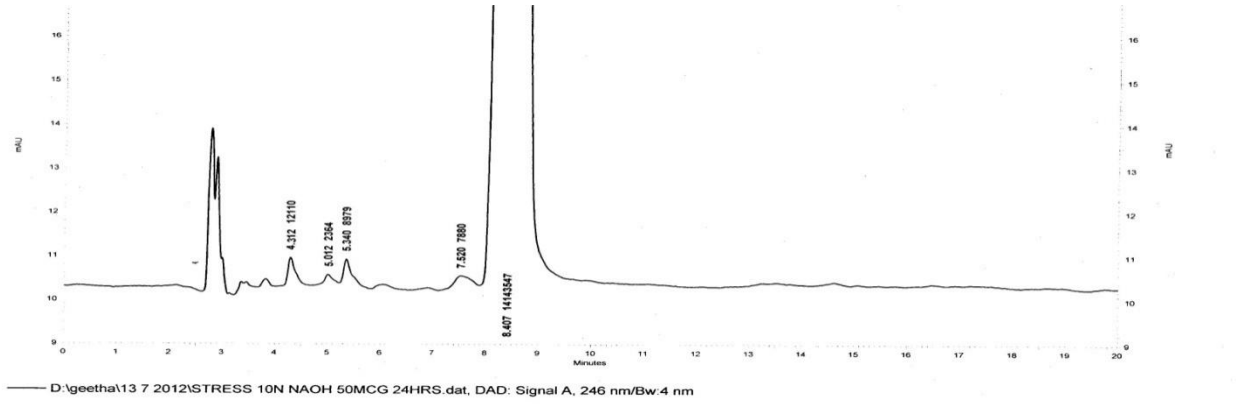

Figure 6 Chromatogram of 10N NaOH degraded sample

Oxidative Degradation:

Accurately weigh tablet powder equivalent to 10mg and transfer in to a 10mL dry volumetric flask. Pipette out 5mL from the above solution in to a 50mL dry volumetric flask and make up to the mark with 10%H_2O_2, sonicate for 5min.Keep aside for 24hrs. After 24hrs 5mL is withdrawn and the sample is neutralized by using HCl and made up to the mark by mobile phase. Filter the solution through 0.45µm filter and inject in to the HPLC system. The chromatogram was shown in the Figure 7.

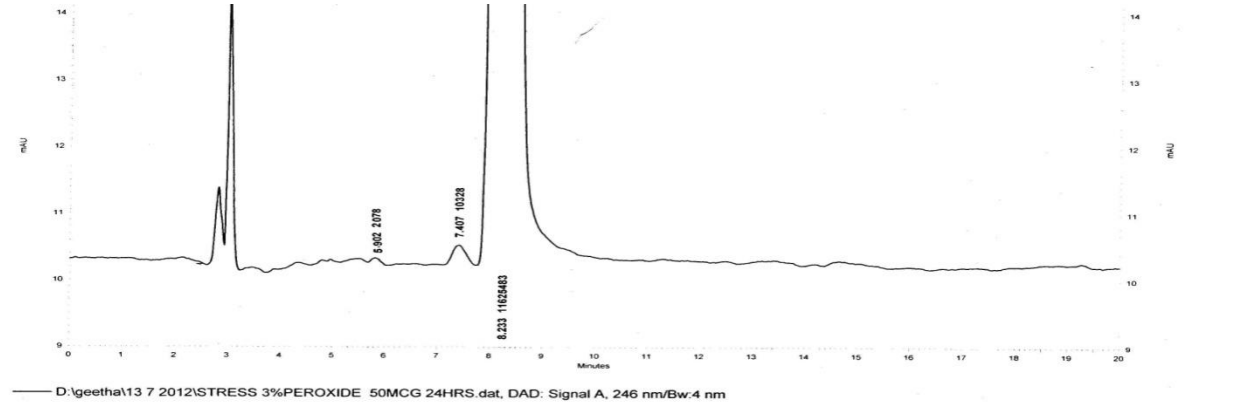

Figure 7 Chromatogram of 10%peroxide degraded sample

Thermal Degradation:

Solid:

100mg of the drug was weighed and exposed to a temperature of 70^0C in hot air oven.10 mg of the drug was taken and then made up to the volume to obtain a conc. of 1000µg/mL. From this 100µg/mL solution was prepared and final concentration was prepared and injected. The analysis was done for 72hrs.

Liquid:

Accurately weigh tablet powder equivalent to 10mg and transfer in to a 10mL dry volumetric flask. Pipette out 5mL from the above solution in to a 50mL dry volumetric flask and make up to the mark with water, sonicate for 5min. These solutions were exposed to 60^0C in water bath and 2mL was withdrawn made to the final volume and injected. The analysis was done for 72hrs.

Photolytic Degradation:

Solid:

100mg of the drug was weighed kept in a petridish and exposed to the UV light. The drug was withdrawn and diluted to suitable concentration with the mobile phase at different intervals like 0hr, 4hr, and 12hr up to 48hrs.

Liquid:

Accurately weigh tablet powder equivalent to 10mg and transfer in to a 10mL dry volumetric flask. Pipette out 5mL from the above solution in to a 50mL dry volumetric flask and make up to the mark with water, sonicate for 5min.Now place the solution in a petri dish and expose to the UV light.2mL of the sample was withdrawn made to the final volume and injected in to HPLC system. The summary of Degradation studies were tabulated in Table 8.

Table 8 Summary of degradation Studies

S .No	Stress condition	Duration (hrs)	Result	No. of degradants
1	Acidic degradation (5N HCl)	24	Unstable (19.79% degraded)	5
2	Alkaline degradation (10N NaOH)	24	Unstable (17.02% degraded)	4
3	Oxidative degradation (10%H_2O_2)	24	Unstable (14.68% degraded)	2
4	Thermal degradation			
	(i) Liquid	72	Stable	--
	(ii)Solid	72		
5	Photo degradation			
	(i) Liquid	72	Stable	--
	(ii)Solid	72		

CONCLUSION

An accurate, precise, economic stability-indicating RP-HPLC method has been developed and validated for the analysis of Erlotinib HCl in bulk and tablet dosage forms. The results of stress testing reveal that the method is specific and stability indicating. The proposed method has the ability to separate the analyte from their degradation products, related substances; excipients found in bulk and tablet dosage forms and can be applied to the analysis of samples obtained during stability experiments.

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