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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING ASSAY METHOD AND STRESS DEGRADATION STUDY OF ADAPALENE BY HPTLC METHOD

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ABSTRACT

To develop a new, sensitive, and precise stability indicating high performance thin layer chromatographic method was developed and validated for quantitative determination of Adapalene. The current study deals with development of validated and stability indicating HPTLC method for estimation of Adapalene.Stability study on Adapalene was carried out on hydrolytic conditions such as Acidic, alkaline, oxidative, thermal, photolytic conditions in accordance with International Conference on Harmonization (ICH) guidelines Q1 (R2). The chromatographic separation was performed on aluminium plate pre-coated with silica Gel $60F_{254}$ by the use of Tetrahydrofuran: Ethyl acetate: Methanol (7:2:1 v/v/v) as a mobile phase. The wavelength selected for densitometric scanning was 267 nm. Linearity range for Adapalene was revealed that 100-600 ng $spot^{-1}$. The R_f value of Adapalene was found to be 0.51 (±0.02). The LOD & LOQ were found to be 14.05 and 42.59 ng $spot^{-1}$ respectively. The developed method was precise and robust, % RSD was found less than 2%. The proposed method can be used for routine quality control analysis of pharmaceutical formulation containing Adapalene.

KEYWORDS: Development, Validation, Degradation, Characterization, HPTLC, Adapalene.

INTRODUCTION

Adapalene (Fig 1) is chemically known as the 6 [3 - (1-adamantyl) - 4 - methoxy - phenyl] naphthalene - 2 - carboxylic acid is an anti-acne drug is a third-generation synthetic topical retinoid used to treat acne vulgaris. Acne vulgaris was not cured, but it will control the acne with the use of Adapalene. This results also will be clearly seen after the prolong usage. Treatment with topical retinoids, such as Adapalene.[1-3] Literature survey revealed several analytical method spectrophotometric, RP-HPLC, HPLC, HPTLC, Flourimetric and LC-MS/MS methods for the determination of Adapalene either as single or in combination with other drug.[4-10] So the aim of this work was to develop and validate a new stability indicating HPTLC method for determination and quantitative estimation of Adapalene.

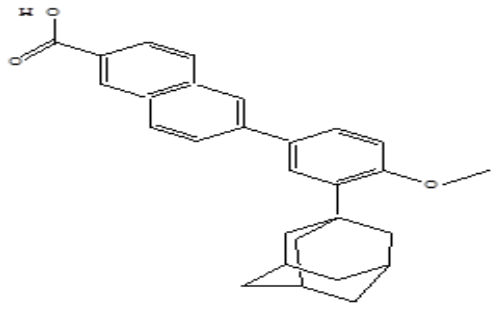

Fig. 1: Structure of Adapalene.

MATERIAL AND METHOD

Materials

Adapalene was obtained as gift sample from Glenmark Pharmaceuticals ltd. Malegaon, Nashik. Adaferin gel (Laboratories Galderma) was purchased from local medical store, containing 0.1% Adapalene. Tetrahydrofuran, Methanol, Toluene, Ethyl acetate were used of HPLC grade.

Chromatographic conditions and Instrumentation

The HPTLC system (CAMAG) consisting a Linomat 5 connected to a nitrogen cylinder, a twin trough glass chamber (10cm×10cm CAMAG) with stainless steel lid,a derivatization chamber, &a plate heater. Pre-coated silica gel 60 F_{254} TLC plates (10×10cm, layer thickness 0.2mm (Merck, Germany) were used as the stationary phase. TLC plates were prewashed with 10 ml of methanol and activated at 80^0C for 10 min prior tosample application. Densitometric analysis was carried out using a TLC scanner IV with WINCATS software. Soniacator and Electronic Balance Shimadzu, were used in the study.

Preparation of standard solutions

For preparation of standard stock solution and working standard solution of Adapalene wasprepared by dissolving 10 mg of pure drug in THF. Then mixture was

sonicated for 20 min. Volume was made up to the 10 ml with THF to get the concentration upto100 µg/ml.

Preparation of Sample Stock Solution

10 mg of adapalene gel were weighed and transferred to 10 mL dried volumetric flask. The solution was sonicating for 10 minutes and filtered through Whatmann filter paper. Volume was made upto 10 ml with THF.

Selection of wavelength

From standard stock solutions of drugs were prepared separately in tetrahydrofuran and scanned over the range of 200-400 nm and spectra was obtained individually. It was observed that adapalene showed considerable absorbance at 267 nm respectively. (Figure.2.)

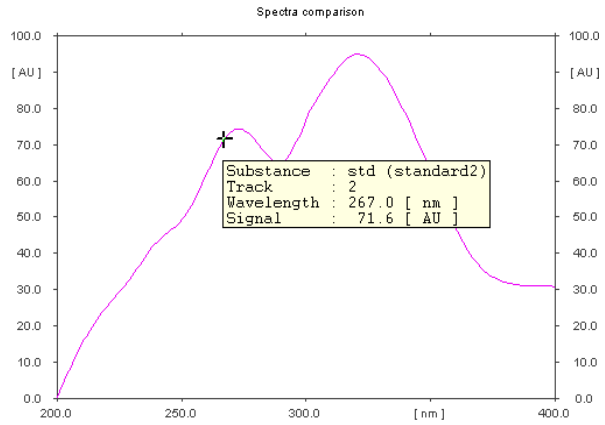

Fig. 2: Spectrum for selection of wavelength (267nm).

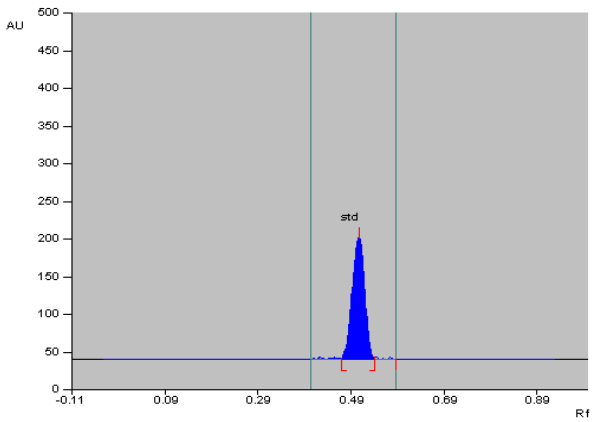

Fig. 3: Densitogram of standard solution of Adapalene 100ng/band.

RESULT AND DISCUSSION

Method Validation

The method validation was carried out for various parameters in accordance with ICH guidelines.[11-13]

Linearity

Aliquots of standard solution were applied in the concentration range of 100-600 ng spot^{-1}. The densitogram was developed under above optimized condition. Calibration curve as shown in the (Figure 4-5). The calibration curve was plotted as the concentration of the drug vs the mean of the response at each level. The linearity of the calibration curve was calculated by linear regression analysis and the statistical data were calculated. The developed method was found to be linear in the range of 100-600 ng/band for Adapalene shown in figure 3. For Adapalene, the linear regression equation was y = 9.18x + 486.99. The correlation coefficient of Adapalene were found to be R^2 = 0.997.

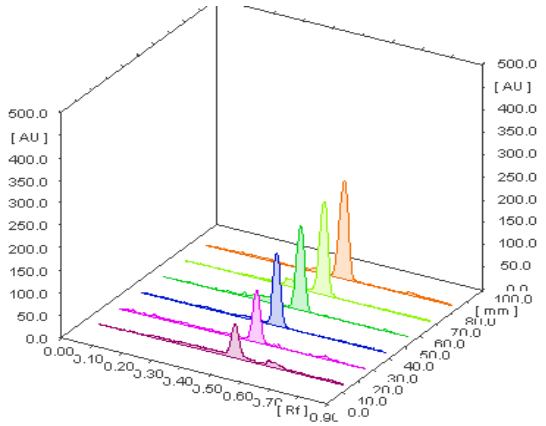

Fig. 4: 3D Spectra of Adapalene using wincats software.

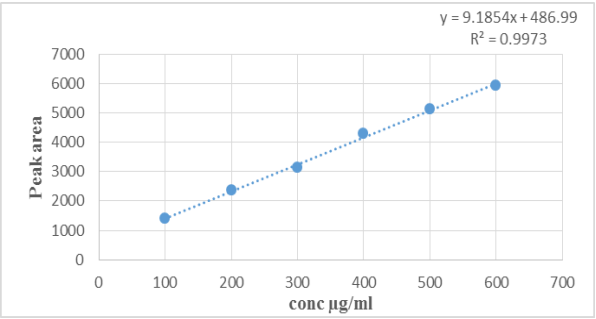

Fig. 5: Calibration curve for Adapalene.

Precision

For Adapalene, the intra-day and inter day were conducted under development chromatographic conditions three times using 400 ng spot^{-1} concentrations. From the obtained data,the method was found to be precise and Adapalene were found to be less than 2%.

Table 1: Intra-day precision.

Sr. No.	Conc (ng band)	Mean Peak area	% RSD
1	400	4305.44	1.30
2	400	4300.02	0.91
3	400	4175.91	0.52

Table 2: Inter-day precision.

Sr. No.	Conc (ng band)	Mean Peak area	% RSD
1	400	4314.19	0.96
2	400	4274.93	0.66
3	400	4256.70	0.88

Accuracy (Recovery study)

The accuracy of the proposed method was established by calculating the recoveries of Adapalene by standard addition method at three concentration levels i.e 80% , 100%, 120%. The samples were analysed by three times at each level. The percentage recoveries of Adapalene were in between range of 98-102% (Table 3). This is in accordance with the ICH guidelines, hence the proposed method was found to be accurate.

Table 3: Accuracy results of Adapalene at 267nm.

Sr. No.	Spike level (%)	Amount added (mg)	Amount recovered(mg)	% Recovery
1	80	8	7.97	99.72%
2	100	10	10.13	101.28%
3	120	12	12.13	101.18%

Method sensitivity (limit of detection and limit of quantitation)

The minimum amounts detected by the developed chromatographic conditions were estimated in terms of LOD. LOD and LOQ were calculated using the regression equation as 3.3*σ/S and 10*σ/S.By area LOD was found to be 14.05 ng/band. Lowest possible quantity to be quantified by the proposed method was found to be 42.59 ng/band.

Robustness

The robustness of an analytical method is its capacity to remain in unaffected by small but deliberate variations in method parameters, like the saturation time of the mobile phase, the amount of the mobile phase were evaluated in this study. Effect of these changes on % drug content was estimated in terms of SD and % RSD. The % RSD were found to be less than 2 which established the proposed method was robust. (Table 4.).

Table 4: Robustness results for Adapalene.

Sr. No.	Parameters	Robust condition	% RSD
1	Mobile phase saturation time (10 min) ± 5min	5 min	1.62
		15 min	1.42
2	Amount of mobile phase (10ml) ± 1ml	9 ml	1.76
		11 ml	1.91

Analysis of Marketed Formulation

The marketed formulation was evaluated by the developed method. The Rf value were found to be 0.51 ±0.02 for Adapalene. The % drug content (Table.5) was calculated and found to be 97 % for Adapalene.

Table 5: Results of analysis of Gel formulation.

Sr.No	Weight of Gel taken (mg)	Amount of pure drug estimated (mg)	% Label claim	Mean % label claim	SD	%RSD
1	10	9.95	101.6%	101.23%	0.99	0.97
2	10	9.90	100.20%			
3	10	9.91	101.33%			
4	10	10.14	100.06%			
5	10	9.46	101.46%			
6	10	9.75	99.90%			

Stress degradation studies

Stress degradation studies were carried on the standard drug to examine stability indicating properties of the developed method. The API were subjected to acid, base and neutral hydrolysis, oxidative, thermal and photolytic degradation to ensure the effective separation of degradation peaks and main peak as per ICH guidelines.

Acid induced degradation

In acidic stress degradation 10 mg of Adapalene were transferred to 10 ml dried volumetric flask and then dissolved in 3 ml of 0.1 N HCL. The mixtures was refluxed at 80^0C for zero to three hours and the volume was made up with THF. The resulting solution was applied on TLC plates and analyzed by developed method.

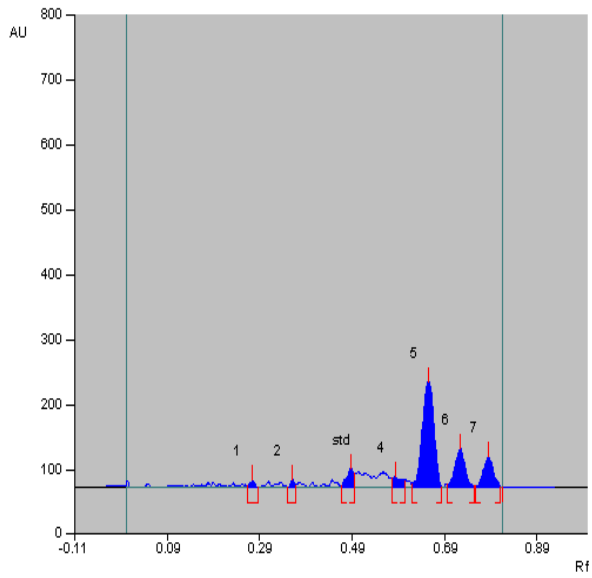

Fig. 6: HPTLC Densitogram of Adapalene in Acidic stress condition.

Base induced degradation

In alkaline stress degradation 10 mg of Adapalene were transferred to 10 ml dried volumetric flask and then dissolved in 3 ml of 0.1 N NaOH and these mixtures were refluxed for 0 h to 3 h at 80^0C the volume was made up with THF. The resulting sample was applied on TLC plate and analyzed by developed method.

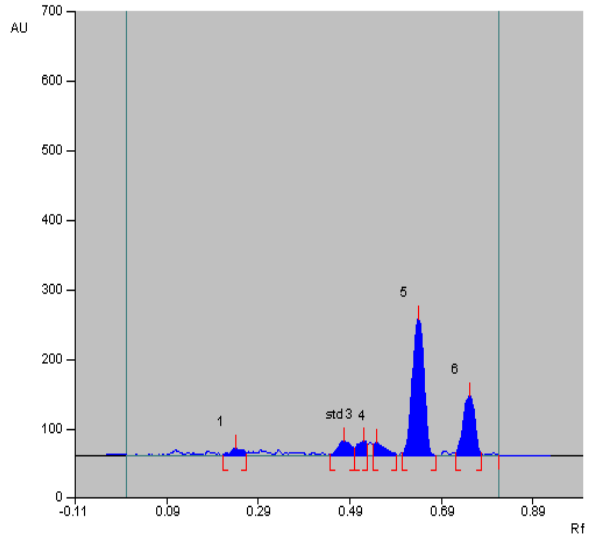

Fig. 7: HPTLC Densitogram of Adapalene in Alkaline stress condition.

Oxidative stress degradation

In oxidative stress degradation 10 mg of Adapalene were transferred to 10 ml dried volumetric flask and then dissolved in 3% H_2O_2 and these mixture were refluxed for 0 h to 3 h at 80^0C the volume was made up with THF. The resulting sample was applied on TLC plate and analyzed by developed method.

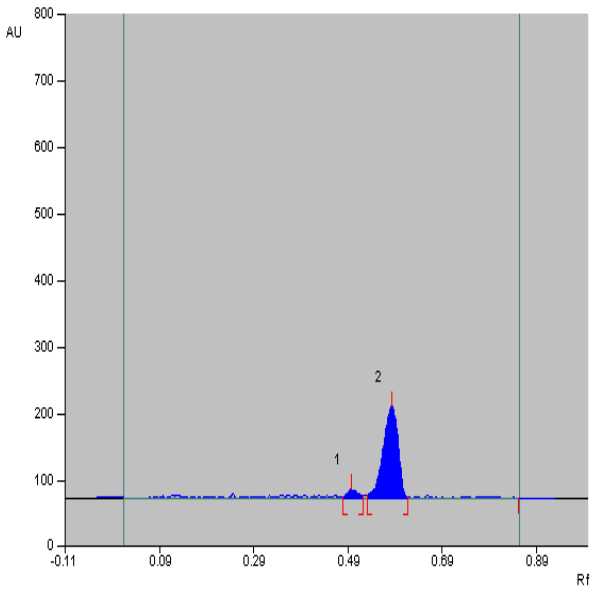

Fig. 8: HPTLC Densitogram of Adapalene in Oxidative stress condition.

Hydrolytic stress degradation

In hydrolytic stress degradation 10 mg of Adapalene were transferred to 10 ml dried volumetric flask and then dissolved in 3 ml distilled water and these mixture were refluxed for 0 h to 3 h at 80^0C the volume was made up with THF. The resulting sample was applied on TLC plate and analyzed by developed method.

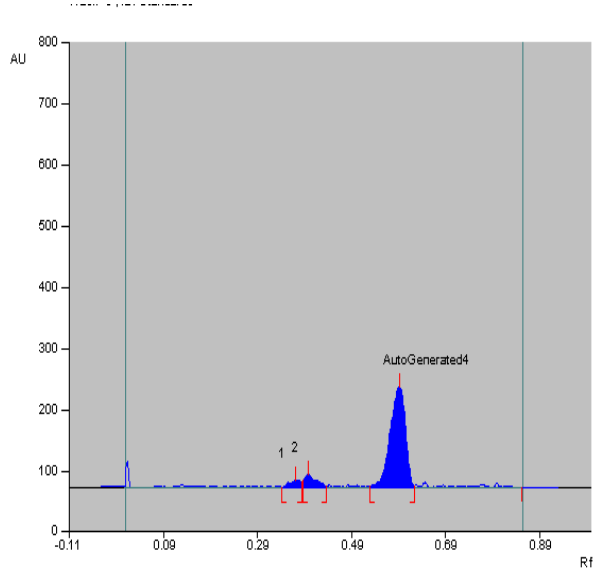

Fig. 9: HPTLC Densitogram of Adapalene in Hydrolytic stress condition.

Photolytic stress degradation

Photolytic stress degradation was analyzed by spreading of Adapalene in a petridish as a thin film separately and kept in ultraviolet chamber having a spectral distribution of 254nm. The volume was made up with THF. The resulting sample was applied on TLC plate and analyzed by developed method.

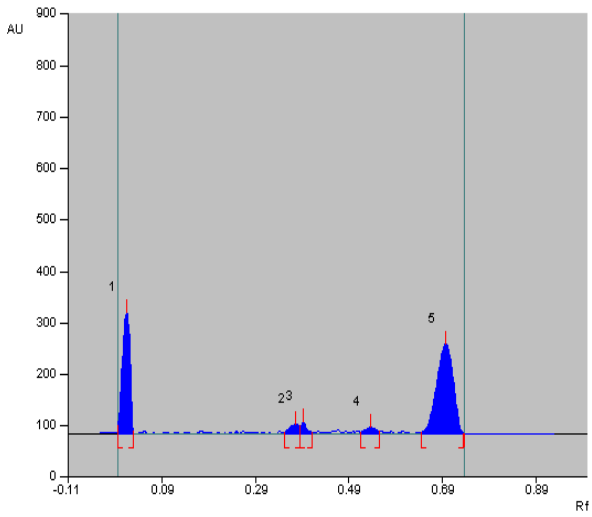

Fig.10: HPTLC Densitogram of Adapalene in Photolytic stress condition.

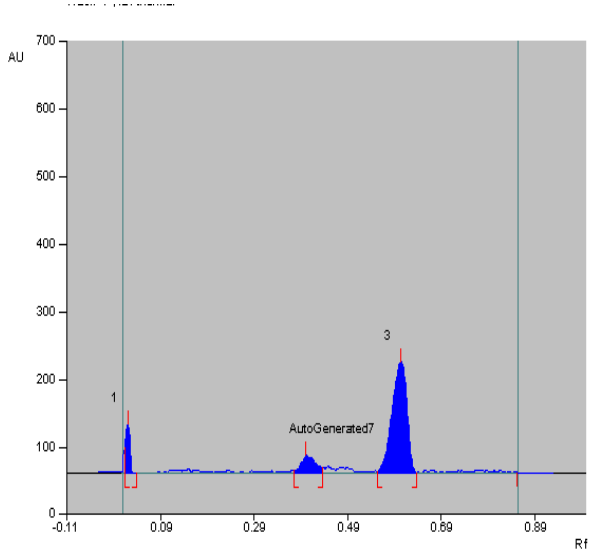

Fig. 11: HPTLC Densitogram of Adapalene in Thermal stress condition.

Thermal stress degradation

Thermal stress degradation was analyzed by spreading in petri dish as a thin film separately and exposed to 80^0C oven in for 30 min. the volume was made up with THF. The resulting sample was applied on TLC plate and analyzed by developed method.

Result of stress degradation Studies

The standard drug were subjected to acid, base and neutral hydrolysis, oxidative, thermal and photolytic degradation as per ICH guidelines. Results of stress Degradation Studies are presented in Table 6. Adapalene was found to be more susceptible to oxidative, acidic and photolytic condition and less susceptible to hydrolytic, thermal and and alkaline stress condition. The peaks of the degradants in each condition were well resolved from main peak (400ng/band). Degradation peaks did not show any interference with the drug peaks. Hence, the method is stability indicating.

Table 6: The results of the stress degradation studies of the Adapalene.

Sr. No	Stress test Conditions	Solvents	Temp.	% Recovery	% Degradation
1	Acidic	0.1 N HCL	80^0C	79.69%	20.31%
2	Alkaline	0.1 N NaOH	80^0C	93.85%	6.15%
3	Oxidative	3% H_2O_2	80^0C	86.63%	13.37%
4	Hydrolytic	Distilled water	80^0C	94.04%	5.06%
5	Thermal	--	80^0C	97.23%	2.77%
6	Photolytic	--	UV Light	83%	17%

CONCLUSION

The HPTLC method was developed and validated for determination Adapalene on pre-coated silica gel HPTLC plates using Toluene: Ethyl acetate: Methanol in the ratio 7:2:1 v/v/v as mobile phase with densitogram detection at 267 nm.This study found that the proposed HPTLC methods well symmetric peaks for Adapalene. Based on the obtained results it is concluded that these method are sensitive, accurate, precise and reproducible. The proposed HPTLC method was also able to selectively quantitate Adapalene in presence of the degradation product obtained in stability study. ICH guideline were followed throughout method validation and the suggested methods can be applied for routine quality control analysis of pharmaceutical formulation containing Adapalene.

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