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Development and Validation of A Stability Indicating HPTLC Method For Determination of Adapalene In Bulk Drug

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ABSTRACT

To develop a new, economical, precise and accurate stability indicating HPTLC method was developed and validated for the determination of Adapalene in bulk drug. The present study deals with development and validation of stability indicating HPTLC method for estimation of Adapalene. Chromatographic separation was performed on aluminium plate pre-coated with Silica Gel 60 F_{254} using Tetrahydrofuran: 2-Propanol: Water (3:3:3 v/v/v) as a mobile phase. The wavelength selected for densitometric scanning was 230 nm. Regression plots revealed linear relationship in the concentration range of 20-120 ng $spot^{-1}$. The R_f value of Adapalene was found to be 0.76 (±0.02).The LOD and LOQ were found to be 3.15 and 9.57 ng $spot^{-1}$respectively. The method was validated as per International Conference on Harmonization (ICH) guidelines, demonstrating to be accurate and precise within the corresponding linearity range of titled analytes. Inherent stability of the drug was studied by exposing drug to acid, alkali, oxidative, photolytic and thermal conditions. Relevant degradation was found to take place under these conditions. The proposed method has been validated as per ICH Q2 (R1) guidelines. This method can be used for routine quality control analysis of Adapalene in bulk drug.

Keywords: Validation, Adapalene, HPTLC, Stability indicating method.

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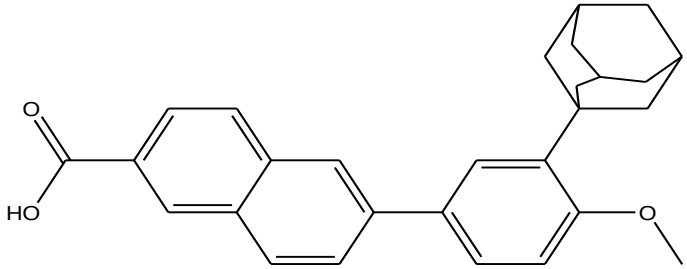
INTRODUCTION

Adapalene is a synthetic naphthoic acid derivative with retinoid activity. The chemical name of Adapalene is 6-[3-(1-adamantyl)-4-methoxyphenyl]–napthalene-2-carboxylic acid (Figure. 1). Adapalene is a white to off-white powder which is soluble in Tetrahydrofuran, sparingly soluble in ethanol, and practically insoluble in water. The molecular formula is $C_{28}H_{28}O_3$ and molecular weight is 412.52 g mol^{-1}. [1-3].



6-[3-(1-adamantyl)-4-methoxy-phenyl] naphthalene-2-carboxylic acid

Figure 1: Chemical Structure of Adapalene

Literature survey revealed few Spectrophotometric, RP-HPLC, HPLC, Flourimetric and LC-MS/MS methods for the determination of Adapalene either as single or in combination with other drug [4-9]. But, to the best of our knowledge, a simple, specific and economical method for estimation of Adapalene by HPTLC for routine laboratory analysis is not yet reported. So the aim of the present work was to develop and validate stability indicating HPTLC method for determination and quantitative estimation of Adapalene.

MATERIALS AND METHOD

Instrumentation

Pre-coated silica gel 60 F_{254} aluminium plates (10 x 10 cm, 250 µm thickness; Merck, Germany), Automatic TLC sampler 4 (Camag, Switzerland), twin trough chamber (10 x 10 cm Camag, Switzerland), UV chamber (Camag, Switzerland), TLC scanner 4 (Camag, Switzerland), win CATS version 1.4.6 software (Camag, Switzerland) were used in the study. Ultrasonic bath and Electronic Balance Shimadzu AX200, were used in the study.

Chemicals and reagents

Tetrahydrofuran and 2-Propanol were used of analytical grade. Adapalene was obtained as gift sample from Glenmark Pharmaceuticals, Mumbai, India. Adapen gel (Intas Pharmaceuticals) was purchased from local medical store, containing 0.1% Adapalene.

Experimental

Preparation of standard solutions

For preparation of standard stock and working standard solutions Adapalene (10 mg) was weighed accurately and transferred into a 10 ml volumetric flask and dissolved in THF. Then mixture was sonicated for 20 min. Volume was made up to the mark with THF to give the concentration of (100 ng $spot^{-1}$).

Prewashing of plates

Densitometric estimation was carried out on (20 × 10) pre-coated silica gel 60 F_{-254} plates from E. Merck. The plates were pre-washed with methanol, dried and activated for 30 min at 110°C.

Selection of solvent

Tetrahydrofuran was selected as a solvent for preparing drug solutions.

Selection of stationary phase

Identification and separation of Adapalene was carried out on (20 cm × 10 cm), pre-coated silica gel aluminium plates 60 F_{-254} (250 µm thickness E. Merck, Darmstadt, Germany).

Application of standard solutions

The standard and working standard solution of Adapalene were spotted on pre-coated TLC plates in the form of narrow bands of length 10 mm from the bottom and left margin and 10 mm distance between two bands. Samples were applied under continuous drying stream of nitrogen gas at constant application rate of 150 nl s^{-1}.

Selection of wavelength

Evaluation was performed by linear regression of peak areas determined by UV absorption as a function of sample analysis at 230 nm using Tetrahydrofuran as a blank solution. The detection wavelength was selected at 230 nm and the spectrum of the drug is depicted in (Figure. 2).

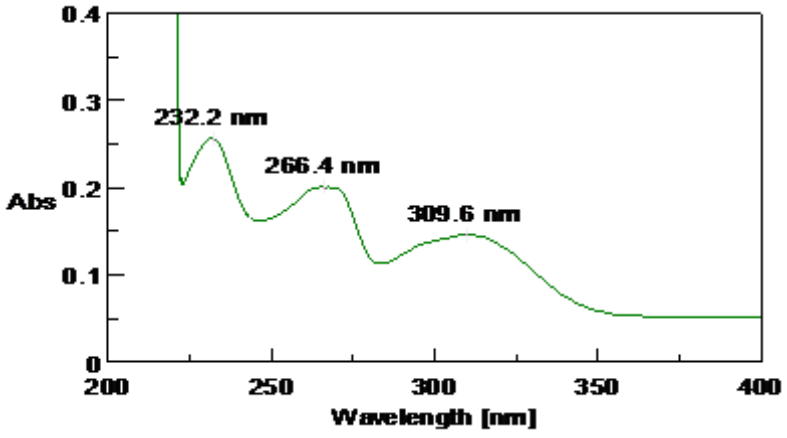


Figure 2: Spectrum for selection of wavelength (232 nm)

Selection of mobile phase

A trial and error method was used to optimize the mobile phase on which different mobile phase were tried. The solvent system of Tetrahydrofuran: 2-Propanol: Water in the ratio 3:3:3 was the most appropriate mobile phase for the HPTLC analysis of Adapalene.

Development of spot

Twin trough chamber containing 9 ml of mobile phase system was used for developing the spotted plate and saturated for 30 min. The plate was dried after development and viewed under UV lamp to evaluate the spot obtained. The spot were uniform and there was no tailing.

METHOD A: METHOD VALIDATION

The method was validated for linearity, accuracy and intra-day and inter-day precision, in accordance with ICH guidelines Q2 (R_1) [10-12].

Linearity

The appropriate volume of standard solution was spotted on a TLC plate to cover the range of 20-120 ng $spot^{-1}$. The analytes were resolved under optimized chromatographic conditions, and the standard calibration graph of peak area versus concentration was plotted. The whole procedure was repeated thrice starting from weighing of analytes to preparation of standard solution as described under standard solution preparation. The linearity of the method was evaluated by linear regression analysis, using the least square method. The slope and intercept were calculated.

Accuracy (Recovery study)

Recovery studies were performed by the standard addition method where known amount of standard substance were spiked to the analyzed dosage form in triplicate. The resulting mixtures were analyzed by the developed method. The amount of Adapalene was estimated by substituting values in the regression equation. The % RSD (relative standard deviation) and the mean recovery were calculated.

Precision

The precision of proposed analytical method was demonstrated by repeatability (Intraday) and intermediate (Interday) precision studies. The intra and inter-day variations were determined using three different concentration levels 40, 80 and 120 ng $spot^{-1}$of Adapalene (n = 3). The precision of the developed method was evaluated by performing repeatability of the sample application and peak area measurement in six replicates of the same spot. The results are expressed in terms of percent relative standard deviation (% RSD) and standard error (SE).

Method sensitivity (Limit of detection and limit of quantification)

The LOD and LOQ of the developed method were calculated using the regression equation. A series of standard preparation containing 20-120 ng $spot^{-1}$ were prepared over different levels. Calibration graphs were plotted for the obtained area under the curve of each level against concentration. The LOD and LOQ were calculated using the equations, LOD =3.3 x σ/S and LOQ =10 x σ/S, respectively where σ is the standard deviation of the y-intercept and S is the slope of the calibration curve. Thus, obtained LOD and LOQ values were further confirmed by applying different volumes of stock solution 10 µg ml^{-1} of Adapalene in three replicate separately on a TLC plate and % RSD values were calculated.

Specificity

The specificity of the method was ascertained by analysing the R_f values and spectra pattern of standard drug and formulation. The marketed formulation, Adapen gel BP (0.1 % w/w) from Intas Pharmaceuticals Ltd was sonicated (1 mg in 10 ml Tetrahydrofuran) for 20 min. The volume was made up to 100 ml by adding Tetrahydrofuran. The resulting solution was centrifuged and the supernatant was filtered. The amount of Adapalene was determined by developing the chromatogram (100 ng $spot^{-1}$) in triplicate by maintaining the chromatographic conditions. The spot for Adapalene in formulation was confirmed by comparing the R_f and densitogram of the spot with that of standard.

Robustness

The robustness of an analytical method is its capacity to remain unaffected by small but deliberate variations in method parameters, like the composition of the mobile phase, the volume of the mobile phase, time from spotting to development, and time from development to scan were evaluated in this study. One factor at a time was changed and the effect on the R_f and the peak area of the drug was studied.

METHOD B: METHOD STRESS DEGRADATION

The study was intended to ensure the effective separation of Adapalene and their degradation peaks. Forced degradation studies were performed to evaluate stability indicating properties and specificity of the method. The objective of stress study was to generate the degradation products under various stress conditions and to verify that the degradation peaks are well resolved from the main peaks by the developed method [13-16].The stock solution was prepared separately in Tetrahydrofuran that contains 100 µg ml^{-1} of Adapalene. This solution was subjected to stress degradation such as acidic (0.1 N HCl), alkaline (0.1 N NaOH), oxidative (3% H_2O_2) for 6 h, photolytic degradation for 24 h, and thermal conditions separately as per the procedures described below. For thermal and photolytic degradation, the solutions were prepared using 10 mg of

exposed sample. The resulting solutions were applied on TLC plates, and the chromatograms were run under the conditions described above.

Acid and base induced degradation

To 5 ml solution of Adapalene 10 ml of (0.1 N HCl) and (0.1 N NaOH) were added separately. These mixtures were refluxed separately for 0 h to 6 h at 80°C respectively. 1 µl (100 ng $spot^{-1}$of ADA) of resultant solutions were applied on TLC plate and developed.

Hydrogen peroxide induced degradation

To 5 ml solution of Adapalene, 10 ml of 3% H_2O_2 was added. This solution was heated in boiling water bath for 20 min to remove completely the excess of hydrogen peroxide and refluxed for 4 h at 80°C. 1 µl (100 ng $spot^{-1}$of ADA) of resultant solutions were applied on TLC plate and developed.

Dry and Wet heat induced degradation

Dry heat degradation of Adapalene was carried out by placing the bulk drug into a hot air oven at 80ºC for 8 h.10 mg of Adapalene powder was spread as a thin layer on petri dish (50 mm diameter). The petridish was heated in an oven at 80 °C for 8 h. For wet heat induced degradation, 1 ml of working standard solution of Adapalene, 5 ml of HPLC grade water was added. The solution was refluxed at 60 °C for 6 h. The resultant solutions were applied on TLC plate and developed.

Photolytic degradation

10 mg of Adapalene powder was spread as a thin layer on a petri dish (50 mm diameter). The first set of petridish was exposed to direct sunlight for 24 h in chamber.

RESULTS AND DISCUSSION

Development and optimization

For the selection of an appropriate mobile phase for the effective separation of ADA, several runs were made by using mobile phase containing solvents of various polarities, at different concentration levels. Various solvent systems like mixture of (a) Water: THF (5: 5 v/v) (b) Water: THF (7: 3 v/v) (c) Water: THF: acetic acid (5:5:5 v/v/v) and (d) THF: 2-Propanol (7: 3 v/v) were tried to separate and resolve spot of ADA from its impurities. The mixture of THF: 2-propanol: Water (4:3:3 v/v) resolved ADA but there was tailing in the peaks. To improve peak symmetry, the ratio was changed and spots were observed. Finally, the mixture of THF: 2-propanol: Water (3:3:3 v/v/v) showed well resolved peak with better peak shape. The drug was resolved with R_f value of

0.76 ± 0.02. Pre-saturation of TLC chamber with mobile phase for 35 min assured better reproducibility in migration of ADA and better resolution (Figure. 3).

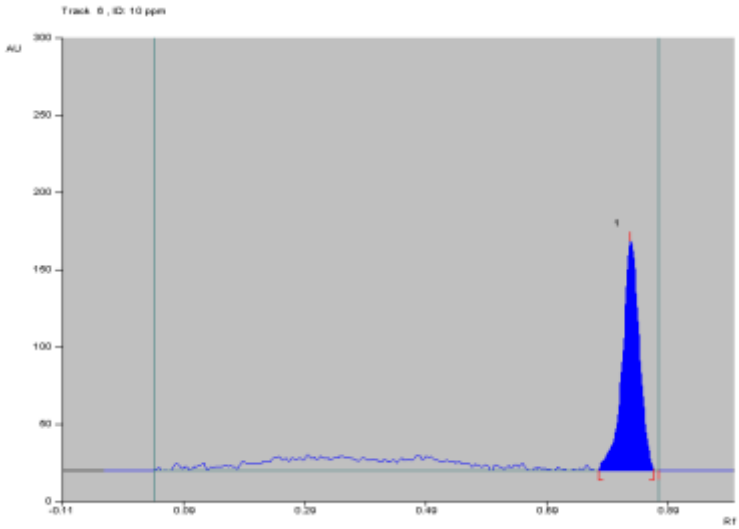


Figure 3: Densitogram of Adapalene (standard)

Validation of developed stability indicating method

Linearity

Aliquots of standard solution were applied in the concentration range of 20-120 ng spot^{-1}and densitogram was developed under above optimized condition. Linearity curve as shown in the (Figure 4-5).

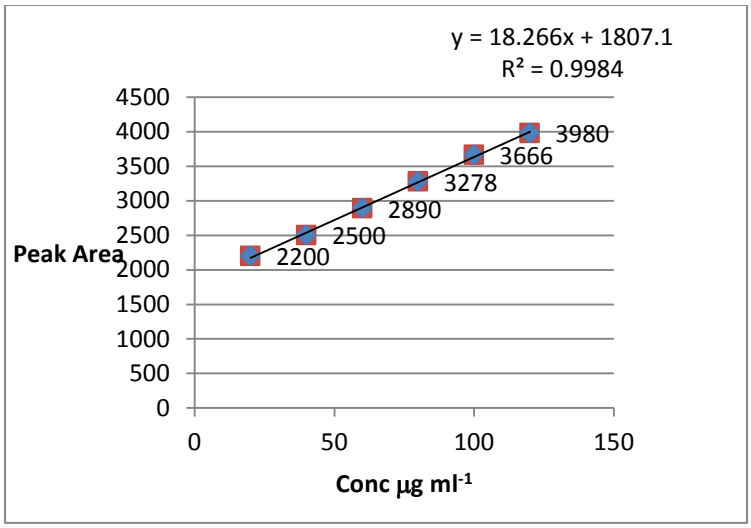


Figure 4: Calibration curve of Adapalene

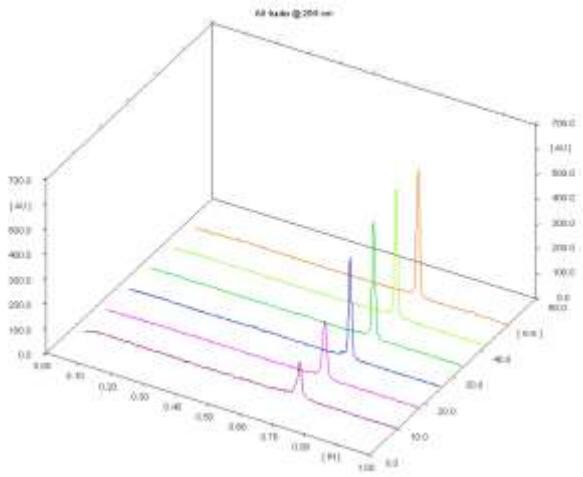


Figure 5: 3D Densitogram of linearity of Adapalene

The calibration curve was plotted as the concentration of the drug versus the mean of the response at each level. The proposed method was evaluated by its correlation coefficient and intercept value calculated in the statistical study and were represented by a linear regression equation. For Adapalene, the linear regression equation was y = 18.26x + 1807. The correlation coefficient of Adapalene was found to be R^2 = 0.998.

Precision

For Adapalene, the intra-day and inter-precision were conducted under developed chromatographic conditions three times using 40, 80, 120 ng spot^{-1}concentrations. The method was found to be precise by exhibiting percentage estimation of about 100 % and RSD less than 2 % (Table 1).

Table 1: Precision results for Adapalene

Conc ng spot^{-1}	Intraday precision (n=3)		Interday Precision (n=3)	
	Mean ±SD	% RSD	Mean ±SD	% RSD
40	2496.0 ± 4.29	0.179	2488 ± 4.12	0.165
80	3182.3 ± 1.78	0.038	3180 ± 2.77	0.098
120	3932.6 ± 4.09	0.104	3945 ± 2.44	0.061

SD= Standard Deviation, % RSD= Relative Standard Deviation

Limit of detection and limit of quantification

The minimum amounts detected by the developed chromatographic conditions were estimated in terms of LOD. By area LOD was found to be 3.15 ng spot^{-1}. Lowest possible quantity to be quantified by the proposed method was found to be 9.57 ng spot^{-1}.

Accuracy

The accuracy of the developed method was established by standard addition method by adding known standard concentration solutions to the pre-analyzed samples in different levels i.e 80, 100

and 120 %. The samples were analyzed for three times at each level. The mean recovery and RSD values were calculated. Recoveries of Adapalene were in between 98-102 % (Table 2). This is in accordance with the ICH guidelines. Hence method was found to be accurate.

Table 2: Accuracy results for Adapalene

Spike level (%)	Amount added (ng spot^{-1})	Amount recovered(ng spot^{-1})	% Recovery
80	180	179.3	99.44
100	200	202.4	101.00
120	220	223.3	101.03

SD= Standard Deviation, % RSD= Relative Standard Deviation

Robustness

The robustness of an analytical method is its capacity to remain unaffected by small but deliberate variations in method parameters, like the composition of the mobile phase, the volume of the mobile phase, time from spotting to development, and time from development to scan were evaluated in this study. One factor at a time was changed and the effect on the R_f and the peak area of the drug was studied. The % RSD was calculated (Table 3).

Table 3: Robustness results for Adapalene

Parameters	SD	SE	%RSD
Mobile phase composition (±) 0.1ml	7.261	1.2	1.230
Amount of mobile phase (±) 0.1ml	8.136	1.3	1.405
Temperature (±) 5ºC (During Sonication)	9.960	1.6	1.772
Time from spotting to chromatography (±) 5 min	9.031	1.5	1.548
Time from chromatography to scanning (±) 5 min	10.48	1.7	1.797

SD= Standard Deviation, SE= Standard Error, % RSD= Relative Standard Deviation

Table 4: Summary of Validation parameters

Parameter	Adapalene
Linearity range (ng spot^{-1})	20-120
Correlation co-efficient 0.998	
Slope (m)	18.26
Intercept (c)	1807
Precision (intraday) % RSD	0.038
Precision (interday) % RSD	0.098
Accuracy (mean % recovery)	100.53
LOD (ng spot^{-1})	3.15
LOQ (ng spot^{-1})	9.57
Robustness	Robust
Specificity	Specific

Forced degradation studies

The results of forced degradation studies are summarized in (Table 5). Under the optimized chromatographic conditions degradation products of analytes were well resolved and the percent degradation was calculated by area normalization technique (Figure 6-10).

Table 5: Forced degradation results

Sr.No	Stressed Condition	Adapalene		Degradation Product		Figure
		%	R_f	%	R_f	
1	Acid, 10 ml (0.1 N HCl reflux for 6 h at 80ºC)	82.36%	0.76	17.64%	0.46, 0.83	Fig. 6
2	Base, 10 ml (0.1 N NaOH reflux for 6 h at 80ºC)	88%	0.76	12%	0.49, 0.79, 0.79, 0.86	Fig. 7
3	Hydrogen peroxide, 10 ml, 3% v/v H_2O_2(reflux for 1 h at 80ºC)	91.63%	0.76	8.37%	0.05, 0.16, 0.45, 0.52, 0.54	Fig. 8
4	Dry heat (8 h at 80ºC)	97.79%	0.76	3.21%	0.02, 0.50	Fig. 9
5	Wet heat (6 h at 60 °C)	100 %	0.76	-------	Not Detected	Fig. 10
6	Photochemical stability (Daylight, 24h)	99.2%	0.76	0.8 %	0.51	Fig. 11

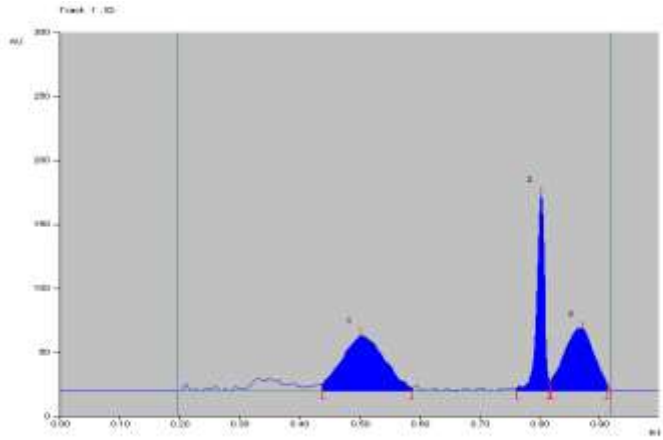


Figure 6: Densitogram of acid degradation condition

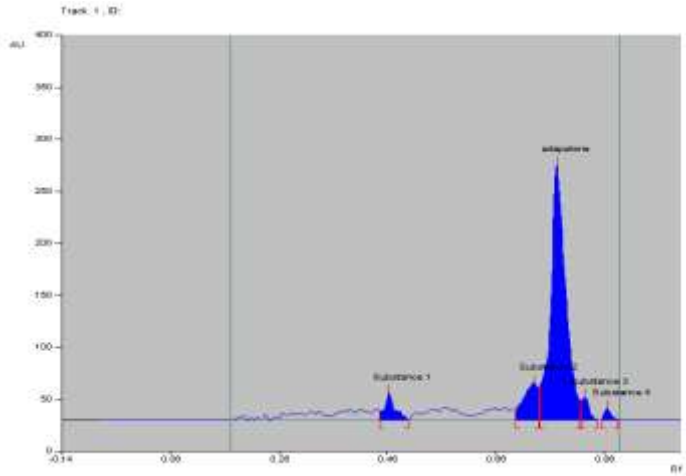


Figure 7: Densitogram of alkaline degradation condition

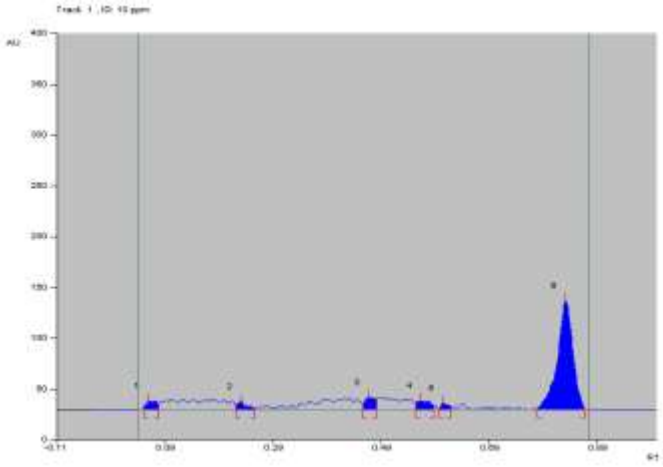


Figure 8: Densitogram of oxidative degradation condition

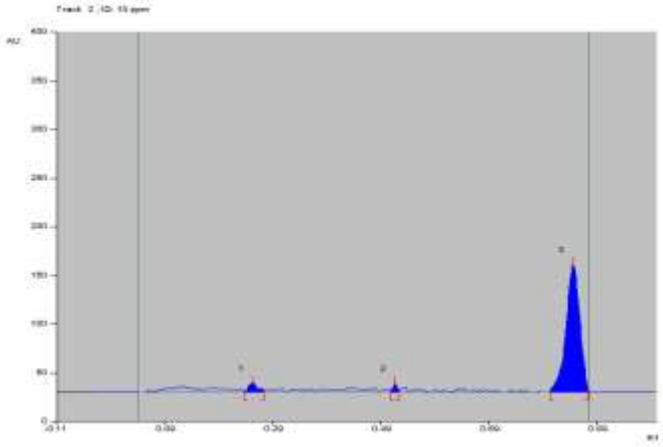


Figure 9: Densitogram of Dry heat degradation condition

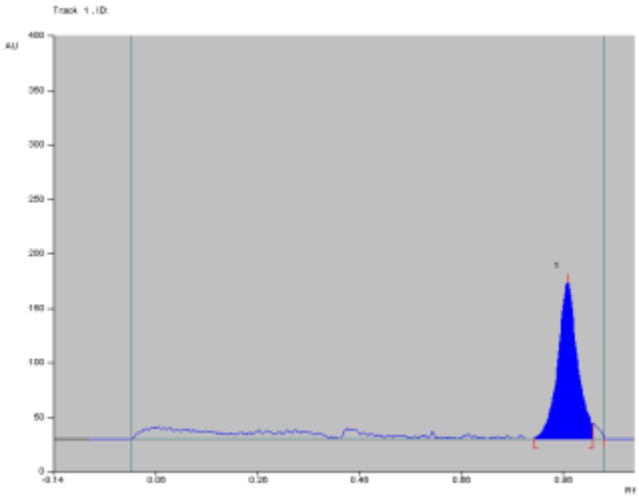


Figure 10: Densitogram of Wet heat degradation condition

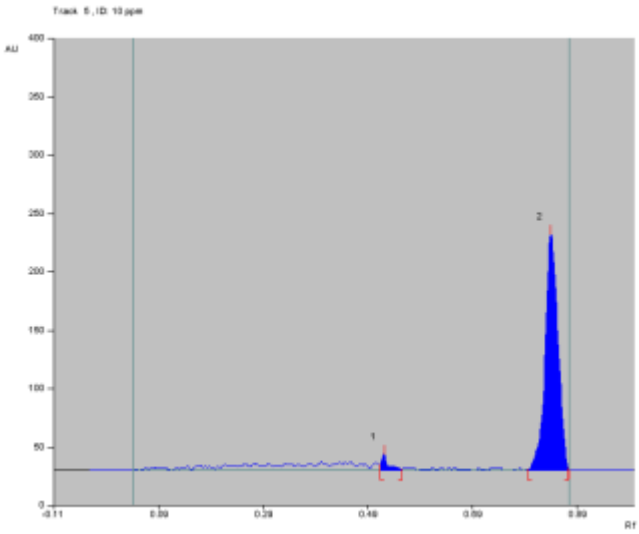


Figure 11: Densitogram of Photolytic degradation condition

During stress degradation experiments, it was observed that Adapalene was more sensitive towards acidic, alkaline and oxidative degradation than compared to photolytic and thermal degradation. The analyte was found quite stable with wet heat and photolytic condition with almost negligible degradation. Overall, Adapalene was found to be more sensitive toward degradation conditions but was more susceptible to alkaline hydrolysis.

CONCLUSION

The HPTLC method was developed on pre-coated silica gel plates using Tetrahydrofuran: 2-Propanol: Water in the ratio 3:3:3 v/v/v as mobile phase with densitometric detection at 230 nm. This study found that stability indicating HPTLC method for determination of Adapalene in bulk form as well as formulation is accurate, precise, linear, highly sensitive, specific and robust. The forced degradation carried out as per recommendations of ICH guidelines shows that Adapalene undergoes degradation to a different extent under different, mentioned stress conditions. The developed HPTLC method was found to be capable to resolve analytes in presence of their degradation products. In the peak purity profile studies, it was confirmed that the peak of the degradation product was not interfering with the response of the analytes. From the above study, we can conclude that the developed method can be employed in the preformulation studies, stability studies and in routine analysis of pharmaceutical dosage form. Thus, the proposed method can be employed in industry and research laboratories.

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REFERENCES

1. www.medicineindia.org
2. Adapalene monograph. European Pharmacopoeia 7.0. 1324–1326.
3. Srinivasan M. High-Performance Thin Layer Chromatography. DOI 10.1007/978-3-642-14025-9, Springer, Heidelberg Dordrecht, London, New York.
4. Suleyman P. and Erol U. A Review of the use of Adapalene for the treatment of acne vulgaris. Therapeutics and Clinical Risk Management.Aug 2007; 3(4):621-624.
5. Khyatik. P, Atul B. RP-HPLC method development and Validation for simultaneous estimation of Nadifloxacin and Adapalene in Bulk and dosage forms. Journal of Pharmaceutical Science and Bioscientific. Research.2016; 6(3): 276-282.
6. M.Mudasir, N.Tabassum, J.Ali. Qualitative and Quantitative estimation by HPLC method in Transdermal formulations-A technical note. Research Journal of Pharmaceutical, Biological and Chemical sciences. 2011; 2(1):231-250.
7. R.Mailvelan, P.Selvamani. HPLC method development and Validation for the estimation of Adapalene in Pharmaceutical formulations. Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry.2013; 1(3):166-171.
8. M.M.Tolba and R.M.El-Gamal. Determination of Adapalene in gel formulation by conventional and derivative synchronous flourimetric approaches. Application to stability studies and *in vitro* diffusion test. Chemistry Central Journal. 2016; 10(33):150-177.
9. Vladimir D, Natasa B. Development and validation of an LC-MS/MS method for the determination of Adapalene in Pharmaceutical forms for skin application. Journal of the Serbian Chemical Society. 2016; 81(0): 1-14.
10. ICH, Q2B. Validation of analytical procedure: Methodology. International Conference on Harmonization, Geneva: 1996; 1-10.
11. International Conference on Harmonization, Validation of Analytical Procedures: Text and Methodology Q2 (R1). Geneva: IFPMA; 2005; 1-20.
12. Huber L. Validation of analytical methods and processes. In: Nash RA, Wachter AH. Editor. Pharmaceutical Process Validation. 3rd Edition, New York: Marcel Dekker, Inc.; 2003; 5-20.

13. Bakshi M, Singh S. Development of validated stability indicating assay methods—critical review. Journal of Pharmaceutical and Biomedical Analysis.2002; 28(10): 11–40.
14. International conference on harmonization, Stability testing of new drug substances and products (revision 2) Q1A (R2). Geneva: IFPMA; 2003; 16-31.
15. Sethi PD. HPTLC: Quantitative analysis of Pharmaceutical formulations. 1st Edition, New Delhi: CBS Publishers and Distributors; 2013; 589-590.
16. Monika B, Saranjit S. Development of validated stability- indicating assay methods-critical review. Journal of Pharmaceutical and Biomedical Analysis.2002; 28:1011- 1040.